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Full Length Research Paper

Effects of fungicides on pollen germination peach and Nectarine *in Vitro*

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Alive pollen with good viability and germination ability for fruit setting in peach and nectarine is necessary. For pollen viability test and aware of pollen quality in these fruits, experiment was done *IN VITRO* conditions based on effects of eight fungicides including Sumi-eight, Cupravit, Karatane, Topsin-M, Vitavax thiram, Beam, Benlate and Tecto at commercially recommended concentrations and at double concentrations on pollen germination two peach (Anjeri) and nectarine (Shalil Moghan) cultivars in basic medium or Control (100 mg/L boric acid, 15% sucrose and 1% agar) at 24°C in dark conditions in 2011 at seed plant improvement institute (SPII), Karaj, Iran. Results showed that all fungicides reduced the percentage of pollen germination and length of germ-tube elongation, regardless of cultivar. The negative effects fungicides on pollen germination percentage and germ-tube elongation were variable and dependent on fungicide concentration and type concentration. The highest pollen germination average (100%) was recorded in basic medium (control). The lowest germination percentage average (0.0%) was found in basic medium containing fungicides Beam and Karatane.

Key words: Fungicides, pollen germination peach, nectarine, *in vitro*.

INTRODUCTION

The timing of fungicide and antibiotic applications in fruit crops often overlaps flowering and pollination. Numerous studies report detrimental effects of chemical applications on pollination, fruit set and yield (Olien et al., 1995; Yi et al., 2003c). Excessive use of pesticides, fungicides and antibiotics also their wrong applications have been adverse effects on environment, living organisms and agricultural plants (Mayer and Lunden, 1986). They inhibit pollen germination and pollen tube formation; and thus affect the fruit production (Tort et al., 2005).

Numerous studies has been done on the detrimental effects of fungicides on pollen germination (Eaton, 1961; Wodehouse,1965; Church and Williams, 1977; Redalen, 1980; Marcucci et al., 1983; Butt et al., 1985; Bristow and Windom, 1987; Watters and Sturgeon, 1990; He et al., 1995;Wetzstein, 1990; Mussen and Montague, 2004; Holb, 2008) and pollen tube growth (Marcucci et al., 1983; He et al., 1996, Bound and Jones, 2004.; 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). Similarly some pesticides and fungicides have been reported to reduce pollen vitality in many peach cultures (Church and Williams, 1977). As many fungal diseases of peach such as brown rot blossom blight of peach caused by *Monilinia fructicola* and some pest are controlled by annual treatment with fungicides in flowering period bloom such as pollen eating (Layne and Bassi, 2008). Consequently, the timing of fungicides applications often simultaneous with blooming period. According to Yi et al. (2003) the pollen germination in apples treated with Captan decreases by 20% as compared to the control. Similarly, a decrease in the pollen germination and pollen tube growth has been recorded in tomato flowers as well when treated with 3 Chlorothalonile 75% WP (3.200 ppm of a.i.),Mancozeb 80% WP (2.400 ppm of a.i.), Mancozeb ppm of a.i.) and 48% WP (1,680 ppm of a.i.)

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+Metalaxyl 10% WP (350 Dibrom 86% EC (1.030 ppm of a.i.) (Lacerda et al., 1994). Pollen germination *in vitro* of raspberry cvs Norna and II-2LGP was reduced by 150, 15 or 1.5 g/100 L captan or 500, 50 or 5 g/100 L 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 (Redalen, 1980). Facteau and Chestnut (1983) have reported the toxicity of various air pollutants to pollen germination and pollen tube growth in apricot and sweet cherry. Olien et al. (1995) showed effects of combined applications of ammonium thiosulphate and fungicides on fruit load and blossom blight and their phytotoxicity to peach trees. The objective of this research was to determine the *in vitro* toxicity of several fungicides on pollen of peach and nectarine.

MATERIALS AND METHODS

This research was conducted in 2011 at seed plant improvement institute (SPII), Karaj, Iran. Branches with unopened flowers were pruned from two varieties of peach and nectarine trees ('Anjiri' and 'Mogan') growing in experimental orchard. Branches with unopened flowers were pruned from cultivars of peach and nectarine trees. 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 razor blade. Pollens were then collected used directly or stored in 1.5 ml microfuge tubes. The fungicides used during this study are 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 10% Sucrose, 2% Agar and 100 ppm 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 were incubated in the dark at 24°C for 24 h; the percentage of germination was determined by using a light microscope. Pollen grains which produced a tube equal to their own diameter were counted as germinated (Imani et al., 2011). 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, 1990) and means were compared using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Results from effects of fungicides on pollen germination of two cultivars peach ('Anjiri') and nectarine ('Mogan') *in vitro* showed that fungicides treatments on pollen germination of these cultivars varied (Table 1). All fungicides reduced the percentage of pollen germination. Percentage of pollen germination was 100% in medium without fungicide (control), while the lowest germination percentage was found 2 g/L Karatane. There were almost no pollen germination (0.0%) in 100 mg/L boric acid, 15% sucrose and 1% agar medium contain Beam (1 and 2 g/L), Karatane (1 and 2 g/L), Vitavax thiram (2 g/L) (Table 2).

On the other hand, considerable differences in peach and nectarine cultivars in the ability for germination were not observed (Table 3). Different fungicides showed significantly different effects with regard to their germination percentage of two peach and nectarine cultivars pollen grain following *in vitro* culture (Table 4).

Our result fit in with report of Marcucci and Filiti (984), Redalen (1980), Yi et al. (2003b, c) and Tort et al. (2005). They obtained similar results with pollen germination inhibitory in pollen culture media content different compounds in some fungicides tested, particular, 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). 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 some fungicides (Lacerda et al., 1994).

In this study, we tested the pollen germination of peach and nectarine affected different fungicide in basic medium which can be used for further studies. Since many fungal diseases of stone fruits particularly almond, peach and nectarine, 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; Layne and Bassi, 2008) but pollen of these fruits is very sensitive to number of fungicides commonly used for disease control on these trees. The optimum fungicides concentration for germination varied from fungicide to other. 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 stone fruits (Yi et al., 2002; Layne and Bassi, 2008).

Examination of the effects of the fungicides used in the

Table 1. Fungicides used and their concentration.

Fungicide	Fungicide attributes	Fungicide concentration used in basic medium or control
Diniconazole-M	General name	1 and 2 g/ L
Triazole	Chemical group	
Sumi-eight	Commercial name	
$C_{15}H_{17}Cl_2N_3O$(326/4)	Formula and molecular weight	
Copper oxychloride	General name	1.5 and 3 g/L
Inorganic	Chemical group	
Cupravit	Commercial name	
$Cl_2Cu_4H_6O_6$(427/1)	Formula and molecular weight	
Dinocap	General name	1 and 2 g/ L
Dinitrophenol	Chemical group	
Karatane	Commercial name	
$C_{18}H_{24}N_2O_6$(364/3)	Formula and molecular weight	
Thiophanate-methyl	General name	5 and 10 g/10 L
Benzimidazole	Chemical group	
Topsin-M	Commercial name	
$C_{12}H_{14}N_4O_4S_2$(342/4)	Formula and molecular weight	
Carboxin thiram	General name	1 and 2 g/ L
Carboxamide-dimethyl dithiocarbamate	Chemical group	
Vitavax thiram	Commercial name	
$C_6H_{12}N_2S_4+C_{12}H_{13}NO_2S$	Formula and molecular weight	
Tricyclazole	General name	1 and2 g/ L
Reductase	Chemical group	
Beam(50WP)	Commercial name	
$C_9H_7N_3S$(189/3)	Formula and molecular weight	
Benomyl(50WP)	General name	1 and2 g/ L
Benzimidazole	Chemical group	
Benlate	Commercial name	
$C_{14}H_8N_4O_3$(290/3)	Formula and molecular weight	
Thiabendazole	General name	1 and 2 g/ L
Benzimidazole	Chemical group	
Tecto	Commercial name	
$C_{10}H_7N_3S$(201/2)	Formula and molecular weight	

[1]Basic medium=100 mg/l boric acid, 15% sucrose and 1% agar.

present study on germination of pollens showed that the values obtained in both the treatments were lower than those in the control (Table 1). Such diminish in germination of pollens in media containing fungicides suggest that fungicides may interfere with nutrient uptake or pollen metabolism (Lacerda et al., 1994; He et al., 1996; Pavlik and Jandurova, 2000; Holb, 2008).

CONCLUSION AND RECOMMENDATIONS

It is found that on *in vitro* pollen germination of peach and nectarine was affected by fungicides treatments. The best pollen germination rates was obtained in basic medium or control relate to all the investigated the fungicides. These results are very similar to those reported by Marcucci and Filiti (1984), Abbott et al. (1983), Marcucci et al. (1983), Bristow and Windom

(1987), Wetzstein (1990); He et al. (1995), Embree and Foster (1999), Fairbanks et al. (2002) and Mussen and Montague (2004). The fungicides used in our studies showed that the peach and nectarine pollen viability is seriously affected by fungicides used *in vitro* pollen culture. This could lead to a decrease in the productivity

Table 2. Effects of fungicides on pollen germination, of peach and nectarine *in vitro*.

Fungicide used in basic medium or control	Concentration	Pollen germination (%)	
		Peach ('Anjiri')	Nectarine ('Mogan')
C[1]	100 mg/l boric acid, 15% sucrose and 1% agar	95.12[a]	90.12[a]
Sumi-eight	2 g/ L+C	55.36[d]	35.54[e]
	1 g/ L+C	83.24[b]	74.12[b]
Cupravit	1.5 g/ L+C	23. 27[f]	18.37[f]
	3 g/ L+C	13.84[fg]	11.24[fg]
Karatane	2 g/ L+C	0[g]	0[g]
	1 g/ L+C	0[g]	0[g]
Topsin M	5 g/10L+C	71.53[c]	61.12[c]
	10 g/10L+C	44.26[e]	50.23[d]
Vitavax thiram	1 g/ L+C	19.5[f]	17.53[f]
	2 g/ L+C	0[g]	0[g]
Beam	2 g/ L+C	0[g]	0[g]
	1 g/ L+C	0[g]	0[g]
Benlate	2 g/ L+C	11.56[fg]	8.21[fg]
	1 g/ L+C	17.19[f]	15.12[f]
Tecto	1 g/ L+C	15.36[f]	21.76[f]
	2 g/ L+C	7.23[g]	8.65[g]

[1]C = Control or basic medium: 100 mg/l boric acid, 15% sucrose and 1% agar, means followed by the similar letter(s) in each column are not significantly different by Duncan test (P<0.05).

Table 3. Average of germination percentage of peach and nectarine cultivars pollen as affected by the different treatments of the fungicides.

Cultivar	Average of germination (%) in the different treatments of the fungicides
'Anjiri'	26.19[a]
'Mogan'	24.58[a]
‘Average	25.38

*Means followed by the similar letter(s) are not significantly different by Duncan test (P<0.05).

Table 4. Average of percent germination of peach and nectarine cultivars pollen as affected by the fungicides.

Fungicide used in basic medium or control	Average germination (%)
C[1]	85.12
Sumi-eight+C	45.45
Sumi-eight+C	78.68
Cupravit+C	18.37
Cupravit+C	12.54
Karatane+C	0
Karatane+C	0
Topsin M+C	68.325
Topsin M+C	48.245
Vitavax thiram+C	18.515
Vitavax thiram+C	0
Beam+C	0

Table 4. Contd.

Beam+C	0
Benlate+C	9.885
Benlate+C	16.155
Tecto+C	18.56
Tecto+C	7.94
Average	25.16

[1]C =15% sucrose and 1% agar (control); B1=50 mg/L boric acid; B2=100 mg/L boric acid; N1=50 mg/L naphthalene acetic acid; N2=100 mg/L naphthalene acetic acid, [*]Means followed by the similar letter(s) are not significantly different by Duncan test (P<0.05).

of fruits. Therefore, according to the results of this study, growers should be advised to apply fungicides pre-bloom or try to delay fungicide treatments for as long as possible during bloom.

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