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SHORT COMMUNICATION

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Phosphite fertilisers as inhibitors of *Hymenoscyphus fraxineus* (*anamorph Chalara fraxinea*) growth in tests *in vitro*

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Abstract

This study is designed to test the potential for reducing the growth of the mycelium of the fungus *Hymenoscyphus fraxineus* (*anamorph Chalara fraxinea*) by using phosphite preparations at various concentrations *in vitro*. The study shows that adding pure phosphite to potato dextrose agar media inhibits the development of the fungus, but if the preparation is applied in the form of ammonium phosphite (Actifos), the growth of fungus will be accelerated. Probably the addition of nitrogen contained in the product Actifos has positive effect on the mycelial growth, but pure phosphite restricts its development. These studies are preliminary and only show the potential use of phosphite to reduce the development of *H. fraxineus*; however, to completely confirm its operation, further research is needed in this area.

Key words

ash, Actifos, phosphite, *Chalara fraxinea*, mycelium, development

Introduction

Until recently, ash (*Fraxinus excelsior* L.) in Poland was considered to be a species resistant to biotic as well as abiotic factors (Grzywacz 1995). In the past decade of the twentieth century, this situation changed and ash stands began to decline in all age classes. Initially, this phenomenon was observed only in the north-eastern part of Poland, but now, it has spread within the whole country. The cause of ash dieback was prescribed to *Hymenoscyphus fraxineus* (anamorph *Chalara fraxinea*) – a newly described fungus (Kowalski 2006). The study conducted by Kowalski (2009) showed that in all areas of Poland, ash dieback was very common. Currently, this phenomenon occurred in many other European countries, and much research concentrated on the growth inhibition of the pathogen and improving the vitality of ash trees. One of the methods proposed in this paper is the application of phosphites. These compounds being a part of commercial fertilisers are recognised as stimulants of tree resistance, in consequences leading to a reduction

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of activity of pathogens (Tkaczyk et al. 2016). Such studies have been conducted on a large scale in horticulture, where apart from improving the health status of trees, these fertilisers stimulated their growth (Orlikowski 2006; Muszyńska and Orlikowski 2010; Wieczorek et al. 2010; Schroetter et al. 2006; Tkaczyk et al. 2014). The goal of our research was to verify this hypothesis in the experiment *in vitro*.

Methodology

An attempt to inhibit the growth of the fungus *H. fraxineus* by using products containing ammonium phosphite (Actifos) and potassium phosphite (Kalex) was investigated in the Department of Forest Protection of the Forest Research Institute in Sękocin Stary. This experiment was carried out *in vitro* on potato dextrose agar medium amended with $(NH_4)_2PO_3$ (0.6%; 1.2%) and K_3PO_3 (0.6%; 1.2%). On such medium, the fungus *H. fraxineus* originating from the FRI collection (KY613993) was implanted on 10 Petri dishes for each variant. Over a period of 20 days, its daily mycelial growth was measured, and for statistical analysis, the nonparametric Kruskal–Wallis test was used.

Results

Potassium phosphate (K_3PO_3) inhibited the growth of fungus *H. fraxineus*. Its average radial mycelial growth in Petri dishes after 20 days was significantly lower than that in the control (without the addition of K_3PO_3). The difference between variants of potassium phosphite concentrations (0.6% and 1.2%) was not noticed, which means that the protective treatment performed at a lower concentration (0.6%) is already sufficient to slow down the growth of the fungus. The use of the preparation Actifos (0.6% and 1.2%) shows statistically significant differences between both the concentrations but do not differ from the control (Tab. 1).

In the case of Actifos, the stimulation of the growth of *H. fraxineus* was noticed. The highest pathogen mycelial growth was found in the case of 0.6% Actifos (Fig. 1). This phenomenon may be related to the presence of nitrogen (10.2%) and other microelements (B, 0.02%; Cu, 0.008%; Fe, 0.06%; Mn, 0.04%; Mol, 0.004%; Zn, 0.02%) in Actifos being sold as a fertiliser.

Table 1. The Kruskal–Wallis test probability values values of fungal growth on media amended with phosphites

	Control	K_3PO_3 (0.6%)	K_3PO_3 (1.2%)	Actifos (0.6%)	Actifos (1.2%)
Control		0.002	0.000	0.118	1.000
K_3PO_3 (0.6%)	0.002		1.000	0.000	0.03
K_3PO_3 (1.2%)	0.000	1.000		0.000	0.000
Actifos (0.6%)	0.118	0.000	0.000		0.006
Actifos (1.2%)	1.000	0.030	0.000	0.006	

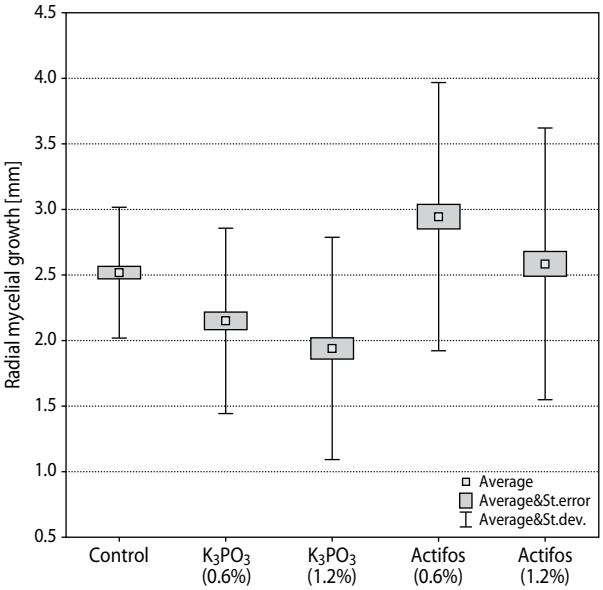

Figure 1. Comparison of an average radial mycelial growth of *H. fraxineus*

Conclusions

Our study shows that the use of phosphorus in the form of potassium can limit the development of the fungus. Moreover, the higher concentration of the preparation significantly decreases the growth of the mycelium. However, the application of Actifos formulation (phosphite in the ammonium form) resulted in the stimulation of mycelial growth. In the concentration variant of 0.6%, Actifos stimulated the growth of the mycelium of *H. fraxineus*

compared to the control. In double concentration (1.2%), the growth of the mycelium was similar to the control.

This experiment proved that the potassium form of phosphate limits the fungal growth in the test *in vitro*. In contrast, ammonium form of phosphite stimulates the growth of mycelium, probably, because of the action of nitrogen and other microelements. In the next step, the effect of potassium phosphate should be checked in tests *in planta*. Possible successful results of the product performance on the plants infected with *H. fraxineus* can be proposed in order to control the ash dieback phenomenon observed across Europe.

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