# Effect of compounds used for soaking narcissus bulbs on mycelium coverage of bulbs and substrate

Adam Tadeusz Wojdyła $^{1*}$ , Jacek Stanisław Nowak $^{1}$ , Jan Bocianowski $^{2}$ , Monika Kałużna $^{1}$ , Jacek Wiśniewski $^{3}$ , Emilia Waszkiewicz $^{4}$ 

Citation: Wojdyła A.T., Nowak J.S., Bocianowski J., Kałużna M., Wiśniewski J., Waszkiewicz E. (2025): Effect of compounds used for soaking narcissus bulbs on mycelium coverage of bulbs and substrate. Hort. Sci. (Prague), 52: 103–110.

**Abstract:** In the experiments, two compounds were used: hydrogen peroxide with silver  $(H_2O_2\text{-}Ag^+)$  and captan, pyraclostrobin + boscalid and methyl thiophanate + tetraconazole to soak narcissus bulbs before planting for the period of 20 minutes. The bulbs were planted in such a way that 2/3 of the bulb was above the surface of the substrate. All the pots with bulbs were subsequently placed in plastic boxes and transferred to a cooling chamber adjusted precisely for 9 °C and 94–99% air humidity. During the rooting of the bulbs in the cold room after 4, 8, 12 and 15 weeks, and then in the greenhouse after cutting the above-ground parts, the observations of the bulbs and percentage substrate coverage by the fungi most frequently isolated in the previous observations. The research showed that  $H_2O_2$ - $Ag^+$  and fungicides used for soaking significantly limited the fungal development on bulbs and also on the substrate during the rooting period in the cold store and after they were placed in the greenhouse until the flowering.

Keywords: bulb rooting; hydrogen peroxide; fungicides; inhibition; fungi on bulb surface and substrate

The existing literature data show that the fungi *Penicillium* spp., *Trichoderma viride* Pers. and *Rhizopus stolonifer* (Ehrenb.) Vuill. (Horst 2008), *Botryotinia narcissicola* (P.H. Greg.) N.F. Buchw. (O'Neill et al. 2005) may be the cause of the rotting of narcissus bulbs, but *Fusarium oxysporum* f. sp. *narcissi* (Cooke & Massee) Snyder & Hansen has been mentioned most frequently (Byther 1987; Hanks 1996; Łabanowski et al. 2006; Horst 2008; Vasić et al. 2021).

Careful selection and elimination of diseased bulbs at the time of planting is one of the ways to reduce the occurrence of the disease, but it does not bring complete success. So far, before planting narcissus, it was recommended to treat bulbs with fungicides, among others from the group of benzimidazoles, imidazoles, chloronitriles and phthalimides (Byther 1987; Hanks 1996; Łabanowski et al. 2006). The research attempted to check the possibility of using other

Supported by the European Union under the "Cooperation" measure of the Rural Development Program 2014–2020 "Implementation of an improved product, innovative technology and production organisation methods in the production of bulbous ornamental plants using high-pressure fogging of refrigerated rooms with silver-stabilised hydrogen peroxide".

<sup>&</sup>lt;sup>1</sup>The National Institute of Horticultural Research, Skierniewice, Poland

<sup>&</sup>lt;sup>2</sup>Department of Mathematical and Statistical Methods, Poznań University of Life Sciences, Poznań, Poland

<sup>&</sup>lt;sup>3</sup>Horticulture Farm Jacek Wiśniewski General, Wiązowna, Poland

<sup>&</sup>lt;sup>4</sup>Emi Agro Emilia Waszkiewicz, Otwock, Poland

<sup>\*</sup>Corresponding author: adam.wojdyla@inhort.pl

<sup>©</sup> The authors. This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International (CC BY-NC 4.0).

fungicides not recommended so far and compounds that are not protective agents, such as hydrogen peroxide, for dressing. A particular advantage of the use of hydrogen peroxide with silver in research is that it has a different action mechanism on pathogens in comparison to the fungicides used so far. Moreover, it is entirely safe for people, animals and the environment. Another problem occurring during the 15-week rooting period of the narcissus in the cold room is the high air humidity above 95%, which causes various types of fungi to develop on the surface of the bulbs and the substrate. Unfortunately, after removing such plants from the cold store, they are often not suitable for trade or require very laborious manual cleaning of the bulbs and the substrate from the mycelium. The research showed that these species were Neopestalotiopsis foedans (Sacc. & Ellis) Maharachch., K.D. Hyde & Crous [syn. Pestalotiopsis foedans (Sacc. & Ellis) Steyaert], Penicillium olsonii Bainier & Sartory, Rhizopus stolonifer (Ehrenb.) Vuill., Trichoderma asperellum Samuels, Lieckf. & Nirenberg (Wojdyła et al. 2022b). They sometimes covered over 40% of bulb surfaces and the substrate with abundant mycelium and spores (Wojdyła et al. 2022a). Therefore, narcissi producers are waiting for the recommendation of new preparations that are possibly safe for plants and the environment.

Attempts to explain the action mechanism of hydrogen peroxide on plant pathogens have shown that active oxygen released during the decomposition of H<sub>2</sub>O<sub>2</sub> can disrupt the functioning of cell membranes, cause oxidative changes in DNA and disrupt cellular metabolism (Hoffman, Meneghini 1979; Imlay, Lin 1988; Mayer at al. 2001). In turn, in the plant, hydrogen peroxide can induce disease resistance by deposition of callose in cell walls, formation of nodules that hinder pathogen infection, and increase in the production of hydroxyproline and phenolic compounds (Mazau, Esguerré-Tugayé 1986; Kotchoni et al. 2007). Hydroxyl radicals (HO•), formed during the decomposition of hydrogen peroxide, cause the entire degradation of the lipid membrane after just 17 minutes (Anzai et al. 1999). As far as anaerobic organisms are concerned, the core cytotoxic factor is the final result of hydrogen peroxide decomposition, i.e. molecular oxygen (Marshall et al. 1995). H<sub>2</sub>O<sub>2</sub> molecules, owing to their small size, penetrate cell membranes easily and show a biocidal effect against a wide spectrum of organisms, including bacteria, fungi and viruses (Miyasaki et al. 1986). Hydrogen peroxide (H2O2) is regarded as a reactive oxygen species (ROS), which can damage various cellular structures. Moreover, recent work has shown that  $\rm H_2O_2$  can act as a powerful signalling molecule and mediate different physiological and biochemical processes in various plants (Nurnaeimah et al. 2020).

The existing literature data show the possibility of using hydrogen peroxide in plant protection against pathogens. In experiments conducted by Meng et al. (2019), H<sub>2</sub>O<sub>2</sub>-Ag<sup>+</sup> was highly effective in inhibiting in vitro growth of radial mycelium and in vivo lesion development of inoculated Penicillum italicum Wehmer and Penicillum digitatum Sacc. causing the growth of mycelium on citrus fruits. Dipping fruit in H<sub>2</sub>O<sub>2</sub>-Ag<sup>+</sup> at concentrations of 1-2% before orange storage decreased the decay incidence of fruits after 30 and 60 days of cold storage. H<sub>2</sub>O<sub>2</sub>-Ag<sup>+</sup> brought about a disinfectant effect on the pericarp, clearly indicated by a significant decrease in total bacteria, mould and yeast counts. This study suggests that H<sub>2</sub>O<sub>2</sub>-Ag<sup>+</sup> can be an alternative to chemical fungicides, which, although more effective, pose problems owing to the residue levels after their use, as well as other health concerns, especially for the organic fruit industry (Meng et al. 2019). In addition, the efficiency of 5 and 10% hydrogen peroxide in growth inhibition of Penicillium expansum Link caused blue mould rot of apples in agar diffusion assay was complete, and its minimum inhibitory concentration (MIC) determined by the use of agar and broth dilution assays was less than 0.025%. These outcomes show that the application of small quantities of hydrogen peroxide to apple skin could be an alternative to fungicides in eliminating P. expansum from apples (Venturini et al. 2002).

However, under *in vivo* conditions,  $H_2O_2$ - $Ag^+$  at the concentration of 0.5%, used to dip eggplant and pepper fruits before storage, decreased fruit rot, leading to almost full inhibition of the development of such pathogens as *Botrytis cinerea* Pers. and *Alternaria alternata* (Fr.) Keissl. (Fallik et al. 1994).

Literature data indicate that silver possesses a strong antimicrobial effect both in ionic and nanoparticulate form, thanks to which it is used in the control of diseases in plants caused by biotic and abiotic factors (Aziz et al. 2015). McShan et al. (2014) report that silver nanoparticles penetrate into cells and are internalised. Nanosilver is a compound that often functions as a source of Ag<sup>+</sup> inside the cell. The major mechanism of toxicity is the fact that it causes

oxidative stress by generating reactive oxygen species and causing damage to cellular components, including DNA damage, antioxidant enzyme activation, antioxidant molecules depletion (e.g. glutathione), protein binding and disabling, as well as cell membrane damage.

There are relatively few studies in the literature on the use of silver in combating plant diseases (Jo et al. 2009; Zalewska et al. 2016). Research by Kim et al. (2012) carried out *in vitro* showed that nanoparticle silver at the concentration of 100 ppm strongly inhibited the growth of cultures of 18 tested fungi pathogenic to plants. On the other hand, studies conducted on ryegrass (*Lolium perenne* L.) showed that in the case of pathogens *Bipolaris sorokiniana* (Sacc.) Shoem. and *Magnaporthe grisea* (T.T. Hebert) M.E. Barr, both ionic and nano silver sprays significantly reduced the intensity of pathological symptoms/disease severity.

Lamsal et al. (2011a) evaluated the effect of silver nanoparticles on the development of powdery mildew symptoms in pumpkin and cucumber in some *in vitro* and *in vivo* studies. In field conditions, the highest effectiveness was found for nanoparticle silver at a concentration of 100 ppm applied both before and after the onset of the disease. Similarly, *in vivo* tests of silver nanoparticles at the concentration of 100 ppm showed maximum inhibition of fungal hyphae growth and conidia germination.

Unfortunately, the accessible literature is short of information on the effects of the fungicides used in our research to treat narcissus bulbs on the occurring species of fungi during the rooting period (Hanks 1996; Łabanowski et al. 2006) and on the growth and the development of plants. Literature data on the effectiveness of the agents that we reviewed/assessed on narcissus, carried out by various authors, showed varying effectiveness depending on the plant species and the pathogen to be controlled (Sallato et al. 2007; Serey et al. 2007). In some in vitro studies, captan proved to be extremely effective in the reduction of mycelium growth, as well as spore germination of *Rhizopus* oryzae Went & Prinsen Geerligs (Amadioha 1996), and at concentrations above 500 µg/mL, the growth of mycelium of Trichoderma viride Pers., T. harzianum Rifai, T. koningii Oudem, T. pseudokoningii Rifai and T. virens Mill, Giddens & Foster (Bhale, Rajkonda 2015). On the other hand, thiophanate methyl at the concentration of 0.1% to 0.2% in vitro provided full inhibition of the growth of Pestalotiopsis disseminata (Thüm.) Steyaert mycelium (Ray et al. 2016).

Under *in vivo* conditions, captan was highly effective in the reduction of rot caused by *R. oryzae* on potato (Amadioha 1996) and eggplant (Youssef et al. 2015). On the other hand, captan and boscalid + pyraclostrobin used during the growing season prevented grapevine fruit rot caused by *P. expansum* and *R. stolonifer* (Serey et al. 2007). Boscalid + pyraclostrobin used during the growing season for the protection of strawberries between flowering and harvest visibly reduced the occurrence of grey mould (*B. cinerea*) and soft rot (*R. stolonifer*) on stored fruits (Sallato et al. 2007).

#### MATERIAL AND METHODS

Experimental design. The experiments were conducted in 2021–2022 at the Horticultural Farm Jacek Wiśniewski Spółka Jawna and the Institute of Horticulture – National Research Institute in Skierniewice. The materials and methods used in the present work are similar to those used in earlier research on hyacinth (Wojdyła et al. 2022a; Wojdyła et al. 2025). Narcissus bulbs were treated with the plant growth stimulator Bisteran (50% hydrogen peroxide + 0.32 g of silver in 1 kg) and the fungicides Biszop 80 WG (80% captan), Signum 33 WG (67 g of pyraclostrobin in 1 kg + 267 g boscalid in 1 kg) and Yamato 303 SE (233 g thiophanate methyl in 1 L + 70 g tetraconazole in 1 L). The control bulbs were soaked in water.

*Narcissus* L. 'Tete-a-Tete' bulbs packed in 3 kg Raschel bags were immersed in the tested agents for 20 min according to the following scheme:

- 1 Control (water);
- 2 Bisteran 1%;
- 3 Bisteran 2%;
- 4 Bisteran 3%;
- 5 Bisteran 5%;
- 6 Bisteran 10%;
- 7 Yamato 303SE 0.5%;
- 8 Signum 33 WG 0.5%;
- 9 Biszop 80 WG 1%.

For soaking the bulbs in plastic buckets, 10 L of suspensions of preparations were prepared. The bags full of bulbs were taken out, drained in order to get rid of the excess liquid, and dried outdoors for several minutes. Then, the bulbs were planted in pots with a diameter of 8 cm and a volume of 0.3 L. Three narcissus bulbs with a circumference

of 11–12 cm were planted in each pot in the substrate so that 2/3 of the bulb was visible above the surface of the substrate. Within the pot, the bulbs occupied approximately 80% of the surface. The substrate consisted of de-acidified raised peat and sand mixed in a ratio of 9:1, was slightly acidic (pH 6.5), contained small amounts of nutrients and had very good air and water properties, which in the case of rooting bulbous plants is an advantageous feature (Wojdyła et al. 2022a).

All the pots with bulbs were subsequently placed in plastic boxes (crates) size  $60 \times 40 \times 20$  cm (length × width × height). The boxes were covered with an upholstery sponge measuring  $60 \times 40 \times 2.5$  cm and purposefully pressed with multiplates measuring  $60 \times 40$  cm so that the bulbs could not be pushed out of the ground by the developing root system. The boxes were stocked on wooden pallets in the following way: five boxes on one level with 10-11 layers and transferred to cooling chambers adjusted precisely for 9 °C and 94-99% air humidity. After 4 weeks of the experiment, the pressure sponge covers were removed from the crates with the narcissus bulbs.

Measurements and observations. During the rooting of the bulbs in the cold room after 4, 8, 12 and 15 weeks, and then in the greenhouse after cutting the above-ground parts, the observations of the bulbs and percentage substrate coverage by the fungi most frequently isolated in the previous observations Neopestalotiopsis foedans (syn. Pestalotia foedans, Pestalotiopsis foedans), Penicillium olsonii, Rhizopus stolonifer, Trichoderma asperellum (Wojdyła et al. 2022b) were carried out. After the sprouted bulbs were taken out from the cold store (second stage), they were transferred to the greenhouse of the National Institute of Horticulture Research and placed on flooding tables in controlled conditions, where the temperature was kept at about 9 °C for several days (5-7 days) to undergo acclimatisation. After this period, the temperature in the greenhouse was increased to 16 °C and kept at this level until the end of cultivation. If necessary, the plants were watered in such a way that the surface of the substrate in the pot was slightly moist. After flowers beheading, the observations of the bulbs and percentage substrate coverage by the fungi were carried out.

**Statistical analysis.** The normality of the distribution of the observed bulbs covered with mycelium in five terms (after 4, 8, 12 and 15 weeks and after flowers beheading) and substrate mycelium cover-

age in five terms (after 4, 8, 12, and 15 weeks and after flowers beheading) was verified with Shapiro-Wilk's normality test to check whether the analysis of variance (ANOVA) met the assumption that the ANOVA model residuals followed a normal distribution. After confirming the normal distribution, a one-way analysis of variance (ANOVA) was performed to verify the null hypotheses of a lack of combination effect on the ten observed traits, independently for each one. The arithmetic means, and standard deviations were calculated. Moreover, Fisher's least significant differences (LSDs) were estimated at a significance level of  $\alpha = 0.05$ . The relationships between observed traits were estimated using Pearson's linear correlation coefficients. The GenStat version 23 statistical software package (VSN International) was used for the analyses.

#### **RESULTS**

ANOVA indicated that the main effect of combinations was significant for all examined traits (Table 1). The highest mean values of bulbs covered with mycelium in all five terms were observed for the control. The highest mean values of substrate mycelium coverage were observed after 8 and 12 weeks in the cold room and after flowers beheading. The highest mean value of substrate mycelium coverage after 4 weeks was observed for treatment with Bisteran 10%; however, after 15 weeks for treatment with Bisteran 2%. On the other hand, the lowest mean values of bulb mycelium coverage were observed for Biszop 80 WG (after 4 weeks and after 12 weeks), Bisteran 10% (after 8 weeks and after flowers beheading) and Bisteran 5% (after 15 weeks). In the case of substrate mycelial coverage treatment with Biszop 80 WG was characterised by the lowest mean values after 4, 8 and 12 weeks. Bisteran 10% had the smallest mean value of substrate mycelial coverage after 15 weeks (1.00). After flowers beheading, mean values equal to zero were obtained for all treatments except the control (Table 1).

Bulbs covered with mycelium observed in all terms were significantly positively correlated. The significant positive correlations for substrate mycelium coverage were observed between: after 8 and 12 weeks (0.71), after 8 weeks and after flowers beheading (0.68), after 12 and 15 weeks (0.67), as well as after 12 weeks and after flowers beheading (0.94) (Table 2, Figure 1).

Table 1. Results of one-way analysis of variance, mean values and standard deviations (SD) as well as homogeneous groups for coverage of bulbs and substrate with mycelium in %

Treatment -	After 4 weeks		After 8 weeks		After 12 weeks		After 15 weeks		After flowers beheading	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Bulbs covere	d with myo	elium (in	%)							
1	17.00 <sup>a</sup>	19.20	16.60 <sup>a</sup>	11.40	9.21 <sup>a</sup>	10.30	$16.70^{a}$	8.07	$19.20^{a}$	9.63
2	7.66 <sup>cd</sup>	10.10	$10.30^{\mathrm{bc}}$	10.20	$7.25^{a}$	10.10	$14.40^{a}$	9.90	$12.00^{b}$	6.44
3	$11.80^{b}$	12.50	$12.90^{b}$	13.70	$8.50^{a}$	11.30	$14.40^{a}$	8.42	$11.00^{\mathrm{bc}}$	7.22
4	$5.15^{\mathrm{def}}$	7.82	$6.56^{\mathrm{de}}$	8.66	$3.25^{\mathrm{bc}}$	6.52	7.69 <sup>d</sup>	7.20	8.19 <sup>de</sup>	7.30
5	6.13 <sup>de</sup>	7.95	$9.81^{bc}$	9.36	$4.75^{b}$	9.54	14.60 <sup>a</sup>	9.35	$7.44^{e}$	7.80
6	$2.81^{ef}$	5.15	$5.44^{\rm e}$	8.54	1.88 <sup>c</sup>	4.80	$8.38^{\rm cd}$	7.66	$2.31^{\rm f}$	4.28
7	$11.10^{bc}$	12.10	$8.50^{\rm cd}$	8.43	$3.00^{\mathrm{bc}}$	5.07	$11.50^{b}$	9.36	$10.20^{\mathrm{bcd}}$	7.69
8	$8.31^{\mathrm{bcd}}$	15.30	$7.44^{\mathrm{cde}}$	9.35	$2.56^{\mathrm{bc}}$	5.09	8.81 <sup>bcd</sup>	7.13	6.69 <sup>e</sup>	6.16
9	$2.56^{\mathrm{f}}$	4.90	5.88 <sup>de</sup>	7.02	$1.25^{\rm c}$	3.33	$11.00^{bc}$	10.48	$8.81^{\rm cde}$	7.81
$\mathrm{LSD}_{0.05}$	3.56	_	3.04	_	2.44	_	2.70	_	2.26	_
F-ANOVA	13.09***	_	11.03***	_	11.47***	_	11.05***	_	31.87***	_
Substrate my	ycelium cov	erage (in	%)							
1	$1.34^{\mathrm{ab}}$	3.70	$3.25^{a}$	5.34	$3.19^{a}$	5.64	$4.50^{ab}$	5.93	$0.38^{a}$	1.91
2	$0.50^{\mathrm{bc}}$	2.33	$1.19^{bc}$	3.92	$1.06^{b}$	3.05	$3.50^{a-d}$	5.06	$0.00^{b}$	0.00
3	$1.94^{a}$	3.94	$1.81^{b}$	4.15	$1.13^{b}$	3.56	$4.63^{a}$	5.20	$0.00^{b}$	0.00
4	$1.86^{a}$	4.23	$2.31^{ab}$	4.97	$0.38^{b}$	1.91	$1.88^{ef}$	4.24	$0.00^{b}$	0.00
5	1.75 <sup>a</sup>	3.82	$2.06^{ab}$	4.34	$0.75^{b}$	2.65	$2.75^{\rm cde}$	4.49	$0.00^{b}$	0.00
6	$2.00^{a}$	4.40	$1.75^{b}$	3.99	$0.38^{b}$	1.91	$1.00^{\rm f}$	3.02	$0.00^{b}$	0.00
7	$0.63^{\mathrm{bc}}$	2.16	$1.81^{b}$	4.23	$0.69^{b}$	2.48	$3.75^{ab}$	6.49	$0.00^{b}$	0.00
8	$1.38^{ab}$	3.81	$1.13^{\mathrm{bc}}$	3.08	$0.31^{b}$	1.46	$3.00^{b-e}$	4.54	$0.00^{b}$	0.00
9	$0.00^{c}$	0.00	$0.25^{\rm c}$	1.58	$0.25^{b}$	1.57	$2.13^{\mathrm{def}}$	4.12	$0.00^{b}$	0.00
$\mathrm{LSD}_{0.05}$	1.06	_	1.27	_	0.92	_	1.52	_	0.20	_
F-ANOVA	3.57***	_	3.36***	_	7.65***	_	4.95***	_	3.08**	_

1-9 – control (water), Bisteran 1%, Bisteran 2%, Bisteran 3%, Bisteran 5%, Bisteran 10%, Yamato 303SE 0.5%, Signum 33 WG 0.5%, Biszop 80 WG 1%, respectively, LSD – least significant difference; \*\*,\*\*\*significant levels at P < 0.01, 0.001, respectively;  $a^{-1}$  values in columns followed by the same letters are not significantly different

Table 2. Correlation coefficients between observed traits, bulbs covered with mycelium (OCwM) and substrate mycelium coverage (SMC) in 4, 8, 12 and 15 weeks as well as after flowers beheading (4w, 8w, 12w, 15w, afb, respectively)

Trait	OCwM 4w	OCwM 8w	OCwM 12w	OCwM 15w	OCwM afb	SMC 4w	SMC 8w	SMC 12w	SMC 15w	SMC afb
OCwM 4w	1.00									
OCwM 8w	0.90***	1.00								
OCwM 12w	0.78*	0.94***	1.00							
OCwM 15w	0.66	0.88**	0.84**	1.00						
OCwM afb	0.83**	0.87**	0.78*	0.77*	1.00					
SMC 4w	0.07	0.13	0.19	-0.14	-0.27	1.00				
SMC 8w	0.64	0.64	0.56	0.35	0.48	0.59	1.00			
SMC 12w	0.84**	0.92***	0.81**	0.77*	0.88**	0.06	0.71*	1.00		
SMC 15w	0.90***	0.86**	0.81**	0.77*	0.80**	-0.12	0.33	0.67*	1.00	
SMC afb	0.72*	0.76*	0.58	0.55	0.79*	0.04	0.68*	0.94***	0.46	1.00

<sup>\*,\*\*,\*\*\*</sup>significant levels at P < 0.05, 0.01, 0.001, respectively

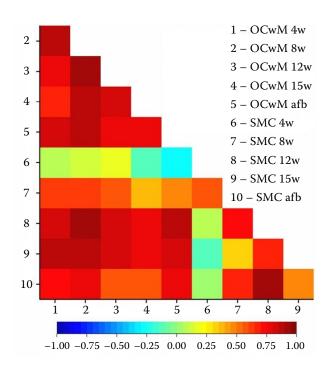


Figure 1. Heatmaps for linear Pearson's correlation coefficients between the observed traits, bulbs covered with mycelium (OCwM) and substrate mycelium coverage (SMC) in 4, 8, 12 and 15 weeks as well as after flowers beheading (4w, 8w, 12w, 15w, afb, respectively)

#### DISCUSSION

In the available literature, there is no data on the possibility of using agents for treating narcissus bulbs and their impact on limiting the development of fungi on the surface of bulbs and substrate, as well as on growth and flowering. Most of our observations indicated a visible effect of hydrogen peroxide with silver (H2O2-Ag+) and fungicides used for the treatment of narcissus on reducing the occurrence and development of fungi on bulbs and the substrate. Previous studies carried out during the cold storage period of hyacinth, narcissus, and tulip showed that the occurrence of N. foedans, P. olsonii, R. stolonifer and *T. asperellum* was the cause of problems, reducing the commercial quality of plants (Wojdyła et al. 2022b). Due to the use of freshly prepared substrate and containers for planting, it should be assumed that the main source of fungi that appeared during the cold storage of narcissus was dead bulb scales. The possibility of using H<sub>2</sub>O<sub>2</sub>-Ag<sup>+</sup> and fungicides to treat narcissus bulbs in order to reduce the occurrence of fungi developing on the surface of the bulbs and the substrate has been demonstrated by our own in vitro studies (Wojdyła et al. 2022b). These studies showed that  $H_2O_2$ - $Ag^+$  and fungicides used to treat bulbs limited the growth of fungal cultures isolated from bulbs and substrate during the forcing period (growing period). *In vitro*,  $H_2O_2$ - $Ag^+$  at a concentration of 0.05% to 0.2% strongly inhibited the growth of the mycelium of *N. foedans* and *P. olsonii*, and slightly stimulated or limited the growth of *R. stolonifer* and *T. asperellum* (Wojdyła et al. 2022b).

The high effectiveness of hydrogen peroxide (Bisteran) in reducing the growth of mycelium and spore germination of the types of fungi we evaluated has also been reported in the literature. In some in vitro studies, high effectiveness of hydrogen peroxide in reducing the growth of mycelium and germination of spores was reported by the following authors: R. oryzae as the cause of tobacco leaf rot (Kortekamp 2006), P. expansum causing apple rot in storage (Venturini et al. 2002), B. cinerea, R. stolonifer, P. digitatum and P. italicum isolates from strawberry and orange (El-Mougy et al. 2008) and from citrus fruit (Meng et al. 2019), P. expansum responsible for rotting cherry fruit (Sehirli et al. 2020), Pestalotia psidii Pat. responsible for the rotting of guava fruit (Youssef et al. 2015).

On the other hand, the second component of the Bisteran agent we tested, nanoparticle silver, at a concentration of 100 ppm *in vitro*, was highly effective in inhibiting the growth of mycelium and spore germination of various species of *Colletotrichum* (Lamsal et al. 2011b), and at a concentration of 150 ppm: *A. alternata, Alternaria citri* Ellis & Pierce and *P. digitatum* (Abdelmalek, Salaheldin 2016). Whereas, nanoparticle silver used prophylactically turned out to be highly effective against powdery mildew on pumpkins and cucumbers caused by *Golovinomyces cichoracearum* (DC.) V.P. Heluta or *Sphaerotheca fusca* (Fr.) S. Blumer (Lamsal et al. 2011a).

In each observation, the tested fungicides significantly reduced bulb coverage with mycelium and sporulation in comparison to the control. In the case of the substrate, a significantly lower percentage of mycelium coverage and sporulation was noted after 8, 12, and 15 weeks and after cutting flowers. The high effectiveness of the tested fungicides in inhibiting the development of mycelium has been confirmed by literature data regarding various species of fungi; however, they are not always present in our research but belong to the same genus. Own research conducted *in vitro* has confirmed

high efficiency in inhibiting the growth of mycelium by: captan (*N. foedans*, *P. olsonii*, and *R. stolonifer*, *T. asperellum*); pyraclostrobin + boscalid (*N. foedans* and *P. olsonii*, *T. asperellum* and *R. stolonifer*); thiophanate methyl + tetraconazole (*N. foedans* and *T. asperellum*) (Wojdyła et al. 2022b). Similarly, literature data indicate that captan in some *in vitro* laboratory tests turned out to be highly effective in limiting the growth of mycelium and germination of *R. oryzae* (Amadioha 1996; Chaurasia et al. 2017) and *Trichoderma* spp. (*T. viride*, *T. harzianum*, *T. koningii*, *T. pseudokoningii* and *T. virens*) (Bhale, Rajkonda 2015).

On the other hand, *in vivo* conditions, captan showed high effectiveness in limiting the development of *R. oryzae* on potato (Amadioha 1996) and eggplant (Youssef et al. 2015), and boscalid + pyraclostrobin (*P. expansum* and *R. stolonifer*) on vines (Serey et al. 2007).

#### **CONCLUSION**

Studies have shown that Bisteran (H<sub>2</sub>O<sub>2</sub>-Ag<sup>+</sup>) and fungicides Biszop 80 WG (captan), Signum 33 WG (pyraclostrobin + boscalid) and Yamato 303 SE (thiophanate methyl + tetraconazole), used to treat narcissus, limited the development of fungi on bulbs and the substrate during the rooting period of the bulbs in the cold room and after placing them in the greenhouse until the flowering period. Based on our own research and previous information included in the Bisteran label, it seems that, in practice, a concentration of 2–3% should be used for dressing bulbs.

## **REFERENCES**

- Abdelmalek G.A.M., Salaheldin T.A. (2016): Silver nanoparticles as a potent fungicide for citrus phytopathogenic fungi. Journal of Nanomedicine Research, 3: 136–140.
- Amadioha A.C. (1996): Control of storage rot of potato caused by *Rhizopus oryzae*. International Journal of Pest Management, 42: 311–314.
- Anzai K., Ogawa K., Goto Y., Senzaki Y., Ozawa T., Yamamoto H. (1999): Oxidation-dependent changes in the stability and permeability of lipid bilayers. Antioxidants and Redox Signaling, 1: 339–347.
- Aziz N., Faraz M., Pandey R., Sakir M., Fatma T., Varma A., Barman I., Prasad R. (2015): Facile algae-derived route

- to biogenic silver nanoparticles: Synthesis, antibacterial and photocatalytic properties. Langmuir, 31: 11605–11612. Bhale U.N., Rajkonda J.N. (2015): Compatibility of fungicides and antagonistic activity of *Trichoderma* spp against plant
  - pathogens. Bioscience Methods, 6: 1–9.
- Byther R.S. (1987): Narcissus Recommendations for control of basal rot of daffodils. Ornamentals Northwest Archives, 11: 18–19.
- Chaurasia S., Chaurasia S., Chaurasia A.K., Chaurasia S., Chaurasia R.K. (2017): *In vitro* inhibitory effect of fungicides on mycelial growth of *Rhizopus oryzae* (Went & Prins Geerl.). International Journal of Advanced Research in Biological Sciences, 4: 176–183.
- El-Mougy N.S., El-Gamal N.G., Abdalla M.A. (2008): The use of fungicide alternatives for controlling postharvest decay of strawberry and orange fruits. Journal of Plant Protection Research, 48: 385–395.
- Fallik E., Aharoni Y., Grinberg S., Copel A., Klein J.D. (1994): Postharvest hydrogen peroxide treatment inhibits decay in eggplant and sweet red pepper. Crop Protection, 13: 451–454.
- Hanks G.R. (1996): Control of *Fusarium oxysporum* f. sp. *narcissi*, the cause of narcissus basal rot, with thiabendazole and other fungicides. Crop Protection, 15: 549–558.
- Hoffman M.E., Meneghini R. (1979): DNA strand breaks in mammalian cells exposed to light in the presence riboflavin and tryptophan. Photochemistry and Photobiology, 29: 299–303.
- Horst R.K. (2008): Westcott's Plant Disease Handbook. 7<sup>th</sup> Ed. Ithaca, Springer.
- Imlay J.A., Linn S. (1988): DNA damage and oxygen radical toxicity. Science, 240: 1302–1309.
- Jo Y.K., Kim B.H., Jung G. (2009): Antifungal activity of silver ions and nanoparticles on phytopathogenic fungi. Plant Disease, 93: 1037–1043.
- Kim S.E., Jung H.J., Lamsal K., Kim Y.S., Min J.S., Lee Y.S. (2012): Antifungal effects of silver nanoparticles (AgNPs) against various plant pathogenic fungi. Mycobiology, 40: 53–58.
- Kortekamp A. (2006): Effectiveness of calcium salts, hydrogen peroxide, azoxystrobin, and antagonistic bacteria to control post-harvest rot on tobacco caused by *Rhizopus oryzae*. International Journal of Pest Management, 52: 109–115.
- Kotchoni O.S., Torimiro N., Gachomo E.W. (2007): Control of *Xanthomonas campestris* pv. *vignicola* in cowpea following seed and seedlings treatment with hydrogen peroxide and *N*-heterocyclic pyridinium chlorochromate. Journal of Plant Pathology, 89: 361–367.
- Lamsal K., Kim S.W., Jung J.H., Kim Y.S., Kim K.S., Lee Y.S. (2011a): Inhibition effects of silver nanoparticles against powdery mildews on cucumber and pumpkin. Mycobiology, 39: 26–32.

- Lamsal K., Kim S.W., Jung J.H., Kim Y.S., Kim K.S., Lee Y.S. (2011b): Application of silver nanoparticles for the control of *Colletotrichum* species *in vitro* and pepper anthracnose disease in field. Mycobiology, 39: 194–199.
- Łabanowski G., Orlikowski L., Saniewska A., Skrzypczak C., Soika G. (2006): Ochrona Ozdobnych Roślin Cebulowych i Bulwiastych. Kraków, Plantpress. (in Polish)
- McShan D., Ray P.C., Yu H. (2014): Molecular toxicity mechanism of nanosilver. Journal of Food and Drug Analysis, 22: 116–127.
- Marshall M.V., Cancro L.P., Fischman S.L. (1995): Hydrogen peroxide: A review of its use in dentistry. Journal of Periodontology, 66: 786–796.
- Mayer A.M., Staples R.C., Gil-ad N.L. (2001): Mechanisms of survival of necrotrophic fungal plant pathogens in hosts expressing the hypersensitive response. Phytochemistry, 58: 33–41.
- Mazau D., Esquerré-Tugayé M.T. (1986): Hydroxyproline-rich glycoprotein accumulation in the cell walls of plants infected by various pathogens. Physiological and Molecular Plant Pathology, 29: 147–157.
- Meng X., Huang Z., Fan C. (2019): Postharvest treatment with hydrogen peroxide to control orange fruit decay caused by *Penicillum digitatum* and *Penicillum italicum*. International Journal of Applied Agricultural Sciences, 5: 114–119.
- Miyasaki K.T., Genco R.J., Wilson M.E. (1986): Antimicrobial properties of hydrogen peroxide and sodium bicarbonate individually and in combination against selected oral, gram-negative, facultative bacteria. Journal of Dental Research, 65: 1142–1148.
- Nurnaeimah N., Mat N., Mohd K.S., Badaluddin N.A., Yusoff N., Sajili M.H., Mahmud K., Adnan A.F.M., Khandaker M.M. (2020): The effects of hydrogen peroxide on plant growth, mineral accumulation, as well as biological and chemical properties of *Ficus deltoidea*. Agronomy, 10: 599.
- O'Neill T.M., Hanks G.R., Wilson D.W. (2005): Control of smoulder (*Botrytis narcissicold*) in narcissus with fungicides. Annales of Applied Biology, 145: 129–137.
- Ray M.K., Mishra P.K., Baruah P.K. (2016): Control of fungal pathogen *Pestalotiopsis disseminata* causing grey blight disease in som (*Persea bombycina* Kost.): An *in vitro* study. International Journal of Pure and Applied Bioscience, 4: 180–185.
- Sallato B.V., Torres R., Zoffoli J.P., Latorre B.A. (2007): Effect of boscalid on postharvest decay of strawberry caused by *Botrytis cinerea* and *Rhizopus stolonifera*. Spanish Journal of Agricultural Research, 5: 67–78.

- Sehirli S., Karabulut O.A., Ilhan K., Sehirli A. (2020): Use and efficiency of disinfectants within a hydrocooler system for postharvest disease control in sweet cherry. International Journal of Fruit Science, 20: 1590–1606.
- Serey R.A., Torres R., Latorre B.A. (2007): Pre- and post-infection activity of new fungicides against *Botrytis cinerea* and other fungi causing decay of table grapes. Ciencia e Investigacion Agraria, 34: 215–224.
- Vasić T., Jevremović D., Krnjaja V., Živković S., Trkulja V. (2021): Morphological and molecular identification of *Fusarium oxysporum* f. sp. *narcissi* on poet's daffodil (*Narcissus poeticus* L.) in Serbia. Journal of Plant Diseases and Protection, 128: 1357–1361.
- Venturini M.E., Blanco D., Oria R. (2002): *In vitro* antifungal activity of several antimicrobial compounds against *Penicillium expansum*. Journal of Food Protection, 65: 834–839.
- Wojdyła A.T., Nowak J.S., Bocianowski J., Kowalski A., Wiśniewski J., Waszkiewicz E. (2025): Effect of treatment of narcissus bulbs with hydrogen peroxide with silver and fungicides on plant growth and development. Hort. Sci. (Prague), 52: 81–90.
- Wojdyła A.T., Nowak J.S., Bocianowski J., Wiśniewski J., Waszkiewicz E. (2022a): Effect of hyacinth treatment by hydrogen peroxide stabilized with silver and some fungicides on fungal development on the substrate and bulbs and on plant growth and development. Agronomy, 12: 2894.
- Wojdyła A.T., Orlikowski L.B., Wiśniewski J., Waszkiewicz E. (2022b): *In vitro* effect of plant protection products on saprotrophic fungi developing on bulbs during forcing. Zeszyty Naukowe Instytutu Ogrodnictwa, 30: 35–46. (in Polish)
- Youssef K., Mustafa Z., Mounir G., Aborehab M.E.A. (2015): Preliminary studies on fungal species associated with guava fruit drop disease and possible management. Egyptian Journal of Phytopathology, 43: 11–23.
- Zalewska E.D., Machowicz-Stefaniak Z., Król E.D. (2016): Antifungal activity of nanoparticles against chosen fungal pathogens of caraway. Acta Scientiarum Polonorum Hortorum Cultus, 15: 121–137.

Received: March 11, 2024 Accepted: August 1, 2024 Published online: April 22, 2025