

## Licorice-root extract and potassium sorbate spray improved the yield and fruit quality and decreased heat stress of the 'osteen' mango cultivar

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Heat stress, low mango yields and inconsistent fruit quality are main challenges for growers. Recently, licorice-root extract (LRE) has been utilized to enhance vegetative growth, yield, and tolerance abiotic stresses fruit trees. Potassium sorbate (PS) also plays a significant role in various physiological and biochemical processes that are essential for mango growth, quality and abiotic stress tolerance. This work aimed to elucidate the effects of foliar sprays containing (LRE) and (PS) on the growth, yield, fruit quality, total chlorophyll content, and antioxidant enzymes of 'Osteen' mango trees. The mango trees were sprayed with (LRE) at 0, 2, 4 and 6g/l and PS 0, 1, 2, and 3mM. In mid-May, the mango trees were sprayed with a foliar solution, followed by monthly applications until one month before harvest. The results showed that trees with the highest concentration (6 g/L) of LRE exhibited the maximum leaf area, followed by those treated with the highest concentration (3 mM) of PS. Application of LRE and PS to Osteen mango trees significantly enhanced fruit weight, Number of fruits.tree<sup>-1</sup>, yield (kg/tree), yield increasing%, and reduced No. of sun-burned fruits compared to the control. LRE and PS foliar sprays to Osteen mango trees significantly enhanced fruit total soluble solids Brix, TSS/acid ratio, and Vitamin C content compared to the control. Meanwhile, Total acidity percentage in 'Osteen' mango fruits significantly decreased after both LRE and PS foliar sprays. 'Osteen' mango trees showed a significant increase in leaf area, total chlorophyll content, total pigments, and carotenoids of leaf. Our results suggest that foliar sprays containing LRE and PS significantly improved growth parameters, yield, fruit quality, antioxidant content, and total pigment concentration in 'Osteen' mango trees. Moreover, the most effective PeerJ reviewing PDF | (2024:05:100914:2:0:NEW 9 Aug 2024)

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treatments were 3mM KS and 6g/L LRE. LRE and PS foliar spray caused a significant increase in yield percentage by 305.77%, and 232.44%, in the first season, and 242.55%, 232.44% in the second season, respectively.



## 1 Licorice-Root Extract and Potassium Sorbate Spray

### 2 Improved the Yield and Fruit Quality and decreased

### **3 heat stress of the 'Osteen' Mango Cultivar**

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### **Abstract**

- Heat stress, low mango yields and inconsistent fruit quality are main challenges for growers.
- 25 Recently, licorice-root extract (LRE) has been utilized to enhance vegetative growth, yield, and
- 26 tolerance abiotic stresses fruit trees. Potassium sorbate (PS) also plays a significant role in
- 27 various physiological and biochemical processes that are essential for mango growth, quality and
- 28 abiotic stress tolerance. This work aimed to elucidate the effects of foliar sprays containing
- 29 (LRE) and (PS) on the growth, yield, fruit quality, total chlorophyll content, and antioxidant
- and enzymes of 'Osteen' mango trees. The mango trees were sprayed with (LRE) at 0, 2, 4 and 6g/l
- and PS 0, 1, 2, and 3mM. In mid-May, the mango trees were sprayed with a foliar solution,
- 32 followed by monthly applications until one month before harvest. The results showed that trees
- with the highest concentration (6 g/L) of LRE exhibited the maximum leaf area, followed by
- 34 those treated with the highest concentration (3 mM) of PS. Application of LRE and PS to Osteen
- 35 mango trees significantly enhanced fruit weight, Number of fruits.tree-1, yield (kg/tree), yield
- 36 increasing%, and reduced No. of sun-burned fruits compared to the control. LRE and PS foliar
- 37 sprays to Osteen mango trees significantly enhanced fruit total soluble solids Brix, TSS/acid
- ratio, and Vitamin C content compared to the control. Meanwhile, Total acidity percentage in
- 39 'Osteen' mango fruits significantly decreased after both LRE and PS foliar sprays. 'Osteen'



- 40 mango trees showed a significant increase in leaf area, total chlorophyll content, total pigments,
- and carotenoids of leaf. Our results suggest that foliar sprays containing LRE and PS
- 42 significantly improved growth parameters, yield, fruit quality, antioxidant content, and total
- 43 pigment concentration in 'Osteen' mango trees. Moreover, the most effective treatments were
- 44 3mM KS and 6g/L LRE. LRE and PS foliar spray caused a significant increase in yield
- 45 percentage by 305.77%, and 232.44%, in the first season, and 242.55%, 232.44% in the second
- 46 season, respectively.
- 47 Keywords: *Mangifera indica* L.; bio-stimulates; leaf area; yield increasing%; carotenoids;
- 48 sunburned

### 49 Introduction

- 50 Ensuring global food security for a growing population is a major challenge for modern
- 51 agriculture (Fróna at al., 2019). This challenge is compounded by several factors. Climate
- 52 change has reduced the productivity of fruit trees. Additionally, the decline of usable land and
- 53 the intensification of agricultural practices further complicate the situation. (Pandey, 2020). To
- 54 achieve sustainable farming, there is a growing interest in finding environmentally safe organic
- 55 materials (Durán-Lara, Valderrama & Marican, 2020).
- Among tropical fruits, the mango stands out as one of the most essential globally, prized for its
- 57 flavor and nutritional value (Hussain et al., 2021). This fruit may be enjoyed sparkling, juiced, or
- 58 maybe integrated into diverse meals products. Mangoes boast an outstanding nutritional profile,
- 59 filled with both macro and micronutrients (Yahia et al., 2023). Low mango yields and
- 60 inconsistent fruit are main challenges for growers. These issues with flowering and fruiting,
- along with abnormal flowering, terrible fruit set, and inconsistent fruit development (Shivran et
- 62 al., 2020). This changes to lower yields, culmination of subpar exceptional and restrained
- availability at some stage in the year. Fortunately, research suggests a promising solution:
- 64 strategic application of nutrients and growth regulators (Ram et al., 2020). By influencing
- 65 flowering and fruiting patterns, these tools can assist us release the whole potential of various
- 66 mango cultivars ('Keitt', 'Tommy Atkins') (Ramírez & Davenport, 2010). This method has the
- 67 energy to seriously boom and stabilize mango production (Tirado-Kulieva et al., 2022).
- 68 Biostimulants have been recognized as a promising approach that utilizes natural products
- 69 (Santini et al., 2021). Biostimulants are a flexible, cost-effective, and widely applicable solution
- 70 that can improve agricultural productivity in a sustainable manner and mitigate the effects of
- 71 climate change (Fallovo et al., 2008; Rouphael & Colla, 2018). Generally, biostimulants have the
- 72 potential to enhance crop productivity through three key mechanisms: (i) optimizing root system
- 73 architecture to improve water and nutrient uptake, (ii) maximizing photosynthetic capacity to
- 74 promote growth, and (iii) reducing oxidative stress in plants by enhancing the antioxidant
- 75 defense system (De Pascale & Rouphael, 2018; Rouphael & Colla, 2018).
- 76 Recent research has explored the potential of plant extracts, like licorice root, to enhance fruit
- 77 tree growth and yield (Nasir et al., 2016). Licorice (Glycyrrhiza glabra), belonging to the
- 78 Leguminoseae family, thrives in Egypt and many other regions globally (Vlaisavljević et al.,
- 79 2018). The licorice root boasts some nutritional value and unique chemical compounds,



- 80 including gleserezin, glycyrrhejel. Additionally, rich in various elements and nutrients (Husain et
- al., 2021). Licorice extract is believed to stimulate cellulose enzyme activity, crucial for cell
- 82 expansion, ultimately accelerating plant growth. It might also help reduce transpiration rates,
- 83 minimizing water loss and maintaining cell turgor (Peng et al., 2023).
- Potassium is a vital mineral nutrient for plants (Wang et al., 2013). This element is crucial for
- 85 numerous functions within plants, influencing growth, yield, fruit development, quality, and even
- 86 their ability to withstand stress (Shahid et al., 2020). Studies have shown that potassium
- 87 improves fruit quality parameters like sugar content, color, and overall taste (Hernández-Pérez et
- 88 al., 2020). Beyond its role in fruit development, potassium is essential for plant life itself
- 89 (Sardans & Peñuelas, 2021). It activates numerous enzymes involved in photosynthesis, sugar
- 90 production, and overall plant metabolism, ultimately leading to increased crop yields (Nieves-
- 91 Cordones et al. 2016). Potassium also regulates various physiological processes like water
- 92 movement, respiration, and nutrient translocation within the plant. Additionally, it activates
- 93 enzymes like nitrate reductase and starch synthetase, which contribute to a healthy balance of
- 94 protein and carbohydrate production (Iqbal et al., 2022). Potassium even influences how plants
- open and close their stomata (tiny pores on leaves). This, in turn, affects photosynthesis, nutrient
- 96 uptake, and overall plant function, ultimately impacting crop yield and fruit quality(Devin et al.,
- 97 2023). Studies have shown that KNO<sub>3</sub> increases the proportion of flowering shoots, flower
- 98 clusters, and vegetative growth, key to better yields and decreased alternate bearing (Alshallash
- 99 et al., 2023: Alebidi et al., 2023).
- 100 Studies show potassium is vital for healthy mango trees and delicious fruit. It improves fruit size,
- sweetness, and color by strengthening the tree and boosting sugar production (Andreotti et al.,
- 102 2022; Ahmad et al., 2023). Field trials confirm that applying potassium, especially through
- leaves, significantly increases mango yield and fruit quality of 'Hindi' mango trees (Baiea et al.,
- 104 2015).
- Many studies have investigated mango cultivation, but none have looked at how licorice root
- extract and potassium sorbate affect the growth, yield, fruit quality, and antioxidant enzymes of
- 107 'Osteen' mangoes. Moreover, this study aimed to elucidate the effects of licorice root extract and
- 108 potassium sorbate on the growth, yield, fruit quality, total chlorophyll content, and antioxidant
- 109 enzymes of 'Osteen' mango trees.

#### Materials & Methods

- 112 Licorice root extract Preparation
- 113 To prepare the Licorice root extract for commercial use (obtained from the Sekem Group in
- 114 Cairo, Egypt), it was mixed with water at a concentration of 250 g L<sup>-1</sup>. The mixture was left at
- 115 room temperature for 24 hours, and then thoroughly blended before being filtered through
- Whatman No. 42 filter papers. This filtration process resulted in obtaining a condensed brown
- 117 liquid extract. To enhance the properties of the filtered solution, gelatin from Sigma-Aldrich (at a
- 118 concentration of 2 g L<sup>-1</sup>) was added. This step followed the methodology described by Younes,
- et al. (2019); Younes et al. (2020). The resulting mixture was heated at a temperature of  $30 \pm 2$



- 120 °C while stirring was done to ensure proper mixing following by storage for application on
- mango trees. The chemical composition of the Licorice root extract was also analyzed, in Table 121
- 1, as reported by (Desoky et al., 2019; Rady et al., 2019; Younes et al., 2021) 122

- 124 2.1. Experimental site
- 125 This study was conducted on 7-year-old Osteen mango trees in a private farm located in the El
- 126 Salheya El Gedida district, El-Sharqia Governorate, Egypt (30.6547° N, 31.8733° E) weather
- data Table 2. The trees are planted in sandy soil at a distance of 2m ×4m (1250 tree/ha) and 127
- irrigated using a drip system. Management practices included regular irrigation, fertilization, and 128
- pest and disease control. From mid-May to one month before harvest, the trees were sprayed 129
- 130 monthly interval with licorice root extract and potassium sorbate solutions as follows in Table 3.
- 131 Moreover, at 7:00 AM, we used backpack sprayers on each tree, applying 5 liters (6250 L/ha) of
- 132 both solutions to the point of runoff.
- 2.2. Measurements: 133
- 134 2.2.1. Leaf area:-Twenty leaves below panicles of the spring growth cycle according to
- Walworth and Sumner (Walworth & Sumner, 1987) were taken (2nd week of June) for 135
- measuring leaf area according of (Ahmed & Morsy, 1999) as the following: 136

137

138 Leaf area =  $0.70 (L \times W) - 1.06 = \dots cm^2$ 

139

140 Where: L and W = leaf length and width, respectively.

141

- 142 2.2.3. Total pigments
- Leaf total chlorophyll content and total carotenoids were extracted from fresh leaves of 'Osteen' 143
- 144 mango cultivar, following the method of Brito et al., (Brito et al., 2011), and the results were
- calculated according to (Wellburn, 1994). 145

146

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- 2.2.4. Determination proline content
- To estimate proline content a rapid colorimetric method was used (Bates et al., 1973). Plant 148
- tissue (0.5 g) was homogenized in a solution and filtered. The filtrate was then mixed with a 149
- 150 freshly prepared acid solution and heated. The reaction was stopped by cooling, and a specific
- 151 organic solvent was used to extract a colored compound. The amount of color formed was
- 152 measured using a spectrophotometer, and the proline content was determined using a standard
- 153 curve.

- 2.2.5. Determination relative water content. 155
- Leaf relative water content (RWC) was determined following the method of (Yamasaki & 156
- Dillenburg, 1999). Two leaves were randomly selected from the middle portion of each plant 157
- replicate. The fresh weight (FM) of each leaf was measured after separation from the stem. To 158
- 159 determine the turgid weight (TM), leaves were rehydrated in closed containers with distilled



- water for 24 hours at 22°C. After rehydration, the leaves were weighed again. Finally, dry weight
- 161 (DM) was obtained by oven drying at 80°C for 48 hours. All weights were measured with a
- balance accurate to 0.001 g. The relative water content was calculated as follows:
- 163 RWC (%) =  $[(FM-DM)(TM-DM)] \times 100$ .

- 165 2.3.7. Antioxidant enzyme activity
- 166 To extract polyphenol oxidase (POX), peroxidase (PPO), and catalase CAT, fresh leaves (0.5 g)
- of Osteen mango cultivar were mashed in a mortar with 5 mL of 0.1 M cold phosphate buffer (pH
- 168 7.1), and the mixture was centrifuged at 15,000xg for 20 minutes at 4 °C. The supernatant was
- used in an enzyme activity experiment (Esfandiari et al., 2007).
- 170 Peroxidase activity (POX) was measured using an approach that was based on (Amako et al.,
- 171 1994). Polyphenol oxidase (PPO) activity was measured in compliance with (Kavrayan &
- 172 Aydemir, 2001). The activity of the catalase (CAT) enzyme was measured in compliance with
- 173 (Aebi, 1984).

174

- 175 Total phenolic and total flavonoid
- 176 According to (Singleton et al., 1999), the total phenolic content of Osteen mango tree leaves was
- measured using the Folin–Ciocalteu colorimetric method and represented as (mg/g) using gallic
- acid as a standard. According to (Escriche & Juan-Borrás, 2018), the total flavonoid
- 179 concentration in fresh leaves of the Osteen mango cultivar was measured using the aluminum
- 180 chloride (AlCl<sub>3</sub>) colorimetric method, with Rutin serving as a standard and being expressed as
- 181 mg/g.
- 182 2.8. Tree Yield
- We harvested the fruit from each experimental tree when the flesh turned yellow and the
- shoulders rounded or flattened according to (Ahmed et al., 2023) Moreover, on the 15th of
- August in both seasons of the 'Osteen' mango cultivar according to (Khattab et al., 2021).
- According to Elshahawy et al., (2022), the Osteen mango cultivar exhibits regular fruiting
- 187 behavior.
- 188 Then, the average yield was calculating in terms of number of fruits/tree and weight (kg). The
- vield increasing percentage was computed using the equation of (Abd El-Naby, Mohamed & El-
- 190 Naggar, 2019).

191

192 Fruit Yield increment (%) = 
$$\frac{[Yield (treatment) - Yield (control) \times 100}{Yield (control)}$$

193

- 194 To assess the percentage of sunburned fruit, we counted the number of sunburned fruits on each
- tree at harvest. Then calculated the sunburn rate as a percentage using a formula developed by
- 196 (Mohsen & Ibrahim, 2021) equation as follows:
- 197 Sunburned fruits =  $\frac{No.of sunburned fruit/tree}{Total No. of fruits/tree} \times 100$

198

199 2.9. Fruits Physical properties.



- Fruit weight (g) was calculated at harvest time using a sample of five Osteen cv. mangoes. The
- samples were then chosen and transferred to the horticulture lab.
- 202 2.10. Chemical characteristics of fruits.
- 203 Mango juice Extraction
- 204 10 g of mango pulp was weighed and added to a 100 mL beaker. The pulp was then
- 205 homogenized with distilled water using a blender to achieve a uniform mixture. The
- 206 homogenized mixture was subsequently filtered to remove any particulates. The filtrate was then
- 207 quantitatively transferred to a 100 mL volumetric flask. Distilled water was further added to the
- 208 flask to bring the final volume to the mark.

- 210 Fruit total soluble solids (TSS) of Osteen mango pulp was determined in two to three drops of
- each sample juice by using a digital refractometer (force-Gouge ModelIGV-O.SA to FGV-100A.
- 212 Shimpo instruments) and expressed as °Brix according to (McKie & McCleary, 2016)

213

- 214 Total acidity percentage
- 215 A 10 mL aliquot of the pulp solution was measured into a conical flask. Two to three drops of
- 216 phenolphthalein indicator were added, and the flask was then swirled vigorously to ensure
- 217 thorough mixing. The solution was immediately titrated with 0.1 N NaOH solution from a
- 218 burette until a permanent pink endpoint was reached. The volume of NaOH solution consumed
- 219 was recorded, and the percent titratable acidity was expressed as citric acid (%) according to the
- 220 method of (McKie & McCleary, 2016).

221

- Total soluble solids/acid ratio was calculated from the values of total soluble solids divided by
- values of total acids. (McKie & McCleary, 2016).

224

- 225 The ascorbic acid content (vitamin C) of fruit juice was determined in triplicate (n=3) for each
- treatment, with a sample volume of 2.0 ml per replicate. The analysis followed the method
- described by McKie & McCleary (2016), which is based on the oxidation of ascorbic acid with
- 228 2,6-dichlorophenolindophenol dye. The results are expressed as (mg/100 ml) of juice and
- 229 represent the average value for each treatment.

230

- 231 Statistical Analysis
- The design of the present study was a randomized complete block design (RCBD). The
- 233 information used to create the charts comes from three separate times when each treatment (9)
- 234 trees/treatments was applied. The analysis of variance as one-way ANOVA was used through
- 235 Costat software (Ridgman, 1990), and means of different treatments were compared using the
- 236 Duncan test ( $p \le 0.05$ ).

237

#### 238 **Results**

239 2.1. Effect of (LRE) and (PS) on leaf area of 'Osteen' mango under heat stress:



- 240 The results in Fig. 1 showed that spraying with licorice root extract and potassium sorbate in
- mid- May significantly increased the leaf area of 'Osteen' mango trees in both studied seasons in 241
- comparison to control. Additionally, a trend emerged where leaf area increased as the 242
- concentration of licorice root extract and potassium sorbate increased. Leaf area promotion was 243
- 244 most dependent on the application of licorice root extract at 6 g/L, followed by 3 mM potassium
- sorbate. Untreated trees consistently had the lowest leaf area values. This trend was observed in 245
- both growing seasons. 246

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- 248 2.2. Effect of (LRE) and (PS) on fruit physical properties:
- The results in Figures (2a, and b) clearly showed that adding licorice root and potassium sorbate 249
- extract at various phenological stages significantly increased fruit weight (g), and No. of 250
- fruits/tree of Osteen mango trees in comparison to those of control in the two studied seasons. 251
- 252 These results are in line with findings of (Baiea et al., 2015) who found that spraying Hindi
- 253 mango trees four times with different types of potassium were very effective in improving
- 254 number of fruits or weight (kg/tree) comparing with the control. Fruit weight (g), and No. of
- fruits/tree promotion were most dependent on the application of licorice root extract at 6 g/L. 255
- followed by 3 mM potassium sorbate. Trees treated with 6 g/L licorice root extract exhibited the 256
- 257 maximum fruit weight (g), and No. of fruits/tree, followed by those treated with 3 mM potassium
- sorbate. Additionally, a trend emerged where fruit weight (g), and No. of fruits/tree increased as 258
- the concentration of potassium sorbate and licorice root extract increased. 259
- 261

2.3. Effect of (LRE) and (PS) on fruit yield.tree-1:

- The results illustrated in Figure (3A and B) revealed notable enhancements in fruit yield (kg/tree) 263
- and yield increasing (%) when 'Osteen' mango trees were subjected to the addition of licorice 264
- root extract and potassium sorbate as foliar spray at different phenological stages. These 265
- 266 improvements were observed across various growth stages and were found to be significantly
- higher compared to the control group in both seasons under investigation. Specifically, the 267
- application of 3mM potassium sorbate or 6.g-1 licorice root extract resulted in a more pronounced 268
- increase in fruit yield (kg/tree) compared to other treatments or the control group. Licorice root 269
- 270 extract and potassium sorbate foliar spray caused a significant increase in yield percentage by
- 305.77%, and 232.44%, in the first season, and 242.55%, 232.44% in the second season, 271
- 272 respectively.

- 274 2.4. Effect of (LRE) and (PS) on No. of sun-burned fruits:
- Figure 4 represents the response of the number of sun-burned fruits of 'Osteen' mango trees to 275
- licorice root extract and potassium sorbate foliar spray. Both potassium sorbate and licorice root 276
- extract foliar spray significantly reduced the number of sun-burned fruits of 'Osteen' mango 277
- 278 trees in comparison to the control group in the two studied seasons. The lowest number of sun-
- 279 burned fruits of Osteen mango trees were possessed from LER6 (6g/l licorice root extract) and or



280 PS3 (3mM PS). Meanwhile, the control group had the highest number of sun-burned fruits of Osteen mango trees in both seasons. Additionally, a trend was observed: as the level of 281 potassium sorbate and licorice root extract increased, the number of sun-burned fruits of Osteen 282 283 mango trees decreased. 284 285 286 2.5. Effect of (LRE) and (PS) on some fruit chemical characteristics: 287 288 Foliar application of both (LRE) and (PS) significantly increased TSS (Brix°), TSS/acid ratio,

and Vitamin C in comparison to the control group, as shown in figures 5a, 5c, and 5d. 289 Conversely, total acidity% in 'Osteen' mango fruits significantly decreased in response to the 290

foliar application during both study seasons (Fig. 5b). Concerning fruit chemical characteristics. 291

292 the data revealed that higher levels of (LRE) and (PS) applied as foliar sprays were superior to

293 the control group and to treatments with lower spraying concentrations. Among all treatments,

294 3mM potassium sorbate (PS3) and 6g/l licorice root extract (LER6) resulted in fruits with the

highest TSS%, TSS/acid ratio, and Vitamin C content, compared to the control group and other 295

treatments. On the other hand, 'Osteen' mango fruits from treatments T3 and T6 showed the 296

297 lowest values of total acidity compared with fruits from other foliar spray treatments or the untreated group. 298

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300 301

2.6 The photosynthetic Pigments Leaf of 'Osteen' mango cv. Under heat stress

It is clear from Figure 6 that the photosynthetic pigments of leaf Osteen mango CV were significantly influenced by foliar spray with licorice root extract (LRE) and (PS) during the 2022

and 2023 seasons under heat stress (sunburn). The application of different LRE and PTS 304

concentrations on mango tree leaves resulted in an increase in photosynthetic pigments under 305

306 heat stress. As shown in Figure 6, increasing the LRE concentrations from 2-6 g/L and (PS)

from 1-3 mM led to an increase in the values of chlorophylls (a and b) and total chlorophyll, 307

carotenoids, and total pigments. With a LRE concentration of 6 g/L (LER6), the highest values 308

for chlorophyll a (1.389), chlorophyll b (0.899), total chlorophyll (2.267), carotenoids (0.433), 309

310 and total pigments (2.689 mg/g fresh weight) were obtained compared to the control, which had

the lowest values for these characteristics under heat stress during both seasons. Additionally, 311

312 spraying (PS) at 3 mM (PS3) had the greatest impact on the photosynthetic pigment contents

compared to other PTS concentrations and the control treatment. The value for total pigments 313

314 (2.219 mg/g fresh weight) was higher than that of the control (non-treated) (1.111 mg/g fresh

weight) under heat stress during the 2022-2023 seasons (Figure 6E). 315 316

317 318

2.7 Antioxidant activity leaf of Osteen mango cv. under heat stress



319 Polyphenol oxidase (PPO), peroxidase (POX), and catalase (CAT) activities were significantly influenced by the foliar spray of (LRE) and (PS) on Osteen mango leaves under heat stress 320 (sunburn) during the 2022 and 2023 seasons (Figure 7). Based on the results, the highest 321 activities of PPO (6.76 U/g F.Wt), POX (15.66 U/g F.Wt), and CAT (24.81 U/g F.Wt) enzymes 322 323 were found under control conditions (non-treated). According to Figure 7 A-C, the enzyme activities decreased with increasing concentrations of LRE and PTS sprayed on mango leaves. 324 The lowest activities of PPO (2.75 U/g F.Wt) and POX (5.39 U/g F.Wt) enzymes were observed 325 when spraying Osteen leaves with 6 g/l of LRE (T3), while the lowest activity of CAT (8.35 U/g 326 F.Wt) was recorded at 3mM of (PS) (PS3). Agricultural production is adversely affected by 327 328 global warming and the anticipated rise in temperatures (Challinor et al., 2014; Fahad et al., 329 2017). 330 331 2.7 Total flavonoids, total phenolic and proline of Leaf Osteen mango cv. Under heat stress 332 The content of total flavonoid in leaves of Osteen mango is shown in (figure 8A). The highest 333 (66.90 mg/g F.W) and lowest (24.03 mg/g F.W) values were observed with control (heat stress non-treated) and T3 6 g/l LRE, respectively in 2022 and 2023 seasons. Total flavonoid content 334 decreased by 26%, 32.66%, and 63% at 2, 4, and 6 g/L LRE, respectively, and by 2.46%, 32.2%, 335 336 and 48.28% at 1, 2, and 3 mM PS, respectively. Figure 8B illustrates data about the total phenolic content. The highest amounts of total phenolic (50.67 mg/g F.W) were found in leaves 337 under control and the lowest amount (15.46 mg/g F.W) was treated with T3 6 g/l LRE. Total 338 phenolic decreased by 69.43% and 59.57% at T3 6 g/l LRE and PS3 3 mM PS, respectively. 339 Within the kingdom of plants, flavonoids and phenolics are the most extensively dispersed 340 341 secondary metabolites. These compounds play a vital role in plant growth and defense mechanisms. The results for proline variation in leaves of treated and non-treated Osteen mango 342 trees under heat stress are presented in Figures 8C. Osteen mango leaves exposed to high 343 temperatures showed higher proline content. The highest accumulation of proline (0.280 mg/100 344 345 g F.W) belonged to control leaves. According to (Bernardo et al., 2018), raising temperatures to extremely high levels led to excessive proline production in grapevine. The trees sprayed with 346 (T3) 6 g/l LRE showed lower proline content (0.026 mg/100g F.W) compared to control and 347

348 349

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#### Discussion

other treatments.

Heat stress causes many disorders in mango growth, which led to a reduction in leaf area.

Spraying with (LER) and (PS) in our study showed a mitigating effect on heat stress. These results are consistent with the findings in numerous studies support the use of foliar potassium to enhance plant growth. (Abd El-Rahman, 2021) observed this when added potassium silicate to 'Sedika' mango trees, (Ayed et al., 2022) with 'Keitt' mango trees, and (EL-Gioushy, 2021) with orange trees. All these studies found that foliar application of potassium significantly improved vegetative growth compared to the control group. The leaf area increasing might be due to



358 potassium spray (Rouphael & Colla, 2018). Potassium plays a vital role by activating enzymes 359 for organic substance synthesis, promoting photosynthesis, and transporting carbohydrate assimilates to storage organs (Fallovo et al., 2008). It's also involved in several basic 360 361 physiological functions. Similarly licorice root extracts improving the vegetative growth of plants (Nasir et al., 2016). The increase in fruit weight of 'Osteen' mango trees could be 362 attributed to the increase in leaf area caused by potassium sorbate and licorice root extract 363 application and subsequently stimulation of photosynthesis intensity shared in increasing the 364 365 fruit weight. Similarly, the study focused on licorice root extract as a potential vegetarian alternative to synthetic growth regulators. This extract is gaining interest due to its reported 366 367 ability to improve plant growth and production in practical applications. Licorice root contains 368 glycyrrhizin, a compound composed of calcium and potassium salts of glycyrrhizic acid, a trihydroxy acid (Rady et al., 2019). Moreover, it contained a wide range of elements and 369 nutrients (Hamam et al., 2021). As a matter of fact, adding a lot of potassium sorbate as foliar 370 371 spray at different fruit growth stage may be attributed to the physiological role of potassium which is needed for many biochemical processes. Potassium also regulates various physiological 372 373 processes like water movement, respiration, and nutrient translocation within the plant. 374 Additionally, it activates enzymes like nitrate reductase and starch synthetase, which contribute 375 to a healthy balance of protein and carbohydrate production(Iqbal et al., 2022).

376 Despite being one of the important activities in tropical regions, mango production has been largely ignored in systematic impact analyses of climate change (Nath et al., 2019). Heat stress 377 378 can reduce fruit yield in mango tree cultivars, leading to a decline in overall tree productivity. This study aligns with previous findings by (El-Merghany, et al., 2019) who reported that 379 380 licorice root extract application to 'Ferehy' date Palm trees significantly increased fruit weight and yield per tree. Similarly, (Ahmed et al., 2023) found that adding 4-8 g/L of the extract to red 381 globe grapevines in April to June increased yield per tree compared to the control group. 382 Similarly, in a study by Yassin, et al. (2023), applying potassium silicate at concentrations of 383 100, and 200 ppm increased the number of fruits and fruit yield per tree of Wonderful and H116 384 385 pomegranate compared to the control group. Additionally, (Silem, Ismail & Mohamed, 2023) 386 reported that applying potassium silicate at a concentration of 500 ppm to mango trees at the beginning of growth, after fruit setting, and one month later increased fruit yield per tree 387 compared to the control group. The increasing in yield /tree of 'Osteen' mango trees might be 388 389 due to licorice root extract has been found to be an amazing biostimulant that increases not only growth but also the yields of various crops (Alshallash et al., 2022). According to (Diab & Abd 390 El-hmied, 2022) they found that foliar spraying the 'Kitte' cv. mango with licorice root extract at 391 392 5 or 10 g/l concentration on three occasions, at the beginning of growth, after fruit set, and three 393 weeks after fruit set, significantly increased tree yield in comparison to the control group. 394 It could be concluded that Application of (LRE) and (PS) to Osteen mango trees at different 395 growth stages significantly enhanced fruit weight, number of fruits per tree, yield (kg/tree), and 396 yield increase percentage, compared to the control. The most effective treatments were 6g/L 397 (LRE) and 3mM (PS). 398 Our results align with the findings of (Ahmed et al., 2023), who reported that foliar spraying the

'Red Glob' cv. grapevine with licorice root extract at a concentration of 4 or 8 g/L, applied three



- 400 times in mid-April. May, and June, significantly reduced the number of sun-burned fruits. The
- presence of various beneficial compounds in licorice root extract, including phenolics, 401
- triterpenes, saponins, amino acids, polysaccharides, vitamins, and growth-promoting 402
- phytohormones, likely contributes to enhanced vegetative growth. These compounds may 403
- 404 stimulate activity in the apical meristem, the plant's growth center, by promoting cell division
- and elongation(Pandey, 2017). The reduction in No. of sun-burned fruits might be attributed to 405
- the enhanced vegetative growth in mango trees caused by potassium application (Baiea, et al., 406
- 2015). Additionally, potassium may influence a tree's canopy size, which could in turn help the 407
- tree withstand environmental challenges like drought and high radiation. (Hamdy et al., 2022; 408
- 409 Alharbi et al., 2022). It could be concluded that Application of potassium sorbate and licorice
- root extract to Osteen mango trees significantly reduced No. of sun-burned fruits, compared to 410
- the control. The most effective treatments were 6g/L licorice root extract and 3mM potassium 411
- 412 sorbate.
- 413 The mango fruit quality results in this study were consistent with those reported by (Baiea, El-
- Sharony & El-Moneim, 2015) found that spraying Hindi mango trees four times with Potassium 414
- were very effective in improving enhanced fruit quality. In addition, fruit total acidity was 415
- reduced compared with the control. Likewise, (El-Morsy, et al. 2017) on Red Globe where the 416
- highest values of fruit chemical characteristics were obtained with addition of licorice extract 417
- foliar spraying treatments 20 and 15 g/l. Similarly, (Obenland et al., 2015) found that potassium 418
- sorbate foliar spray at concentration 1.3g/l significantly increased fruit Flame seedless SSC in 419
- comparison to control. Potassium treatments may be responsible for the enhanced chemical 420
- 421 characteristics of 'Osteen' mango fruits. This is likely because potassium acts as a catalyst for
- 422 numerous biological processes within the trees, leading to improved overall tree health and
- nutrient status (Amtmann et al., 2005). 423
- 424 Licorice extract application may be responsible for the observed improvements in physical and
- chemical fruit quality, as well as increased yield. This effect could be attributed to the presence 425
- 426 of mevalonic acid in the extract. Mevalonic acid acts as a precursor to gibberellin, a plant
- hormone that stimulates leaf cell expansion. These results in increased leaf area and chlorophyll 427
- content, ultimately leading to improved fruit set and yield (Petoumenou & Patris, 2021). We can 428
- conclude that application of (LRE) and (PS) to Osteen mango trees significantly enhanced fruit 429
- 430 TSS%, TSS/acid ratio, and Vitamin C content compared to the control. Meanwhile, total acidity
- percentage in 'Osteen' mango fruits significantly decreased in response to the foliar spray. The 431
- most effective treatments were 3mM (PS) and 6g/L (LRE). 432
- High temperatures or severe heat waves are among the abiotic stresses that inhibit mango 433
- growth, development, and crop quality(Muthuramalingam et al., 2023). A water shortage, often 434
- caused by heat and intense light, harms plants' ability to photosynthesize by inducing stomatal 435
- closure, mesophyll compactness, and photoinhibition. Mango leaf photosynthesis, transpiration, 436
- and water potential can all be impaired when exposed to high temperatures and low air relative 437
- 438 humidity (Faria et al., 2016; Khanum et al., 2020).



439 Spraying LRE led to a reduction in sunburn and improved the photosynthesis system because it behaves similarly to gibberellin, which promotes vegetative growth. This extract contains 440 nutritive components including N, Mg, Zn, Cu, and Fe. Since nitrogen aids in the creation of 441 chlorophyll, these minerals play a significant role in the process of chlorophyll synthesis. 442 443 Furthermore, iron contributes to the crucial steps that lead to chlorophyll production by increasing the number and size of chloroplasts and grana(Venzhik, Shchyogolev & Dykman, 444 2019). Spraying mango leaves with (PS) at different concentrations enhances chlorophyll content 445 and plant pigments because potassium is a major nutrient that directly participates in the vital 446 functions of plants, most notably regulating the photosynthesis process and osmotic regulation of 447 448 stomatal activity and transpiration. It also plays a crucial role in plant survival under various stresses (Araujo et al., 2015; Tränkner, Tavakol & Jákli, 2018). (Alsalhy & Aljabary, 2020) 449 discovered that grape cv. Halawany leaf chlorophyll content increased when LRE was applied as 450 a spray at a level of 2.5 g/l. (Silem et al., 2023) reported that the application of potassium silicate 451 452 as a spray to Keitte mango tree leaves at 500 ppm elevated total chlorophyll contents in leaves during the 2021 and 2022 seasons under salinity stress compared to the control group. These 453 findings align with those of .(Younes et al., 2021) and (Abdel-Mola et al., 2022). 454 We can conclude that application of (LRE) and (PS) to Osteen mango trees significantly 455 increased leaf Chl A, Chl B, total chlorophyll content, carotenoids and total pigments under heat 456 stress compared to the control. The most effective treatments were 3mM (PS) and 6g/L (LRE). 457 Elevated temperatures can cause harm to plant cells in various ways, including interfering with 458 protein synthesis and function, enzyme deactivation, and membrane rupture. These processes 459 affect physiological functions such as respiration and photosynthesis. One common issue is an 460 excess of harmful substances, like reactive oxygen species (ROS), leading to oxidative stress 461 (Hasanuzzaman et al., 2013). In response to stressful conditions, plants exhibit an internal 462 defensive mechanism through ROS scavenging, which is demonstrated by the activities of 463 enzymatic antioxidants such as superoxide dismutase (SOD), peroxidase (POX), and catalase 464 465 (CAT) (You & Chan, 2015; Rossi et al., 2017). Under heat stress (sunburn), PPO, POX, and CAT enzyme activities increased with increasing 466 ROS levels(You & Chan, 2015). Our study's results showed that all treatments decreased the 467 activities of antioxidant enzymes PPO, POX, and CAT (Figure 7A-C). This suggests that Osteen 468 469 mango trees treated with LRE and PTS are more stable, producing fewer ROS and, therefore, requiring less of these enzymes. In contrast, leaves of untreated mango trees are exposed to heat 470 stress, which increases ROS emission and necessitates the upregulation of antioxidant enzyme 471 activities to maintain cellular balance. Azab et al. (2013) discovered that foliar application of 472 potassium reduced SOD, CAT, and PPO activities in well-watered Squash plants(Azab et al., 473 2022). However, under drought stress, tomato plants responded more favorably to potassium 474 application in terms of antioxidant enzyme activity than to a well-watered treatment. Rady et 475 al.(Rady et al., 2019) found that licorice root extract (LRE) application increased the activity of 476 antioxidant enzymes (POX, CAT) and decreased hydrogen peroxide (H2O2) and superoxide 477 478 radical (O2•–) levels in common bean plants under salt stress when compared to the control



479 without LRE. (Hamdy et al., 2022) found that leaf Keitt mango content of antioxidants, such as CAT, POX, and PPO enzyme activities, decreased under solar radiation (sunburn) following 480 kaolin application. These findings are consistent with (Cabo et al., 2020). It could be concluded 481 that Enzyme activities of PPO, POX, and CAT all declined with increasing concentrations of 482 483 LRE and PTS sprayed on mango leaves. The lowest activities of PPO (2.75 U/g F.Wt) and POX (5.39 U/g F.Wt) were observed at the highest concentration of LRE (6 g/L, T3). Meanwhile, the 484 lowest CAT activity (8.35 U/g F.Wt) was recorded at the highest concentration of (PS) (3 mM, 485 486 According to (Tohidi, et al., 2017), these substances have a wide range of biochemical and 487 molecular functions in plants, including those of signaling molecules, plant defense, regulating 488 auxin transport, antioxidant activity, and free radical scavenging. Phenols and flavonoids, which 489 are non-enzymatic antioxidants, accumulate in different tissues and scavenge free radicals, 490 helping plants tolerate salt stress (Sirin & Aslım, 2019). According to our findings, control (heat 491 492 stress non-treated) significantly increased the amount of total flavonoids and total phenolic (figure 8A-B). One of the defense strategies employed by plants against oxidative stress is the 493 rise of antioxidants, such as total phenolics and flavonoids, under high temperatures (Wen et al., 494 2008). From these results, we suggest that the increase in phenolic compounds may be due to the 495 activity of phenylalanine ammonia-lyase under high temperatures (Gebauer, Strain & Reynolds, 496 1997). However, spraying the leaves with LRE and PTS at all concentrations caused all the 497 values of flavonoids and phenolics to decrease, likely due to a reduction in the harmful effects of 498 high temperature on the leaves, which lessened the need for phenylalanine to be directed towards 499 synthesizing these compounds. According to Rady et al., 2019), common bean plants 500 grown under salt stress can benefit from the application of LRE as a natural biostimulant that can 501 effectively boost their salt tolerance. Our outcomes were in agreement with those attained by 502 (Dinis et al., 2018; Younes et al., 2021). Plant cells exposed to any stress exhibit proline 503 accumulation, which is an indicator of the damage (Per et al., 2017; Dinis et al., 2018). Plant 504 505 antioxidant systems contain a variety of low-molecular-weight substances, including proline and ascorbic acid (Rady et al., 2019). All treatments with LRE and PTS resulted in reduced proline 506 levels compared to control. This decrease is likely due to LRE and PTS mitigating the negative 507 effects of heat stress on Osteen mango leaves through improved physiological performance. 508 509 Potassium is essential for physiological processes in plants, which helps them endure stressful environments (Wang et al., 2013). The findings suggested that spraying LRE and PTS reduced 510 the oxidative damage to cell membranes, as seen by the decrease in proline. These results are in 511 agreement with (Cabo et al., 2020), (Dbara, et al., 2022), and (Shehzad et al., 2020), who found 512 that adequate external supply of potassium led to a significant reduction in the activities of 513 antioxidant enzymes and proline in drought-stressed plants. (Younes et al., 2021) found that 514 applying LRE as a biostimulant may be useful to improve bulb quality and, eventually, the 515 productivity of onion cultivars in field conditions. 516 It could be concluded that Flavonoid content significantly decreased following treatments. 517 518 Levels dropped by 26%, 32.66%, and 63% at increasingly higher concentrations (2, 4, and 6 g/L)



of (LRE), respectively. Similarly, (PS) treatments resulted in a decrease of 2.46%, 32.2%, and 519 48.28% at concentrations of 1, 2, and 3 mM, respectively. Total phenolic content also showed a 520 substantial reduction. The greatest decline (69.43%) was observed in leaves treated with 6 g/L 521 LRE (T3), while the highest concentration of PTS (3 mM, T6) caused a 59.57% decrease. 522 523 Proline accumulation was highest in untreated leaves (control). Conversely, proline levels were significantly lower in leaves treated with 6 g/L LRE (T3), reaching only 0.026 mg/100g fresh 524 weight (FW) compared to the control value of 0.280 mg/100g FW. This suggests that this 525 concentration of LRE may inhibit proline production. 526 These results are consistent with the findings in numerous studies support the use of foliar 527 528 potassium to enhance plant growth. (Abd El-Rahman, 2021) observed this when added potassium silicate to 'Sedika' mango trees, (Ayed et al., 2022) with 'Keitt' mango trees, and (EL-529 Gioushy, 2021) with orange trees. All these studies found that foliar application of potassium 530 significantly improved vegetative growth compared to the control group. The leaf area increasing 531 532 might be due to potassium spray (Rouphael & Colla, 2018). Potassium plays a vital role by activating enzymes for organic substance synthesis, promoting photosynthesis, and transporting 533 carbohydrate assimilates to storage organs (Fallovo et al., 2008). It's also involved in several 534 basic physiological functions. Similarly licorice root extracts improving the vegetative growth of 535 536 plants (Nasir et al., 2016).

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### **Conclusions**

539 This study demonstrated that foliar application of (LRE) and (PS) at specific concentrations (3) 540 mM potassium sorbate and 6 g/L licorice root extract) significantly improved fruit yield, quality, and antioxidant enzyme activity in Osteen mango trees compared to the control. These 541 treatments increased fruit weight, number of fruits, yield per tree, soluble solids content (TSS), 542 543 TSS/acid ratio, and vitamin C content, while reducing total acidity and fruit enzyme activities (PPO, POX, and CAT). However, both licorice root extract and potassium sorbate caused a 544 decrease in flavonoid and phenolic content, with the highest concentration (6 g/L LRE and 3 mM 545 PTS) showing the most significant reductions. Additionally, proline accumulation was inhibited 546 by the highest concentration of licorice root extract (6 g/L LRE). These findings suggest that 547 548 licorice root extract and potassium sorbate can be effective growth promoters for Osteen mango 549 trees, but their influence on certain fruit quality parameters like fruit weight and yield should be considered. 550

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## Table 1(on next page)

Table 1

Chemical analysis of licorice root extract (LRE) according to Rady et al., desoky, et. al., and Younes et al. [35–37]



1 Table 1. Chemical analysis of licorice root extract (LRE) according to Rady et al., desoky, et. al.,

### 2 and Younes et al. [35–37]

Constituents	Value
Na+ (mg 100mg-1 dr.wt.)	101.54
K+ (mg 100mg-1 dr.wt.)	376.96
Ca2+ (mg 100mg-1 dr.wt.)	754.90
Mg2+ (mg 100mg-1 dr.wt.)	524.78
Fe+ (mg 100mg-1 dr.wt.)	32.88
Zn2+ (mg 100mg-1 dr.wt.)	0.91
Total phenols (mg of GAE g-1 dr.wt.)	4.38
Total flavonoids (mg of QE g-1 dr.wt.)	2.14
Total antioxidant capacity(mg of AAE g-1	72.99
dr.wt.)	
Tannins (mg 100 g-1 dr.wt.)	33.98
Saponins (mg 100 g-1 dr.wt.)	21.00
Ascorbic acids (mg 100 g-1 dr.wt.)	2.77
Ferric reducing antioxidant power (mg 100-1	43.90
Dr.wt.)	

Aae, L-ascorbic acid equivalent; dr.wt, dry weight; GAE, gallic acid equivalent; QE, quercetin equivalent; mg, milligram.



## Table 2(on next page)

Weather data of El Salheya El Gedida district, El-Sharqia Governorate, Egypt (means 2022 and 2023)



- 2 Table 2: Weather data of El Salheya El Gedida district, El-Sharqia Governorate, Egypt
- 3 (means 2022 and 2023)

Months	T2M	TMIN	TMAX	RH2M	RAIN	SRAD
January	12.11	6.68	19.42	51.78	2.20	13.08
February	13.64	7.56	21.63	57.93	5.60	15.29
March	15.81	9.19	23.99	57.68	11.00	19.55
April	19.69	12.34	28.40	51.01	2.40	23.47
May	26.35	17.31	36.70	39.58	0.10	27.32
June	29.57	21.53	38.58	44.71	0.00	27.62
July	30.65	22.43	39.82	44.28	0.00	28.26
August	30.71	22.88	39.60	45.50	0.00	26.21
September	27.69	20.80	36.26	53.63	0.00	22.95
October	24.72	18.75	32.49	61.41	19.40	17.80
November	20.72	15.16	28.72	58.28	0.00	14.73
December	14.91	10.08	21.60	63.37	12.80	12.05
Average and Sum	22.21	15.39	30.60	52.43	53.50	20.70

4

### Interpretation

T2M Temperature Average at 2 Meters (°C)
TMIN Temperature at 2 Meters Minimum (°C)
TMAX Temperature at 2 Meters Maximum (°C)
RH2M Relative Humidity Average at 2 Meters (%)

Precipitation

RAIN (mm)

SRAD Solar Radiation (MJ/m^2/day)



Table 3(on next page)

Foliar sprays on mango trees



### 1 Table 3. Foliar sprays on mango trees:

Treatments	Foliar spray material	Concentrations
Control	Water spray	0.0
LRE2	Licorice root extract	2g/l
LRE4	Licorice root extract	4g/l
LRE6	Licorice root extract	6g/l
PS1	potassium sorbate	1mM
PS2	potassium sorbate	2mM
PS3	potassium sorbate	3mM

2

3

Figure 1. Effect of licorice-root extract and Potassium sorbate spray on leaf area of the 'Osteen' mango cultivar in 2022 and 2023 seasons. Control: water spray, LRE2, LRE4, LRE6,: 2, 4 and 6g/l Licorice root extract, PS1, PS2 and PS3.: 1, 2 and 3mM potas

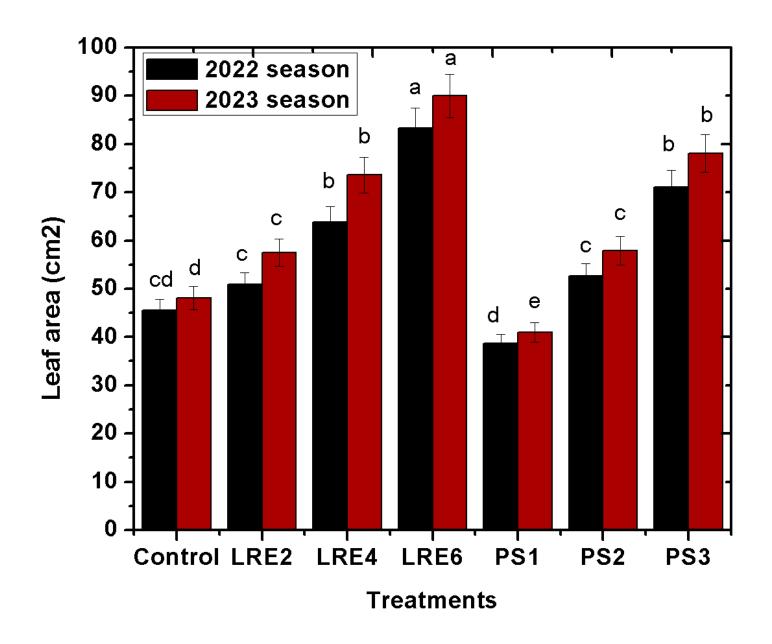


Figure 2. Effect of licorice-root extract and Potassium sorbate spray on fruit weight and No. of fruit/tree of the 'Osteen' mango cultivar in 2022 and 2023 seasons. Control: water spray, LRE2, LRE4, LRE6,: 2, 4 and 6g/l Licorice root extract, PS1, PS2 and

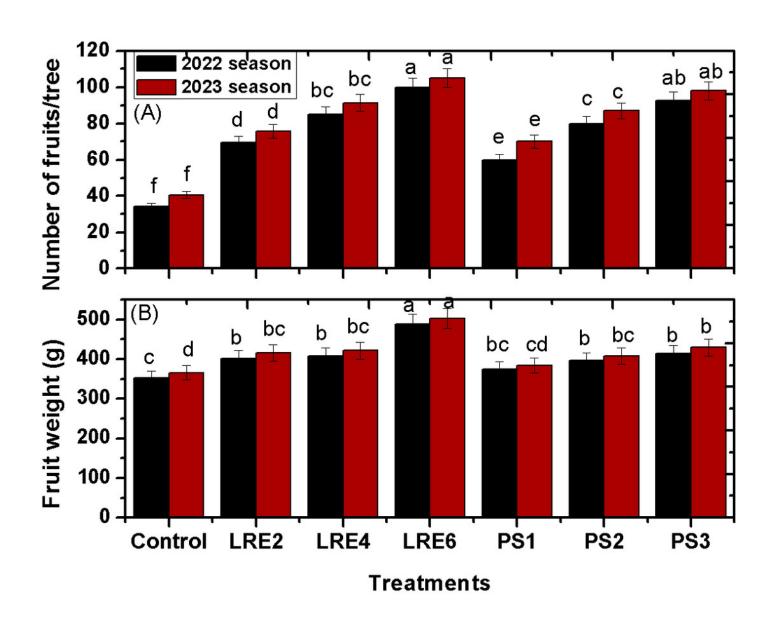


Figure 3. Effect of licorice-root extract and Potassium sorbate spray on yield /tree and yield increasing (%) of the 'Osteen' mango cultivar in 2022 and 2023 seasons. A): yield component/tree (kg) and B): yield increasing %. Control: water spray, LRE2, LR

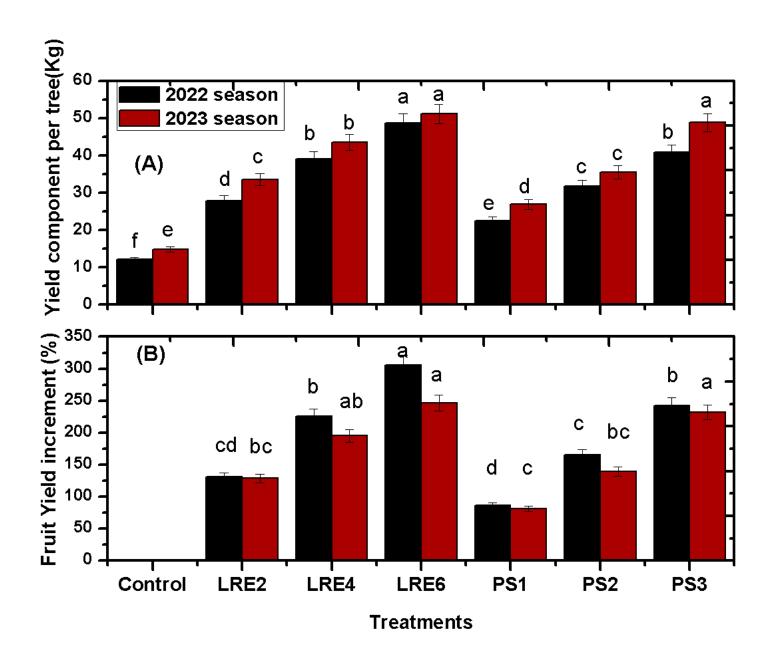


Figure 4. Effect of licorice-root extract and Potassium sorbate spray on No. of sunburned fruits of the 'Osteen' mango cultivar in 2022 and 2023 seasons. Control: water spray, LRE2, LRE4, LRE6,: 2, 4 and 6g/l Licorice root extract, PS1, PS2 and PS3.: 1,

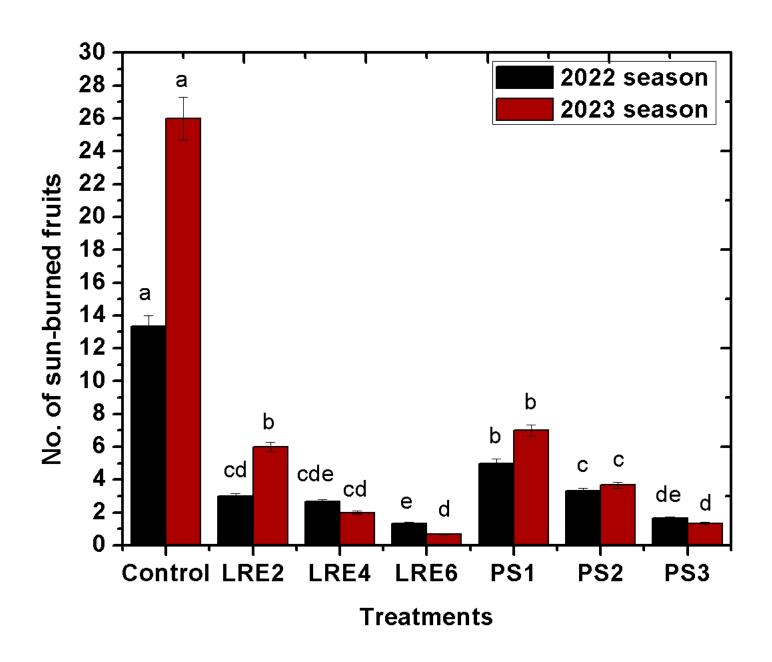




Figure 5. Effect of licorice-root extract and Potassium sorbate spray on some of fruit chemical characteristics of the 'Osteen' mango cultivar in 2022 and 2023 seasons. A): TSS Brix°, B): Total acidity%, C):TSS/acid ratio and Vitamine C. Control: water sp

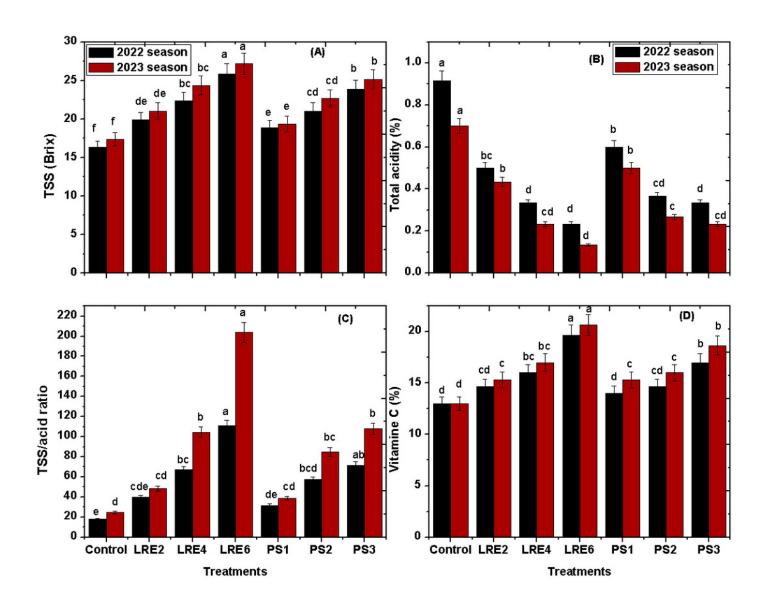




Figure 6. Effect of licorice-root extract and Potassium sorbate on the photosynthetic Pigments Leaf of the 'Osteen' mango cv. under heat stress in 2022 and 2023 seasons. Control: water spray, LRE2, LRE4, LRE6,: 2, 4 and 6g/l Licorice root extract, PS1, PS

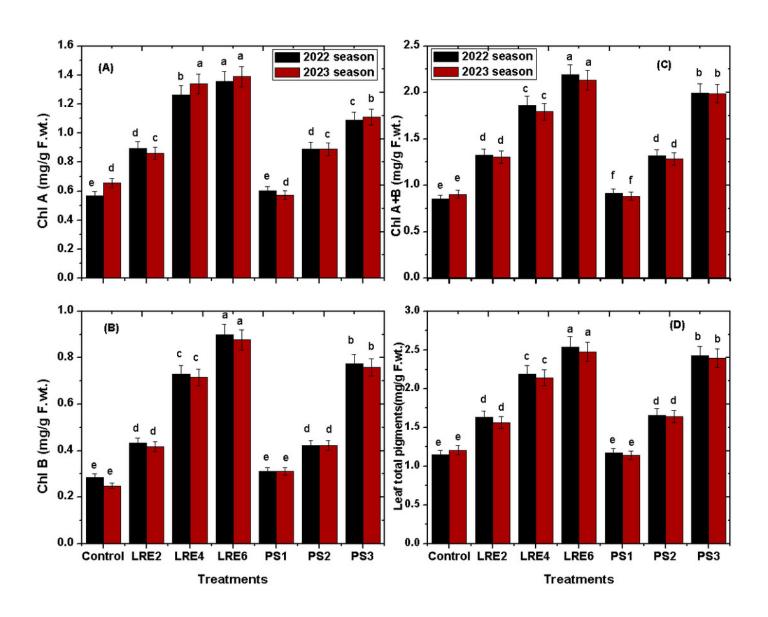




Figure 7. Effect of licorice-root extract and Potassium sorbate on carotenoids, catalase activity, peroxidase activity and polyphenol oxidase activity of leaves osteen mango cv. under heat stress in 2022 and 2023 seasons. Control: water spray, LRE2, LRE4,

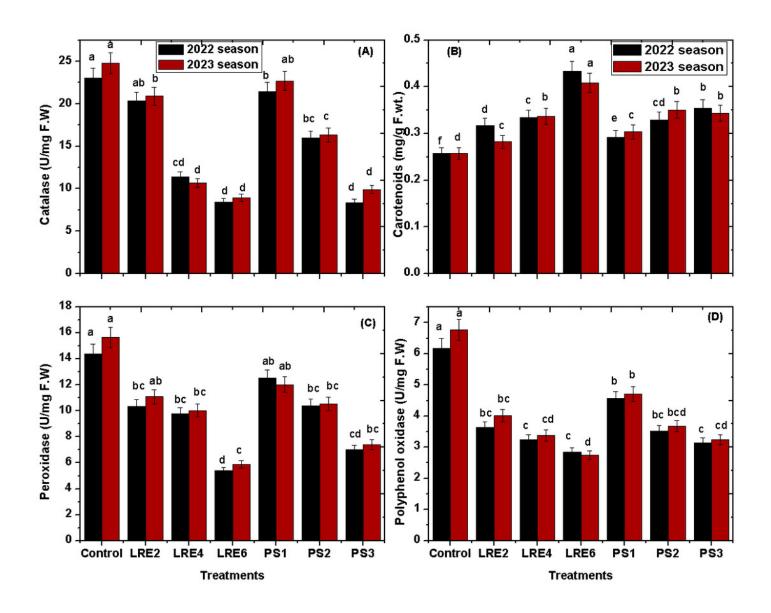




Figure 8. Effect of licorice-root extract and Potassium sorbate on Total flavonoids, total phenolic and proline of leaves osteen mango cv. under heat stress in 2022 and 2023 seasons. Control: water spray, LRE2, LRE4, LRE6,: 2, 4 and 6g/l Licorice root ext

