Mycopath (2020) 18(1): 19-23 **Research Article**

Chemical control of *Penicillium expansum,* the cause of blue mold disease in apple

Ifrayeem Butt, Muhammad Abrar Ul Hassan, Iqra Haider Khan and *Arshad Javaid

Institute of Agricultural Sciences, University of the Punjab, Quaid-i-Azam Campus, Lahore 54590, Pakistan
**Corresponding author's email: arshad.iags@pu.edu.pk, arshadjpk@yahoo.com*

Abstract

Penicillium expansum is a common soil-borne fungus occurring worldwide and causes post harvest diseases in a wide range of plant species especially in apple. Three fungicides namely metalaxyl+mencozeb 72% WP, thiophenate methyl 70% WP and fosetyl-Al 80% WP were evaluated *in vitro* against this fungal pathogen. Five concentrations of each fungicide *viz.* 50, 100, 150, 200 and 250 ppm, were tested by food poisoning technique using malt extract agar as a growth medium. All the three fungicides significantly reduced the pathogen growth with variable extents. Among these, thiophenate methyl was the most effective one causing 65–90% reduction in fungal growth. Metalaxyl+mencozeb also showed a very similar effect resulting in 73–88% suppression in the fungal growth. Fosetyl-Al was comparatively less effective fungicide and reduced fungal growth by 73–88%, over control. This study concludes that thiophenate methyl and metalaxyl+mencozeb are highly effective fungicides against *P. expansum,* whose 50 ppm concentration can control fungal growth by 65 and 73%, respectively.

Keywords: Blue mold, metalaxyl+mencozeb, *Penicillium expansum,* thiophenate methyl.

Introduction

Penicillium expansum is an aggressive cosmopolitan fungus and is soil-borne in nature (Rodríguez-Chávez *et al.,* 2019). It is one of the most important decay causing post-harvest fungal pathogen that can tolerate many adverse environmental conditions (Ma *et al.,* 2020). It is associated with blue mold infection on stored apples in Pakistan and worldwide (Khan and Javaid, 2020). It also causes post harvest infections in grapes (Khan *et al.,* 2021). Usually, after harvest, apples are stored at low temperature in a controlled environment for at least 8 months. During this period, infection can occur and proceeds slowly at cold storage temperatures whereas a rapid progression is found under warm conditions (Wallace *et al.,* 2017). Conidia are the main source of inoculum, which may be present in fruit storage rooms, flotation-tank water, orchards, dump-tank and packing facilities. Moreover, fungal spores adjacent to water flumes or in drench solutions increase the amount of inoculum and decay (Yu *et al.,* 2020). Besides causing economic losses, it is also important for public health as it produces a mycotoxin named patulin in excessive amounts on rotted fruits (Zhong *et al.,* 2018). Although some cultural management strategies are in practice to prevent the growth of *P. expansum,* however, each has some limitations (Tannous *et al.,* 2020). So far, pre- and post-harvest chemical treatment remains the main measure to control the pathogen attack (Yu *et al.,* 2020).

Beside certain strategies applied to reduce the amount of *P. expansum* inoculum, the most effective one is the use of fungicides during harvest and packaging (Reki *et al.,* 2020). In general, many synthetic fungicides based on dicarboximide and imidazole groups are commercially available for the control of blue mold pathogen (Wang *et al.,* 2018). However, the efficacy of several fungicides has been reduced due to resistance development mechanism in *P. expansum* populations (Lichtner *et al.,* 2020). Some of the fungicides such as thiabendazole (TBZ), benomyl, pyrimethanil, fludioxonil and thiophanatemethyl are used commonly prior to storage against the pathogen (Cerioni *et al.,* 2017). In addition, thiophanate methyl is considered as the most effective fungicide in inhibiting the germ-tube elongation and conidial germination of *P. expansum* (Samaras *et al.,* 2020). Therefore, the objective of present study was to determine the efficacy of different concentrations of fungicides namely metalaxyl+mencozeb, thiophenate methyl and fosetyl-Al against the blue mold pathogen.

Materials and Methods

To evaluate the potential of three fungicides namely metalaxyl+mencozeb 72% WP, thiophenate methyl 70% WP and fosetyl-Al 80% WP, the poison food technique was used. For this purpose, 100 mL of 2% malt extract agar (MEA) medium was prepared in 250-mL conical flasks for each concentration of the three fungicides, and autoclaved at 121 ºC for 30 minutes. The medium was cooled at room temperature and added appropriate quantities of fungicides (while the medium was in molten state) to prepare 50, 100, 150, 200 and 250 ppm concentrations. To avoid bacterial growth, streptomycin was added in the medium. Control treatment was without fungicides. The growth medium was poured in 9-cm diameter Petri plates (25 mL per plate) and allowed to cool (Khan and Javaid, 2015).

Penicillium expansum was obtained from Biofertilizers and Biopesticide Lab, Faculty of Agricultural Sciences, University of the Punjab Lahore, Pakistan. Originally the fungus was isolated from blue mold suffering apple. Fresh culture of the fungus was prepared on MEA plates. A disc of 5 mm diameter of the actively growing fungal colony was transferred in the centre of each Petri plate. Each treatment was replicated four times and the experiment was done using a completely randomized design. After one-week, radial growth of the fungus in each plate was measured. Standard errors of the means were calculated. 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.

Results and Discussion

Metalaxyl+mencozeb showed a remarkable activity against *P. expansum.* All the concentrations of this fungicide significantly ($P \leq 0.05$) decreased the fungal growth and the antifungal activity was increased with an increase in concentration of the fungicide (Fig. 1A). The lowest concentration (50 ppm) reduced the fungal growth by 73% that was gradually increased 77, 79, 85 and 88% by increasing the fungicidal concentration to 100, 150, 200 and 250 ppm, respectively (Fig. 1B). The relationship between concentration and fungal growth was a polynomial with $R^2 = 0.841$ as shown in Fig. 1C. The fungicide metalaxyl+mencozeb is a mixture of two fungicides *viz.* mencozeb (64%) and metalaxyl (8%). The mixture has a dual action *i.e.*, preventive and curative. Mencozeb acts through its contact action. After its exposure to air, it is converted to an isothiocyanate that results in inactivation of sulphahydral groups of fungal enzymes. Moreover, exchange of metals may take place between the fungicide and fungal enzymes, resulting in interference in functions of fungal enzymes. Metalaxyl hinders synthesis of protein, and also inhibits growth and reproduction of the fungi (Sukul and Spiteller, 2000). Metalaxyl+mencozeb is useful in controlling soil-borne as well as foliar diseases caused by oomycetes. This fungicide can be applied as pre-harvest sprays to control post-harvest diseases. It is recommended by the manufacturer for the control of downy mildew of grapes, late blight of potato, damping off of tobacco, Phytophthora root rot of black pepper, and white rust of mustard. Platt (1985) reported better disease control by metalaxyl+mencozeb as compared to metalaxyl alone. Türkölmez and Dervis (2017) reported significant control of crown and root rot diseases of apricot and cherry caused by *Phytophthora palmivora*, due to application metalaxyl+mencozeb. Recently, Khan *et al.* (2020) reported that mencozeb application significantly reduced the incidence of collar rot disease in chickpea caused by *Sclerotium rolfsii.*

Thiophenate methyl exhibited the highest activity against *P. expansum.* The effect of all the concentrations was statistically significant ($P \leq 0.05$) and concentration dependant (Fig. 2A). The lowest concentration of 50 ppm reduced fungal growth by 65% that increased 90% due to the 250-ppm concentration (Fig. 2B). There was a polynomial relationship between concentration and fungal growth with $R^2 = 0.875$ as given in Fig. 2C. This broad-spectrum systemic fungicide belongs to benzimidazole group. It has both protective and curative actions against a variety of fungal pathogens causing diseases in a number of fruits, vegetables and cereal crops (Anonymous, 2007). It is useful against Sigatoka leaf spot and anthracnose diseases of banana in Brazil (Vieira *et al.,* 2017). There is report of control of mango decline disease in Pakistan due to application of thiophanate methyl together with Bion (Arif *et al.,* 2015).

Fosetyl-Al demonstrated the least activity against the targeted pathogen. However, the antifungal effect of all the concentrations was significant ($P \leq 0.05$) and concentration dependent (Fig. 3A). There was 32–78% reduction in growth of *P. expansum* due to range of applied concentrations (Fig. 3B). A polynomial relationship was recorded between the concentration and the fungal growth with $R^2 = 0.995$ as demonstrated in Fig. 3C. Fosetyl-Al is an aluminum salt of the diethyl ester of phosphorous acid. It is a systemic fungicide that is generally used to control diseases caused by oomycetes (Cohen and Coffey, 1986). It ionizes into phosphonate inside the plants. Phosphorous acid is a breakdown product of this fungicide and is translocated from aerial parts to the roots and has effects on oomycetes (McGrath, 2004). A mixture of 260 μg mL^{-1} of fosetyl-Al inhibited formation of lesion by *Phytophthora capsici* on detached leaflets of tomato (Fenn, 1985). This fungicide significantly reduced the development of esca disease of grapevine in greenhouse as well as under field conditions (Di Marco *et al.,* 2011).

Conclusion

This study concludes that metalaxyl+mencozeb and thiophenate methyl are very effective against *P. expansum* even at very low concentration of 50 ppm.

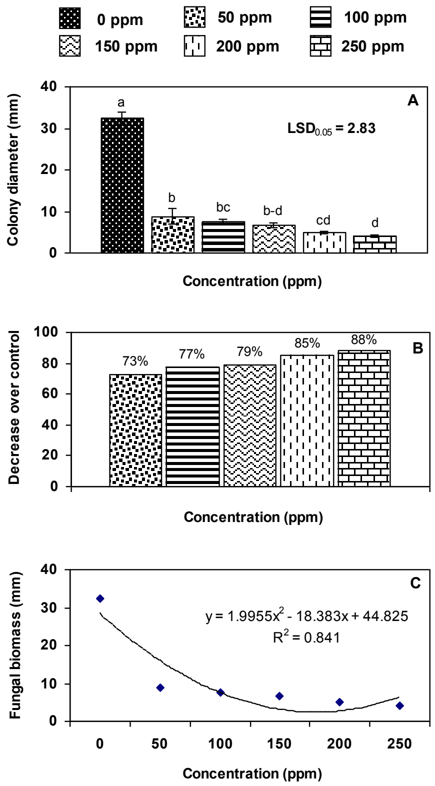

Fig. 1: Effect of metalaxyl+mencozeb on growth of *Penicillium expansum*. Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by LSD Test.

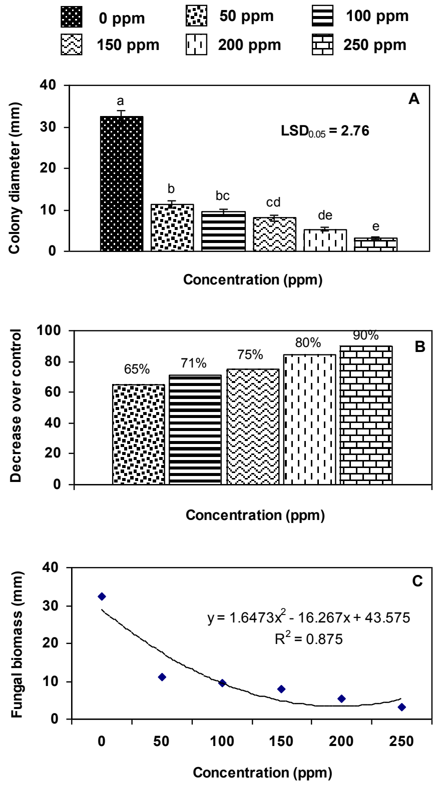

Fig. 2: Percentage decrease in biomass of *Fusarium oxysporum* f. sp. *cepae* (FOC) due to methanolic extracts of different parts of *Chenopodium murale*. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by LSD Test.

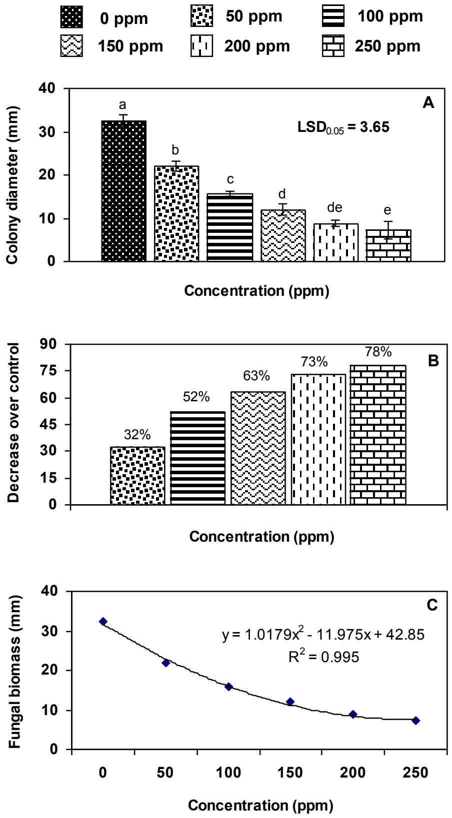

Fig. 3: Effect of fosetyl-Al on growth of *Penicillium expansum*. Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by LSD Test.

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