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EFFICACY OF THIOPHENATE METHYL, METALAXYL+MENCOZEB AND FOSETYL-AL FUNGICIDES FOR *IN VITRO* CONTROL OF *SCLEROTIUM ROLFSII*

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A B S T R A C T

Sclerotium rolfsii Sacc. is a soil-borne necrotrophic plant pathogen that causes diseases in over 500 plant species including vegetables, fruits and ornamental plants. An *in vitro* study was undertaken to assess the potential of thiophenate methyl 70% WP, metalaxyl+mencozeb 72% WP and fosetyl-Al 80% WP to control this pathogen. Five concentrations (50, 100, 150, 200 and 250 ppm) of each fungicide were prepared by mixing the appropriate quantities of each product in autoclaved malt extract agar. For comparison, a control treatment (0 ppm) without fungicides was also included. Metalaxyl+mencozeb was found the best fungicide with 29–84% control of fungal growth in different concentration. The other two fungicides *viz.* thiophenate methyl and fosetyl-Al were less effective and controlled the fungal pathogen by 10–35% and 30–48% over control, respectively. Regression analysis showed a linear or a polynomial relationship between the concentration and the fungal growth with R^2 = 0.9631, 0.9797 and 0.9106 for thiophenate methyl, metalaxyl+mencozeb and fosetyl-Al, respectively. This study concludes that metalaxyl+mencozeb is a highly effective fungicide for the control of *S. rolfsii.*

Keywords: Chemical control, Collar rot, Fungicides, Laboratory bioassays, Metalaxyl+mancozeb.

INTRODUCTION

Sclerotium rolfsii is an important soil-borne necrotrophic plant pathogen, causing diseases in over 500 plant species (Sana *et al.,* 2017; Sharf *et al.,* 2021). It has the ability to cause extensive damage to various horticultural and agricultural crops in tropical, subtropical and temperate areas of the world (Tarafdar *et al.,* 2018; Javaid *et al.,* 2020). It primarily attacks on host stem which under favorable environmental conditions infects other plant parts such as fruits, roots, leaves and petioles (Bosamia *et al.,* 2020). The first observed symptoms include the production of dark-brown lesions on plant surface, which later on results in progressive yellowing, wilting and eventually leads to plant death (Sahu *et al.,* 2019). It produces sclerotia which overwinter in the soil and can survive for long periods on plant debris causing diseases in the following season (Gandhi *et al.,* 2017). Thus, the complete eradication of this devastating pathogen is very critical (Atri and Kaur, 2021). Different integrated management strategies such as cultural and biological control have been investigated before including chemical practices (Ali *et al.,* 2020; Khan *et al.,* 2020). The application of synthetic chemicals in agriculture is a common practice for the effective control of plant diseases. These are not only used as a disease management strategy but also to improve the crop yield (Kerchev *et al.,* 2020). So far, several fungicides namely carbendazim, quintozene, thiram, fenhexamid, fluazinam and fludioxonil have been reported to control the diseases caused by soil-borne pathogens (Chauhan *et al.,* 1988; Matheron and Porchas, 2004).

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Many fungicides such as carbendazim, mancozeb, hexaconazole, thiophanate methyl, carboxin and propiconazole have been found effective against *Fusarium oxysporum* f. sp. *ciceri* (Sahane *et al.,* 2021), *Macrophomina phaseolina* (Khamari and Patra, 2018), *Rhizoctonia bataicola* (Khaliq *et al.,* 2020) and *S. rolfsii* (Khan and Javaid, 2015). Among them, carbendazim belonging to benzimidazole group is the most popular fungicide used widely in crop protection program to inhibit the mitosis in pathogenic fungi (Verma and Srivastava, 2018). It also has the potential to exert antifungal action by suppressing microtubule polymerization, which consequently results in fungal cell divisions (Goyal *et al.,* 2018). Likewise, carboxin is applied as an inhibitor to dysfunction the mitochondrial electron transport chain and tricarboxylic cycle to terminate the activities of respiratory fungal cells (Haq *et al.,* 2020). The present study was carried out to further investigate the possible potential of thiophenate methyl 70% WP, metalaxyl+mencozeb 72% WP and fosetyl-Al 80% WP against *in vitro* growth of *S. rolfsii*.

MATERIALS AND METHODS

The preserved culture of *S. rolfsii* that was previously isolated from chili (*Capsicum annuum* L.) was acquired from Biofertilizers and Biopesticide Lab at Department of Plant Pathology, Faculty of Agricultural Sciences, University of the Punjab, Lahore, Pakistan. Fungal culture was revived on malt extract agar (MEA) growth medium. For this purpose, 2% MEA was prepared in 250-mL conical flasks having 100 mL of distilled water. The Petri plates (9-cm diameter) and the flasks were then autoclaved at high temperature (121 °C) by steam sterilization method under the pressure of about 103.4 kPa for a time of 30 min to make them sterilized from harmful pathogens and their spores. Then the growth medium was allowed to cool down to 50 °C. Before pouring into the Petri plates, the antibiotic streptomycin (10 mg 100 mL^{-1}) was added to inhibit the bacterial growth. Moreover, different quantities of the fungicides were also added at this stage to prepare 50, 100, 150, 200 and 250 ppm concentrations of each fungicide.

Using a cork borer, a 5 mm disc of mycelium was transferred to the center of each media plate from an actively growing culture under sterilized conditions in laminar flow. Four replicates of each treatment were designed and arranged in a completely randomized design under laboratory condition at 25–27 °C. The plates without fungicide served as control (0 ppm). The growth of the fungal pathogen in each plate was measured after 4 days growth.

Statistical analysis

All the data were analyzed by analysis of variance (ANOVA) followed by application of LSD test at 5% level of significance using Statistix 8.1. Inhibition in growth of the fungal pathogen over control was measured by applying the following formula:

$$\text{Growth inhibition (\%)} = \frac{\text{Colony diameter in control - Colony diameter in fungicide}}{\text{Colony diameter in control}} \times 100$$

RESULTS AND DISCUSSION

All the doses of thiophenate methyl significantly ($P \leq 0.05$) reduced *in vitro* growth of *S. rolfsii.* A gradual decrease in diameter of fungal colony was observed with an increase in concentration of the fungicide. The lowest concentration (50 ppm) suppressed fungal growth by 10% while the highest concentration (250 ppm) reduced fungal colony growth by 35% over control. A linear relationship between concentration and colony diameter was recorded with $R^2 = 0.9631$ (Figure 1). Although, this fungicide controlled the growth of *S. rolfsii* significantly, however, it was found the least effective among the three fungicides used in this study. Hassan *et al.* (2020) also reported a similar moderate effect of this fungicide on growth of *Curvularia lunata.* Same concentrations of this fungicide reduced biomass of *C. lunata* by 15–26%. By contrast, its effect against *Penicillium expansum* was far better than against *C. lunata* and *S. rolfsii*, where 69–90% reduction in growth of *P. expansum* was recorded due to the same concentrations of this fungicide (Butt *et al.,* 2020). It shows that thiophenate methyl has differential affectivity against different fungal species. It is a broad-spectrum and systemic fungicide of benzimidazole group that possesses protective as well as curative properties against many fungal pathogens of vegetable, fruits and cereal crops (Anonymous, 2007). Together with Bion, this fungicide is reported to control decline disease of mango in Pakistan (Arif *et al.,* 2015). Although, broad spectrum in its action, thiophenate methyl has a specific mode of action. It controls different fungal species by binding to tubulin that result in blocking of mitosis (Pscheidt, 2021).

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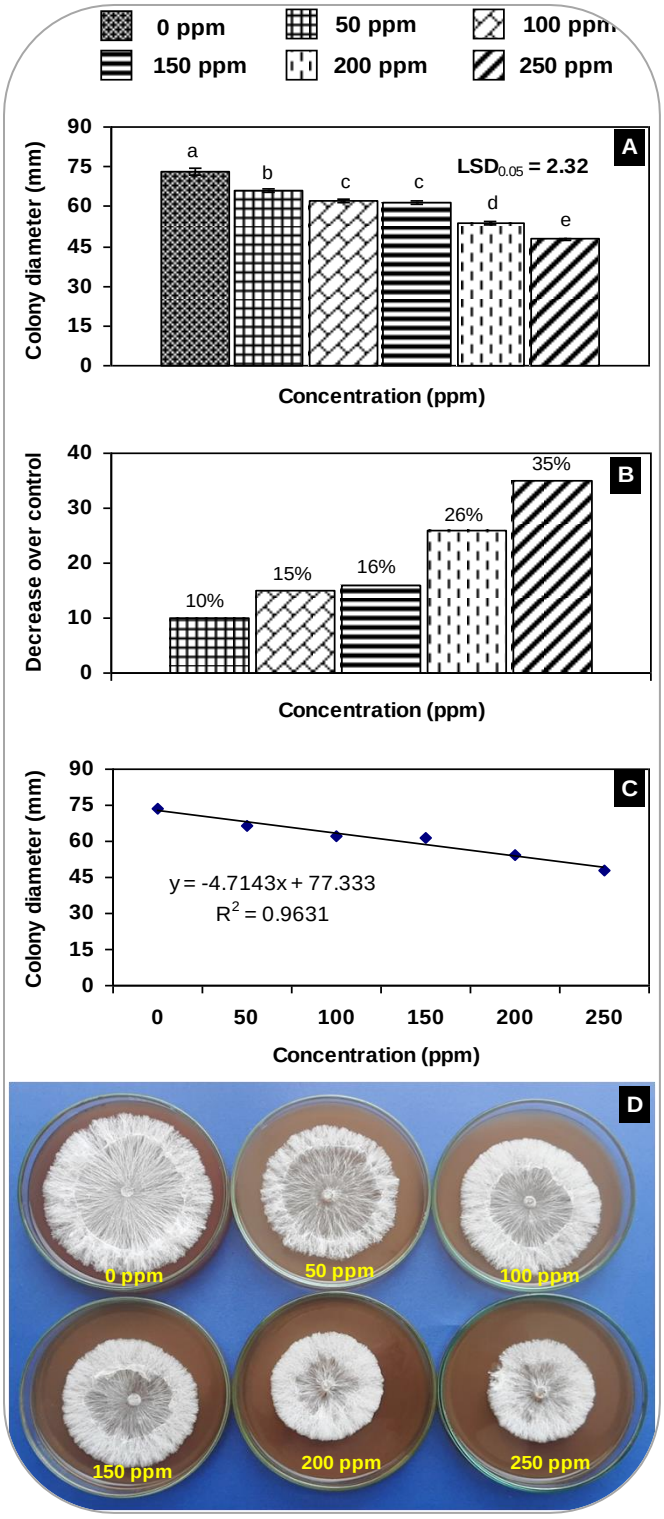

Figure 1. Effect of thiophenate methyl on growth of *Sclerotium rolfsii*. Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by LSD Test.

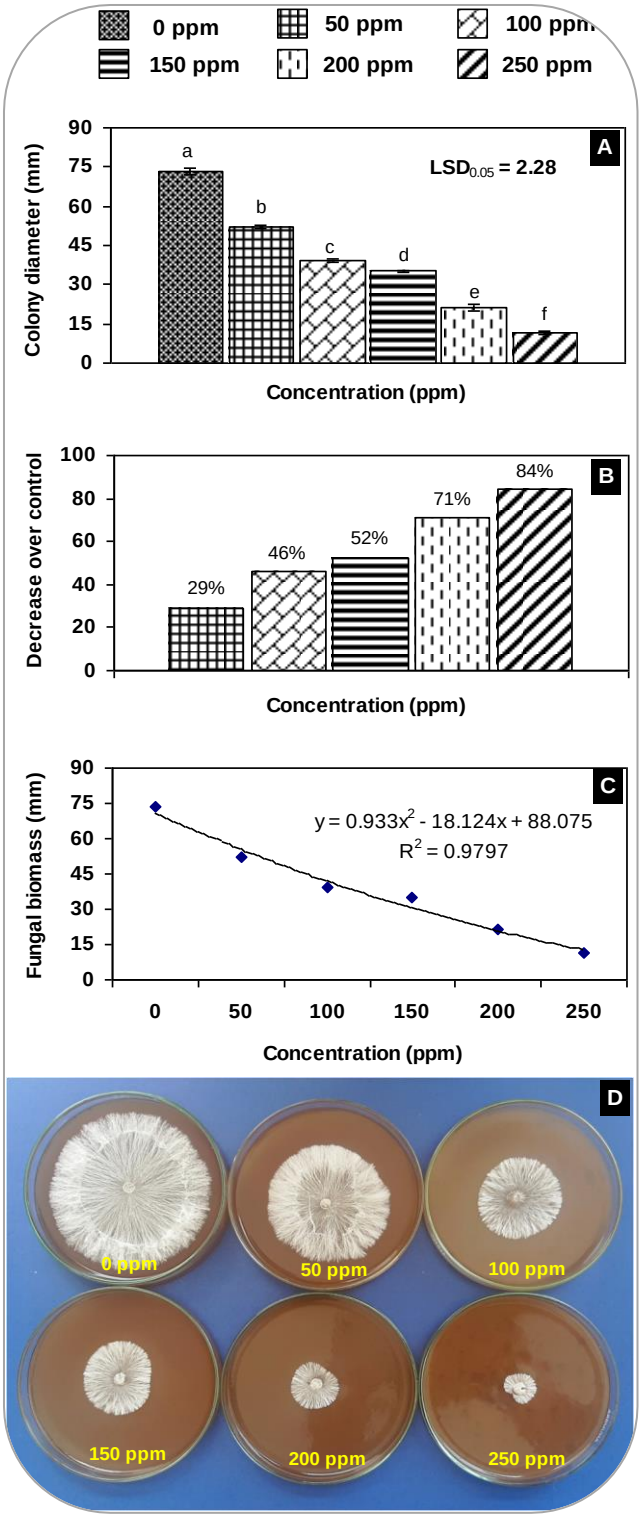

Figure 2. Effect of metalaxyl+mencozeb on growth of *Sclerotium rolfsii*. Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by LSD Test.

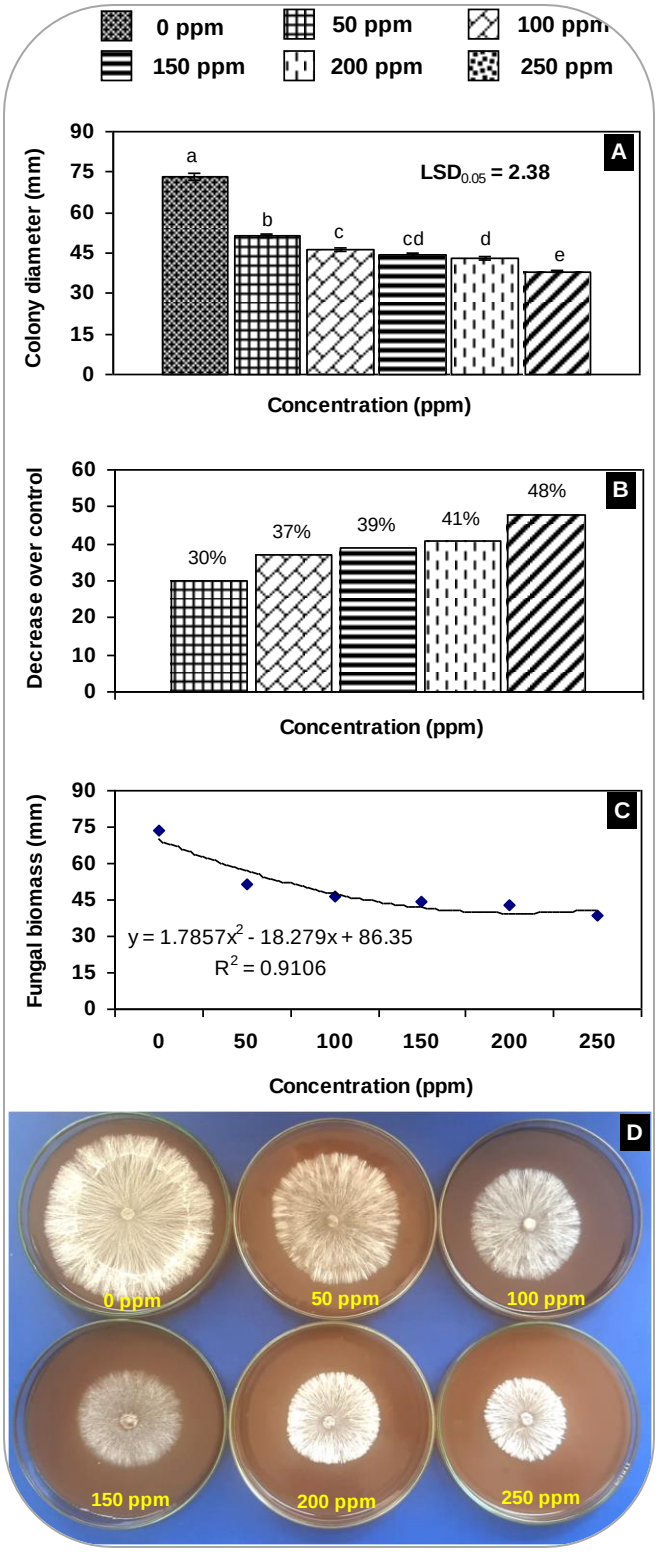

Figure 3. Effect of fosetyl-Al on growth of *Sclerotium rolfsii*. Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by LSD Test.

Metalaxyl+mencozeb was found the most effective fungicide in the present study. Similar to that of thiophenate methyl, different concentrations of this fungicide also reduced fungal growth significantly and the efficacy of this fungicide was gradually increased by increasing its concentrations in the growth medium. However, it proved far more effective against *S. rolfsii* than that of thiophenate methyl. Its lowest concentration (50 ppm) suppressed fungal growth by 29% while the highest concentration caused 84% reduction in colony diameter (Figure 2A, B and D). Regression analysis showed a polynomial relation between concentration of the fungicide and the radial growth of *S. rolfsii* with R^2 = 0.9797 (Figure 2C). This fungicide was also highly effective against *C. lunata* and *P. expansum* causing and 69–90% reduction in their *in vitro* growth due to a concentration range of 50 to 250 ppm (Butt *et al.,* 2020; Hassan *et al.,* 2020). Khan *et al.* (2020) reported that mencozeb significantly reduced the incidence of collar rot disease caused by *S. rolfsii.* Similarly, Türkölmez and Dervis (2017) reported significant control of fungal diseases of apricot and cherry caused by *Phytophthora palmivora*, due to application metalaxyl+mencozeb. This fungicide is a mixture of metalaxyl (8%) and mencozeb (64%) having a dual action, curative and preventive. Mencozeb is converted to isothiocyanate after exposure to air and causes inactivation of fungal enzymes belonging to sulphahydral groups. In addition, exchange of metals may occur between fungal enzymes and the fungicide that interferes in functions of fungal enzymes. On the other hand, metalaxyl obstructs protein synthesis, and also hinders fungal growth and reproduction (Sukul and Spiteller, 2000). Mencozeb was found very effective in controlling collar rot of chickpea caused by *S. rolfsii* (Khan and Javaid, 2015). Metalaxyl+mencozeb can be used against foliar as well as soil-borne diseases caused by oomycetes. It is recommended to control various diseases including late blight of potato, downy mildew of grapes, damping off of tobacco, white rust of mustard, and Phytophthora root rot of black pepper (Butt *et al.,* 2020).

Fosetyl-Al showed moderate efficacy against the *S. rolfsii.* Its effectiveness was in between the efficacy of the other two fungicides used in the present study. Its different concentrations significantly reduced fungal growth by 30–48% over control. A polynomial relationship was recorded between fungicide concentration and the fungal growth with R^2 = 0.9106 (Figure 3). This fungicide also proved less effective against *P. expansum* and *C. lunata*

(Butt *et al.,* 2020; Hassan *et al.,* 2020). This systemic fungicide is an aluminum salt of the diethyl ester of phosphorous acid and is mostly used against diseases caused by oomycetes (Cohen and Coffey, 1986). Breakdown product of this fungicide is phosphorus acid that is translocated from shoot to the root and controls oomycetes (McGrath, 2004).

CONCLUSION

This study clearly indicated that metalaxyl+mencozeb is an exceedingly effectual fungicide to control *in vitro* growth of *S. rolfsii.* Further studies are recommended to assess its efficacy against in vivo growth of *S. rolfsii* using various hosts of this pathogen.

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Contribution of Authors:

Ifrayeem Butt	:	Conducted the experiments
Arshad Javaid	:	Supervised the work, analyzed the data, prepared graphs and finalized the paper
Muhammad A. U. Hassan	:	Conducted the experiments
Iqra H. Khan	:	Contributed in write up
Shahbaz Ahmad	:	Contributed in write up