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Full Length Research Paper

Effects of fungicides on *in-vitro* pollen germination, tube growth and morphology of almond (*Prunus dulcis*)

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Eight fungicides namely Sumi-eight, Cupravit, Karatane, Topsin-M, Vitavax thiram, Beam, Benlate and Tecto at commercially recommended concentrations and at double concentrations were evaluated for their effects on pollen germination, tube growth and morphology of almond cultivar (Ferragness) in basic medium or control (100 mg L^{-1} boric acid, 15% sucrose and 1% agar) at 24°C in dark condition. Results showed that different treatments had significant effects on the germination, tube growth and morphology of pollen. All fungicides reduced the percentage of pollen germination, length of germ-tube elongation and morphological features of almond pollens. The negative effects of fungicides on pollen germination percentage and germ-tube elongation and view of pollen morphology were variable and dependent on type and material concentrations. The highest pollen germination (100%) for almond cultivar (Ferragness) was recorded in 100 mg L^{-1} boric acid, 15% sucrose and 1% agar medium (control). The lowest pollen germination percentage (0.0%) was found in 100 mg L^{-1} boric acid, 15% sucrose and 1% agar medium contain 2 g L^{-1} Karatane.

Key words: Almond, culture pollen, fungicide, germination.

INTRODUCTION

Almond (*Prunus dulcis*) is a self-incompatible nut crop that for nut production depends upon transfer by pollinators of viable, compatible pollen between healthy flowers (Mussen and Montague, 2004). Therefore, influence of fungicide sprays on pollens and pollination is of concern, and identification of chemicals having the least detrimental effects would be desirable (Holb, 2008). Many fungal diseases of almonds, such as brown rot, blossom rot, shot hole and anthracnose, are controlled by annual treatment with fungicides just previous to, during, or immediately following bloom (Mussen and Montague, 2004). Consequently, the timing of fungicide applications often overlaps flowering and pollination. Excessive use of fungicides as well as their wrong applications have had adverse effects on pollen germination and pollen tube formation; and thus affect the fruit production.

Several researchers have been carried out on the detrimental effects of fungicides on pollen germination (Eaton, 1961; Church and Williams, 1978; Redalen, 1980; Marcucci et al., 1983; Bristow and Windom, 1987; Watters and Sturgeon, 1990; Wetzstein, 1990; He et al., 1995; Mussen and Montague, 2004; Holb, 2008) and pollen tube growth (Marcucci et al., 1983; He et al., 1996; Tort et al., 2005; Ozturk and Candan, 2010) for commercially important plants. In pollens treated with fungicides under *in-vitro* conditions, a decrease in pollen germination deformation and cracks in pollen tubes have been reported (Lacerda et al., 1994; Pavlik and Jandurova, 2000; Holb, 2008). The excessive use of some fungicides on some fruit trees during the flowering period has been observed to cause a negative effect on pollen germination and fruit formation (Marcucci and Filiti, 1984; Redalen, 1980). Yi et al. (2003b, c) evaluated the effect of *in-vitro* fungicide sprays on pollen germination and growth. Captain and azoxystrobin were the most toxic to the pollens, the germination percentages were less than 1% of the untreated control. Similarly, the pollen germination in apples treated with Captan decreases by 20% as compared to the control (Yi et al., 2003a). A decrease in the pollen germination and pollen tube growth has been recorded in tomato flowers as well, when treated with 3Chlorothalonile 75% WP (3.200 mg L^{-1}

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of a.i.), Mancozeb 80% WP (2.400 mg $L^{-1}$of a.i.), Mancozeb 48% WP (1,680 mg L^{-1} of a.i.)+Metalaxyl 10% WP (350 mg L^{-1} of a.i.) and Dibrom 86% EC (1.030 ml L^{-1} of a.i.) (Lacerda et al., 1994).

According to the report of Redalen (1980), pollen germination *in-vitro* of raspberry cvs Norna and II-2LGP was reduced by 150, 15 or 1.5 g 100 L^{-1} captan or 500, 50 or 5 g 100 L^{-1} dichlofluanid (that is normal, 1/10 and 1/100 normal concentrations), but 1/100 normal concentration of benomyl did not reduce germination and 1/10 had less effect than the other fungicides. Tube growth was also reduced after germination. According to the reports, application of ammonium thiosulphate at 2.0% to 'Hunter' apricot at 20, 40, 60, 80 or 100% bloom, fruit set reduced regardless of the time of application (Bound and Jones, 2004). Also, based on results from ammonium thiosulphate and fungicides application on peach, it was demonstrated that fruit number per tree was affected only by ammonium thiosulphate and the numbers of blossom blight cankers per tree only by fungicide (Olien et al., 1995). Facteau and Chestnut (1983) have reported the toxicity of various air pollutants to pollen germination and pollen tube growth in apricot and sweet cherry. The aim of this study was to know the germination of pollen grain *in-vitro* treated with different fungicides.

MATERIALS AND METHODS

This research was conducted in 2011 at Seed Plant Improvement Institute- Karaj, Iran. Branches with unopened flowers were pruned from variety of almond trees Ferragness growing in experimental orchard. The cuttings, which were collected prior to applications of fungicides, were clipped under water and stored in tap water under laboratory conditions. After 24 h, pollen grains were collected from freshly opened blossoms from the branches with a sharp razor blade. Pollens were collected then used directly or stored in 1.5 mL microfuge tubes. The fungicides used during this study were shown in Table 1. The fungicide applications were prepared in 1 L of water and applied at dosages recommended by the manufacturer and double the recommended dosage.

Medium

The pollen germination basic medium or control contained 15% sucrose, 1% agar and 100 mg L^{-1} boric acid was autoclaved and cooled to 50°C. The fungicides were added before it was poured into Petri dishes. A pH meter was used to determine the pH of the medium for each treatment. After pollen culture, they incubated in the dark at 24°C for 24 h; the percentage of pollen germination was determined using a light microscope. Pollen grains which produced a tube equal to their own diameter were counted as germinated (Imani et al., 2011).

Pollen germination percentage was determined by dividing the number of germinated pollen grains per field of view by the total number of pollen grains per field of view. Pollen tube equal to at least twice the diameter of pollen grains were counted as germinated, burst pollen were not counted as germinated. The statistical analysis was performed using Microsoft Excel (2007) and SAS software (SAS Institute Inc, 1996) and means were compared using Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

Results from effects of different fungicidal treatment on *in-vitro* pollen germination, tube growth and morphology of almond were varied and shown in Table 1. All fungicides reduced the percentage of pollen germination and length of germ-tube elongation over control. The highest number of pollen germination (100%) was obtained from the control medium (without fungicide). No pollen grain germination were found from the treatment of Karatane, Beam and Vitavax thiram @ 1 and 2 g L^{-1}, 100 mg L^{-1} borric acid, 15% sucrose and 1% agar (except 1 g L^{-1} of Vitavax thiram) and Vitavax thiram @ 2 g L^{-1} fungicide application (Table 2). Our result fit in with the report of Redalen (1980); Marcucci and Filiti (1984); Yi et al. (2003b,c); Tort et al. (2005). They obtained similar results with pollen germination inhibitory in pollen culture media content different compounds in some fungicides tested, particularly the excessive use of some fungicides on some fruit crops during the flowering period has been observed to cause a negative effect on pollen germination and fruit formation. It was reported that Captan and some chemicals reduced pollen viability in many apple cultures (Church and Williams, 1977). Similarity, the pollen germination in apples treated with Captan decreases by 20% as compared to the control (Yi et al., 2003a). A decrease in the pollen germination and pollen tube growth has been recorded in tomato flowers as well, when treated with some fungicides (Lacerda et al., 1994).

In this study, we tested the pollen germination of almond affected different fungicide in basic medium which can be used for further studies. Since many fungal diseases of almonds, such as brown rot, blossom rot, shot hole and anthracnose, are controlled by annual treatment with fungicides just previous to, during, or immediately following bloom (Mussen and Montague, 2004) but pollen of almond is very sensitive to the number of fungicides commonly used for disease control on this tree. The optimum fungicides concentration for germination varied from one fungicide to another. Always in the higher concentrations of fungicides as simple or compound were consistently inhibitory. The negative effects of high concentration of fungicides on pollen germination suggest that a definite level of these materials is necessary for normal germination of pollen grains. High concentrations appeared toxic and could have a negative effect on fruit productivity and quality of almond in the future. Also, some low concentrations may have been inadequate for controlling many fungal diseases of almonds. Examination of the effects of the fungicides used in the present study on the morphology and views of pollens showed that the values obtained in both the treatments were lower than those in the control (Table 1). Such diminish in growth of pollens of almond in media containing fungicides, which suggest that fungicides may interfere with nutrient uptake or pollen metabolism. Additionally, distorted and abnormally thin

Table 1. Fungicides used and their concentration.

Fungicide	Fungicide attributes	Fungicide concentration used in basic medium or control (100 g L^{-1} boric acid, 15% sucrose and 1% agar)
Diniconazole-M	General name	1 and 2 g L^{-1}
Triazole	Chemical group	
Sumi-eight	Commercial name	
$C_{15}H_{17}Cl_2N_3O$(326/4)	Formul and molecular weight	
Copper oxychloride	General name	1.5 and 3 g L^{-1}
Inorganic	Chemical group	
Cupravit	Commercial name	
$Cl_2Cu_4H_6O_6$(427/1)	Formul and molecular weight	
Dinocap	General name	1 and 2 g L^{-1}
Dinitrophenol	Chemical group	
Karatane	Commercial name	
$C_{18}H_{24}N_2O_6$(364/3)	Formul and molecular weight	
Thiophanate-methyl	General name	5 and 10 g $10L^{-1}$
Benzimidazole	Chemical group	
Topsin-M	Commercial name	
$C_{12}H_{14}N_4O_4S_2$(342/4)	Formul and molecular weight	
Carboxin thiram	General name	1 and 2 g L^{-1}
Carboxamide-dimethyl dithiocarbamate	Chemical group	
Vitavax thiram	Commercial name	
$C_6H_{12}N_2S_4+C_{12}H_{13}NO_2S$	Formul and molecular weight	
Tricyclazole	General name	1 and 2 g L^{-1}
Reductase	Chemical group	
Beam (50WP)	Commercial name	
$C_9H_7N_3S$(189/3)	Formul and molecular weight	
Benomyl (50WP)	General name	1 and 2 g L^{-1}
Benzimidazole	Chemical group	
Benlate	Commercial name	
$C_{14}H_8N_4O_3$(290/3)	Formul and molecular weight	
Thiabendazole	General name	1 and 2 g L^{-1}
Benzimidazole	Chemical group	
Tecto	Commercial name	
$C_{10}H_7N_3S$(201/2)	Formul and molecular weight	

tubes were seen by some of the fungicides (Table1). It is possible that the very thin tubes actually had ruptured, as reported by He et al. (1996).

In conclusion, it is found that on *in-vitro* pollen germination, tube growth and morphology of almond was affected by fungicides treatments. In recent years, the studies on pollen germination of almond support our findings (Mussen and Montague, 2004). The best pollen germination rates were obtained in basic medium or control related to all the investigated fungicides. These results are very similar to those reported by Marcucci et al. (1983), He et al. (1996) and Mussen and Montague (2004). The fungicides used in our treatments led to some changes in the morphological features of almond pollens. Our studies show that the almond pollen viability is seriously affected by fungicides used *in-vitro* pollen

Table 2. Effects of fungicides on pollen germination, tube growth and morphology of almond *in-vitro*.

Fungicides	[1]Fungicidal concentrations (g L^{-1})	Pollen germination (%)	Remarks
Control (plain water)	-	100[a]	Long, obvious, strong pollen tube
Sumi-eight	1	85.12[a]	Long strong pollen tube, and sub obvious, pollen grain
	2	45.36[c]	Long strong pollen tube, and sub obvious, pollen grain
Cupravit	1.5	20.47[d]	Short, weak pollen tube
	3	12.14[e]	Short to medium pollen tube and obscure pollen grain
Karatane	1	0.00[f]	No pollen tube and obscure pollen grain
	2	0.00[f]	No pollen tube and obscure pollen grain
Topsin-M	0.5	75.12[a]	Long, weak to medium pollen tube
	1	62.23[b]	Long to medium pollen tube sub obvious pollen grain
Vitavax thiram	1	10.54[e]	Short weak pollen tube and obscure pollen grain
	2	0.00[f]	Short weak pollen tube and obscure pollen grain
Beam	1	0.00[f]	No pollen tube and obscure pollen grain
	2	0.00[f]	No pollen tube and obscure pollen grain
Benlate	0.5	20.12[d]	Long ,weak pollen tube
	1	10.21[e]	Short weak pollen tube and abnormal pollen grain
Tecto	1	22.76[d]	Long ,weak pollen tube
	2	10.75[e]	Long ,weak pollen tube

[1]Fungicidal concentrations used in basic medium or control 100 mg L^{-1} boric acid, 15% sucrose and 1% agar. *Means followed by the similar letter(s) are not significantly different by Duncan multiple range test (DMRT) at ($P<0.05$).

culture. This could lead to a decrease in the productivity of fruits. Therefore, according to the results of this study and previous studies (Vezvaei and Jackson, 1995; Mussen and Montague, 2004) growers should be adviced to apply fungicides pre-bloom or try to delay fungicide treatments for as long as possible during bloom.

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