

Examination of different fungicides against *Macrophomina phaseolina* in laboratory conditions

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SUMMARY

In Hungary, sunflower is the third most important arable crop, which has a lot of pathogenic fungi. One of these fungi is the *Macrophomina phaseolina*, which is a well-known fungus in all over the world, since this pathogen has more than 700 host plants. In Hungary, several host plants can be found as well. The *M. phaseolina* produces microsclerotia, which can survive in the soil and residues for almost 10 years. For now, there is no efficient treatment against this pathogen because of this fungus, since it is extremely resistant and cannot be destroyed easily. The only effective treatment against the fungus is genetic defence. In this study, three different fungicides were tested in vitro against the fungus. The Mirage (prochloraz) seemed to be the most effective fungicide as it completely arrested the hyphal growth. In contrast, the Amistar Xtra (azoxystrobin and ciproconazol) has only a minor effect on the growth of *M. phaseolina*. Thirdly, the Retengo (pyrachlostrobin) arrested the hyphal growth of the fungus with 71% at 100 ppm, in other words, the use of this fungicide seems promising.

Keywords: *Macrophomina phaseolina*, in vitro, fungicide, azoxystrobin, ciproconazol, prochloraz, pyrachlostrobin

INTRODUCTION

Sunflower (*Helianthus annuus*) is the third most important field crop in Hungary and it is grown on more than 500.000 hectares. This plant has a number of pathogenic fungi: *Plasmopara halstedii*, *Phoma macdonaldi*, *Diaporthe helianthi*, *Alternaria sp.*, *Septoria helianthi*, *Sclerotinia sclerotiorum* and *Macrophomina phaseolina*. These fungi cause huge damage to the health of the sunflower crop. In Hungary, Békési (1970) was the first who described the *M. phaseolina* on sunflowers. This fungus is hosted by approximately 700 species (Békési et al., 2014).

The pathogen was identified in *Zea mays* (Vörös and Manninger, 1973), *Glycine max* (Érsek, 1979). From 1987 to 1991 Simay found this fungus in *Solanum tuberosum*, *Helianthus tuberosus*, *Phaseolus vulgaris*, *Vicia faba*, *Allium sativum*, moreover it was also identified in *Beta vulgaris* (Koppányi, 1993), *Cannabis sativa*, *Valeriana officinalis* (Simay and Kadlicskó, 1993), *Capsicum annuum* (Fischl et al., 1995), *Citrullus lanatus* (Békési et al., 1995) *Prunus armeniaca* (Vajna and Rozsnyai, 1995) and *Picea pungens* (Fischl et al., 2008). It has 2 anamorph forms. The name of the pycnidia form, which constitute conidiophores *M. phaseolina*. Another form is *Rhizoctonia bataticola* constitute microsclerotia. The fungus constitutes microsclerotia both on and within the stem. Pycnidia are usually in host tissue.

Taxonomic Description of *Macrophomina phaseolina* (Wheeler, 1975)

Division	<i>Eumycota</i>
Sub Division	<i>Deuteromycotina</i>
Class	<i>Coelomycetes</i>
Order	<i>Sphaeropsidales</i>
Family	<i>Sphaeropsidaceae</i>
Genus	<i>Macrophomina</i>
Species	<i>phaseolina</i>

M. phaseolina is primarily a seed, soil and stubble borne pathogen. This fungus causes charcoal rot, root rot and seedling blight in young plants; and in adult plants shows wilting, premature dying and loss of vigor. On sunflower after flowering this fungus cause early maturing. If the temperature is high and there is a high level of drought around flowering time, the symptoms are dramatic and progressive. After flowering, the lower stem and top of the taproot will show grey and black discoloration, and also shredding of the plant tissue. The epidermis is usually removed and in the stem there are numerous microsclerotia. (Sinclair, 1982; Yang and Owen, 1982; Hoes, 1985; Kolte, 1985).

The oil content is higher when infected with *M. Phaseolina* than in the healthy sunflower. The head weight and diameter are less in infected sunflowers when compared with healthy sunflowers. (Raut, 1983; Kolte, 1985). The microsclerotia can survive in the soil almost 10 years.

There is no effective treatment against the pathogen. Békési (2007) claimed that dressing and the late showing can provide protection as the fungus probably prefer early and dense showing. However, the most effective solution would be the development of genetic protection against the pathogen. Further protective methods could include rotation crop. However, due the polyphagic trait of *M. phaseolina* wide host range related to the fungus, the establishment of crop rotation is difficult not only in Hungary, but also all over the world. According to Ndiaye et al. (2008) the amount of microsclerotia in the soil decrease if *Panicum miliaceum* or *Digitaria sp.* are integrated in the crop rotation. Kending et al. (2000) claimed that appropriate amount of water supply can push back the amount of microsclerotium in soil. There are other biological protection methods against the *M. phaseolina* as well: *Aspergillus sp.* (Eswaran and Mishra, 2004), *Trichoderma sp.* (Dinakaran et al., 1995; Prashanthi et al., 2000), pl. *Actinomycetes sp.*

(Herbar et al., 1991), *Pseudomonas* sp. (Kavitha et al., 2005) and *Bacillus subtilis* (Siddiqui and Mahmood, 1993).

MATERIALS AND METHODS

We tested 3 different pesticides at 4 different concentrations for the growth mycelial and sclerotial formation of this pathogen by using a poisoned media technique.

Amistar Xtra (200 g L⁻¹ azoxystrobin + 80 g L⁻¹ ciproconazol, 1 L ha⁻¹): „Both active ingredients act as an inhibitor of mitochondria respiration by disrupting the energy cycle within the fungus and interrupting the biosynthesis of ergosterol. This interferes in the fungal life cycle mainly during spore germination, infection and hyphen growth. Both active ingredients are transported acropetally and translaminary in the xylem and therefore gradual uptake in to the leaves of the plants.” (I1). Retengo (200 g L⁻¹ pyraclostrobin 1 L ha⁻¹): „Pyraclostrobin has a sustained inhibiting effect on spore germination, the formation of infection structures, mycelial growth and sporulation of harmful fungus. The fungicide is taken up via the leaves and shows primarily locally systemic and translaminar activity. In small volumes, the active ingredient is transferred through the plant via the sap.” (I2). Mirage 45 EC (450 g L⁻¹ prochloraz, 1 L ha⁻¹) „Broad-spectrum with protectant and eradicant properties. Disrupts membrane function.” (I3).

We made a number of stock solutions with various concentrations of fungicides, testing at 10 ppm, 20 ppm, 50 ppm and 100 ppm. On each plates 1 ml stock solutions was pipetted and mixed with 20 ml 50°

(liquid) media. After the solidification of media, 0.5 cm diameter *M. phaseolina* disc was placed into the centre of poisoned plates. These discs were taken from 7 days old pure culture. The poisoned mediums with 0.5 mm diameter mycelial discs were incubated under dark condition at 25 ± 1 °C for 6 days. We used 130 Petri dishes (10 dishes/ppm). Fungicide was not added to control plates. The linear growth of *M. phaseolina* colonies was measured on the third and on the sixth day. The measurement of the colonies was two different angels (mm) and after the average values calculated.

The per cent inhibition of growth of *M. phaseolina* was calculated using the following formula (Vincent, 1947):

$$I = \frac{C - T}{C} \times 100$$

Where, I= Per cent inhibition

C= Colony diameter in control (mm)

T= Colony diameter in respective treatment (mm)

RESULTS AND DISCUSSION

The three tested fungicides produced different results (Table 1). The result of the first measurement was the Mirage (prochloraz) completely arrested the hyphal growth (Figure 1, Figure 2). The *M. phaseolina* did not produce form of microsclerotia just only in control plates. The Amistar Xtra did not arrest the fungus. The Retengo arrested the pathogen but not completely. The control at the first measurement produce microsclerotia and the hyphal system of the pathogen completely overran the media.

Table 1

The *M. phaseolina* hyhpal growth at first measurement

	10 ppm	20 ppm	50 ppm	100 ppm
Amistar Xtra Mycelia (mm)	52.8	50.5	44	38.1
Mirage Mycelia (mm)	0	0	0	0
Retengo (mm) Mycelia	36.5	30.1	25	22
Control Mycelia (mm)			90	
Control Mycosclerotia (mm)			66.72	

Figure 1: The hyphal growth in 10 and 20 ppm at the first measurement

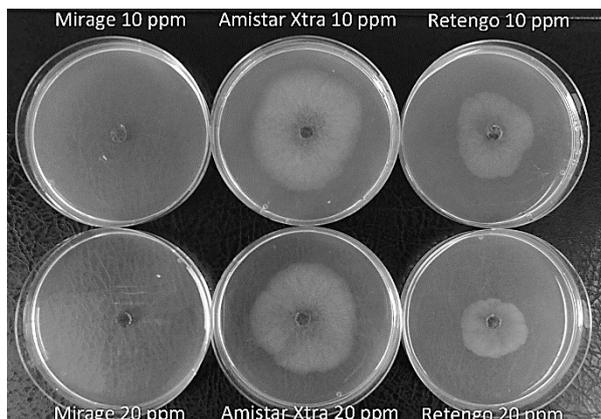
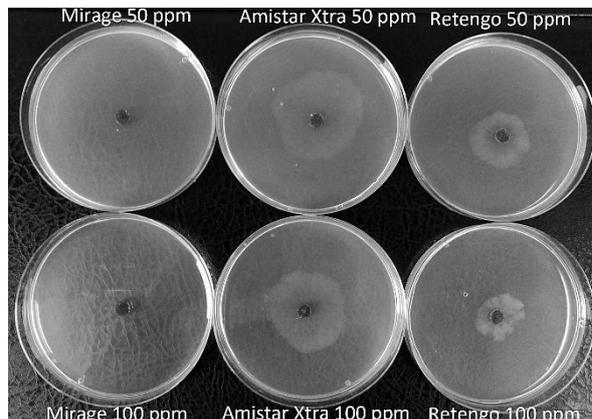


Figure 2: The hyphal growth in 50 and 100 ppm at the first measurement



At the second measurement the *M. phaseolina* produced microsclerotia (Table 2). The hyphal system of fungus overran the entire media at 10 ppm, 20 ppm and 50 ppm on the Amistar Xtra poisoned media (Figure 3, Figure 4). At 100 ppm the hyphal system of pathogen grew only 70.5 mm. Similarly to the first measurement the prochloraz did not arrest any hyphal

and microsclerotial growth of the pathogen. Due to the influence of Retengo, the growth of *M. phaseolina* fungus was observed on each ppm treatment, however, the rate of the growth did not exceed the efficiency of Amistar Xtra, because in the case of treatment with the Amistar Xtra fungicide, the growth of the hyphal and microsclerotia was even more intense.

Table 2

The *M. phaseolina* hyphal growth at second measurement

	10 ppm	20 ppm	50 ppm	100 ppm
Amistar Xtra (mm) Mycelia	90	90	90	70.5
Amistar Xtra (mm) Microsclerotia	65.1	57.2	52.6	46.6
Mirage (mm) Mycelia	0	0	0	0
Mirage (mm) Microsclerotia	0	0	0	0
Retengo (mm) Mycelia	58.6	39.6	34	25.8
Retengo (mm) Microsclerotia	37.1	29.1	31.8	22.6
Control Mycelia (mm)	90 mm			
Control Mycosclerotia (mm)	90 mm			

Figure 3: The hyphal and microsclerotia growth in poisoned media with pyraclostrobin

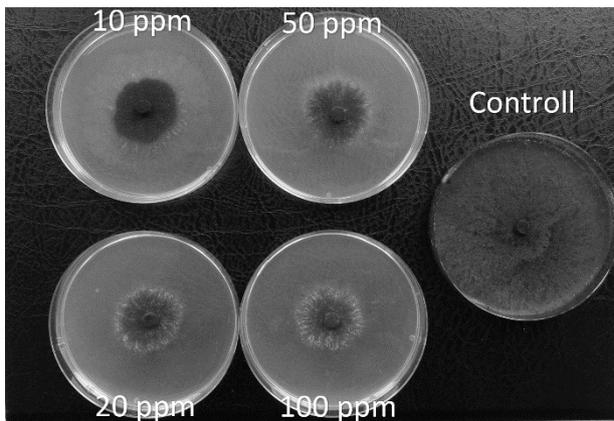
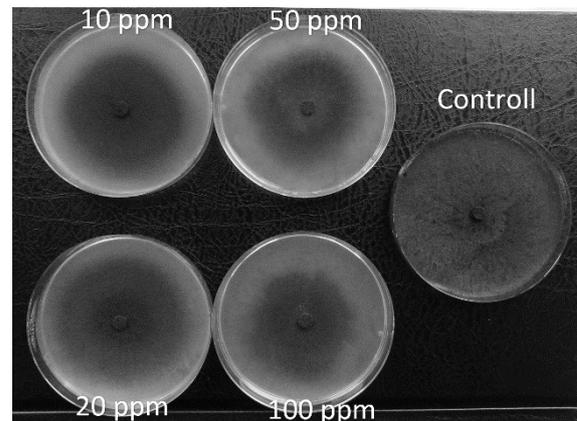


Figure 4: The hyphal and microsclerotia growth in poisoned media with azoxystrobin and ciproconazole



The following table shows the first measurement of the per cent inhibition of growth of *M. phaseolina* (Table 3). The Mirage was the most effective fungicide. At 100 ppm the pyraclostrobin arrested 75% the hyphal growth of fungus, while the Amistar Xtra arrested only 58%.

At the second measurement the prochloraz did not allow growth of the pathogen, while the Amistar Xtra

and the pyraclostrobin did allow it (Table 4). The hyphal system completely overran the media at 10 ppm, 20 ppm and 50 ppm poisoned media with Amistar Xtra. The pathogen produced microsclerotia form at every concentration of Amistar Xtra and Retengo. At 100 ppm the microsclerotia form was arrested supremely.

Table 3

At the first measurement the inhibition of the growth in percentage

	10 ppm	20 ppm	50 ppm	100 ppm
Amistar Xtra (%)	42	44	52	58
Mirage (%)	100	100	100	100
Retengo (%)	59	67	72	75

Table 4

At the second measurement the inhibition of the growth in percentage

	10 ppm	20 ppm	50 ppm	100 ppm
Amistar Xtra Mycelia (%)	0	0	0	18
Amistar Xtra Microsclerotia (%)	28	36	42	49
Mirage Mycelia (%)	100	100	100	100
Mirage Microsclerotia (%)	100	100	100	100
Retengo Mycelia (%)	35	56	62	71
Retengo Microsclerotia (%)	58	68	67	75

CONCLUSIONS

M. phaseolina fungus is a prevalent pathogen worldwide, including Hungary. Unfortunately, there is no effective treatment against this fungus. In the field there are no effective fungicides that farmers can use against the pathogen. In Hungary, there are no sunflower species, which are resistant to this fungus. In this study we tested 3 fungicides and only the

prochloraz was able to arrest the hyphal and microsclerotial growth. Conversely the azoxystrobin and ciproconazol (Amistar Xtra) cannot arrested the hyphal and microsclerotial growth enough. The pyraclostrobin had a moderate effect. The *M. phaseolina* could grow on the poisoned media with pyraclostrobin, but not too much. At present, the only reliable treatment is the genetic protection.

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