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Examination of the efficacy of different fungicides against *Macrophomina phaseolina* and *Sclerotinia sclerotiorum* in laboratory conditions

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SUMMARY

Macrophomina phaseolina and Sclerotinia sclerotiorum are two significant fungal pathogens of sunflower. M. phaseolina causes charcoal rot and ashy stem blight in several important crop species. Sclerotinia sclerotiorum causes white mold disease which can occur as middle stalk rot, head rot and premature plant death. Due to the wide host range of the two pathogens and their survival structures, crop rotation cannot provide sufficient protection against them. In our experiment, we selected two fungicides, Mirage and Prosaro, which are widely used in practice, and we tested their efficacy against the two pathogens. The efficiency of these fungicides was tested at a concentration of 10; 20; 50; 100 and 500 ppm. The Prosaro totally inhibited the mycelial growth of both pathogens at a concentration of 50 ppm, 100 ppm and 500 ppm. The Mirage caused total mycelial growth inhibition in all treatments against both pathogens.

Keywords: *Macrophomina phaseolina, Sclerotinia sclerotiorum, prochloraz, prothioconazole, tebuconazole*

INTRODUCTION

The two fungal pathogens included in our studies *Macrophomina phaseolina* and *Sclerotinia sclerotiorum* are among the most important pathogens of sunflowers. *Macrophomina phaseolina* (Tassi) Goid.; syn.: *Rhizoctonia bataticola* (Taubenh.) E.J. Butler causes charcoal rot and ashy stem blight of several major crops. This fungus has more than 500 host plants worldwide (Ghias et al., 2021). Host plants of the disease belong to dicotyledonous and monocotyledonous as well, moreover, they can be herbaceous or woody plants. *Sclerotinia sclerotiorum* (Lib.) de Bary causes white mold disease which can occur as middle stalk rot, head rot, and premature plant death. This pathogen has more than 600 host plants, involving a high number of cultivated plants and weeds (Boland and Hall, 1994). *M. phaseolina* is favored by dry, warm summer, high temperature, and winter with low precipitation (Marquez et al., 2021). The *Rhizoctonia bataticola* form can produce microsclerotia, which is the primary source of infection and which can preserve germination ability for years under dry conditions. In contrast, *Sclerotinia sclerotiorum* occurs when the weather is rainy, wet, and cold. The fungus can germinate from sclerotia even after 6–8 years. Due to the wide host range of the two pathogens and their persistent formulas crop rotation cannot provide sufficient protection against them (Nelson, 1998). The practical application of two agrotechnical control methods would be necessary in the case of *M. phaseolina*, these are late sowing and irrigation. General agrotechnical methods such as an optimal plant density, harmonic nutrient supply, and weed control are important protection methods against *S. sclerotiorum*. Biological preparations are also available. In the case of *M. phaseolina*, biological protection methods are used as well. For instance, *Aspergillus sp.* (Eswaran and Mishra, 2004), *Trichoderma sp.* (Dinakaran et al., 1995; Prashanthi et al., 2000), *Actinomycetes sp.* (Herbar et al., 1991), *Pseudomonas sp.* (Kavitha et al., 2005) and *Bacillus subtilis* (Siddiqui and Mahmood, 1993). Against *S. sclerotiorum*, biological protection methods can also be used, such as *Erwinia herbicola*, *Bacillus polymyxa* (Yuen et al., 1992), *Phaeosphaeria minitans* syn: *Paraconiothyrium minitans* and *Trichoderma harzianum* (Smolińska and Kowalska, 2018). However, neither biological nor other ways of protection can provide satisfying results, thus chemical control is necessary against the two pathogens. Because of the life cycle of the two pathogens using chemical methods to treatboth problems simultaneously is difficult. The efficiency of fungicides against pathogens is different. It is important to find and use the most effective fungicides against these two important pathogens. In an earlier experiment, it was found that of the 6 different fungicides they tested, only the Pictor was inhibiting the formation of microsclerotia (Csüllög et al., 2020). The Propulse inhibited microsclerotia formation only at concentrations of 50 and 100 ppm. The Amistar Xtra and Peretrix-Bordeaux mixtures did not inhibit the hyphal growth and the production of microsclerotia. Kaur et al. (2019) showed that the Propulse (active ingredient fluopyram and prothioconazole), the Fontelis (penthiopyrade active ingredient) and the Omega (fluazinam active ingredient) can inhibit totally the mycelial growth of *S. sclerotiorum*. PoSlušná (2018) showed that Prosaro fungicide at registered dose rates was fully effective in inhibiting the mycelial growth of *S. sclerotiorum* on poisoned media.

MATERIALS AND METHODS

In this study, two fungicides were selected which are widely used in practice and tested those efficiencies

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against *Macrophomina phaseolina* and *Sclerotinia sclerotiorum*. The fungicides were the Prosaro (125 g L^{-1} prothioconazole and 125 g L^{-1} tebuconazole) and the Mirage 5 EC (450 g L^{-1} prochloraz). In the experiment, these 2 different pesticides were tested at 5 different concentrations for the mycelial growth and sclerotia formation of the two pathogens by using a poisoned media technique in vitro. 6 stock solutions were made with various concentrations of fungicides. Fungicides were tested at final concentrations of 10 ppm, 20 ppm, 50 ppm, 100 ppm 200 ppm, and 500 ppm. In the first step, 1 ml of every stock solution was pipetted and mixed with 20 ml 50 °C potato-dextrose-agar media in 50 ml Falcon tubes. In the second step, in the Falcon tubes, the liquid media was mixed with the fungicide by vortex for 4 sec. In the next step, the 21 ml media with fungicide was filled in 90 mm Petri-dishes. After the solidification of media, 5 mm diameter *Macrophomina phaseolina* or *Sclerotinia sclerotiorum* discs were taken from 7-day-old pure culture and were placed into the center of poisoned plates. The Petri-dishes with the cultures were incubated under dark conditions at 25 ± 2 °C for 3 days when the controls growths reached 90 mm

The growth of fungal colonies was measured on the third day. The percentage inhibition of the growth of the fungus was calculated using the following formula (Vincent, 1947):

$$I = \frac{C - T}{C} x100$$

Remarks:
I= percentage inhibition
C= diameter of the fungal colony in the control culture (mm)
T= diameter of the fungal colony in the appropriate treatment (mm)

Every concentrations and the controls were tested in 10 repetitions. As statistical analysis we used Kruskall-Wallis non parametric test which, was supported with Mann-Whitney U-test

RESULTS AND DISCUSSION

The measurement of the growth of fungal colonies was done on the third day. The two tested fungicides produced different results on the two examined fungi. The results of the measurement are shown in *Figure 1*. In the case of *M. phaseolina* using Prosaro, the hyphal growth was arrested above a concentration of 50 ppm. Against *M. phaseolina*, Prosaro showed good results and has blocked the growth of hypha above 50 ppm concentration. At the same time, Mirage completely blocked the growth (I=100%) of the fungus The pathogen was only able to produce microsclerotia in control cultures, and the mycelium of the fungus could grow undisturbed. The average diameter of control mycelia was 90 mm and the average diameter of microsclerotia was 68 mm.

Figure 1: **The results of Kruskal-Wallis (p<0.05) and Mann-Whitney statistical analyzis of the efficacy of the Prosaro against *M. phaseolina***

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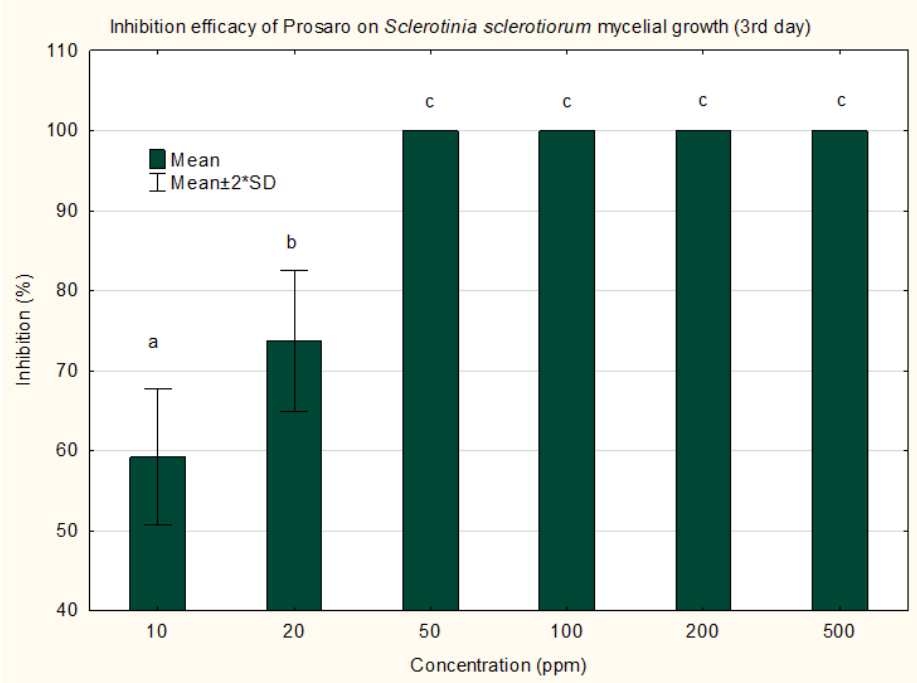
In the case of *S. sclerotiorum* Mirage completely blocked the growth of the fungus. In contrast, Prosaro caused only minimal inhibition in hyphal growth at concentrations of 10 ppm and 20 ppm (*Figure 2*) bAt 50 ppm, 100 ppm, and 500 ppm concentrations, the hyphal growth was totally blocked by Prosaro. The control cultures were completely overgrown with the mycelium of *S. sclerotiorum* on 3rd day.

Figure 2: **The results of Kruskal-Wallis (p<0.05) and Mann-Whitney statistical analyzis of efficacy the Prosaro against *S. sclerotiorum***



CONCLUSIONS

M. phaseolina and *S. sclerotiorum* can cause huge economic damage, not only to sunflowers but also to other plants. In addition to agrotechnical and biological control, it is necessary to use chemical active substances that are fully capable of inhibiting the growth and damage of the two pathogens. In this experiment, the Prosaro inhibited the mycelial growth of both pathogens at a concentration of 50 ppm, 100 ppm, and 500 ppm. The Mirage (prochloraz) caused total mycelial growth inhibition in all treatments against both pathogens. Both the fungicides inhibited the formation of microsclerotia. In terms of our former and current results, prochloraz is considered to have excellent effects against both of the examined dangerous pathogens. Additional chemical and biological pesticides should be tested against the two pathogens.

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