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Effects of Copper and Leadon Pollen Germination Traits in Almond Cultivars

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Abstract

There is minimal information about the effect of heavy metals such as lead and copper on pollen grains and pollen tubes of fruit trees. Fruit set of these plants are affected by different environmental, biological, physical and chemical factors. If one of these factors be abnormal, pollination, fertilization and fruit set and orchard yield will decrease. In polluted cities, this phenomenon is affected by the stresses of heavy metals. Almond is one of the most important nut fruits of Iran. Pollen germination and tube growth are the main basic factors of fruit set in almond. In this study, the effects of heavy metals, copper and lead, on pollen germination and tube growth of five almond cultivars included Azar, Mamaei, Shahroud 21, Shahroud 18 and Shokofeh, were studied *in vitro*. The results showed that both traits were affected significantly by different levels of metals and cultivars. Increasing the concentrations of heavy metals resulted in a decrease in pollen germination and tube growth of all cultivars. In the concentrations, 250 ppm pollen germination and tube growth neared to zero in the most cultivars. Lead showed the highest toxicity on pollen germination and tube growth. However, Shahrodi 21 and Shahrodi 18 cultivars showed the highest sensitivity to copper and lead among the cultivars.

Keywords: Almond, Copper, Lead, Pollen germination, Tube growth.

Introduction

Increasing the concentration of air pollutants in the environment as a result of human industrial activities has become a problematic issue. There has been a dramatic increase in environmental contamination with heavy metals, causing hazardous effects on human, animal, and plant organisms (Sawidis and Reiss, 1995).High concentrations of heavy metals can affect plant reproduction, resulting in anomalies in gamete development, embryogenesis and, consequently, a decrease in seed production and loss of biodiversity (Sawidis, 1995, 1997 and 2008).
Pollen is a haploid organism that consists of only two or three cells and ensures the formation and delivery of male gametes to the site of fertilization and fruit set. The effects of heavy metal stress on pollen grain germinating of angiosperms, especially fruit trees, is one of the most interesting yet poorly studied events.

However, among plant organs, pollens are considered to be highly sensitive to various air pollutants. Pollen germination and tube growth are inhibited by various heavy metals, such as copper, lead, cadmium, cobalt and mercury. Thus, the *in vitro* culture of pollen can provide a sensitive standard system, with which the biological activity of various toxic metals at the cellular level can be

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investigated (Sawidis, 2008; Gur and Topdemir, 2005). In most previous studies, various plant species pollen have been used to determine the cytotoxic effects of environmental pollutants (Sawidis, 2008).

Some researchers have reported the inhibitory effect of heavy metals, including nickel, on pollen germination (Mohsenzadeh *et al.,* 2011; Gu¨r and Topdemir, 2008; Sabrine *et al.,* 2010; Sawidis and Reiss, 1995). In other studies, intracellular effects of cadmium, chromium and lead on pollen tubes were described. These metals caused apex expansion and affected the pattern of structural polymer distribution along the tube cell wall (Sawidis, 2008). Effects of heavy metals on the ultra-structure of organelles and their distribution in pollen tubes have been previously reported. Chromium causes chromatin condensation, mitochondria swelling, cytoplasm vacuolization and perturbed arrangement of EPR cisternae. Lead causes partial disassembly of longitudinally oriented pollen actin bundles in some plants (Breygina *et al.,* 2012).

Currently, some reports exist regarding heavy metals effects on ornamental and forest trees but less information has been reported about the effect of lead and copper on pollen grains and pollen tubes of fruit trees (Chancy and Strckland, 1984; Cox, 1983; Holuk and Ostrolucka, 1984; Kaalbande *et al.,* 2008; Kapler and Kristen, 1987; Shkarleto, 1972).

The effects of heavy metals on pollen germination and tube growth of apples has been studied (*Malus silvestris* Miller cv. Golden) (Munzuroglu and Gur, 2000). There is also a report on the effects of simulated acid rain on the pollen germination and pollen tube growth of apple Golden cultivar (Munzuroglu *et al.,* 2003).

Almond is one of the most important nut crops of the Rosaceae family. Most almond cultivars and genotypes are self (cross)-incompatible. Thus, pollination, fertilization and commercial production require compatible pollen. Selecting cross-compatible cultivars with high quality pollen is the most important practice in almond orchard (Sharafi, 2011 a and b). In addition, fruit set in almond is affected by different environmental, biological, physical and chemical factors. If one of these factors is abnormal, fruit set and orchard yield will decrease. Pollen germination and tube growth are the main basic factors of fruit set in almond. In polluted cities such as Tehran, this phenomenon is affected by stresses of heavy metals (Wolters and Martens, 1987).

The objective of this research was to identify the effects of copper and lead on pollen germination and tube growth of five almond cultivars *in vitro*.

Materials and methods

Five cultivars of almond, including “Azar”, “Mamaei”, “Shahroud 21”, “Shahroud 18” and “Shokofeh”, which are grown in different regions of Tehran, were selected. In the spring of 2014, flower buds in balloon stage were gathered and transmitted to the laboratory in Shahed University. Petals and sepals were separated and anthers isolated from flower buds and placed 72 hours in Petri dishes for releasing pollens. Then, pollens were gathered in distilled glasses. Pollen germination percentage and pollen tube growth of cultivars were immediately tested. Pollens planted in the standard *in vitro* medium (containing 1% agar, 15% sucrose) were treated by 0 (control), 50, 100, 150, 200 and 250ppm of copper ($CuCl_2$) and lead ($PbCl_2$) solutions for 24 hours at 24°C. Chlorophorm was added to top pollen tube growth. Pollen germination percentage (PGP) and pollen tube length (PTL) were measured under lightmicroscope. Seven microscopic areas were randomly counted for evaluation of PGP and PTL.

Measurements of pollen tube length were directly recorded by an ocular micrometer fitted to the eyepiece on microscope based on micrometer (µm). Factorial experiment was carried out with two factors (1; copper and lead by 6 level and 2; almond cultivars) based on acompletely randomized design (CRD) with six replications (6 Petri dishes). Data were analyzed using SAS software and comparison of means was carried out with Duncan's multiple rangetests.

Results

Analysis of variance in Table 1 indicated significant differences for pollen germination percentage and pollen tube length among the five almond cultivars in different concentrations of copper and lead. Interaction between cultivars and different concentrations of copper and lead had a significant effect on pollen germination percentage and pollen tube length (Table 1).

Table 1. Analysis of variances of pollen germination percentage and pollen tube length in five almond cultivars.

Source of variation (SOV)	df	Mean Square			
		Pollen germination percentage		Pollen tube length (µm)	
		Cupper	Lead	Cupper	Lead
Cultivar	4	587**	1831.1**	1721.2**	7918.7**
Heavy metal	1	3965.1**	2971.2**	1902.9**	9241.3**
Cultivar*Metal	4	63.2**	1413.5**	20.1**	8.7**
Error	48	6.1	17.1	2.5	1.4
Total	59				
CV		7.8	15.7	9.7	10.3

**: Significant in P<0.01% level.

Increasing the concentration of both copper and lead caused to a significant decrease in pollen germination percentage and pollen tube length, nearly to zero in all cultivars (Table 2).

Among almond cultivars, means of pollen germination percentage and pollen tube length in a Copper-treated medium ranged between 32.8 to 44.7% and 7.3 to 12.1µm, respectively. However, in lead-treated medium, the mean of pollen germination percentage and pollen tube length ranged between 30.2 to 36% and 5.1 to 8.4 µm in almond cultivars, respectively.

Table 2. Mean comparison of pollen germination percentage and pollen tube length among different concentrations of copper and lead.

Concentration (ppm)	Pollen germination percentage		Pollen tube length (µm)	
	Cupper	Lead	Cupper	Lead
0 (control)	86.3[a]	92.4[a]	214.1[a]	160.4[a]
50	71.3[b]	58.9[b]	150.3[b]	94.5[b]
100	45.4[c]	33.1[c]	80.5[c]	51.3[c]
150	28.9[d]	13.5[d]	69.2[d]	42.7[d]
200	10.2[e]	9.3[e]	41.7[e]	23.1[e]
250	7.1[f]	2[f]	13.6[f]	4.3[f]

Same letters show no difference among genotypes of each column.

In copper treatments, the maximum and minimum pollen germination percentage was observed in cultivars “Azar” (44.7%) and “Shahroud 21” (32.8%), respectively. The maximum and minimum pollen tube length was in “Azar” (12.1 μm) and “Shahroud 18” (7.3 μm), respectively (Table 3). In lead treatments, the maximum and minimum pollen germination percentage was observed in cultivars “Mamaei” (36%) and “Shahroud 18” (30.2%), respectively. The maximum and minimum pollen tube length was in “Shahroud 21” (8.4 μm) and “Shokofeh” (5.1 μm), respectively (Table 3). Furthermore, in all cultivars, lead highly decreased both traits of pollens in compared with copper, indicated the higher poisonous effect of lead (Table 3).

Table 3. Mean comparison of pollen germination percentage and pollen tube length among five almond cultivars.

Cultivar	Pollen germination percentage		Pollen tube length (μm)	
	Cupper	Lead	Cupper	Lead
Azar	44.7 [a]	34.4 [b]	12.1 [a]	7.4 [d]
Mamaei	36.1 [c]	36 [a]	10.7 [b]	8.1 [c]
Shahroud 21	32.8 [c]	32.1 [b]	10.8 [b]	8.4 [c]
Shahroud 18	36.1 [b]	30.2 [c]	7.3 [c]	6.2 [e]
Shokofeh	37.1 [b]	33.7 [b]	8.6 [c]	5.1 [f]

Same letters show no difference among genotypes of each column.

Interaction between cultivars and different concentrations of copper and lead significantly decreased both pollen germination percentage and pollen tube length of all almond cultivars (Table 4).

Table 4. Comparison of means of pollen germination percentage and pollen tube length (interaction between five almond cultivars and different concentrations of cupper and).

Cultivar	Cupper			Lead	
	Concentrate (ppm)	PGP (%)	PTP (μm)	PGP (%)	PTP (μm)
“Azar”	0	90.6 [a]	0.4 [a]	98.0 [a]	6.7 [a]
	50	68.1 [b]	0.3 [b]	69.4 [b]	4.6 [b]
	100	41.4 [c]	0.3 [c]	69.4 [c]	2.6 [c]
	150	25.0 [d]	0.3 [d]	69.4 [d]	2.2 [d]
	200	6.0 [e]	0.2 [e]	5.6 [e]	1.5 [e]
	250	1.9 [f]	0.1 [f]	1.3 [f]	0.4 [f]
“Mamaei”	0	83.6 [a]	1.2 [a]	84.6 [a]	8.1 [a]
	50	52.6 [b]	0.8 [b]	45.7 [b]	5.5 [b]
	100	25.9 [c]	0.6 [c]	19.0 [c]	3.2 [c]
	150	9.5 [d]	0.6 [d]	6.7 [d]	2.7 [d]
	200	4.3 [e]	0.5 [e]	6.5 [e]	1.5 [e]
	250	0.8 [f]	0.4 [f]	1.5 [f]	0.3 [f]
“Shahroud 21”	0	82.4 [a]	2.1 [a]	79.5 [a]	8.7 [a]
	50	46.8 [b]	2.0 [b]	48.6 [b]	6.0 [b]
	100	20.1 [c]	1.9 [c]	21.9 [c]	3.4 [c]
	150	6.7 [d]	1.8 [d]	7.7 [d]	2.9 [d]

Table 4 continued

	200	4.4 e	1.5 e	6.8 e	1.4 e
	250	0.7 f	1.2 f	1.7 f	0.3 f
"Shahroud 18"	0	75.5 a	2.7 a	79.5 a	4.7 a
	50	54.4 b	2.6 b	50.8 b	3.2 b
	100	27.7 c	2.5 c	24.1 c	1.9 c
	150	11.3 d	2.4 d	8.9 d	1.6 d
	200	6.2 e	2.1 e	7.0 e	0.9 e
	250	0.5 f	2.1 f	1.8 f	0.1 f
"Rabi"	0	81.8 a	3.5 a	80.9 a	6.0 a
	50	56.6 b	3.2 b	52.7 b	4.1 b
	100	29.9 c	3.0 c	26.0 c	2.4 c
	150	13.5 d	2.9 d	9.6 d	2.0 d
	200	6.6 e	2.8 e	7.4 e	0.9 e
	250	0.9 f	2.7 f	1.9 f	0.2 f

Same letters show no difference among genotypes of each column.

Discussion

The results confirmed that by increasing the concentration of both copper and lead, pollen germination percentage and pollen tube length were significantly decreased, nearly to zero in all cultivars (Tables 2, 3 and 4).Compared to the control medium, traits of pollens in all studied cultivars decreased at list three times. However, the difference in means of pollen germination percentage and pollen tube length among the studied cultivars showed higher variety in pollen germination percentage compared to pollen tube length (Tables 2, 3 and 4).

In this study, lead decreased both traits of pollens in all cultivars, which proved the high poisonous effect of lead in almond in comparison to copper. However, it was reported that copper had the highest inhibition on pollen germination and tube growth in apricot cultivars (Gür and Topdemir, 2008).The effects of lead (Pb) pollution from vehicles on the pollen germination and pollen tube growth of apricot has been studied (*Prunus armeniaca* cv. Sekerpare) (Semra *et al.*, 2009).

Copper has an essential role in many physiological pathways in plants, including photosynthesis, respiration, and carbohydrate distribution, and protein metabolism. It is toxic to plants at high concentrations. The toxicity of copper on plants is higher than most of heavy metals, such as nickel, zinc and chromium. Studies have demonstrated that copper was the second most toxic metal on the seed germination, root elongation and coleoptile and hypocotyls growth in *Triticum aestivum* and *Cucumis sativus* among mercury, cadmium, cobalt, lead and zinc (Gür and Topdemir, 2008).Other studies demonstrated that high concentration of copper caused chromosome anomalies in plant organs (Kaalbande *et al.*, 2008; Kapler and Kristen, 1987).

However, different effects of heavy metal application has been reported in fruit trees, which reported that copper weakly affected pollen germination and tube growth. These different observations may be related to different toleration of different plants to heavy metal stress (Gür and Topdemir, 2005).

Another study showed the effects of heavy metals on pollen germination and tube growth of apples (*Malus silvestris* Miller cv. Golden) (Munzuroglu and Gur, 2000).

Copper is an essential micronutrients and its adequate supply for growing plants should be ensured through artificial or organic fertileness. The appropriate content of copper in plants is essential both for the health of the plant and for the nutrient supply to humans and animals. Certain plant species have a great tolerance to increased concentrations of copper and this metal can accumulate to extremely high amounts in their tissues.

Furthermore, copper, nickel and mercury are the most toxic elements on pollen germination in tobacco plant (Breygina*et al.,* 2012). Tuna *et al.,* (2002) have reported similar results in other tobacco cultivars.

Lead is a common heavy metal pollutant that is released from loaded gasoline and industrial processes. It has not been shown to be essential in plant metabolism. It was demonstrated that lead stimulates free radical and reactive oxygen formation, which can damage plant cells (Wolters and Martens, 1987; Sharma and Dubey, 2005).

Therefore, cultivar "Azar," which had the highest pollen germination percentage, could be used for almond orchard establishment as a pollinizer for pollination of commercially growing cultivars in polluted cities such as Tehran.

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