Comparative Impacts of Salt Stress on Survival and Leaf Anatomy Traits in Olive Genotypes

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An experiment was conducted to investigate the tolerance of olive cultivars namely Picual, Hamed, Kalamata and Toffahi and two hybrids H61 (Picual x Hamed) and H69 (Kalamata x Toffahi) to saline irrigation water at total concentration 6000 and 9000 ppm and sodium adsorption ratio (SAR) 12 during two successive seasons (2014 & 2015). The harmful effect of salinity on survival ratio and leaf anatomical characteristics in the total plants at the end of experiment showed that the survival percentage was significantly varied depending on salinity level and olive genotypes. The highest survival percentage (100%) was recorded with H61 in both seasons and Picual seedlings at the second season. Meanwhile, H69 seedlings gave the lowest significant survival percentage (84.45 and 80 %) in tested seasons.

Relative salt tolerance could be arranged ascendingly as follows H69, Toffahi, Kalamata, Hamed, Picual and H61 in two seasons. Leaf blade thickness upper and lower epidermis and spongy tissues of the olive plant clearly increased in plants received the high saline water (9000 ppm) compare to the control plants irrigated with tap water. The highest increase due to saline treatment was recorded for H61 and the least for H69 compared to their maternal parents Hamed and Toffahi. Meanwhile, thickness of palisade tissue, midrib, xylem, phloem and fibers thickness clearly reduce as salinity treatment. The above mentioned survival percentage and anatomical changes in response to the salinity level could be considered as function to adapt and tolerate the cultivar Hamed and its hybrid H61 more than the cultivar Toffahi and its hybrid H69 to salinity.

Key words: Olea europea L., Olive genotypes, Salt stress, Survival, Anatomy

INTRODUCTION

Olive (Olea europea L.) is one of the major fruit cultivated trees in Egypt. Olive is a glycophytic species of intermediate tolerance to salinity (Gucci and Tattini, 1997). However, significant differences in olive salt tolerance have been reported depending on the genotype (Chartzoulakis, 2005). Salinity is an environmental stress that limiting plant growth and productivity around the world. This problem is more severe in arid and semi-arid regions (Munns, 2002). High soil salinity can lead alteration of composition and structure of membranes (Zhang and Shi, 2013) such as leaf thickness (Çavuşoğlu et al., 2008), distance between vascular bundles and epidermis cell number (Çavuşoğlu et al., 2007), diameter and number of xylem vessels (Hassani et al., 2014). Plants generally develop salt resistance mechanism and unique structures to survive under high saline-stress conditions (Roy et al., 2014 and Eltarawy, 2017). Therefore, a better understanding of the structural variations in plants induced by salinity should facilitate the identification of saline tolerance mechanisms (Roy et al., 2014). The aim of this study was to compare the
tolerance of two olive hybrids H61 and H69 and their maternal parents Hamed and Toffahi. Respectively to salt stress and its influence on their growth and leaf anatomy.

**MATERIALS AND METHODS**

Field experiments of this study were carried out at the Horticulture Research Institute at Giza Governorate, Egypt during the two successive seasons (2014 and 2015). This study mainly aimed to investigate the tolerance of olive cultivars (*Olea europaea* L.) namely Picual, Hamed, Kalamata and Toffahi and 2 hybrids (H61 and H69) to saline irrigation water at total concentration 6000 and 9000 ppm and SAR 12.

**Plant material:**

Screening for salt tolerance of olive transplants conducted from cuttings propagation of 4 genotypes (Picual, Hamed, Kalamata and Toffahi) and 2 F1 hybrids trees, derived by crossings between these olive cultivars, which varied in their salinity tolerance, through Genetic Improvement of Olive, (CFFC)/ IOOC, project 001. Parents and selected hybrids were as follows:

<table>
<thead>
<tr>
<th>Pollinator (♂)</th>
<th>Receptor (♀)</th>
<th>Tree No. in the hybrids project map</th>
</tr>
</thead>
<tbody>
<tr>
<td>Picual</td>
<td>Hamed</td>
<td>61</td>
</tr>
<tr>
<td>Kalamata</td>
<td>Toffahi</td>
<td>69</td>
</tr>
</tbody>
</table>

**Experimental procedure (salinity treatments and growth conditions):**

Uniform and healthy one-year-old of olive transplants of all experimented olive genotypes (parents and hybrids), raised by leafy cuttings under mist, were planted individually each in plastic bag (30 cm in diameter and 35 cm height) that had been filled with about 6 kg of ultimately washed sandy soil. After transplanting had been done in the first week of March of each 2014 and 2015 seasons, irrigation was successively applied at 2 days intervals till first week at April, whereas, investigated treatments included in this study were started in both experimental seasons. Selected transplants were similar in growth at the start of experiment and they were pruned to single main shoot per plant. Irrigation was conducted three times each week by adding 500 cm from the next mentioned sex mixture salts (Based on bringing the soil about 25% over the holding field capacity to prevent salt accumulation in the growth medium and avoid salt chock (Moya et al., 2002). Salt solutions used were derived from six stock solutions NaCl, Na₂SO₄, CaCl₂, MgSO₄, K₂SO₄, and KCl mixture to yield a balance of cations and anions with SAR of 12 and two concentrations 6000 and 9000 ppm. The different saline solutions were prepared as shown in the following Table (1), Abou El Khashab (1997). The used design was a complete randomize with three replicates.

**Survival percentage study:**

They were recorded at the end of each experimental season (1st November) as percentage of survival rate.

**Anatomical study:**

Samples necessary for these studies were obtained from plants grown in the pots of different treatments during the second season only. Leaf samples were collected for the replicated at 210 days after the onset of the treatments from the mature leaves sprouted on the fifth node of shoots, then the samples were taken from the middle portion of the leaf of one square centimeter. The collected samples were thoroughly washed with water and dried with plotting paper, and immediately kept in FAA solution for killing and fixation. Afterward, samples were dehydrated with normal butyl alcohol and paraffin wax (m. p. 58 - 60°C) for infiltration and embedding (Johansen, 1940). Transverse sections of 10–12 micron thickness were obtained using a rotary microtome. Saffranin T and fast green (F. C. F.) technique were used in staining, then washed in absolute ethanol and cleared in xylene and mounted in Canada balsam (Johansen, 1940). Slides were oven dried at 40°C for one week. The prepared slides were examined microscopically for identifying the various layers of treated leaves, thickness (depth) of each tissue (layer) were estimated and described. Whereas thickness of all epidermis layers for upper and lower leaf surfaces, mesophyll, palisade and spongy tissues, midrib, xylem and phloem in vascular bundle and fibers in vascular bundle were determined for each transverse section, (Osman, 2005).

**Statistical analysis of the data:**

The statistical analysis was carried out as described by analysis of variance (ANOVA) according to (Snedecor and Cochran, 1980). Differences between treatments were compared by Duncan's multiple range tests SAS (SAS, 1994).
Results and Discussion

Survival percentage (%):

The present study involved two main factors: the first factor is the cultivars and its hybrid (Picual x Hamed, H61 and Kalamata x Toffahi, H69) and the second factor is the salinity irrigation water (tap water, 6000 and 9000 ppm). The actual treatments involved all the possible combinations of the two main factors. Data presented in Tables (2&3) showed that survival percentage was significantly varied depending on salinity level and olive genotypes (hybrids and their parents). The survival percentage of olive hybrids and their parents was adversely associated with salinity concentration in both seasons of investigation. The significant highest survival (%) recorded in control treatments and decreased with raising salinity level till reach the lowest value (83.33%) with 9000 ppm in the two seasons. Concerning the response of olive genotypes to salinity treatments, it was evident that the highest survival percentage (100%) was recorded with H61 hybrid (Hamed x Picual) in the two seasons and Picual seedlings at the second season. It was followed by Hamed, Kalamata, Toffahi and H69 (Kalamata x Toffahi) and seedlings in descending order. Meanwhile, H69 hybrid (Kalamata x Toffahi) seedlings gave the lowest significant survival percentage (84.45 and 80%) in both seasons of study. The tolerance of H69 hybrid to salt stress (84.45 and 80%) was less than its parents, specially the maternal parent Toffahi (86.67%); meanwhile the tolerance of H61 hybrid to the same salt stress was more than its parents (100%) specially its maternal one Hamed (95.56%).

The interaction between these genotypes and salinity concentrations, revealed that either of Picual, Hamed and H61 could tolerate salinity level at 6000 ppm (100% survival), but at 9000 ppm only H61 had the maximum tolerance at both seasons, besides Picual at the second season (100% survival). On the opposite, H69 hybrid seedlings had the least tolerance at both seasons especially at the high tested salinity level (66.67 and 60%).

Such tolerant of H61 hybrid may due to its mechanism to have a low rate of sodium and chloride transport to leaves and the ability to compartmentalize these ions into vacuoles in order to avoid salt toxicity as mentioned by Munns (2002). Generally, survival percentage under salt stress considered as important indicator for salt tolerance. These results are in agreement with the observation of (Sherin, 2002 and Darweesh, 2006), concluded that saline water of olive seedlings led to decrease the survival percentage. As well as these results may indicate that salinity tolerance in olive hybrids is a quantitatively inherited trait originated from tolerance or sensitively of its parents and accumulated in their progeny to give more salt tolerant or sensitive genotype in comparison with their parents as previously reported by Ait et al., (2002).

Leaf anatomical characteristics:

From the microscopic scrutinization of cross sections, a typical mature olive leaf processes the following tissues: the upper epidermis, the mesophyll (palisade cells and spongy tissue), leaf veins and lower epidermis. Palisade cells of the mesophyll are very much elongated more or less cylindrical in one to two layers, relatively uniform or less compact; small intercellular spaces exist. These cells are characterized by the presence of abundant chloroplast which gives them a green color. The spongy mesophyll lies between the palisade cells and the lower epidermis. It consists mainly of parenchyma cells; however, they are variable in shape and contacts (Few layers of relatively compact parenchyma cells containing a moderate number of chloroplasts exist, irregular large parenchyma cells with no spaces follows and give a pronounced spongy appearance). The lower epidermis looks much like the upper epidermis except that the cells are smaller, coated by thin cuticle layer and number of stomata is more. The vascular system consists of one midrib (mid-vein) from which arise lateral veins. These in turn branch to finer veins thus forming a mesh work through the entire blade. Anyhow, in the leaf midrib, the vascular bundles of the secondary vein consisted of fibers, xylem and phloem.

Table 1: Diluted solutions as (ml/l) were prepared from stock solutions as M/L of water were added to irrigate olive plants in soil experiment during 2014 and 2015 growing seasons.

<table>
<thead>
<tr>
<th>Salt Conc. (ppm)</th>
<th>CaCl₂</th>
<th>MgSO₄</th>
<th>KCl</th>
<th>K₂SO₄</th>
<th>Na₂SO₄</th>
<th>NaCl</th>
<th>Cl:SO₄</th>
<th>SAR</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>6000</td>
<td>1.050</td>
<td>1.250</td>
<td>0.050</td>
<td>0.250</td>
<td>1.550</td>
<td>1.850</td>
<td>1:1</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>9000</td>
<td>1.499</td>
<td>1.598</td>
<td>0.103</td>
<td>0.350</td>
<td>2.500</td>
<td>2.950</td>
<td>1:1</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

Chloride to sulphate were adjusted 1:1 as meq. SAR It is the ratio of the Na concentration divided by the square root of one-half of the Ca+Mg concentration.
Table 2: Effect of saline water on the survival percentage (2014).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Picual</th>
<th>Hamed</th>
<th>H61</th>
<th>Toffahi</th>
<th>Kalamata</th>
<th>H69</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>6000 PPM</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>86.67</td>
<td>93.33</td>
<td>86.67</td>
<td>94.44</td>
</tr>
<tr>
<td>9000 PPM</td>
<td>93.33</td>
<td>86.67</td>
<td>100.00</td>
<td>73.33</td>
<td>80.00</td>
<td>66.67</td>
<td>83.33</td>
</tr>
<tr>
<td>Mean</td>
<td>97.78</td>
<td>95.56</td>
<td>100.00</td>
<td>86.67</td>
<td>91.11</td>
<td>84.45</td>
<td></td>
</tr>
<tr>
<td>LSD at 0.05</td>
<td>Cvs. = 6.647</td>
<td>Treat. = 9.401</td>
<td>Cvs. x Treat. = 16.283</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Effect of saline water on the survival percentage (2015).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Picual</th>
<th>Hamed</th>
<th>H61</th>
<th>Toffahi</th>
<th>Kalamata</th>
<th>H69</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>6000 PPM</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
<td>86.67</td>
<td>86.67</td>
<td>80.00</td>
<td>92.22</td>
</tr>
<tr>
<td>9000 PPM</td>
<td>100.00</td>
<td>86.67</td>
<td>100.00</td>
<td>73.33</td>
<td>80.00</td>
<td>60.00</td>
<td>83.33</td>
</tr>
<tr>
<td>Mean</td>
<td>100.00</td>
<td>95.56</td>
<td>100.00</td>
<td>86.67</td>
<td>88.89</td>
<td>80.00</td>
<td></td>
</tr>
<tr>
<td>LSD at 0.05</td>
<td>for: Cvs. = 5.215</td>
<td>Treat. = 3.687</td>
<td>Cvs. x Treat = 9.032</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The median bundle of midrib has vessels in the xylem and sieve tubes in both layers of internal and external phloem. In this concern, Pfeiffer et al., (2010) detected significant differences between leaf tissue areas of the two olive cultivars, salt sensitive (Leccino) and salt tolerance (Oblicoi), respectively. The present study average thickness of different tissues of leaf blade, in addition to anatomical structure of leaf midrib as affected by salinity treatment are shown in Table (4) and Figures (1&2).

1- Blade thickness (mm):

The cross sections and measurements recorded in Table (4) and Figures (1&2) revealed the increase in the leaves blade thickness of the olive plant applied the saline water 9000 ppm compared to the control plants irrigated with tap water. In this respect, olive leaf blade thickness was: 381.25, 447.25, 329 and 279 mm for Hamed, H61 hybrid, Toffahi and H69 hybrid when the plants received the salinity compared to the corresponding control plants: 352.75, 371.25, 281.25 and 271.87 mm, respectively. The highest increase due to saline treatment was recorded for H61 hybrid (20.47%) followed by H69 hybrid (2.62%) compared to their maternal parents Hamed (8.08%) and Toffahi (16.98%), respectively. The promoting effects of salt stress on leaf thickness have also been recorded by Vijayan et al., (2008) in mulberry. Kchaou et al., (2010) on five olive cultivars detected the increase in leaf thickness after salt application at high salinity treatment. The extent of this increase differed among studied cultivars. Hassani et al., (2014) and Eltarawy (2017) also showed that the salt stress reduced the olive area with highly significant effect of the genotype. Analogical results were previously reported Mohsen et al., 1987 and Abd-El Karim, 1991 and 1997.

Epidermis:

Upper Epidermis:
The increase in the thickness of upper epidermis in the plants irrigated with saline water were the highest (50%) in Hamed cv. and its hybrid H61, meanwhile it was only 32% in Toffahi cv. and its hybrid H69.
Figure 1: Transverse sections for Hamed and its hybrid (H61) of the leaf of salt treated olive plants: (40 xs) (A: Hamed with 9000 ppm, B: Hamed with tap water, C: H61 with 9000 ppm and D: H61 with tap water).
Figure 2: Transverse sections for Toffahi and its hybrid (H69) of the leaf of salt treated olive plants: (40 xs) (A: Toffahi with 9000 ppm, B: Toffahi with tap water, C: H69 with 9000 ppm and D: H69 with tap water).
Table 4: Anatomical structure of olive leaf blade cultivars and hybrids as affected by salinity treatment (9000 ppm).

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Treatments</th>
<th>Leaf blade thickness (mm)</th>
<th>Epidermis thickness (mm)</th>
<th>Palisade thickness (mm)</th>
<th>Spongy thickness (mm)</th>
<th>Midrib thickness (mm)</th>
<th>Xylem thickness (mm)</th>
<th>Phloem thickness (mm)</th>
<th>Fibers thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>upper</td>
<td>lower</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H61</td>
<td>Control</td>
<td>371.25</td>
<td>12.50</td>
<td>6.25</td>
<td>125.00</td>
<td>227.50</td>
<td>537.50</td>
<td>147.50</td>
<td>62.50</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>447.25</td>
<td>18.75</td>
<td>11.00</td>
<td>113.00</td>
<td>241.00</td>
<td>512.50</td>
<td>105.00</td>
<td>58.00</td>
</tr>
<tr>
<td>Hamed</td>
<td>Control</td>
<td>352.75</td>
<td>12.50</td>
<td>6.25</td>
<td>125.00</td>
<td>209.00</td>
<td>525.00</td>
<td>162.50</td>
<td>62.50</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>381.25</td>
<td>18.75</td>
<td>10.50</td>
<td>115.00</td>
<td>225.00</td>
<td>487.50</td>
<td>137.50</td>
<td>59.00</td>
</tr>
<tr>
<td>H69</td>
<td>Control</td>
<td>281.25</td>
<td>12.50</td>
<td>6.25</td>
<td>125.00</td>
<td>137.50</td>
<td>450.00</td>
<td>177.50</td>
<td>62.50</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>329.00</td>
<td>16.50</td>
<td>11.50</td>
<td>103.00</td>
<td>187.50</td>
<td>420.00</td>
<td>125.00</td>
<td>50.00</td>
</tr>
<tr>
<td>Toffahi</td>
<td>Control</td>
<td>271.87</td>
<td>12.50</td>
<td>9.37</td>
<td>125.00</td>
<td>125.00</td>
<td>437.50</td>
<td>200.00</td>
<td>62.50</td>
</tr>
<tr>
<td></td>
<td>Treated</td>
<td>279.00</td>
<td>16.50</td>
<td>12.50</td>
<td>87.50</td>
<td>162.50</td>
<td>375.00</td>
<td>100.00</td>
<td>47.00</td>
</tr>
</tbody>
</table>
Lower Epidermis:
Lower epidermis also showed an increase in the thickness of the olive leaves of the plants applied the saline water than the control plants (irrigated tap water). The highest increase was recorded with hybrid H69 (84%) followed by the hybrid H61 (76%) compared with their corresponding mother plants; Hamed (68%) and Toffahi (33.4%), respectively. Also, Abd el-Karim 1991 on Thomson seedless and George grapevine; Mohsen et al. (1987) on guava and Salem et al. (1990) on apple seedling and Sherin (2002) and Pfeiffer et al. (2010) on olive found that increasing salinity levels was accompanied by increasing the thickness of leaf blade and epidermis.

Mesophyll tissue:

Palisade tissue:
Table (4) and Figures (1 and 2) indicated that thickness of palisade tissue decrease as salinity treatment. Yet, values of palisade tissue were 115, 113, 103 and 87.5 mm in Hamed, H61, H69 and Toffahi respectively compared to control (tap water). The highest increase was recorded with hybrid H69 (84%) followed by the hybrid H61 (76%) compared with their corresponding mother plants; Hamed (68%) and Toffahi (33.4%), respectively. Also, Abd el-Karim 1991 on Thomson seedless and George grapevine; Mohsen et al. (1987) on guava and Salem et al. (1990) on apple seedling and Sherin (2002) and Pfeiffer et al. (2010) on olive found that increasing salinity levels was accompanied by increasing the thickness of leaf blade and epidermis.

Spongy tissue:
Data presented in Table (4) and Figures (1&2) show that Spongy tissue thickness, on the opposite was increased with saline water treatment (9000 ppm) compared with control (tap water). However, the results show that spongy tissue thickness was more increased in the H69 (36.36%) than its mother cultivar Toffahi (30%), meanwhile such increase in H61 (5.93%) was less than that occurred in plants of its mother parent Hamed (7.66%). Karimi et al., (2009) on olive found a marked increase in spongy mesophyll thickness due to salt stress along with a slight reduction in palisade mesophyll length and thickness. Parida et al., (2004) also found that mesophyll thickness of mangrove decreased with salinity due to a decrease in length of palisade cells and number of spongy cell layers. Spongy cell diameter also decreased with salinity, but they were denser. As a consequence, the amount of intercellular space in spongy tissue was lower in salt treated leaves.

Leaf midrib:
From the microscopic scrutinization of cross sections, the mature olive leaf midrib showed some modification of different tissues in response to salinity. Leaf midrib thickness and its area, as well as, number of xylem vessels/section and/or area of vessel were found to decrease due to water salinity treatment. The decrease in midrib thickness was much higher in the olive salt sensitive H69 leaves after salt treatment (14.29%) meanwhile that of the more tolerant one, H61 hybrid showed the least decrease (4.65%). Moreover, xylem thickness of the highest salinity tolerant genotype (H61 hybrid) was the least (147.5 mm) compared to its mother parent (162.5 mm), and the least tolerant genotypes Toffahi (177.5 mm) and its hybrid H69 (200 mm). As for phloem tissue, it was decreased in the leaves of the saline treated plants. However, the more tolerant cultivar Hamed and its hybrid H61 showed least effect by salinity treatment (7.2 and 5.6%) compared to the least tolerant cultivar Toffahi and its hybrid H69 (20 and 24.8%), respectively. Fiber thickness showed the same trend of phloem tissue. In agreement with the present result, the binding of Nomir, 1994; Abd El-Karim, 1997; Sherin, 2002; Çavusoglu et al., 2007; Hui et al., 2015 and Eltarawy 2017. Also, Emtithal et al., (1996) and Lorenzo et al., (2015) reported that the olive tested cv. showed a wide variation in this concern. Similarly, Hassani et al., (2014) noted that the increase of salinity level reduced the xylematic vessel diameter and the reduction was of 10 – 18 % according to the genotype. The above mentioned anatomical differences in response to the highest salinity level show that the greatest loss in palisade tissue, area of the leaf midrib, number of xylem vessels / section were specially noticed at the least Toffahi cv. and H69 (Toffahi x Kalamata), compared to Hamed cv. and H61 (Hamed x Picual) for being the more tolerance to salinity. This may indicate that, the relative salt tolerance (Toffahi and H69) could be injured from salinity; however, Hamed and H61 were the most tolerant salinity.

CONCLUSION
In this respect, it can be concluded that, there was a progressive decrease in the survival percentage of olive genotype seedlings with increasing salinity concentration up to 9000 ppm. Investigation of the
more salt tolerant olive Hamed and its hybrid H61 compared to the less tolerant cultivar Toffahi and its hybrid H69 revealed differences in anatomical characteristics of their leaves in response to high salt treatment. Salt tolerant genotypes showed more increase in leaf thickness and upper epidermis, besides less decrease in palisade thickness and midrib thickness compared to less tolerant genotypes. Such less reduction in thickness of palisade and midrib in high salt tolerant genotypes may suggest their capacity for re-translocation of minerals and assimilates which resulted in their higher growth rates and subsequent higher salt tolerance.

**CONFLICT OF INTEREST**
The authors declared that present study was performed in absence of any conflict of interest.

**ACKNOWLEDGEMENT**
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**AUTHOR CONTRIBUTIONS**
All authors contributed equally in all parts of this study.

**REFERENCES**


