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EFFECT OF NPK FERTILIZERS AND COMMERCIAL BIOFERTILIZERS ON SOUTHERN BLIGHT DISEASE AND PLANT GROWTH IN CHILI

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Abstract

Sclerotium rolfsii Sacc., a soil-borne fungal pathogen, causes southern blight disease in chili pepper (*Capsicum annum* L.) and results in significant yield losses. Disease is generally managed by application of chemical fungicides which also pollute environment and adversely affect human and animal health. The present study was carried out to manage this disease using two commercial biofertilizers, namely Biopower and Feng Shou in combination with recommended and half doses of NPK fertilizers. The highest disease incidence (74%) and plant mortality (61%) were recorded in positive control where *S. rolfsii* inoculation was done without any other treatment. The two biofertilizers either alone or in combination with two doses of NPK fertilizers significantly reduced disease incidence to 7 - 26%. Likewise, plant mortality was reduced to 0 - 26%. Under biotic stress of *S. rolfsii,* both the biofertilizers significantly enhanced shoot growth over positive control either with or without recommended dose of NPK fertilizers. *S. rolfsii* inoculation significantly reduced chlorophyll and protein contents while enhance peroxidase (PO) and polyphenol oxidase (PPO) activities in chili plants. The two biofertilizers further enhanced PO and PPO activities in non-fertilizers treatments.

Introduction

Chili (*Capsicum annum* L.), a world's famous spice of Solanaceae, is native to America. Worldwide, it is extensively consumed as fresh, dried and fermented source of food ingredients (Madhuri *et al*. 2014). It is the widely grown species and at global level it generates huge revenues for producers and therefore contributes to poverty alleviation (Costa *et al*. 2009). Heat in chili is mainly due to presence of secondary metabolites capsaicinoids in chili which are bioactive valued alkaloids also hold importance pharmacologically (Amruthraj *et al*. 2013). Moreover, chili carotenoids, ascorbic acid (vitamin C), tocopherols (vitamin E), appreciable amount of minerals, lipids, proteins and carbohydrates make it the healthiest food amongst vegetables (Núñez-Ramírez *et al*. 2011, Madhuri and Gayathri 2014). Pakistan is the sixth largest exporter, exporting chilies to several countries including USA, Sri Lanka, Bangladesh, Saudi Arabia, UAE and others (Sahar *et al*. 2013). *Sclerotium rolfsii,* the cause of southern blight disease, has become a major constraint in chili production globally (Dagnoko *et al*. 2013, Madhuri and Gayathri 2014). Generally, sclerotia and mycelium in soil or plant is the main source of infection to other plants. Up till now, *S. rolfsii* is considered a difficult pathogen to be managed by available methods due to extensive host range, prolific growth and resistance sclerotia (Sennoi *et al.* 2013). Chemicals such as pentachloronitrobenzene, mancozeb copper oxychloride, difenconazole, tebuconazole, carbendazim, and flutolanil are effective against *S. rolfsii* (Madhavi and Bhattiprolu 2011, Khan and Javaid 2015). However, these chemical fungicides are not considered appropriate because of persistent nature of the pathogen (Yaqub and Shahzad 2009), and ill effects of fungicides on all other form of life (Dias 2012).

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Use of biofertilizers is the best option of management of diseases to fulfill current demand of safe and healthy food along with concerns on environmental pollution (Mishra *et al.* 2015). Biofertilizers are cheaper than chemical fungicides, self-maintaining with long-lasting effects and also having high antifungal activity (Bhattacharjee and Dey 2014). Biofertilizers generally enhance plant growth by increasing availability of primary nutrients to the host plant. They can also be term as biopesticides if exhibit biocontrol potential against a desire pest such as biofungicides made from *Trichoderma* spp. and bacterial strains of *Pseudomonas* and *Bacillus* (Gupta and Dikshit 2010, Mishra *et al.* 2015). Microbial agents in biopesticides function by predations, competition for available nutrient and space, and by disruption of pest biochemical, genetic or structural functions (Hubbard *et al.* 2014). Various forms of biofertilizers and biopesticides are currently available with different trade names. Bacterial biopesticides are most frequently used and claims about 74% of the total biopesticides market followed by fungal biopesticides (10%) (Thakore 2006). In the present study, two biofertilizers, namely Biopower and Feng Shou were used with and without two doses of NPK fertilizers to manage southern blight disease of chili caused by *S. rolfsii*.

Materials and Methods

Two types of commercial biofertilizers, namely Biopower and Feng Shou were used in the present study. Biopower was procured from National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan. Feng Shou was obtained from local market of Lahore. It was a liquid bacterial culture, manufactured by CV. Green life Bioscience, Bogor Indonesia. Hundred grams of Biopower were mixed in 1 liter of water and roots of chili seedlings were dipped in it for 30 min before transplantation in the pots. In a similar way, inoculum of Feng Shou was prepared by adding its 100 ml in 1000 ml of water, and roots of chili seedlings were dipped in it for 30 min before transplantation.

A greenhouse experiment was carried out using plastic pots (25 cm diameter, 30 cm height) filled with sandy loam soil (4 kg/pot) having pH 7.5, nitrogen 0.046%, exchangeable potassium 100 mg/kg and available phosphorus 6.6 mg/kg. N, P and K fertilizers were procured from local market of Lahore as diammonium phosphate, ammonium nitrate and potassium sulphate. Full dose of fertilizer was 63 : 57 : 62 kg/ha N : P : K for chili. Recommended (NPK) and half doses (½ NPK) of fertilizers were applied as basal. Chili var. Tatapuri was procured from Ayub Agricultural Research Institute, Faisalabad, Pakistan. Five chili seedlings were transplanted in each pot. Experiment was carried out in a completely randomized deigned in triplicates. Treatments included a negative control without any application, a positive control with *S. rolfsii* (SR) inoculation in unamended soil, Biopower + SR, Feng Shou + SR, ½ NPK + SR, ½ NPK + SR + Biopower, ½ NPK + SR + Feng Shou, NPK + SR, NPK + SR + Biopower, and NPK + SR + Feng Shou.

Total chlorophyll was extracted and was calculated according to the formula of Lichtenthaler and Buschmann (2001). Total soluble protein contents were estimated by the method of Lowry *et al*. (1951), using bovine serum albumin as standard. Polyphenol oxidase and peroxidase tests were carried out according to methods described by Mayer *et al.* (1965) and Kumar and Khan (1982), respectively.

Plants were harvested at maturity. Data regarding disease incidence, plant mortality, shoot and root length as well as fresh and dry biomass were recorded. Shoot biomass included leaves, stems and fruits. Data were subjected to analysis of variance (ANOVA) and means were compared by LSD test using computer software Statistics 8.1.

Results and Discussion

The highest disease incidence (74%) and plant mortality (61%) were recorded in positive control treatment where only *S. rolfsii* (SR) was inoculated**.** Biopower as well as Feng Shou application, either alone or in the presence of half dose (½ NPK) or recommended dose (NPK) of chemical fertilizers significantly reduced disease incidence and plant mortality to 7 - 26%. Difference in disease incidence among the various biofertilizers treatments was insignificant (Fig. 1).

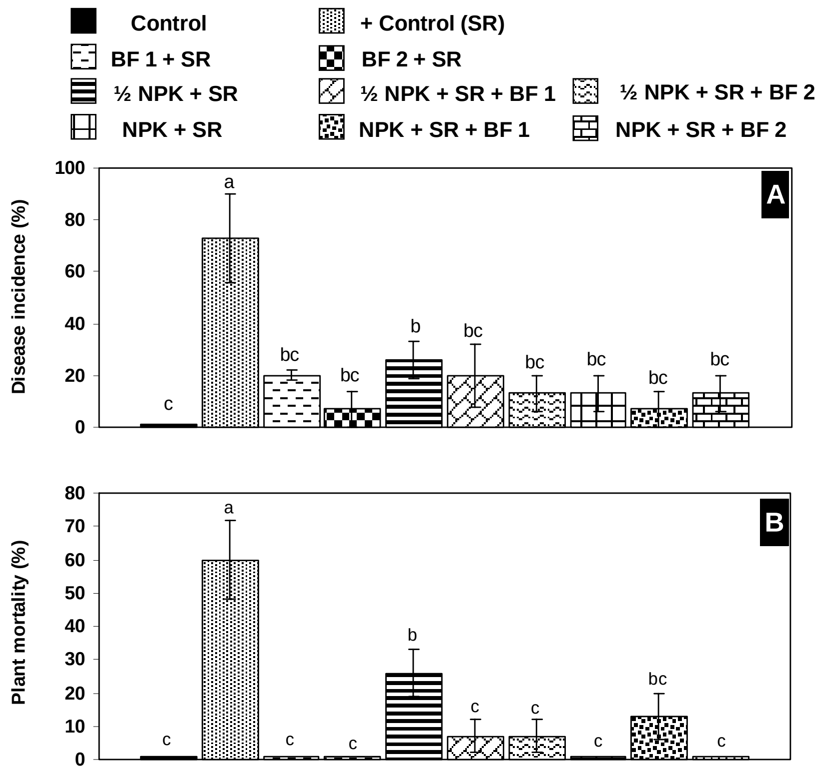

Fig. 1. Effect of NPK fertilizers [recommended (NPK) and half (½ NPK) doses] and biofertilizers (BF) on disease incidence and plant mortality in chili due to *Sclerotium rolfsii* (SR). Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference ($p \leq 0.05$) as determined by LSD test. BF 1: Biopower; BF 2: Feng Shou.

The highest shoot length (54 cm) was recorded in Biopower + SR treatment that was significantly greater than shoot length in positive control, and ½ NPK + SR + Biopower treatments. However, shoot length in different treatments was insignificantly different as compared to positive control treatment (Fig. 2A). *S. rolfsii* inoculation reduced shoot fresh and dry biomass by 30 and 40% over negative control, respectively. Different biofertilizers and chemical fertilizers treatments improved fresh and dry biomass of chili shoot to variable extents. The effect of Biopower + SR on shoot dry biomass was significant with an increase of 73% shoot dry biomass as compared to positive control. In addition, Feng Shou + SR, NPK + SR + Biopower and NPK + SR + Feng Shou treatments also markedly enhanced shoot dry biomass over positive control (Fig. 2B,C).

The effect of various biofertilizers and chemical fertilizers treatments on root length was insignificant as compared to negative as well as positive control treatments. *S. rolfsii* inoculation markedly reduced root dry biomass by 31% over negative control. Root dry biomass in Feng Shou + SR was significantly higher by 114% than that in positive control. The effect of other treatments on root dry biomass was insignificant as compared to negative or positive control treatments (Fig. 3 A-C).

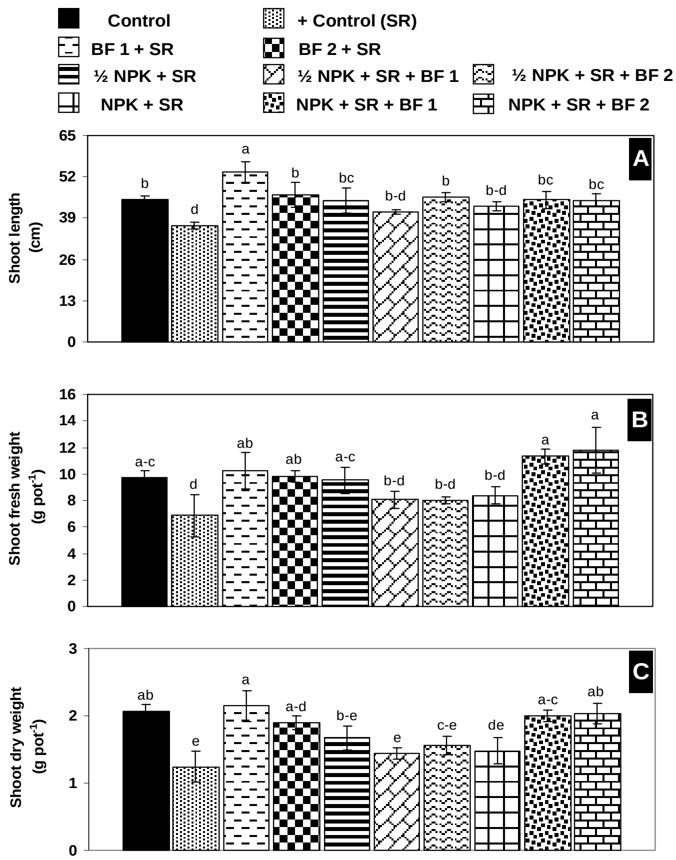

Fig. 2. Effect of *Sclerotium rolfsii* (SR), NPK fertilizers [recommended (NPK) and half (½ NPK) doses] and biofertilizers (BF) on shoot growth of chilli. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference ($p \leq 0.05$) as determined by LSD test. BF 1: Biopower; BF 2: Feng Shou.

A mixture of symbiotic and non-symbiotic bacteria is present in Biopower. These bacteria exhibits potential to produce plant growth hormone and solubilize phosphorus to make available for plant. Feng Shou comprised of combination of *Azospirillum*, *Azotobacter* along with plant growth hormone producing bacteria. Decrease in disease incidence could be attributed to improvement in nutritional supply and by changing in hormone level due to beneficial microorganisms. Besides, induction of systemic resistance in chili plant due to production of antibiosis (4-diacetylphloroglucinol and phenazine-1-carboxylic acid), hydrolytic enzyme (chitinases, dehydrogenase, β-glucanase, lipases, phosphatases, proteases etc.) and siderphores by microorganisms in biofertilizers could be participated to combat against *S. rolfsii* (Gupta *et al*. 2015).

Reduction in disease with improvement in plant growth due to soil fertilization with NPK has already been reported (Siddiqui *et al*. 1999). Positive outcome on plant health and reduction in disease due to combined effect of NPK and biofertilizers might be result of direct effect of NPK in providing fundamental nutrients in a variety of enzymatic, energy transfer and biological processes to chili plant (Farouk *et al*. 2007) and indirect effect of microorganisms on chili growth through enhancement in levels of enzymes, important to photosynthesis and metabolism along with suppression of *S. rolfsii* (El-Sherbeny *et al*. 2007).

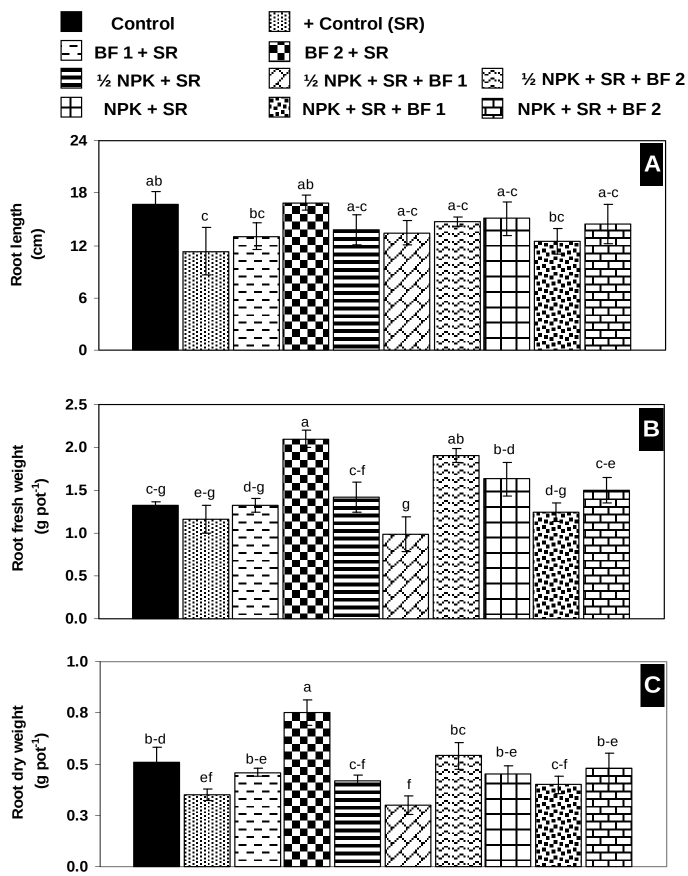

Fig. 3. Effect of *Sclerotium rolfsii* (SR), NPK fertilizers [recommended (NPK) and half (½ NPK) doses] and biofertilizers (BF) on root growth of chili. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference ($p \leq 0.05$) as determined by LSD test. BF 1: Biopower; BF 2: Feng Shou.

There was a great variability in chlorophyll contents in different treatments. The highest chlorophyll contents (6.56 mg/g) were recorded in control. *S. rolfsii* inoculation significantly reduced chlorophyll contents by 49% over negative control. None of the biofertilizers and chemical fertilizers treatment improved chlorophyll contents under *S. rolfsii* biotic stress. Conversely, most of the treatments *viz.,* Biopower + SR, ½ NPK + SR and NPK + SR + Biopower further reduced chlorophyll contents as compared to positive control treatment (Fig. 4A). Presently, it is important to consider that when different biofungicides were provided to soil, they impart differential growth responses in chili plants and affected their vegetative and maturity stages as well. The disappearance and degradation of chlorophyll is the one of the most prominent phenomenon of an advanced plant age. It is generally taken as dependable parameter of age related deterioration and loss of essential plant metabolites (Chopade *et al*. 2007). Alteration in source-sink balance due to change in stomatal conductance possibly be responsible of variable total chlorophyll content of chili plant after soil amendment with different fertilizers (Kasai *et al*. 2008).

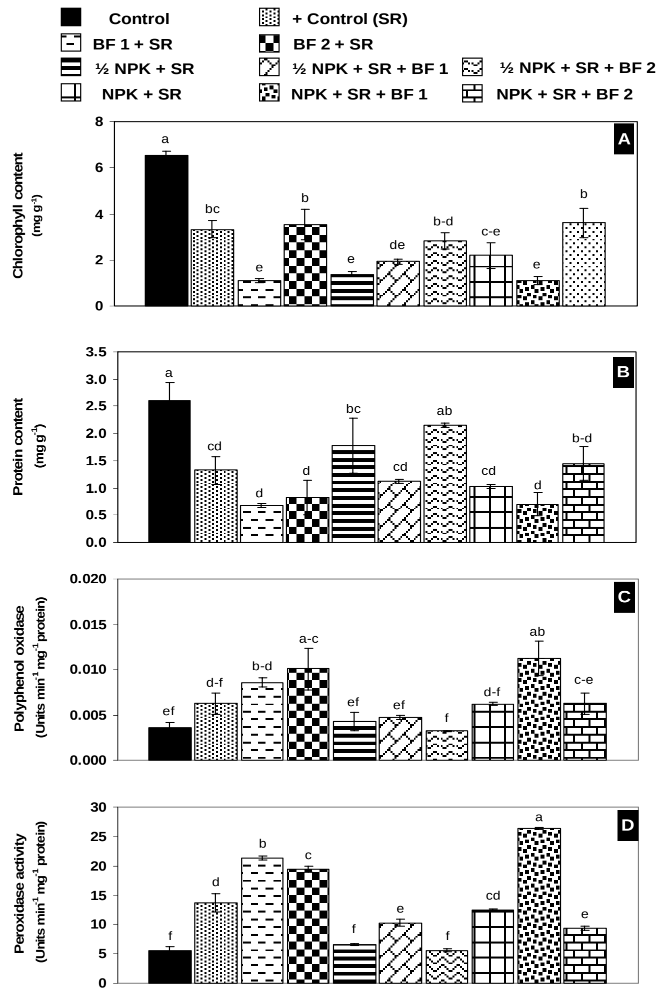

Fig. 4. Effect of *Sclerotium rolfsii* (SR), NPK fertilizers [recommended (NPK) and half (½ NPK) doses] and biofertilizers (BF) on various physiological parameters of chili. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference ($p \leq 0.05$) as determined by Tukey's HSD test. BF 1: Biopower; BF 2: Feng Shou.

The highest protein contents (2.60 mg/g) were recorded in negative control that were significantly reduced by 50% in positive control. ½ NPK + SR + Feng Shou treatment showed significant increase in protein contents over positive control. Conversely, protein contents in Biopower + SR and NPK + SR + Biopower were significantly lower than in positive control. In rest of the treatments, the effect of various combinations of biofertilizers and chemical fertilizers in the presence of *S. rolfsii* was insignificant as compared to positive control (Fig. 4 B). Change in substrate demand required for induction of plant defense mechanisms after *S. rolfsii* inoculation either alone or in combination with fertilizers might consequences with variable protein content in host plant. Besides, reduction in protein content could be attributed to increase in total amino acid pool under stress that probably indictes the mode of adjustment (Parida *et al*. 2004).

PPO activity was enhanced by 72% in positive control as compared to negative control. Except ½ NPK + SR + Feng Shou, all the treatments enhanced PPO activity to variable extents as compared to negative control. The effect of Feng Shou + SR and NPK + SR + Biopower on PPO activity was significant where 177 and 211% increase in PPO activity was recorded over negative control, respectively (Fig. 4C). Increase in PPO may signify its importance in plant defense by oxidizing phenolic compounds into quinones that could create toxic environment for pathogen and could react with it (Duffy and Felton 1991). Beside, PPO could activate plant defense mechanism by inactivating pathogen pectolytic enzyme (Okey *et al*. 1997).

The lowest PO activity (5.60 units/min/mg^{-} protein) was recorded in negative control. All the treatments except ½ NPK + SR and ½ NPK + SR + Feng Shou significantly increased PO activity over negative control. The highest PO activity (26.32 units/min/mg protein) was recorded in NPK + SR + Biopower followed by Biopower + SR (21.38 units/min/mg protein) and Feng Shou + SR (19.45 units/min/mg protein) (Fig. 4D). The alteration in PO of chili plants was in harmony with protein content and PPO due to effect of different biofungicides. Stimulation in its activity after soil amendments may result of phenols oxidation, suberization and lignifications of host plant cells during the defense reaction against pathogenic agents (Ashry and Mohamed 2011).

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