THIOBACILLUS BACTERIA IN CEREALS PROTECTION

BAKTERIE THIOBACILLUS V OCHRANĚ OBILNIN

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ABSTRACT

The laboratory experiment evaluated the effectiveness of the Aqua Almus® biopreparation containing bacteria of *Thiobacillus* sp. on the elimination of the natural microflora (*Alternaria*, *Bipolaris*, *Cladosporium*, *Drechslera*, *Epicoccum*, *Fusarium*, *Nigrospora*, *Rhizopus*) on the grains of winter wheat (*Triticum aestivum* L.) and spring barley (*Hordeum vulgare* L). The increasing concentration of the preparation (1, 2, and 4%) applied on the grains resulted in the decline in ratios of contaminated/non-contaminated grains compared to control samples treated by water solutions by 20, 10, and 42% in wheat, respectively, and by 28, 34, and 40% in barley, respectively. Mainly the saprophytic microflora (*Cladosporium*, *Epicoccum* and *Nigrospora*) was eliminated; the occurrence of pathogens with mycelium that penetrates the inner grain tissues (*Drechslera*, *Fusarium*) was not reduced. This fact is possibly the result of the short duration of the exposition of grains to the active substance of the preparation (especially in case of the hulled barley) and the chosen method of application (spraying/soaking). In upcoming research, effectiveness of the preparation will be tested on grains artificially infected by isolates of pathogens and vitality of germinating plants under modified environmental conditions will be studied.

Key words: biopesticides, pathogens, antagonism, seed treatment, barley, wheat, grain, Aqua Almus®

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INTRODUCTION

Plant nutrition and protection is currently being promoted by chemical inputs – fertilizers and pesticides. In the Czech Republic, plant nutrition is mainly covered by mineral fertilizers. It is estimated that on average 94 kg/ha of macronutrients (NPK) were applied over the last eleven years whereas the application of organic fertilizers was far lower, ranging between 50–60 kg/ha (Budňáková *et al.* 2011). Similarly, when comparing the use of conventional pesticides to the average application of biological preparations in the last two years, treatment with conventional pesticides (1–1.8 kg or l/ha) prevailed over the biological one (0.1 kg/ha) more than ten times. According to the Czech Ministry of Agriculture, the Czech Republic belongs to the countries with a very low pesticide usage; however, this is more a result of the economic situation in the agricultural sector rather than usage of bioactive substances instead of chemical preparations.

Although conventional fertilizers and pesticides allow, in most cases, sufficient nutrition and protection of plants, a long-term usage of these agrochemicals may results in undesirable contamination of soil as well as plants by chemical residues. Therefore the preparations based on biological agens are becoming of high interest not only for the sector of ecological agriculture but also for other sectors (Bailey *et al*. 2010). This fact was also confirmed by the data provided by the State Phytosanitary Administration. Altogether 38,240 ha of crops were treated by biological substances (microbial and macrobial) in the Czech Republic in 2011, which is almost by 12,000 ha more than in the previous year (increase by 46%). The country that is currently considered as a leader in the use of biological preparations among the EU countries is Holland. Worldwide, the market with biopreparations reached approx. 2% (750 mil. USD; Bt alone 375–450 mil., neem based products 20–30 mil., all other biopesticides as microbials, pheromones, plant extracts etc. about 300–350 mil.) of the whole market with agrochemicals (35–37 bil. USD; Mehta 2012).

The purpose of biopesticide application is to eliminate the occurrence of certain pests or pathogens by means of natural antagonism among microorganisms. As fungicide plant protection, preparations based on e. g. *Pythium oligandrum*, *Trichoderma* sp. and *Bacillus* sp. are used. Riungu *et al.* (2008) proved the antagonistic effect of *Epiccocum* sp., *Bacillus* sp., *Alternaria* sp. and *Trichoderma* sp. on *Fusarium graminearum*. The effect is based on the reduction of the growth of the pathogen's colonies on wheat plants by 49–64%. Wheat variants inoculated by *Alternaria* sp. showed decrease of deoxynivalenol in the grains by 91%. The presence of *Bacillus* sp., *Epiccocum nigrum* and *Aspergillus niger* on the wheat leaves reduced the germination capacity of Blotch of Wheat's (*Septoria tritici*) spores by 30–88% and spores of *Drechslera tritici-repentis* by 40–96%. The antagonistic effects of many other microorganisms are currently being evaluated.

The aim of this study was to verify the effectiveness of biological preparation containing *Thiobacillus* bacteria in terms of inhibition of pathogen infection in chemically untreated grains of winter wheat (*Triticum aestivum* L.) and spring barley (*Hordeum vulgare* L.).

MATERIAL A METHODS

Biological material and experimental conditions

Chemically untreated winter wheat (Mulan variety) and spring barley (Prestige variety) grains were used in the laboratory experiment. Ten grains from each species were placed on moist filtration paper into Petri's dishes in five replications. The seed was treated by liquid bacterial preparation Aqua Almus® (content of bacteria *Thiobacillus thiooxidans* and *T. ferrooxidans* 1.2 mil./ml of solution, pH 2.2) of escalating concentration of 1, 2 and 4%. Detection of microorganisms was carried out by microscopic means after five days of cultivation (20–23 °C, light regimen 12/12 hrs).

RESULTS

A microflora of winter wheat grains was represented by several genera (Tab. 1), namely *Alternaria*, *Bipolaris*, *Cladosporium*, *Epicoccum*, *Fusarium* (Fig. 1a), and *Nigrospora*. Concerning the pathogen genera occurred in spring barley grains (Tab. 2), *Alternaria* (Fig. 1b), *Cladosporium* (Fig. 1c), *Drechslera*, *Epicoccum*, *Fusarium*, *Nigrospora* (Fig. 1d), and *Rhizopus* were identified. In case of both crop species, the effectiveness of the Aqua Almus® preparation was proved by decrease of the number of pathogens occurring on the surface of naturally infected grains. However, the antagonistic effects of *Thiobacillus* of the Aqua Almus® seemed to be significant only for saprophytic genera (*Cladosporium*, *Epicoccum*, and *Nigrospora*) which were eliminated. The extent of antagonism among *Thiobacillus* and saprophytic genera was dependent on the concentration of the Aqua Almus®. The increasing concentration of the preparation (1, 2, and 4%) applied on the grains resulted in the decline in ratios of contaminated/non-contaminated grains compared to control samples treated by water solutions by 20, 10, and 42% in wheat, respectively, and by 28, 34, and 40% in barley, respectively. On the other hand, the Aqua Almus® concentration of 1 and 2% led to the increase of the number of saprophytic species of *Alternaria* genus (*A. tenuissima*, *A. alternata*) found on wheat grains. Their presence has no negative impact on germinating plants. Saprophytic representatives may colonize the cereal grains and create a competitive environment for other cereal grain pathogens and consequently inhibit their development. The spores and mycelium of saprophytic *Alternaria* spp. may occur on the grain surface as well as inside grains and their presence is signaled by e. g. blackish colouring of the outer layers of the grains.

The occurrence of pathogens with mycelium penetrating into the inner grain tissues (*Drechslera*, *Fusarium*) was not eliminated by the Aqua Almus®. These pathogens were identified on the grains even after treatment by concentrations higher than the recommended one (0.15%). No effects on these pathogens could be caused by the short exposition time of the grains to the active substance or

by way of application (spraying instead of soaking). In particular, in case of barley, the used method of application is a key factor because the most pathogens occur between the lemma and the outer layers of the grain. Therefore, the active substances (*Thiobacillus* bacteria) could not effectively penetrate through the outer layers, and thus compete with present pathogens.

Fig. 1 Pathogens of Alternaria, Cladosporium, Fusarium, and Nigrospora sp. on cereal grains of wheat and barley

a) Fusarium sp.

b) Alternaria sp.

c) Cladosporium sp.

d) Nigrospora sp.

Photos by Šafránková

Tab. 1 Spectrum of pathogens on winter wheat after cultivation of grains treated by the Aqua Almus® preparation (containing Thiobacillus thiooxidans and T. ferrooxidans)

Concentration	Pathogens	% of grains with pathogens occurrence
Control	*Alternaria*	20
	Alternaria+Cladosporium	2
	Alternaria+Epicoccum	2
	Bipolaris	4
	Cladosporium	10
	Epicoccum	8
	Fusarium	4
	Without microflora occurrence	50
1%	*Alternaria*	20
	Alternaria+Epicoccum	4
	Cladosporium	6
	Without microflora occurrence	70
2%	*Alternaria*	30
	Alternaria+Cladosporium	4
	Bipolaris	4
	Nigrospora	2
	Without microflora occurrence	60
4%	*Alternaria*	8
	Without microflora occurrence	92

Tab. 2 Spectrum of pathogens on spring barley after cultivation of grains treated by the Aqua Almus® preparation (containing Thiobacillus thiooxidans and T. ferrooxidans)

Concentration	Pathogens	% of grains with pathogens occurrence
Control	*Alternaria*	22
	Alternaria+Cladosporium	2
	Cladosporium	10
	Drechslera graminea	10
	Epicoccum	2
	Nigrospora	2
	Without microflora occurrence	52
1%	*Alternaria*	2
	Cladosporium	6
	Drechslera graminea	8
	Drechslera graminea+Fusarium	2
	Rhizopus	2
	Without microflora occurrence	80
2%	*Drechslera graminea*	14
	Without microflora occurrence	86
4%	*Alternaria*	2
	Cladosporium	2
	Drechslera graminea	4
	Without microflora occurrence	92

SUMMARY

Biological preparations have a significant potential in plant protection. The current research is focused on the targeted sampling and proprietary isolation and cultivation of plant associated microbes (rhizosphere, endosphere, phyllosphere) from diverse biotopes and environments around the world. Usage of *Thiobacillus* bacteria seems purposeful and promising in view of the other proved positive characteristics, such as an effective intake of nutrients by plants (transformation into a more acceptable form takes place), regeneration of damaged tissues and increase of plant resistance to low temperatures. In further stages of the research, other ways of Aqua Almus®

application (soaking, duration of exposition) will be tested. Testing of the preparation's effectiveness on the cereal grains artificially infected by isolates of pathogens and the vitality of the germinating plants under modified environmental conditions will be studied.

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