Penicillium decay on stored apple fruits after microbiological treatments

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Abstract

Postharvest diseases cause considerable losses to harvested fruits during storage. Biocontrol of postharvest diseases is an alternative means of management of blue mould pathogen. Control of apple postharvest diseases caused by Penicillium expansum *on apple fruit was evaluated with three different commercial products: Yield Plus®, Trifender® and Polyversum®. Biocontrol agents of these products were yeast like fungus* Cryptococcus albidus, Trichoderma asperellum *and* Pythium oligandrum*, respectively.*

In the first experiment, under field conditions, P. oligandrum *and* T. asperellum *were applied three times during the vegetation season and the last one was made just before harvest. Suspension of both products, in recommended concentration, was introduced into organic apple orchard. After harvest apples were stored in the paper bags in cooling room (4^{0}C, 70%RH). The incidence of blue mould as a percentage of infected of fruit area was assessed after 4 months.*

The second test was conducted in the laboratory on harvested apple fruits. Each bioagents were used at different concentration 10 X, 2,5 X and 1 X of recommended dose by dipping of fruits in water suspension before storage. Treated fruits were storied at 4^{0}C, in the plastic bags to maintain high humidity (98%RH). In test disease assessment was quantified by the four month period of storage as a percent of mean decay area.

As the results were noted that the percentage of decayed fruits after field treatment with T. asperellum was reduced by 10% compared to P. oligandrum*.*

On apples fruits treated with the highest concentration of all biocontrol agents was observed the best reduction effect of P. expansum *(0,93; 0,73 and 0,97% of decay area for* C. albidus*,* T. asperellum *and* P. oligandrum*, respectively). The best result was obtained after Trifender® treatment (0,73; 0,67 and 2,07 % of decay symptoms, respectively for each used concentrations), while 5% of spoilage was observed on untreated by microorganisms fruits. In case of recommended dosage (1X) of* C. albidus *was noted increase of decay symptom area (30,33%) compared to control. Polyversum® in recommended (1X) and 2,5X dose was also recognized as concentration with weak or lack of blue mould reduction.*

Keywords: organic production, apple fruits, blue mould, storage ability, biocontrol

Introduction

Postharvest diseases cause considerable losses to harvested fruits during storage. Decay damage is calculated as 20-30 % of the world fruits production (Sharma *et al*. 2009). The fruit infection can occur either prior to harvest or during harvesting and subsequently during handling and storage. Public awareness on pesticide residues issues as well as European policies are creating the need for searching of alternative control methods of postharvest diseases caused by different bacterial and fungal pathogens. There is no doubt that biocontrol of postharvest diseases is an alternative means of management of blue mould, one of the most common postharvest apple diseases.

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Public awareness on pesticide residues issues as well as European policies are creating the need for searching of alternative control methods of postharvest diseases caused mainly by *B. cinerea* and *Penicilium* sp. Microorganisms to be used for control of post-harvest diseases (the yeast *Candida oleophila, Aureobasidium pullulans, Pseudomonas* sp.*)* are under assessment for inclusion into the list of active substances foreseen in Reg. 1107/2009. According to knowledge of authors only *Coniothyrium minitans* and *Pseudomonas chloraphis* MA342 are included. The aim of investigations was to assess the ability of three different microbiological products, used directly in orchard or as postharvest treatment to reduce blue mould infection and incidence in storage conditions.

Material and Methods

Control of apple postharvest diseases caused by *Penicillium expansum* on apple fruit was evaluated with an three different commercial products: Yield Plus ®, Trifender® and Polyversum®. Biocontrol agents of this products was yeast like fungus *Cryptococcus albidus*, *Trichoderma asperellum* and *Pythium oligandrum,* respectively.

The first experiment was carried out in years 2009-2010 in the organic orchard composed of Gloster cultivar. In the experiment under field conditions, *P. oligandrum* and *T. asperellum* were applied three times during the vegetation season and the last treatment was made just before harvest. Suspension of both products, in recommended by the producers concentration, was introduced into organic apple orchard. The incidence of blue mould as a percentage of infected of fruit area was assessed after 4 months of storage. Antagonists' fungi suspension was sprayed uniformly onto the plants using a shoulder-carried sprayer "Kwazar" (vol. 10 L). Fruits were harvested carefully and put in paper bags and were directly transported to the laboratory where they were immediately cooled to 4^0C in the storage room. After the period of storage (4 month, 4^0C, 70% RH), fruits were checked for appearance of blue mold symptoms. The incidence of *Penicillium* disease was presented as a percentage of fruit decay area.

The experiment in laboratory was carried out in 2009/10 to assess the ability of used products to protect fruits against *Peniclillum* spoilage. The second purpose in this test was to evaluate the proper concentration of used fungi to obtain the best control efficiency. This experiment was conducted on harvested apples of the same Gloster cultivar. Apples were delivered from the organic orchard and before harvest were no treated with any chemicals or products. As a comparative sample some of fruits were treated by captan 14 days before harvest (at dose 1.9 kg per hectare). Only undamaged and unwounded fruits were chosen for the experiment. One day after harvest, organic fruits were dipped in water suspensions containing three different concentrations of spores of used products, i.e. 10 X; 2,5 X and 1 X of recommended dose. According to the producers, the recommended dose for Trifender, Polyversum and YieldPlus is 5×10^8 cell $\cdot ml^{-1}$, 1×10^6 cell $\cdot ml^{-1}$ and 1×10^6 cell $\cdot ml^{-1}$, respectively. The concentrations of the suspension were checked out with Brücker chamber and then adjusted to required concentration. After treatment apples were put on paper towels to remove the excess of the suspension. Fruits were then placed into plastic boxes and stored at 4^0C and 98% RH. In test disease assessment was quantified by the four month period of storage as a percent of spoilage area. As a statistical analysis firstly, the normality of distribution of the trait was tested using Shapiro-Wilk's normality test (Shapiro & Wilk, 1965). Non-normal trait was transformed using the power (Box-Cox) transformation (Quinn & Keough, 2002) with λ=0.45. Two-way fixed model analysis of variance was carried out to determine the effect of treatment, concentration and treatment $\times$ concentration interaction on the variability of the studied trait. The Tuckey's honestly significant differences (HSDs) of trait were calculated, and on this basis homogeneous

groups (not significantly from each other) were determined. One-sample *t*-test was used for statistical verification of the hypothesis of lack of differences between mean value for each level of treatment × concentration interaction and control (s.a. captan). Data analyses were performed using the statistical package GenStat v. 7.1 (Payne *et al*., 2003).

Results

Results of the field experiment indicated that application of microorganisms during vegetation could be important for quality of stored fruits. The best results were obtained after field treatment with *T. asperellum*. Under storage condition the blue mould symptoms was reduced by 10% compared to *P. oligandrum* (Table 1).

Table 1: The influence of antagonistic fungi application into organic orchard on blue mould incidence on apples fruits after storage period.

No.	Treatments	Mean % of dacay area
1	*T. asperellum*	33,31
2	*P. oligandrum*	44,30

As a results of laboratory test analysis of variance indicated that the main effects of treatment (P=0.014) and concentration (P=0.028) as well as treatment × concentration interaction (P=0.045) were significant for studied trait (Table 2). It means that every of used fungi or concentration had significant different influence on development of *Penicilium* spoilage. Also the interaction between every used concentration and antagonist were significant differ. In this case every obtained result could be discussed as important value.

Table 2: Mean squares from analysis of variance for studied effect of treatments of antagonist and used concentration and their interaction.

Source of variation	Number of degrees of freedom	Mean squares
Treatment	2	1.616*
Concentration	2	3.440*
Treatment × Concentration	4	1.225*
Residual	18	9.841
* significant at 0.05 level		

Table 3: The influence of antagonist and used concentration and their interaction on area of spoilage calculated as mean values and standard deviations (SD).

Microorganism	Concentration							
	1X r.e.		2,5X r.e.		10X r.e.		1X, 2,5X and 10X	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
C. albidus	30.333	35.388	1.667	1.528	0.933	0.577	10.978A	22.904
P. oligandrum	13.867	22.633	13.267	18.583	0.967	0.351	9.367A	15.943
T. asperellum	2.067	1.328	0.667	0.577	0.733	0.681	1.156B	1.053
C, P and T	15.422a	24.347	5.200b	11.126	0.878c	0.492		
$HSD_{0.05}$	for treatment: 6.512, for concentration: 4.101, for treatment × concentration interaction: 9.815							

C – *C.albidus*, P- *P. oligandrum* and T- *T. asperellum*
r.e. – recommended dose

In experiment conducted with different antagonistic fungi obtained results were significant differ from obtained with apples immersed in captan control. After four months of storage the decay area of blue mould reached 5 % of captan treated fruits area. In case of some concentrations and microorganisms was noted that 1X of *C. albidus* affected in 30.33% of spoilage area, in case of *P. oligandrum* and its concentration 1X and 2.5X was observed the spoilage area 13.96 and 13.27%, respectively. It was significant higher percent comparing to captan control (5%). Surprising was that in case of recommended dosage of *C. albidus* and in case of *P. oligandrum* in recommended and 2,5X dose was recognized as ineffective treatments. The best result was obtained after *T. asperellum* treatments (0.73; 0.67 and 2.07 % of decay symptoms in different concentrations). The highest used concentration of microorganisms was the most effective (0.93; 0.73 and 0.97% of decay area).

Discussion

One of the major unanswered questions is when and how the antagonistic microorganisms should be applied to assure their survival and maximum control of postharvest diseases. According to this Wiśniewski & Wilson (1992) it is believed that biological control by means of microbial antagonist have greater potential for success when applied after harvest. Obtained results suggested that treatment before or after harvest could be effective in control blue mould. However, very important impact on reduction of *Penicillum* decay had used cell concentration for fruit protection. Zhang *et al.* (2005) also noticed that the concentration of antagonist had significant effects on biocontrol effectiveness. They obtained completely inhibition of oranges Penicilium decay with antagonist spores suspension 1 $x10^9$ cell x ml^{-1}. The results of laboratory trial suggesting that recommended by producers' antagonists' concentration had significant lower influence on decay incidence than commercially used chemical (captan) treatment. The observed spoilage fruit areas after postharvest treatment underline the importance of the suspension spore density to assure the efficacy of the application. We believe that for long term cold storage should be used even ten times higher concentration that is recommended to received the best control of apples blue mould.

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