Does Foliar $CaCl_2$ Application Control Pistachio Endocarp Lesion

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Abstract

Pistachio Endocarp Lesion (PEL), an economic physiological disorder of pistachio fruits, has been reported in Iran, Australia and California. PEL would cause considerable but unpredictable damage in all Iran pistachio growing areas. There is not yet a known method available to even predict or control of the disorder. In the current work, the possibility of controlling PEL development after its natural incidence in developing clusters, using different concentrations and application- timings of $CaCl_2$ sprays were evaluated. Also, the influences of sprays on leaf macro and micro nutrient elements of trees were compared. In 2011, monitoring PEL in orchard condition revealed that the highest damage rate was occurred in the mid of May and decreased during the last week of July. Foliar $CaCl_2$ sprays were highly efficient in controlling PEL in late April so that it decreased PEL from 20% damage in control to less than 2% using one spray of 4 kg $CaCl_2$ 1000 L^{-1} ha^{-1}. Similar results obtained using two sprays of 2 kg ha^{-1} treatment. Between macro-nutrients including phosphorus, potassium, calcium and magnesium; orchard trees showed phosphorus deficiency with levels less than the critical level (0.14%) while others were higher than their critical levels. However Iron, zinc, manganese and copper were sufficient in all treatments. PEL monitoring-based $CaCl_2$ application has no effect on changing calcium content of leaves while it was efficient in controlling PEL disorder during the development stage of pistachio clusters.

Keywords: pistachio, endocarp lesion, $CaCl_2$, foliar spray, disorder

Introduction

Cultivated pistachio, *Pistacia vera* L., (Anacardiaceae) is the only widely cultivated species between eleven *Pistacia* species. It has been grown in Iran from three to four thousands years ago (Abrishami, 1994). For about a decade Pistachio Endocarp Lesion (PEL) is known to cause sever damages in Iran pistachio orchards especially in Kerman province, the world's main pistachio growing area (Mahmoudi-meimand, 2006). PEL has also been reported on Kerman and Trabonella cultivars in California, USA, where it is called as Styler End Lesion. In Australia, a similar physiological disorder has also been reported without reporting an exotic causative agent (Hashemirad, 2005). In association with the causative agent(s) of PEL; it is known that the symptoms are not related to any pistachio arthropod pest (Rice et al., 1996). Studies on the role of pistachio injurious bugs such as *Lygaeus panderus, Apodiphus amygdali, Brachynema* spp., and *Acrostenum* spp. have shown that none of these species is not able to cause such symptoms on pistachio fruits and the signs of feeding bugs on fruits are quietly different from PEL ones. No bacterium, fungi or even virus has not been isolated from the damaged fruits. Observational and comparative researches on climatic conditions like spring-freezing, weather temperature- fluctuations (day-night temp. difference), incomplete degree-day requirements, hailstone, mildew, and even hot summer injuries have also showed that all their symptoms are quite different from PEL ones (Hashemirad and Heidari-nejad, 2006).

The PEL symptoms would change during pistachio cluster development period. In the early stage of endocarp formation, darkening of the endocarp staring from fruit tip along to the 2/3 of its length is observable. Then, the inner endocarp surface turns white while the cutting edge between healthy and infected region gets brown. Afterwards, the green skin of the nuts will turn necrotic and dark brown (Figure 1). After a while, the affected fruit nuts will shrink and fall from the cluster (Hokmabadai, 2011). When endocarp was hardened and cotyledons start developing, the infected endocarp area gets soft and flexible with black spots on the green pericarp (Hokmabadi, 2011).

There are hundreds of literatures available about Calcium roles and effects on plant physiological disorders either on fruits and other organs, most of them related to different apple fruit disorders like Bitter Pit, Cork Spot, Internal Breakdown, and Lentical breakdown. Most others are Blossom End Rot in Tomato, Watermelon and Pepper, Blackheart in Salary and Brussels Sprouts and also Fruit Crack of Sore Cherry and Plum (shear, 1975).

In pistachios, until the current work, however, there was not a scientific results reporting direct calcium efficacy on PEL-affected clusters but it has been conveyed by Mahmoudi-meimand (2006) that endocarp pistachio hardiness is dependent to Ca^{++} availability in fruits. He had also claimed that the calcium absorption is not efficient in the roots of pistachio trees since it is transpiration that determines the calcium absorption and movement from roots to shoot organs like fruits. During calcium shortage condition, because of more leaf transpiration rate, the calcium content that moves to fruits, is lower that the amount which moves to the leaves. In such a condition, no calcium deficiency is detectable in leaves while it is lower than the amount which fruits need to harden their endocarps.

The aim of the current work was to evaluate the efficacy of foliar $CaCl_2$ application in controlling PEL during the fruit development and maturation period. The leaf content of calcium and some other macro and micro-nutrient elements were also compared in all treatments.

Figure 1 Symptoms of pistachio endocarp lesion on a pistachio cluster from Ahmad-aghaei cultivar.

Materials and Methods

The Orchard History and Location

The Orchard where the study was conducted is in Kerman Province, Iran located at 57.067' 30.280" with pistachio trees of about 20 years old from Ahmad-aghaei cultivar (an Iranian widely used commercial cultivar) with no major sign of disease or pest problem but with five year PEL history with the highest damage report in the year before the study year. Trees were cultivated on 50 meter rows with 7 m row-intervals and 1.5 m tree-intervals within a row.

$CaCl_2$ Application

Experiment was conducted according to a factorial design based on a completely randomized block design including 12 treatments and four blocks. Factors were A- $CaCl_2$ concentration including four levels of 1, 2, 3 and 4 kg $CaCl_2$, food-grade $CaCl_2$ (water-soluble, flake form, made in China) dissolved in 1000 L irrigation water and sprayed in a hectare and, B- Spraying replication (timing) factor including one, two or three times foliar spray with 10-day intervals, the first in April, 28th, the second on May, 8th and the last spray was on the May 18th. Sprays were accomplished using a 20 L of liquid-spray-equipment according to the spray schedule and during the evening time. Irrigation water was used as control. All percent data were normalized using Arc-Sin data-transformation and were analyzed using SAS System 6.0 statistical software.

PEL Monitoring

PEL was monitored in orchard condition using randomized sampling of clusters starting from the first incidence of the disorder and it finished until no further PEL has been observed in clusters. Each sample was a cluster per tree, located in the mid of a fruit-bearing branch. The number of total and infected nuts per cluster was recorded through visual inspecting. After recording, infected fruits were carefully eliminated from the clusters, in order to prohibition of their re-counting in next sampling. Data of three sampling occasions including the May 25th, the July 13th and the August 11th were used for analysis.

Leaf Sampling

Fully expanded sub-terminal leaflets (pistachios typically have five leaflets per compound leaf) were randomly collected from non-fruiting branches in middle of the tree. Four to ten leaves were typically collected per tree, and 10-20 trees are sampled in each experiment block. Sampling was accomplished in the August, 12th, 2011. Samples were kept in labeled paper bags in 4ºC refrigerator until element analysis in laboratory.

Leaf Element Analysis

Collected leaves were thoroughly washed and rinsed with distilled water. The samples were then dried in oven at 65°C. The dried samples were then ground into fine powder. Samples were prepared using dry ash method (Ahmed and Chaudhary, 2009). The flame photometric method was used in determining the Potassium (K) content. Spectrophotometric method was used in determining the Phosphorus (P), Iron (Fe), Manganese (Mn), and Copper (Cu) content (Franson, 1985) while complexometric titration method was used in determining calcium (Ca) and magnesium (Mg) content of leaf samples (Lambert, 1976). The experiments were done in triplicate and the results were averaged.

Results and Discussion

Analysis of variance for data from May 25th showed a significant difference between both treatments and the treatments versus control. Changing in both the concentration and the spray-timing factors had significant effects on PEL control. Maximum PEL damage was recorded in control (20.26±26% infected nuts per cluster) that was not significantly different from plots received one spray of 1kh/ha $CaCl_2$. Minimum PEL damage were achieved using 4 Kg/ha spray (2.75±0.27%, 2.00±0.27% and 3.75±2.08% per cluster for one, two and three time sprays, respectively) (Figure 2).

Considering the second monitoring data, July 13th, just the spray-timing factor was statistically significant on mean PEL disorder. Using third monitoring data, August 11th, analysis has not shown significant effect as the PEL damage was decreased below 5 percent even in control plots. In maximum When the PEL damage was high in control plots, the maximum PEL damage was observed in plots with single $CaCl_2$ spray while in two and three times sprayed plots, it was decreased but there was not a significant difference between two and three times spray-replications (Figure 2).

The pistachio leaf content of macro and micro nutrient element in different treatments were given in Tables 1 and 2. The element contents of pistachio leaves were compared with the minimum critical levels which were determined by researchers (Uriu and Pearson, 1981, 1983, 1984, 1986; Uriu, 1984; Uriu et al., 1989; Brown et al., 1993). According to Table 1., between four tested macro nutrients including P, K, Ca and Mg; the orchard trees has shown P deficiency since in both the treatments and control, their levels were less than its critical level (0.14%) while K, Ca and Mg levels were higher than their critical levels (1.6, 1.3 and 0.6% for K, Ca and Mg, respectively). However Fe, Zn, Mn and Cu were sufficient relative to their critical levels. There were significant differences between treatments with regard to the micro-nutrient elements while No significant difference was observed between treatments in P, Ca and Mg but the K that its content was different between treatments (Tables 1 and 2).

In 2011, the first PEL incidence in the selected orchard was recorded during the second week of May that has not related to the spatial situation of the tree. The results of the current study showed that during the developmental period of pistachio clusters in 2011 year, PEL has just a maximum damage point that was during the last week of May.

Table 1 Macro nutrient element contents of pistachio leaves after one to three times spraying of different concentrations of $CaCl_2$.

Concentration (kg 1000 L^{-1} ha^{-1})	Spraying repeat	Content (%)±SE			
		P	K	Ca	Mg
1	One time	0.107±0.018a	1.85±0.50bc	1.97±0.22a	0.60±0.31a
	Two times	0.122±0.028a	2.15±0.41a	1.45±0.23a	0.60±0.08a
	Three times	0.087±0.020a	1.27±0.36c	1.47±0.30a	0.72±0.15a
2	One time	0.07±0.016a	1.07±0.37c	1.40±0.29a	0.67±0.18a
	Two times	0.102±0.015a	1.62±0.30c	1.52±0.17a	0.72±0.17a
	Three times	0.110±0.008a	1.45±0.17c	1.47±0.22a	0.72±0.09a
3	One time	0.112±0.026a	1.80±0.62bc	1.40±0.31a	0.77±0.15a
	Two times	0.110±0.014a	2.05±0.20a	1.52±0.26a	0.72±0.17a
	Three times	0.265±0.056a	1.32±0.32c	1.52±0.32a	0.87±0.26a
4	One time	0.100±0.028a	1.67±0.86c	1.67±0.34a	0.85±0.19a
	Two times	0.135±0.036a	2.27±0.45a	1.30±0.29a	0.77±0.12a
	Three times	0.127±0.017a	2.02±0.28a	1.50±0.25a	0.67±0.17a
Control (water)		0.100±0.008a	1.43±0.37c	1.45±0.17a	0.93±.012a

Table 2 Micro nutrient element contents of pistachio leaves after one to three times spraying of different concentrations of $CaCl_2$.

Concentration (kg 1000 L^{-1} ha^{-1})	Spraying repeat	Content (ppm)±SE			
		Fe	Zn	Mn	Cu
1	One time	120.25±4.34bc	11.25±4.32bc	35.50±15.28bc	6.72±0.51cd
	Two times	158.25±29.12a	17.57±9.27bc	33.50±9.81cd	13.55±4.38a
	Three times	149.00±19.18a	15.32±5.26c	28.00±7.74cd	6.85±3.47cd
2	One time	102.75±24.34bc	11.22±2.26cd	36.50±11.21bcd	5.35±1.23de
	Two times	116.0±25.12bc	14.60±5.09bcd	50.25±9.06a	7.85±1.87cd
	Three times	156.25±26.34a	24.32±7.04a	29.75±3.50cd	9.92±5.40bc
3	One time	100.25±21.79bc	5.80±1.85de	31.50±7.50cd	8.65±2.02bc
	Two times	119.50±6.35vb	12.45±1.66cd	31.50±5.50cd	4.10±0.67de
	Three times	98.75±21.21c	15.17±2.07cd	37.25±8.99bc	6.40±3.18cd
4	One time	97.25±27.87c	4.97±0.36e	53.00±10.73a	7.80±1.53cd
	Two times	103.75±28.17bc	9.72±3.01cd	38.25±13.69bc	6.50±0.90cd
	Three times	144.75±20.36a	12.17±3.89cd	28.75±4.99cd	5.70±1.16de
Control (water)		123.50±12.92b	17.98±5.13bc	45.75±7.93ab	10.20±3.23bc

However it is notable that it could vary from year to year according to the weather condition and the fruit development phenology. The maximum PEL damage was about 20 percent mean infected nuts per cluster in control plots which received one-tree times irrigation water sprays. Then the number of PEL-affected clusters was decreased to minimum level during the third week of July. Further inspecting of clusters showed that there was not any new PEL damage on clusters after the mid-August when all endocarps have gotten hard enough and there were no further growth in nut-size (Figure 3). It was also revealed that $CaCl_2$ application has definite effects in controlling PEL in trees that had started to be affected by the disorder.

Many physiological disorders on fruit and other plant-organs in many other plant species, especially in apple fruit with including blossom end rot, bitter pit, crack spot, internal breakdown and lenticel breakdown, have found to be associated with low levels of calcium (Shear, 1975; Malakoti, 1999; Drazeta, 2001). It has been shown that calcium chloride has increased the calcium concentration of

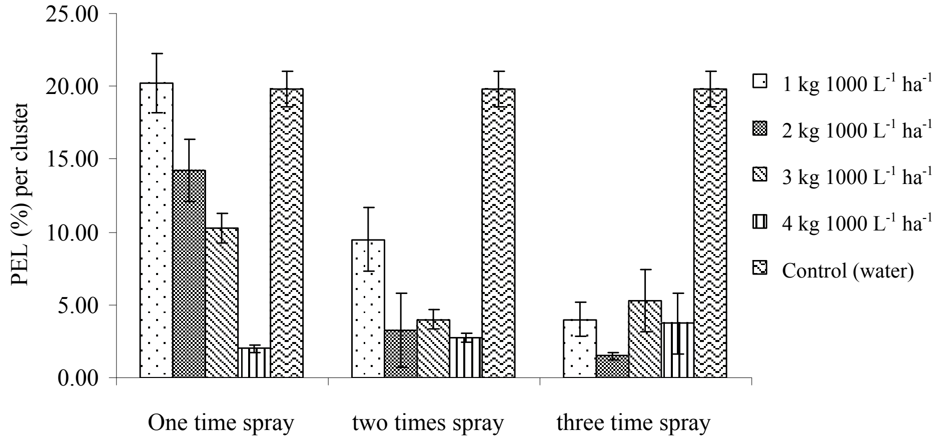

Figure 2 Effects of $CaCl_2$ spraying in controlling pistachio endocarp lesion (PEL). Mean standard errors were shown as error bars.

the apple fruits and decreased the fruit-rot of McIntosh apples during its postharvest (Delahaut and Stevenson, 2004). Foliar calcium application can provide fruit calcium before symptoms appearance on fruit (Mulrooney, 1997). It is notable that the timing of the $CaCl_2$ is very important on its efficacy for PEL control as its foliar spray in the first or the last week of May had significant effect in preventing further disorder development (Figure 3).

Many scientists have tried to increase the Ca level in fruit by providing additional Ca with often limited success. The aim of increasing the Ca content of fruit is to improve fruit stability and especially to prevent Ca-related disorders. However, some authors have realized that even at quite low Ca levels Ca-related disorders must not necessarily occur. In apple, for bitter pit, Ca sprays substantially reduced the amount of bitter pit in one year were not significantly beneficial in the next year, and the reduction of bitter pit by Ca sprays was not always commensurate with the increase in fruit Ca concentration (Stahly, 1986). Ferguson and Triggs (1990) observed that the expected pattern of low calcium/high bitter pit may not apply in particular lines of fruit. In Mandarin, *Citrus reticulate,* foliar calcium nitrate spray in the early stages of cellular fruit enlargement has significantly decreased the percent of cracked fruits (Monselise and costo, 1985; Almela et al., 1994).

With regard to the role of calcium in plants, Ca is being regarded as the important and powerful

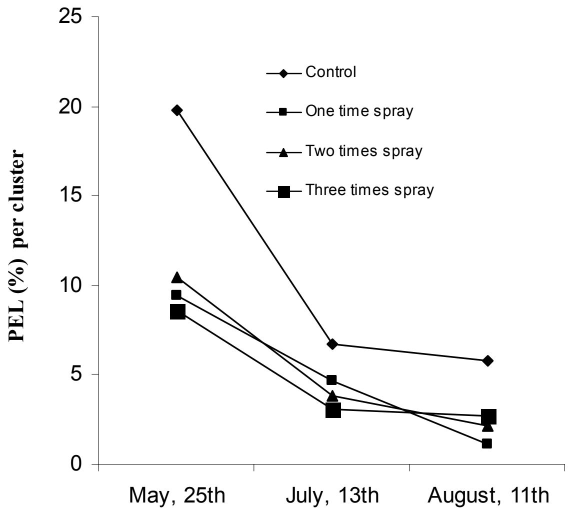

Figure 3 Trend of pistachio endocarp lesion (PEL) damage on fruit clusters during growing season with and without $CaCl_2$ application.

element in postharvest quality of fruits and vegetables that stabilize cell membranes and delaying senescence in horticultural crops (Poovaiah et al, 1988; Pervaiz et al., 2002; Hossain et al., 2005; Abdi et al., 2006; Misra and Gupta, 2006; singh et al., 2006; Hosseini and Thengane, 2007; Naeem et al., 2009). In fruit disorders, the interference of polyphenol oxidase (PPO) with oxidable polyphenol substrates would cause browning of the tissue that is the result of oxidative injury (Hodges, 2003). The high level of calcium is efficient in decreasing ethylene production, electrolyte leakage and flesh browning symptoms that are all directly associated with the calcium content in fruits (Hewajulige et al., 2003). Marschner (1995) pointed out that an increased supply of Ca from the nutrient solution leads to an increase in the Ca content of the leaves, but not necessarily in low-transpiring organs such as fleshy fruits. He conceded that high growth rates of low-transpiring organs increase the risk that the tissue content of Ca falls below the critical level required for cell wall stabilization and membrane integrity. Such a condition may occur for pistachio trees when they bear in "on year" with high number of clusters per shoot and even more dense clusters with higher number of nuts per cluster.

There are many fertilizers containing different forms of calcium that can be used rather than calcium chloride or calcium nitrate. Bramlage et al. (1985) have evaluated some products through multiple year experiments on McIntosh apple. Their results showed that most of them are less efficient than $CaCl_2$ or Calcium nitrate. The most serious problem of those products was the low concentration of calcium so that the efficiency was directly related to calcium concentration (Babalar and Pirmoradian, 2000).

It is of interest that our in-orchard observations showed that the cluster position can substantially influence the PEL damage of clusters and will have a big share in the large differences in the percent of effected nuts per cluster on the same individual tree. Well exposed clusters to sunshine were generally different in color, with more anthocyanin pigments in hulls, than that of fruit from the lower and inner parts of the tree, especially in large trees.

Conclusions

$CaCl_2$ showed obvious effects in controlling PEL but not in increasing the leaf Ca content. What should be kept in mind is that the best management of PEL disorder for orchards with the history of its damage is to careful monitor of the disorder from the early stages of the cluster development. Whenever the PEL incidence is confirmed, the concentration and timing of $CaCl_2$ application will have a tremendous role in its control efficacy on the disorder. It is also suggested that the Ca content to be compared between the pistachio nut hulls of PEL-affected and healthy clusters

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