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Control of pistachio endocarp lesion by optimizing the concentration of some nutrients using taguchi method

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ABSTRACT

Pistachio endocarp lesion (PEL) problem is one of the most common anomalies that has spread to Iran's pistachio areas over the past few years and has caused much damage to Iranian pistachio farmers. Few studies have been conducted to reduce the damage of this problem. In all of these studies, the effects of calcium deficiency alone have been considered, while recent studies, in addition to calcium, have shown other nutrients to be effective in creating this problem. In this study, by optimizing the Taguchi method, optimal levels of fertilizer spray for some of the effective nutrient elements in this problem were determined. To this end, six factors of macro and high microelements (Ca, Mg, Cu, Fe, Zn, and Mn) were selected at three levels and Taguchi design (L_{27}) in the orchard was applied. Response variable in this study, the percentage of contamination in healthy pistachio seeds to PEL problem were selected. Regarding the varied nature of the response, "the lower, the better" index was used in order to analyze the results of Taguchi experiment. The results of this study showed the optimal levels of each of the elements(2 g/L calcium, 2.5 g/L magnesium, 1.5 g/L iron, 0.3 g/L copper, 1 g/L manganese, without the presence of Zinc), which significantly reduces the spread of the PEL problem in healthy seeds. Based on this method, it is predicted, this fertilizer sequence can reduce the intensity of this problem by 1.5%. Among them, calcium, copper, and magnesium respectively ranked first to third in reducing the spread of the PEL problem.

1. Introduction

Pistachio (*Pistacia vera*) is one of the most important exporting products of Iran (The Islamic Republic of Iran Customs Administration (IRICA, 2011). There are currently over 500 thousand hectares of fertilized and unfertilized pistachio orchard in Iran and about 450–500 thousand tons of pistachio is produced in this area (Food and Agriculture Organization of the United Nations (FAO, 2018). Many factors have reduced the quality of this product in recent years. Pistachio endocarp lesion (PEL) is one of the problems that has been occurred in Iran's pistachio orchards and has imposed high losses to the Iranian pistachio farmers. This problem starts with endocarp formation (in Kerman weather conditions in mid-May) and many contaminated pistachios will fall by the end of June. The remained pistachios become soft and flexible, resulting in pistachios deformations at the harvesting step. The most common and famous sign of this problem is blackening the endocarp from the top side toward the downside (Fig. 1).

This problem was first seen in 1984 in the pistachio orchards in California that was called Stylar end lesion (Bolkan et al., 1984; Rice et al., 1985). Zhang (2004) and Hashemi Rad (2005) have identified nutrient deficiencies such as calcium deficiency as one of the causes of this problem, while Sadr et al. (2019), using artificial intelligence techniques among 68 feature of the soil, water, leaf, and fruit in pistachio trees with symptoms of PEL problem, selected some of these features as selective features that have an effect on the PEL problem. The results of this study showed that the ratio of Ca to Mg in soil, soil salinity, phosphorus, and some microelements concentrations in the soil and plant are effective on PEL. So far, few studies have been conducted to apply effective solutions in reducing the destructive effects of this problem. Sajadian and Hokmabadi (2011) showed that this problem will be decreased by applying 40–50 tons/hectare gypsum at March along with spraying calcium solution during the rapid growth of

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Fig. 1. Symptoms of pistachio endocarp lesion on a pistachio cluster (Iran-Rafsanjan, May 2016).

endocarp. Adibfar et al. (2012) studied the effect of calcium chloride in controlling PEL and found that this problem could be reduced by spraying 4 kg/m^3 of calcium chloride solution in pistachio orchards in late May.

So far, in the conducted studies, given a large number of experiments, costs and long periods of these experiments, only the treatment of an element was considered (different calcium concentrations) and the effect of different concentrations of other macro and microelements was not considered in the expansion of this problem. Therefore, in this study, we have evaluated the effect of spraying some macro and micro nutrients on the spread of PEL. In many experiments that have multiple factors and levels, a full factorial design has been considered because it is comprehensive and not only can evaluate the effect of the main variables, but also the trends of the variables. But when the number and level of each factor increases, using a full factorial design will be very time-consuming, costly, and even impossible (Sadeghi et al., 2012). Fractional factorial experiments were developed in order to minimize the number of the required experiments (Rosa et al., 2009). Taguchi method is a fractional factorial experiment which directly conducts the optimization without calculating the mathematical function, and significantly reduces the time and cost of experiments (Chou et al., 2009; Roy, 2001).

Taguchi method has two parts: the design of experiment and optimization. Taguchi has designed some orthogonal arrays as a standard array that makes the design of experiment very efficient (Montgomery et al., 2009; Sadeghi et al., 2012).

In Taguchi method, diagnosing and obtaining of optimal conditions from the experiments is done using S/N ratio (S/N ratio = signal to noise) analysis. S/N analysis specifies that the desirable condition is presented where the signal (controllable factors) to noise (uncontrollable factors) is maximal; in other words, we have an optimal condition in an experiment when the changes in the given variables are more related to the signal than to the noise.

For optimizing different fields like aerospace (Singaravelu et al., 2009), communications (Al-Darrab et al., 2009), sport (Burton et al., 2010), environment (Oztop et al., 2007; Zolfaghari et al., 2011), food (Oztop et al., 2007), material engineering (Dingal et al., 2008), energy (Zeng et al., 2010), industry (Zhang et al., 2007) and dentistry (Geerts et al., 2008), Taguchi method has been used widely and successfully but despite its frequent usage, it has been less used in the agriculture. Among the studies which have used Taguchi method to optimize various agricultural problems, we can mention the effects of silt concentration, surface slope, vegetation cover and earth outcrops on the soil erosion. This study has been conducted using Taguchi orthogonal arrays in the Fars province in Iran. In this study, the silt concentration and the slope have had the highest and the lowest values for soil erosion, respectively, and Taguchi method has been introduced as an accurate method (95%) for soil erosion modeling (Sadeghi et al., 2012). Taguchi method is extremely rare in optimizing the nutrient concentrations to reduce the development of a nutrient problem in the plant. This study has used the Taguchi method for optimizing the concentration of some nutrients to reduce PEL.

2. Material and methods

2.1. The study area

This experiment has been conducted in the pistachio orchard in Ahmadabad Razavi (Rafsanjan-Iran). The study orchard has extended within the geographical range of 55° 54′ 54.726″ to 55° 55′ 189″ to the East and 30° 13′ 435″ to 30° 24′ 375″ to the North. The location of the orchard relative to Rafsanjan city has been shown in Fig. 2. The long-term rainfall average and the height of sampling points are 80 mm and 1490 m above sea level, respectively. PEL problem was observed in 4 consecutive years (Independent from on or off) in this orchard which damaged 30% of the yield. Compound Soil Sample was taken from A (0–40 cm and B (40–80 cm) depths in order to evaluate the soil characteristics of this orchard. Compound Soil Sample was obtained from a combination of six soil samples from two depths in pilot orchard

Soil sample was air dried and passed through 2 mm sieve. The soil texture was measured using hydrometer method (Gee and Bauder, 1986). EC_e (Electrical conductivity in saturated extract) was determined in the extract using an electrical conductivity meter (Model Ohm-644, Metrohm AG Herisus, Switzerland) (Rhoades, 1996). Soil pH

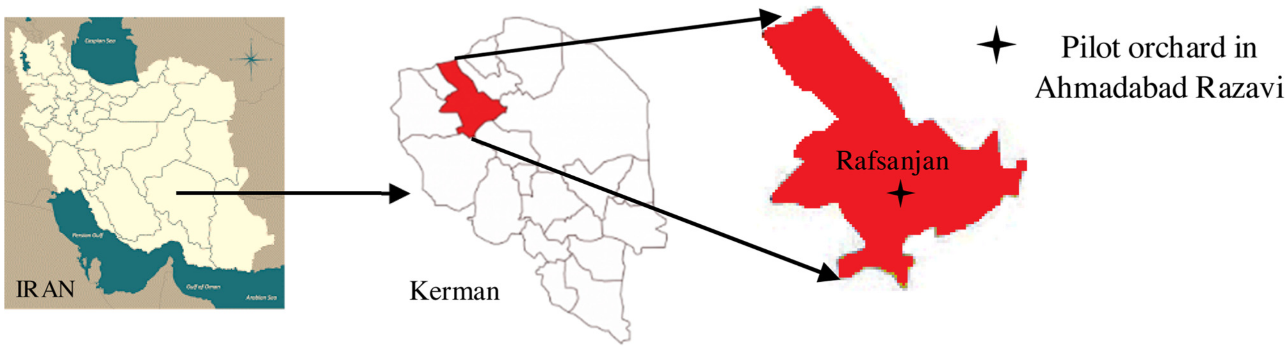

Fig. 2. The location of the selected orchard in Iran and the position of the Rafsanjan city.

was measured in saturated paste using digital pH-meter (Model 691, M0065trohm AG Herisus, Switzerland) (Thomas, 1996). CCE (Calcium Carbonate Equivalent) was measured by neutralization method (Allison and Moodie, 1965). SOC (Soil Organic Carbon) content was measured by Walkley-Black method with dichromate extraction and titrimetric quantization (Walkley and Black, 1934). Available phosphorus was measured using colorimetric method with Olsen extraction (Olsen et al., 1954). Exchangeable potassium was extracted with ammonium acetate and was measured using flame photometer (410 Cornig model) (Knudsen et al., 1982) and DTPA–extractable iron, copper, manganese, and zinc was measured using atomic absorption method (Lindsay and Norvell, 1978).

2.2. Experimental design

In the experimental design, changing the amount of each agent causes the conditions that can be seen in the output. Then, using the experimental data analysis method, the effect of each factor was studied and the output of new factors was predicted and optimized.

In the first step, by identifying the problem, the best response (target) was selected in order to obtain the desired result. This study considered the relative percentage of contamination in healthy pistachio seeds as the response (Eq. 1).

$$M= \frac{A}{B} \times 100 \quad (1)$$

where M, is the percentage of contamination in healthy pistachio seeds to PEL problem, A, is the difference of healthy pistachio seeds before and after treatment and B, is the total number of pistachios before treatment.

In the second step, the given factors and surfaces were selected by considering the time and economic conditions. In this study, 6 nutrient factors were considered (calcium, magnesium, zinc, copper, iron, and manganese from chelate fertilizer source) with each factor in three levels (Table 1). Since the goal is to study the main factor and select the optimal level of essential nutrients in the PEL problem, the number of experiments is $729 = 3^6$ based on Eq. (2).

$$\text{Number of experiment} = (\text{levels number})^{\text{factor number}} \quad (2)$$

Table 1
The factors and levels used in the design of the Taguchi experiment.

Factors	Level 1 g/L	Level 2	Level 3	Source of supply
Calcium	0	1	2	Nano Chelated Calcium 7%
Magnesium	0	1.5	2.5	Nano Chelated magnesium 6%
Iron	0	1	1.5	Nano Chelated Iron 9%
Copper	0	0.3	0.6	Nano Chelated Copper 8%
Zinc	0	1	2	Nano Chelated Zinc 12%
Manganese	0	1	1.5	Nano Chelated Manganese 12%

The number of experiments was very high, and not only the limited time of expanding PEL problem did not allow this experiment to be carried out, but also the number of experimental orchard trees was not enough to implement the design, so the Taguchi design method was used for this experiment.

The number of orthogonal arrays for this experiment was selected L_{27} based on the number of levels and given factors (Table 2). It is worthy to mention that in Taguchi experiments, the replication was not required but the replication provided more reliable results; therefore, two replications were considered for each experiment and the number of experiments was equal to 2*27 and each array was applied to two distinct trees.

2.3. The way of imposing the treatments

In this study before the treatment two clusters of each tree were selected and in each cluster, the number of healthy and seeds with signs PEL problem was counted and recorded. Then, the treatments were imposed according to the Taguchi design as foliar application on April 20, 2018. The number of healthy and contaminated pistachios in each cluster was counted and recorded six weeks after imposing the treatments. For each Taguchi arrays, Eq. (1) was used to calculate the response variable. Table 2 shows the orthogonal arrays along with the response variable for each array.

2.4. Determine optimal conditions in experiment

Generally, there are three quality indicators in S/N analysis:

$$\text{The lower the Better } \frac{\mathbf{S}}{\mathbf{N}} = -10\mathbf{log}_{10}\left[\frac{1}{\mathbf{n}}\sum_{\mathbf{i}=1}^{\mathbf{n}}\mathbf{y}_{\mathbf{i}}^{2}\right] \quad (3)$$

$$\text{Nominal the Better } \frac{\mathbf{S}}{\mathbf{N}} = 10\mathbf{log}_{10}\left[\frac{\mu^2}{\mathbf{R}^2}\right] \quad (4)$$

$$\text{The Larger the Better } \frac{\mathbf{S}}{\mathbf{N}} = -10\mathbf{log}_{10}\left[\frac{1}{\mathbf{n}}\sum_{\mathbf{i}=1}^{\mathbf{n}}(1/\mathbf{y}_{\mathbf{i}}^{2})\right] \quad (5)$$

In the above equations, y_i is the yield variable per i_{th} run, n is the number of replications, μ is mean, and R^2 is variance. In all conditions, the highest S/N ratio is the best condition. Regarding the varied nature of the response, "the lower, the better" index was used in order to analyze the results of Taguchi experiment (Eq. 3). Selection of the arrays and calculations were done in Minitab software (version 16).

3. Result

3.1. Orchard soil characteristics

The results of soil analysis in the study have been shown in Table 3. Based on the results, the salinity of the orchard soil in A and B depths

Table 2
Orthogonal arrays L^{27} and responses after executing any orthogonal arrays.

N	Control factor						M	N	Control factor						M
	Ca	Mg	Fe	Cu	Zn	Mn			Ca	Mg	Fe	Cu	Zn	Mn	
1	1	1	1	1	1	1	32.6	15	2	2	3	1	3	1	58.3
2	1	1	1	1	2	2	13.4	16	2	3	1	2	1	2	3.6
3	1	1	1	1	3	3	37.4	17	2	3	1	2	2	3	42.4
4	1	2	2	2	1	1	20.2	18	2	3	1	2	3	1	10.7
5	1	2	2	2	2	2	31.3	19	3	1	3	2	1	3	11.4
6	1	2	2	2	3	3	1.1	20	3	1	3	2	2	1	7.4
7	1	3	3	3	1	1	9.4	21	3	1	3	2	3	2	6.2
8	1	3	3	3	2	2	2.3	22	3	2	1	3	1	3	18.2
9	1	3	3	3	3	3	17	23	3	2	1	3	2	1	3.6
10	2	1	2	3	1	2	2.9	24	3	2	1	3	3	2	25
11	2	1	2	3	2	3	53	25	3	3	2	1	1	3	10.7
12	2	1	2	3	3	1	28.6	26	3	3	2	2	1	1	17.4
13	2	2	3	1	1	2	41.4	27	3	3	2	1	3	2	12.7
14	2	2	3	1	2	3	4.7	**Rank**[a]	1	3	6	2	5	4	–

M: percentage of contamination in healthy pistachio seeds to PEL problem in two replications.
N: Experiment number.
a: Ranking based on the effect of the factor on increasing S/N ratio.

Table 3
Summary statistics of soil properties from Ahmad Abad Razavi –Rafsanjan – Iran (Pilot orchard).

Parameter	depth			Parameter	depth		
P	A	$(mgkg^{-1})$	10.5	Texture	A		Sandy clay loam
	B		7.90		B		Sandy clay loam
K	A		476	pH	A		8.0
	B		441		B		7.8
Fe	A		3.40	Ca/Mg	A		1.3
	B		3.20		B		1.7
Cu	A		1.50	EC	A	(dSm^{-1})	3.9
	B		1.40		B		4.0
Zn	A		0.44	SOM	A		1.2
	B		0.32		B		0.8
Mn	A		3.70	CCE	A		15

A: Depth of 0–40 cm, B: Depth of.40–80 cm.

was equal to 3.9 and 4, respectively. Mean soil acidity in depths A and B was equal to 8 and 7.8, respectively and the calcium to magnesium ratio in these depths was 1.3 and 1.7 respectively. Soil texture in the samples was light and calcium carbonate percentage in soil was 15% on average.

3.2. Extracted experiments from Taguchi design

Since the number of orthogonal arrays was L_{27}, all 27 experiments were tested on the orchard. Figs. 3 and 4 can be used in order to determine the optimal concentration of each element on the PEL problem. These figures show the signal to noise ratio (S/N) and mean PEL problem spread, respectively. The comparison of these two figures shows that the effects of changes are reverse in two models. For example, if the change in signal to noise ratio in the various levels of iron is ascending (Fig. 3), these changes in the mean are downward trend (Fig. 4). It can be concluded that the number and concentration of levels for each factor is sufficient for determining the main factors on the spread PEL problem and results can be used with higher confidence. If one of these factors show the reverse trend in both figures, this means that the number of selected concentrations was not sufficient to obtain the desired result; therefore, the results are not dependable and higher concentration should be used.

3.3. The optimal concentration of nutrients

Table 4 has shown the best spraying concentration for reducing the spread of PEL problem. Calcium is the most important factor in increasing S/N among the studied factors. The best-reported calcium concentration is 2 g/L. Hashemi Rad (2005) and Adibfar et al. (2012) reported similar concentrations for calcium spraying in their studies.

The selected sequence, as the optimal spraying mix, may or may not exist among the testes orthogonal arrays. In this study, it has been observed that this sequence does not exist among the orthogonal arrays (Table 2), but according to the Taguchi equations, it can be estimated that with the condition of similarity of an orchard soil properties with the test orchard characteristics (Table 3)., and with the application of optimal levels introduced, the percentage of contamination in healthy pistachio seeds (M) decreases to less than 1.5%.

4. Discussion

Calcium and copper were introduced as the most effective factors on the PEL problem among the macro and micronutrient elements in this study. This result was in agreement with the result of Sadr et al. (2019). Calcium is an intermolecular connector for the stabilization of the pectin complex in the middle lamella in plant cells. This element by blocking the production of ethylene that stimulates the activity of the cell wall hydrolysis enzymes, Prevents cell wall destruction and keeps the fruits firmly (Marschner, 1995). This element is effective in the cell division, elongation and the growth of fruit (Antunes et al., 2004). About 60% of calcium remains in the cell wall.

Calcium absorption by the plant from the culture media is often done by root tips in Ca^{2+} inactive cation. The main problem of calcium is not its absorption by the plant, but it is its transfer inside the plant. Calcium transfers in the plant with the apoplastic method through the xylem with low mobility. Based on the studies, calcium has the lowest mobility in the phloem among the nutrients. High pH and the presence of phosphate in the phloem form phosphate salt with low solubility. These types of salts slow down the calcium movement in the phloem. Due to this calcium characteristic, the evapotranspiration from upper organs, such as leaf and fruit, determine the absorption and movement of Ca^{2+} to the upper plant's limb. Due to the fact that the level of transpiration in the leaf surface is higher than fruit surface, in the calcium deficiency conditions, the calcium supplied for the fruit is less than the amount supplied for the leaf. On the other hand, excessive growth of organs or low transpiration, increase the risk of reducing the concentration of calcium in the tissue, and consequently, calcium

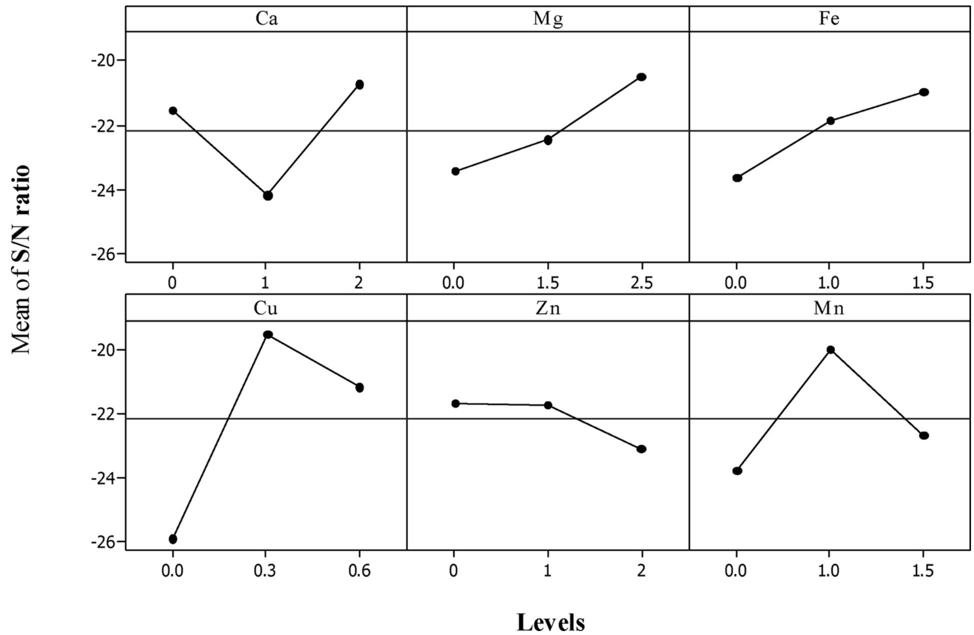

Fig. 3. Effect of main factors on signal to noise ratio in percentage of contamination in healthy pistachio seeds to PEL problem.

deficiency symptoms may not be seen in the leaves, while the fruit and skin are severely deficient in calcium (Fernández et al., 2013; Marschner, 1995; Torre et al., 2001). This is while fruits need higher amounts of calcium than the other parts of the plant (Abbott et al., 1989). Calcium is not transferred from the old tissue to young tissue (it is immobile); therefore, its deficiency is high in the young organs like flowers and fruits (Kleemann, 1999).

Copper is the second effective factor on PEL. This element plays a role in the respiration and photosynthesis. The absorption of the copper by the plants is influenced by the factors such as soil pH, chemical composition and its concentration in the soil. Copper mobility in the plant is average and its re-mobility is low. Copper is not easily transferred from the old leaves to young leaves therefore, its deficiency symptom has been observed in the young organs. The concentration of copper used by plants reduces by increasing pH and its adsorption by carbonates and oxides (Lindsay, 1991). Copper carbonate, after its exchange and soluble forms, is the less common form of copper in the soil (Ma and Uren, 1995). Disturbance in the lignification of the cell wall in the higher plants is the most obvious examples of the structural changes that has been induced with copper deficiency (Marschner, 1995). The prevention of lignification in tissue with copper deficiency relates to the phenols and laccase enzymes which have several copper atoms and contribute in the phenols oxidations, such as paracomaric acid, which is one of the early constituents for the formation of lignin. Phenol accumulation has been seen in the tissues with copper deficiency (Marschner, 1995). Calcium displacement to new growth places is prevented in copper- deficient plants (Brown, 1979).

According to the priority determined in the Taguchi method, magnesium is the third important element in spreading PEL. Sadr et al. (2019) have introduced the calcium to magnesium ratio as one of the factors affecting this problem due to endocarp lesion. They argued that the main cause of spreading this problem is increasing the magnesium

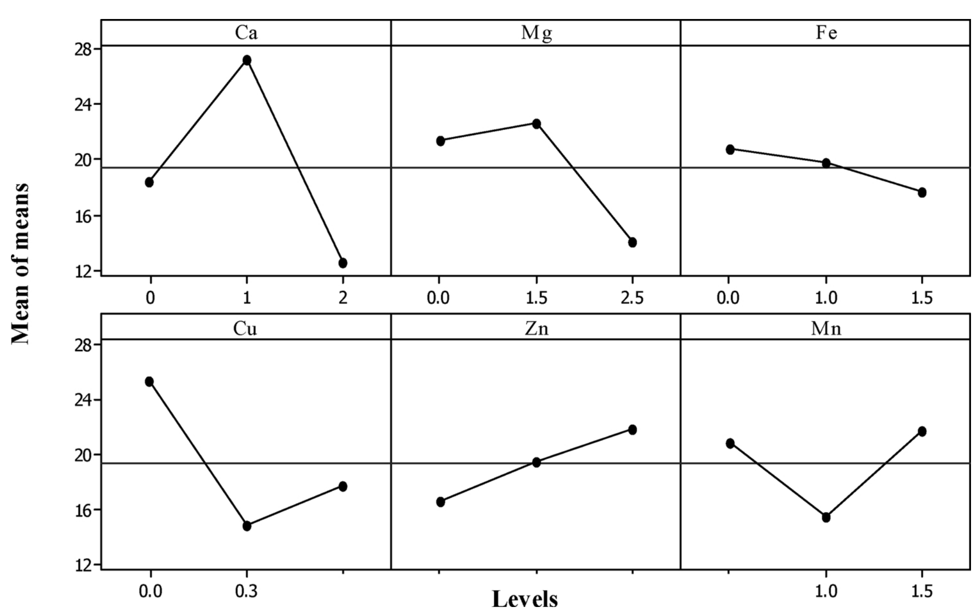

Fig. 4. Effect of main factors on the mean of percentage of contamination in healthy pistachio seeds to PEL problem.

Table 4
Optimum spraying levels to reduce of percentage of contamination in healthy pistachio seeds to PEL problem.

Factor	Ca	Mg	Fe	Cu	Zn	Mn	The predicted percentage for healthy fruits to PEL problem after applying optimal levels
level	3	3	3	2	1	2	1.3
Concentration (gr/L)	2	2.5	1.5	0.3	0	1	

concentration in the irrigation water in recent years and substituting calcium with the magnesium.

Manganese, zinc, and iron are the deficient elements on the alkali and calcareous soils and little attention has been paid to these elements in the pistachio orchards; therefore, supplying these elements at the pistachio formation and filling (when the tree needs these elements) can be effective in increasing the yield. Spraying these three elements can reduce this problem but its importance is lower than the other three factors.

5. Conclusion

Results of this research showed the best effective soluble fertilizer in reducing endocarp lesion for the first time. According to these results, spraying 2 g/L calcium, 2.5 g/L magnesium, 1.5 g/L iron, 0.3 g/L copper, 1 g/L manganese, without the presence of Zinc, has the best effect in minimizing the endocarp lesion. This fertilizer sequence can reduce the intensity of this problem by 1.5%. Among the high-consumption and low-consumption elements studied in this research, calcium has the highest effect in reducing this problem followed by copper and magnesium.

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