PAGV – SPECIAL REPORT NO 18 – 2017, 85-90

Biologicals for the control of *Alternaria solani* under greenhouse and field conditions

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SUMMARY

In this study, the potential of some *Trichoderma* spp. and *Bacillus subtilis* as biological plant protection products has been observed in a first step. Therefor greenhouse trials and an annual field trial were performed. For the greenhouse trial, the potato plants were treated with different biologicals and one day later the plants were inoculated with *Alternaria solani*. In detail, the spore solutions of *T. asperellum*, *T. atroviride* and *T. harzianum*, but also commercial products like TrichoStar®, TrichoMix® and Serenade® were used for the biological control. TrichoStar® and TrichoMix® are mixtures of different *Trichoderma* spp. and Serenade® includes *Bacillus subtilis*. For the field trial with the variety Lady Amarilla, the same treatments as in the greenhouse and additionally *T. hamatum*, were applied in a 7 to 10-day interval. A reduction of early blight could have been observed for most of the treatments in the greenhouse and field trial.

KEYWORDS

Alternaria solani, early blight, early blight control, kernel infection, biological control, *Trichoderma* spp., *Bacillus subtilis*

INTRODUCTION

In recent years the increasing relevance of *Alternaria solani*, the pathogens which causes early blight on potato, leads to several discussions about the best way to control this disease. The most effective way of controlling this pathogen is the use of fungicides. Unfortunately, there is an increasing resistance development, regarding the two main fungicide groups for controlling early blight, the QoIs and the SDHIs due to their frequent use (Pasche et al., 2004; Leiminger et al., 2013; Gudmestad et al., 2013; Bauske et al., 2017). The upcoming question is now, if there are other possibilities to keep the plants healthy and in a second step to stop or at least slow down this resistance development. Referring to this, the biological control as an ecofriendly alternative method has been mentioned several times (Siameto et al., 2010; Soria et al., 2012). Already in 1932, the potential of *Trichoderma* spp. as biocontrol agent was reported by Weindling. There are already studies, which evaluated the antagonistic potential of some *Trichoderma* spp. e.g. against *A. porri* on onions or *A. solani* on tomatoes (Kamal et al., 2014;

Sobia et al., 2015). In this study the potential of different *Trichoderma* strains and some commercial biological products as biocontrol agents against *A. solani* on potatoes has been investigated. Therefor *in vivo* and field trials were performed.

MATERIALS AND METHODS

In vivo trial

Cultivation of Trichoderma strains

The strains, which were used in this trial were provided by the University of Szeged, Hungary. To cultivate the strains, they were put on ¼ PD-agar and incubated under daylight and 25°C for about two weeks.

Execution of the greenhouse trial

For the greenhouse trial the cultivar Kuras was used due to its high susceptibility to *A. solani*. The plants were cut to a three-leaf stadium and then treated with three different spore solutions of *Trichoderma* strains and the commercial products one day before inoculation with *A. solani*. The spore density for the *Trichoderma* spp. was 10^7 spores per ml. For the infection with *A. solani* one day after the treatments, a spore density of 10^3 spores per ml was used. In this trial three plants per treatment were used. Three neither treated nor inoculated control plants were also integrated. After the inoculation with the spore solutions *A. solani*, the plants had to incubate in the mist chamber for 48 h at 100% relative humidity and 20°C. After these 48 h a relative humidity of 70% was pursued until the end of the experiment. The infection with *A. solani* was assessed after two and seven days after inoculation (dai). For the visual assessment the rating schedule from Granovsky and Peterson (1954) was used. This trial was repeated twice.

Field trial

General information

For the first field trial in Freising - Weihenstephan, Germany, the cultivar Lady Amarilla from the early maturity group, known to be very susceptible against early blight was used. The plot size was 4m x 4,5m. The plots were treated with spore solutions of the single *Trichoderma* strains or the commercial products. Altogether the trial consisted of 9 biological treatments, one chemical reference (multisite fungicide) and an untreated control. The trial included four repetitions per treatment. Furthermore, a kernel infection was done to ensure an infection with early blight in the field.

Kernel infection

The used isolates were cultivated on SN-agar for two weeks under near UV-light (12h/12h). To generate the kernel inoculum, 150g of barley kernels were put in an autoclavable bag with 60 ml distilled water and closed with a buckler and rubber band. The bags were autoclaved twice. Half of an overgrown, about 2 weeks old SNA-plate was used to knead the kernels with the grown fungus, to ensure adherence of the conidia on the grains in the autoclaved bag. The infected kernels need to be incubated for four weeks under near UV-light (12h/12h), to support fungal growth. 5 g of the finished kernels were spread equally per m^2 between the potato rows.

Disease assessment

The observation of the disease progress started with emergence until death of the potato plants. In each of the four replications, 10 plants per plot were rated for disease progress of early blight. To exclude the influence of the surrounding plots, only the two rows in the middle of each plot were used for observation. For the visual assessment the potato plant was divided into three leaf levels (lower, middle, upper leaf level) in order to follow disease development. One leaflet per leaf level was examined to determine the percentage of necrotic leaf area. This rating was done by using a scheme for evaluation of the leaf necrosis in percentage from Granovsky and Peterson (1954).

RESULTS AND DISCUSSION

The results for the greenhouse trial showed a clear advantage for the chemical fungicide with 100% efficacy, whereas the biologicals reached efficacies from about 20% in average (Figure 1). Since it wasn´t the goal to get the same effects with the biologicals compared to the chemical fungicide, the potential of these tested substances is visible and need to be analyzed in more detail.

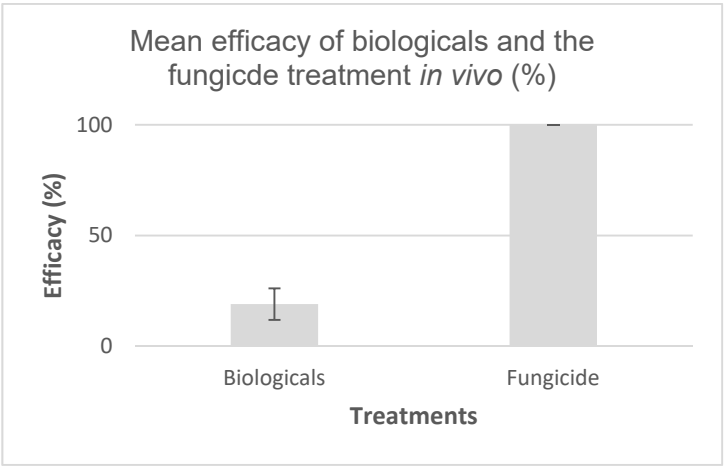

Figure 1. *Mean efficacy of biologicals and the fungicide treatment in vivo (%). For the biologicals, the average of all efficacies was calculated (n=6).*

Regarding the annual field trial, the results from the greenhouse were confirmed. The mean efficacy of the biological treatments at the beginning of the disease progression (11.08.2016) was nearly the same as for the fungicide treatment (44% and 52% respectively). One week later, a clear advantage for the fungicide treatment was observed (78%), but still the average of the efficacies from the biologicals was about 23% (Figure 2).

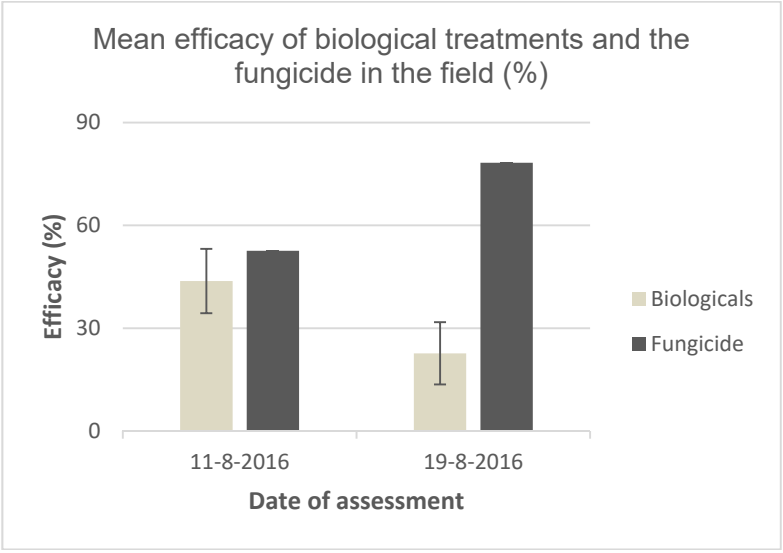

Figure 2. *Mean efficacy of biological treatments and the fungicide in the field (%). For the biologicals, the averages of all efficacies for the two dates were calculated (n=9).*

CONCLUSION

All in all, the potential for *Trichoderma* spp. and some other biological products to play a role in the control of early blight is visible. These biologicals cannot replace the chemical fungicides, but they could be a possibility to reduce the chemical treatments, by alternating use of biologicals and fungicides. Therefore, further research is necessary to end up with an optimized application strategy of these ecofriendly alternatives for conventional and biological farmers.

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