Kaolin particle film alleviates adverse effects of light and heat stresses and improves nut and kernel quality in Persian walnut

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ABSTRACT
Spraying with kaolin is becoming a feasible and cheap treatment to alleviate the destructive effects of light and heat stresses. Here, the effects of spraying with kaolin were investigated on leaf temperature, photosynthesis and gas exchange rate, sunburn and quality of nut and kernel, potassium (K) and sodium (Na) concentrations and proline content of the walnut. Three spraying levels of kaolin (0%, 3% and 6%) were used in 5 replicates during two consecutive years. Results indicate that the kaolin spray increases leaf area and chlorophyll content (up to 14.71% and 11.99%, respectively) comparing to the control. The severity of leaf, husk, and kernel sunburn of kaolin treated trees was reduced up to 50% comparing to the control plants. Considering physiological parameters kaolin treatment reduced leaf temperature (4.4 °C and 5.6 °C, respectively in kaolin 3% and 6% compared to the control), but increased photosynthesis rate (A_max), gas exchange (g_s), internal CO2 partial pressure (C_i) and the water use efficiency (up to 29.85%, 30%, 10.97% and 19.88%, respectively) comparing to the control. Kaolin application also improved nut and kernel weight, kernel percentage and kernel oil content (up to 8.2%, 16.1%, 7.7% and 11.29%, respectively) comparing to the control. Kaolin treatments increased potassium level and K:Na ratio, but decreased Na and proline content, both in leaf and kernel, in comparison with the control. However, there was no significant difference between the results of applying kaolin at concentrations of 3% and 6% in almost all of the measured characteristics, but improved results were obtained with higher levels of kaolin, except for nuts and kernel quantities. Considering global warming, using kaolin application can be recommended as a suitable and cheap method for the acclimation of walnut to high temperatures and solar radiation.

1. Introduction

The Persian walnut (Juglans regia L.) is one of the most important nut crops which is widely distributed around the world, and is cultivated on a large scale (Vahdati et al., 2018). Because of its geographical location and its specific climatic conditions, Iran hosts intense light radiations among countries producing walnuts, while being more vulnerable to temperature fluctuations and droughts. This issue is much more serious in areas with lower geographic latitudes, in southern Iran such as the Fars province.

Generally, sunburn in the walnut is most common on the southwest side of the tree, and on the lower part of the canopy. Different types of walnuts are completely different with respect to sunburn intensity, darkening of the color and shrinkage of the kernel (Lampinen et al., 2006). Therefore, the use of methods that can protect the tree from extreme solar radiation and high temperatures at the lowest cost are necessary. One solution is to use sprinkler irrigation systems to cool down the trees so as to counteract the effects of high summer temperatures in fruit orchards. This system requires high technology, high costs and adequate water, while also having the side-effect of spreading some diseases (Kotzé et al., 1988). Another method is to use colored netting for making the shadow on the trees, although it is very expensive to install them on the orchard (Widmer, 2001). Considering the height of walnut trees, using the two methods mentioned for walnuts is difficult.

In recent years, use of particle films as a new approach has been put forward and has yielded very promising results. Various studies have been concerned the subject, on different fruit crops which confirm its high efficiency for this purpose and kaolin is now widely used in orchards of warm and dry areas (Glenn and Puterka, 2005). In a study...
in South Africa, Gindaba and Wand (2005) used a variety of methods (cooling by sprinkling, kaolin, and black netting as shading) to control sunburn in 'Royal Gala' apple. In Spain, reports suggest positive effects of kaolin treatment on pomegranate sunburn (Mellarejo et al., 2004). Rosati et al. (2006) used kaolin to reduce the damaging effects of high temperatures and drought stress on the photosynthesis of almonds and walnuts, and reported that use of kaolin cannot compensate for the negative effects of high temperatures and drought stress, but its positive effects on some physiological indices of almond and walnut trees were confirmed under drought stress conditions. Also, Rosati et al. (2007) studied the effects of kaolin on the distribution and absorption of light within the canopy of walnut and almond trees. Lampinen et al. (2006) and De Buse et al. (2010) reported that kaolin treatment reduced the temperature of the canopy of trees, resulting in reduced sunburn in the leaf and fruit of walnut trees. But kaolin spraying cannot be economically efficient if it is only applied to reduce sunburn. However, when other goals such as pest control enters the equation, then perhaps it could become economically feasible.

None of the studies on dealing with kaolin spray on walnut are gone deep on the evaluation of kaolin treatments on the nut and kernel characteristics. Therefore, in this research, the effects of different concentrations of kaolin were investigated on several characteristics such as photosynthesis and other gaseous exchanges by the tree, rate of leaf, husk and kernel sunburn, the quantity and quality of nut and kernel, the absorption of certain nutrients, water use efficiency, and also the amount of proline accumulation as indicators of the presence or absence of stress in walnut trees.

2. Materials and methods

2.1. Experimental site

This study was done in a commercial orchard located in Khan-Zenyan region, a district of Shiraz, in Fars province with 1486 m altitude where the climate exhibits dry and hot summers, but cold and semi-humid winters. Average of rainfall is 273 mm a year, and most of this rainfall occurs in late fall and winter. The experiment was conducted on 15-year-old walnut trees with planting distances of 7 × 6 m, which were irrigated by the flood irrigation system. In the selected region for the test, adverse effects of hot summers were confirmed in walnut orchards in the previous years. Regarding this point that walnut trees in the experimented orchard were seedlings and non-uniform, uniform trees with relatively similar vigour, and also nut features (the study of nut features was done in the previous year from the beginning of the experiment) were selected for this purpose in order to increase the accuracy. The trees under the experiment received all the usual cares of a commercial orchard such as fertilization, irrigation, and protection against pests and weeds.

2.2. The experimental design and kaolin spraying treatments

The experiment was performed through a complete randomized block design with three treatments [three levels of kaolin spraying 0% (control) 3% and 6%], with five replications (each replication including one tree) in two subsequent growing seasons (2013 and 2014). The kaolin (Sepidan* WP, Kimia Rahavard Ltd., Tehran, Iran) spraying treatments were applied twice on the same trees each year. In each growing season, the first instance of kaolin spraying was done on all the foliage of the trees in the middle of June (when the fruits were the size of a hazelnut), with appropriate concentrations by a sprayer behind the tractor with a high diffusion mode. The second application of kaolin spraying which had a supplemental mode was carried on one month after the first spray; with half amount of the first dosage (respectively with the densities 0%, 1.5% and 3%) and according to the method of the first application on trees, especially on newly grown branches. All these treatments were also repeated on these trees in a similar way in the second year.

2.3. Measurements

For this purpose, five branches were tagged in different directions on each tree, and other subsequent measurements were done on the leaves and fruits of these branches.

2.3.1. Leaf surface

Ten leaves were taken randomly from each tree in different directions of the tree and from the middle of branches at the end of August. Then, they were moved to the laboratory and the surface area of each leaf was measured by the leaf surface measurement device (Li-Cor, Model Li-1300, USA), thereby recording the average of leaf surface area.

The chlorophyll rate was measured by the SPAD-502 system (made in Japan) at the beginning of August. The obtained number is the average of 8 measurements from different directions of the trees.

2.3.2. The leaf sunburn rate

For this purpose, 20 leaves were removed randomly in different directions of the tree in the middle of branches at the end of August. The sunburn rate of each leaf was investigated by using a coding system as follows; Lack of sunburn (0), mild sunburn (1), medium sunburn (2) severe sunburn (3), very severe sunburn (4), and the averages were recorded.

2.3.3. Photosynthesis rate, leaf temperature, gas exchanges, and water use efficiency

A portable device (ADC BioScientific LCA4 Ltd., UK) was used in order to measure photosynthesis and other gas exchanges. Reading of the parameters was done at the beginning of August, on a sunny day, and measurements were made on 5 leaves located in different directions of each tree between hours 10–12 in the morning. Accordingly, the gas exchange rate of the stomata, the leaf surface temperature, photosynthesis amount, and carbon dioxide rate of intake under the stomata module were measured, while water use efficiency was obtained.

2.3.4. Quantitative and qualitative indicators of nuts

The weight of green husk, nut, kernel, and the ratio between them were measured; for this purpose, all the fruits of tagged branches were harvested in a common time of commercial harvesting. Among the harvested product, 100 fruits per tree were selected randomly and the weight of their green husk was recorded after peeling the fruits. The nuts were dried in a sunshine-shadow place for two weeks, and then they were weighted. Their kernels were brought out and their weight was measured. Finally, the weight ratio of the kernel of the nut was calculated.

2.3.5. Rate of sunburn in green husk and kernel

For measuring the sunburn rate of the green husk, numeric codes of zero (without sunburn) to four (severe sunburn) were used (Fig. 1). For this purpose, 20 fruits were selected randomly from each tree and coded on this scale. Finally, the averages were recorded for each tree.

2.3.6. Percentage of kernel oil

The Soxhlet system was used for measuring the total oil. Accordingly, 20 kernels were selected firstly from each experimental unit at random, and they were placed in the oven (70 °C for 72 h) in order to be dried completely. They were then ground, and 2.5 g of kernel powder was put in the Soxhlet device. The separated oil content in the flask was placed in the oven for three hours at 70 °C after 2 h and 30 min of washing with N-hexane solvent. Finally, it was measured and the total oil content was obtained by subtracting the amount from the first weight of the flask (Parvaneh, 1995).

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2.3.7. Nutritional elements in the kernel and leaf of walnut

For measuring the nutritional elements in leaves, about 50 leaflets were harvested from the completely-extended leaves in each tree in the middle of grown branches in early August, and then they were rinsed several times with water after being completely washed. Also, 10 complete nut kernels were selected randomly from each tree. Leaves and nut kernels were completely dried in the oven (70 °C in 72 h). Then, they were ground, and 1 g of each powdered sample was turned into ash at 550 °C in an electric furnace. The obtained ash was solved in five mm double normal hydrochloric acid and was passed through a filter paper; so that its final volume was reached to 50 mm after adding hot distilled water on the surface of filter paper. The rate of its sodium and potassium was measured using a flame meter device (Jenway PEP7, ELE Instrument Co. Ltd) (Chapman and Pratt, 1961).

2.3.8. Proline

Free proline accumulation was measured using the method described by Bates et al. (1973). Shoot fresh tissues (0.5 g) were homogenized with 3% sulfosalicylic acid. After 72 h, the homogenates were centrifuged for 20 min at 3000 rpm. The supernatants were treated with acetic acid and ninhydrin, boiled for 1 h and then absorbance of colored phase was read at 520 nm using a spectrophotometer (Model T60U, PG Instruments, Leicestershire, UK). Proline concentration (in μg g⁻¹ fresh weight), was calculated using L-proline for the standard curve.

2.4. Statistical analysis

This experiment was performed as a complete randomized block design, consisted of 3 treatments and 5 replicates, and was performed in 2 consecutive years (years 2013 and 2014). At first, data pertaining to two years were compared so as to determine the significance of each year, and since the effect of the year was not significant at 5%, the average data of two years were used. Statistical analysis of data was performed by using SAS version 9.1 and comparison of mean values was done by the LSD test at the 5% level.

3. Results and discussion

3.1. Leaf characteristics

Kaolin spraying increased the leaf area and chlorophyll content compared to the control treatment and decreased leaf sunburns. Although there was no significant difference between the different levels of kaolin, the highest values for leaf area and chlorophyll and the lowest value for leaf sunburns were recorded by the 6% kaolin treatment (Table 1).

Reducing leaf area is one of the mechanisms that plants adapt to avoid drought and reduce the effects of thermal and sunlight radiation stress. By decreasing the leaf area, the amount of transpiration and light interception decrease and, as a result, the plant survival increases (Parsons, 1980). Increasing the concentration of kaolin to 6% showed an increase in leaf area. This increase in leaf area can be cited as an evidence for the reduction in plant stress as a result of kaolin treatments. Aly et al. (2010) also showed that the application of kaolin increased the leaf area, compared to the control, by applying different concentrations (1%–3%) of kaolin on the 'Anna' apple cultivar. In this study, kaolin solubilizing treatments greatly reduced the negative effects of temperature stresses caused by high light intensity on chlorophyll content. Zarco-Tejada et al. (2000) stated that chlorophyll content of the leaf is one of the important physiological characteristics that can change under stress. They considered the chlorophyll of the leaf as one of the most important indicators of environmental stresses on plants and indicated that the amount of chlorophyll in plants under stress decreases, and reduces the absorption of light by the plant. The results of this study confirm the effective role of kaolin in reducing the stresses on the leaves of walnut trees.

Table 1

<table>
<thead>
<tr>
<th>Kaolin levels</th>
<th>Leaf sunburn (0–4)</th>
<th>Leaf area (cm²)</th>
<th>Chlorophyll (SPAD reads)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3 0 a</td>
<td>341.98 b</td>
<td>36.7 b</td>
</tr>
<tr>
<td>3%</td>
<td>1 7 b</td>
<td>378.89 ab</td>
<td>41.0 a</td>
</tr>
<tr>
<td>6%</td>
<td>1 6 b</td>
<td>400.98 a</td>
<td>41.7 a</td>
</tr>
</tbody>
</table>

* Means with the same letters in columns are not significantly different at P ≤ 5% of LSD test.
exchange (gs), internal CO2 partial pressure (Ci), and also the instantaneous water use efficiency at the leaf (WUE). No significant difference was observed between the levels of kaolin treatment in all of the mentioned indices. Although kaolin increased the transpiration (E) from leaf area, this difference was not statistically different with the control treatment (Table 2).

Most of the literature is consistent in proving that kaolin spray decreases the temperature of canopy and leaves in fruit trees. For instance, reduction of 3°C, 4°C, and 2–3°C have been reported respectively in grapefruit (Jifon and Syvertsen, 2003), pecan (Lombardini et al., 2004) and walnut (Rosati et al., 2006), in kaolin treated trees compared to the control. These results are in line with the finding of current research regarding leaf temperature. Kaolin reflects both visible and ultraviolet sunlight and thus reduces the surface temperature of the leaf in situations when temperatures are supra-optimal (Glenn et al., 2001, 2003). It also facilitates better distribution of light to lower shaded parts of the tree canopy, and consequently improves the gaseous exchange rate, pure photosynthesis, and transpiration (Glenn, 2009).

Foliar spray of kaolin is also improved water use efficiency (WUE) comparing to the control with no significant differences between the two levels of kaolin treatments. As leaf E was not affected by kaolin treatments the improved WUE could be due to increased CO2 absorption (Jifon and Syvertsen, 2003). Internal CO2 partial pressure (Ci) measured in the current study was higher in kaolin treatments (211.6 and 228.0 μmol mol−1 in walnut 3% and 6%, respectively) than that of the control (203.0 μmol mol−1), which indirectly proven the increased photosynthesis and WUE rates of kaolin treated leaves comparing to the control.

### 3.3. Quantitative and qualitative traits of nut and kernel

Foliar application of kaolin significantly reduced the amount of sunburn in green husk and nut kernels compared to the control treatment (Table 3; Fig. 2). Different levels of kaolin had no significant difference in the amount of sunburn on the walnut husks, but the amount of sunburn in the nut kernels was significantly different between the two levels of kaolin (Table 3), so that the brightest colored and the healthy kernels belonged to the kaolin treatment of 6%, and most dark kernels were burnt, from the control trees (Fig. 2).

The green husk plays an important role in nourishing and preserving environmental stresses and the radiant heat of the sun. Due to sunburn, thinness and dehydration of these parts will result in the inferior quality of those sensitive parts of the fruit, especially its kernel. In accordance to the results of this study kaolin film were found to protect some crops such as apple (Glenn et al., 2001; Schupp et al., 2002; Wand et al., 2006), pomegranate (Melgarejo et al. 2004) and walnut (Coates and Van Steenwyk, 2002) from sunburn. Kaolin reflects a portion of the direct light reaching the surface of the fruits and prevents overheating, thereby significantly reducing the percentage of fruits that are affected by severe sunburn (Glenn et al., 2001). It is believed that the effectiveness of kaolin sprays in reducing sunburn in different regions, crops and even cultivars could be more ascribed to the reduction in damaging radiation reaching the fruit surface than to the reductions in surface temperature (Gindaba and Wand, 2005).

Kaolin treatment increased green husk weight, nut weight, kernel weight, as well as the percentage of nut kernel. Although there was no significant difference between the two levels of kaolin treatment in the

### Table 2

Effects of kaolin spray on physiological parameters of walnut (average of two years data).

<table>
<thead>
<tr>
<th>Kaolin levels</th>
<th>Tl (°C)</th>
<th>E (mmol m⁻²s⁻¹)</th>
<th>gs (mmol m⁻²s⁻¹)</th>
<th>Amax (μmol m⁻²s⁻¹)</th>
<th>Ci (μmol mol⁻¹)</th>
<th>WUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>34.6 a</td>
<td>3.93 a</td>
<td>0.07 b</td>
<td>5.29 b</td>
<td>203.0 b</td>
<td>1.37 b</td>
</tr>
<tr>
<td>3%</td>
<td>30.2 b</td>
<td>4.27 a</td>
<td>0.10 a</td>
<td>6.96 a</td>
<td>211.6 ab</td>
<td>1.63 a</td>
</tr>
<tr>
<td>6%</td>
<td>29.0 b</td>
<td>4.44 a</td>
<td>0.10 a</td>
<td>7.54 a</td>
<td>228.0 a</td>
<td>1.71 a</td>
</tr>
</tbody>
</table>

* Means with the same letters in columns are not significantly different at P ≤ 5% of LSD test.

* * Tl = Leaf temperature; E = Transpiration; gs = Stomatal gas exchange; Amax = Photosynthesis rate, Ci = Internal CO2 partial pressure; WUE = Water use efficiency.

### Table 3

Effects of kaolin spray on nut and kernel traits of walnut (average of two years data).

<table>
<thead>
<tr>
<th>Kaolin levels</th>
<th>Husk sunburn (0–4)</th>
<th>Kernel sunburn (0–4)</th>
<th>Husk weight (g)</th>
<th>Nut weight (g)</th>
<th>Kernel weight (g)</th>
<th>Kernel percentage (%)</th>
<th>Kernel oil percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.4 a</td>
<td>2.0 a</td>
<td>17.68 b</td>
<td>14.10 b</td>
<td>6.64 b</td>
<td>47.06 b</td>
<td>51.68 c</td>
</tr>
<tr>
<td>3%</td>
<td>1.4 b</td>
<td>1.4 b</td>
<td>24.98 a</td>
<td>15.36 a</td>
<td>7.91 a</td>
<td>51.51 a</td>
<td>58.26 a</td>
</tr>
<tr>
<td>6%</td>
<td>1.2 b</td>
<td>1.0 c</td>
<td>27.74 a</td>
<td>15.18 a</td>
<td>7.71 a</td>
<td>50.78 a</td>
<td>55.04 c</td>
</tr>
</tbody>
</table>

* Means with the same letters in columns are not significantly different at P ≤ 5% of LSD test.
quantitative traits of nuts, but the highest amounts of quantitative traits were observed in kaolin 3% treatment (Table 3). Also, the percentage of total kernel oil increased due to the application of kaolin. The highest oil content (58.26%) was observed at kaolin treatment 3% and the lowest (51.68%) in the control treatment (Table 3).

In line with the results of this study, kaolin spray has been often found to increase yield and its component in many fruit crops such as apple (Glenn et al., 2001), pear (Puterka et al., 2000), high bush blueberry (Spiers et al., 2003) and olive (Saour and Makee, 2004). Kaolin treatment increases the amount of carbohydrates and also improves the overall yield of the fruit trees (Glenn et al., 2001), due to the increase in the duration of photosynthesis throughout the day (Rosati, 2007). Wand et al. (2006) also reported that kaolin increases the weight of apple fruits by decreasing leaf temperature and decreasing the heat and light intensity. Rosati et al. (2006) also stated that kaolin increased fruit weight of walnut and almond in both irrigation and stress conditions, but this effect was more related to drought stress conditions. They also reported that the use of a layer of kaolin could improve the relative quality of nuts, which is consistent with the results obtained in this study. On the other hand, some studies have attributed the increase in yield because of kaolin treatments to pest control, as kaolin was sprayed (Saour, 2005; Saour and Makee, 2004).

### 3.4. The concentration of sodium and potassium in leaf and nut

Kaolin treatment resulted in increased potassium and decreased sodium concentration in both the leaf and nut, compared to the control treatment. Regarding the concentrations of the mentioned elements in walnut leaf, kaolin treatment (3%) did not show a significant difference with both the control and kaolin treatment (6%), but the difference between the treatment of kaolin 6% and control was significant. No significant difference was found between the two concentrations of kaolin in term of the concentration of these two elements in the nut kernel. It should be noted that the amount of potassium in walnut kernel was much higher than that of the walnut leaves, while the opposite of this was observed in the case of sodium. Kaolin treatments also led to an increase in potassium to sodium ratio in the walnut leaves and kernel, which was highest in walnut leaves (0.38) in 3% kaolin treatment and in walnut kernels (0.69) in the 6% treatments of kaolin. Also, the ratio of these two elements in the walnut kernel was much higher than the walnut leaf (Table 4).

Potassium does not play a structural role in plant tissues but, with its enzymatic and co-enzymatic roles, it is an important element in the plant. The intense competition between Na⁺ and K⁺ in various metabolic processes makes it possible for any change in the ratio of these two elements to have a decisive effect on plant growth. Potassium deficiency can happen because of decreased stomatal conductance, increased mesopholic resistance, decreased rubisco activity, and increased active oxygen species which decrease photosynthesis. The need increases for potassium adsorption to enhance the efficiency of the photosynthetic apparatus and to transfer the photosynthetic products to stored organs in severe light conditions (Cakmak, 2005; Hu and Schmidhalter, 2005). Oraei et al. (2009) reported a positive correlation between the pure photosynthesis intensity and the K:Na ratio in two almond rootstocks which are in agreement with the obtained results of this study.

### 3.5. Proline content

Kaolin treatments significantly reduced leaf proline content, but this decrease in proline content was only significant when kaolin was applied at its 6% concentration (Table 4).

Proline accumulation is an indicator of damage to the plant. It is considered due to its rapid appearance following any stress. There have been reports of increases in the proline content by 3–300-fold in different species, by different treatments of osmotic stress (Delaney and Verma, 1993). Lotfi et al. (2009) reported a direct correlation between the degree of drought stress and proline content in walnut and proposed proline concentrations as a biochemical marker of the drought stress level in walnut plants. This finding is in line with the obtained results of the current study. The results of this study showed that proline accumulation can be used as an indicator to show the amount of stress entering the plant and to show the protective role of kaolin in causing tolerance to stress in plants.

### 4. Conclusion

In general, kaolin has significant positive effects on morphological and physiological parameters of the tree, i.e. reduction of leaf, husk and kernel sunburn, besides having beneficial effects on the qualitative and quantitative characteristics of nut and kernel as well as plant photosynthesis and gas exchange. By increasing the kaolin concentration, its positive effects became more prominent. However, in many of the measured traits, the observed effects of 3 and 6% kaolin were not...
statistically different. Therefore, the use of kaolin spray can be a good and inexpensive way to reduce the vulnerability of walnut trees under high temperatures and intense solar radiations. Therefore, this treatment can be recommended in the areas with hot summers.

Acknowledgements

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References


Table 4

<table>
<thead>
<tr>
<th>K Leaf (%)</th>
<th>K Kernel (%)</th>
<th>Na Leaf (%)</th>
<th>Na Kernel (%)</th>
<th>K/Na Leaf</th>
<th>K/Na Kernel</th>
<th>Leaf proline (μmol g⁻¹ FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.22 b</td>
<td>0.42 b</td>
<td>0.40 a</td>
<td>0.26 a</td>
<td>0.32 b</td>
<td>0.56 b 11.90 a</td>
</tr>
<tr>
<td>Levels</td>
<td>3%</td>
<td>0.23 ab</td>
<td>0.45 a</td>
<td>0.38 ab</td>
<td>0.21 b</td>
<td>0.38 a 0.62 ab 10.89 ab</td>
</tr>
<tr>
<td></td>
<td>6%</td>
<td>0.24 a</td>
<td>0.46 a</td>
<td>0.35 b</td>
<td>0.21 b</td>
<td>0.35 ab 0.69 a 8.63 b</td>
</tr>
</tbody>
</table>

* Means with the same letters in columns are not significantly different at P ≤ 5% of LSD test.