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Exogenous Gamma-Aminobutyric Acid (GABA) and Oxalic Acid Enhance Growth and Salinity Tolerance in *Zanthoxylum piperitum* Plants: Anatomical, Biochemical, and Physiological Insights.

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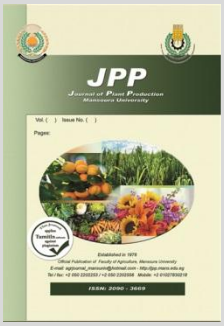

ABSTRACT

Salinity stress, a major abiotic factor, significantly limits global plant productivity. Exacerbated by population growth, global warming, and climate change, it seriously threatens sustainable agriculture. Addressing its impact on plant systems has therefore become a critical priority. In this context, the present study aimed to assess the potential of foliar applications of gamma-aminobutyric acid (GABA) and oxalic acid (OA), at varying concentrations, in alleviating salinity stress in *Zanthoxylum piperitum*. The experiment was conducted using a split-plot arrangement within a randomized complete block design at the ornamental nursery in Giza, Egypt, during two successful seasons in 2023 and 2024. The findings demonstrated that salinity stress at any dose hurts physiological, morphological, and anatomical characteristics. Conversely, the GABA and OA are additions that significantly enhanced all characteristics. OA (0.1 g/l) has been shown to promote plant growth, increase biomass accumulation, and nutrient availability, particularly nitrogen. Additionally, it elevates total carbohydrate concentrations, proline accumulation, and mitigates chlorophyll degradation. It also improves key histological characteristics of leaves, thereby reducing physiological toxicity under conditions of severe salinity stress. Moreover, GABA has demonstrated potential in mitigating the adverse effects of salinity by enhancing nutrient uptake, particularly phosphorus and potassium, improving photosynthetic efficiency, and carbohydrate metabolism under moderate salinity conditions. Overall, the application of OA at low doses in combination with moderate or severe salinity stress demonstrated the potential to enhance morpho-physiological and anatomical traits, thereby supporting the growth of *Z. piperitum* plants. These highlight OA as an effective and environmentally friendly strategy for alleviating salinity-induced stress.

keywords: *Zanthoxylum piperitum*; Global warming; Salinity stress; Anatomical characteristics; Morpho-physiological traits.

INTRODUCTION

Abiotic stress in plants, including salinity, is among the environmental extremes and significantly reduces crop productivity worldwide (Kamran et al., 2020). Salinization of agricultural land is an increasingly serious global issue, particularly in arid and semi-arid regions, owing to increased population growth, global warming, and changing climates, which threaten sustainable agriculture (Abbasi et al., 2015; Kumar et al., 2023; Othman et al., 2023). Salinity refers to the excessive accumulation of toxic ions, particularly chloride (Cl^-) and sodium (Na^+), in the soil, which deteriorates soil structure and significantly reduces or completely inhibits plant growth (Shilev, 2020; Bueno and Cordovilla, 2021). It induces stomatal closure in leaves, causes nutrient imbalances, and reduces photosynthesis (Hafez et al 2020). These effects, along with metabolic disorders and decreased cell division, result from ion toxicity and water deficit due to osmotic stress (Machado and Serralheiro, 2017). Thus, both osmotic stress and ion toxicity trigger oxidative stress by causing an overproduction of reactive oxygen species (ROS), which leads to cellular membrane damage and cell death (Yu et al., 2021). To cope with salinity, many plants commonly respond to salinity through a complex trait involving various biochemical and molecular mechanisms that help mitigate the detrimental effects of osmotic and ionic stress (Aazami et al., 2023). These mechanisms cause disorders in morphological, anatomical, physiological, and biochemical processes (Shalaby and Ramadan, 2024). For example, increasing osmolytes (such as soluble sugars, some organic acids, and amino acids) as antioxidant systems, with increasing the activity of antioxidant enzymes, are key indicators of salt tolerance in plants (Othman et al., 2023). Therefore, the struggle to mitigate the negative impacts of salt on plants through various approaches that have been taken becomes a challenge worldwide and needs critical attention under climate change.

Osmolyte compounds formed by plant metabolism and involved in plant growth processes play a significant role in response to environmental stresses such as salinity (Mohammadrezakhani et al., 2019). These compounds possess antioxidant properties that inhibit the generation of reactive oxygen species (ROS), thereby reducing the toxicity induced by various stresses (Ansari et al., 2021). Gamma-aminobutyric acid (GABA), a plant growth and development regulator, is a non-protein amino acid that plays a role in controlling proline and tricarboxylic acid (TCA) cycle metabolism (Iqbal et al., 2023). It is considered an endogenous signaling molecule in several regulatory mechanisms, including carbon/ nitrogen metabolism, regulation of redox status, and osmotic pressure (Jalil and Ansari, 2020). The accumulation of GABA in plant cells increased under different biotic and abiotic stresses (Ansari et

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al., 2021). In plants, GABA functions as a beneficial osmolyte under salinity stress, promoting growth, facilitating osmoregulation, scavenging free radicals, and reducing oxidative damage without exerting harmful effects (Ullah et al., 2023). Although numerous studies have investigated the effects of salt stress on various plants, the role of GABA, whether endogenously synthesized or applied exogenously, has remained understudied (Khanna et al., 2021). Few studies revealed that exogenous application of GABA enhances the salt tolerance level of *Capsicum annuum* plants by improving antioxidant defense systems, ATPase enzymes, net photosynthetic rate, transpiration rate, stomatal conductance, and intercellular CO_2 concentration (Ramzan et al., 2023). Moreover, exogenous GABA had less damage to membrane stability and H_2O_2 level in the *Origanum vulgare* plant under salt stress (Garoosi et al., 2023). In addition, the germination rate, plant height, and biomass of *Gossypium hirsutum* increased by regulating osmo-protectant levels, maintaining ion homeostasis, and alleviating oxidative stress in GABA+ NaCl-treated plants as compared to only NaCl-treated plants (Dong et al., 2024).

Oxalic acid (OA) is a dicarboxylic acid, crystalline and colorless, that is a low molecular weight organic acid and plays a pivotal role in various metabolic processes during growth and development, such as energy production and adaptation to abiotic stresses (Li et al., 2022). It is also known as ethanedioic acid and is considered a strong acid with notable chelating and reducing properties, which is a reliable source of both protons and electrons (Hasan et al., 2023). Likewise, OA was a key factor in the calcium (Ca^{2+}) reaction to form the salt Calcium oxalate (CaOx) as a complex and developed in crystal idioblasts, specialized cells, in cell vacuoles, then this complex regulates the cytosolic concentration levels, it also immobilizes excess amounts of Ca element (Javkar and Avhad, 2023). Additionally, OA enhances plant antioxidant capacities by regulating pH, maintaining membrane integrity, and promoting the metabolism of osmotic regulators (Osmolovskaya et al., 2018). Recent studies have shown that under stress conditions, OA plays multiple roles in metabolic and physiological responses, including its involvement in both synthesis and degradation processes that contribute to pH regulation within plant cells (Sadak and Orabi, 2015). According to Azhargul et al. (2023), the foliar application of OA has been shown to enhance peroxidase enzyme activity, grain yield, and increase stress tolerance in wheat plants. Moreover, Javkar and Avhad (2023) discovered that OA acts as a dynamic carbon store and triggers an alert during photosynthesis. This alert increases CO_2 production, which supports photosynthesis in the *Portulacaria afra* plant under salinity stress. Wei et al. (2021) found that a 0.5% concentration of OA enhanced root length and biomass, as well as defense enzymes in the *Suaeda salsa* plant, whereas a higher concentration (1.5%) induced increased toxicity under salinity stress.

Zanthoxylum Piperitum, also known as Japanese pepper, belongs to the *Rutaceae* family and is native to East Asian regions, including Japan (Yamasaki et al., 2022). It is a deciduous, aromatic woody shrub that can mature into a small tree, characterized by glossy pinnate leaves that turn yellow in autumn. Also in June, it produces clusters of small, cup-shaped yellowish-green flowers, followed by aromatic, red berry-like fruits, which appear in autumn (Esmail et al., 2021). Japanese pepper is an attractive, low-maintenance shrub that responds well to pruning, making it ideal for small gardens, particularly when planted along walls or borders (Hirata et al., 2019). In recent years, it has been increasingly grown as a container plant for patios, proving particularly well-suited for the bonsai industry owing to its slow growth rate and adaptability (Nooreen et al., 2019). Additionally, it is an attractive pioneer tree with high ecological value, because it supports water conservation by improving the soil's hydraulic properties, soil conservation by carbon sequestration capacity, and so on, as well as playing a key role in managing desertification under climate change (Liu et al., 2021). *Z. piperitum* plants have a high trade value and diverse uses in Ayurveda, pharmacy, and industry as unique spice products (Liu et al., 2019). It is considered a medicinal and aromatic plant because it contains essential oils and many active ingredients, that have been used in traditional medicinal practices to cure several diseases such as abdominal pain, asthma, antispasmodic, cholera, diabetes, antipyretic, anthelmintic, and anticancer activity (Barua et al., 2018; Phuyal et al., 2019). So, Japanese pepper is indeed a shrub that is both aesthetically pleasing and beneficial.

In the literature, very few studies have focused on *Z. piperitum* as an ecological value under environmental stresses, despite its extensive study within its tolerance range under natural conditions. However, further research is needed to better understand the plant's morphological, physiological, and anatomical responses to salinity stress, as well as its underlying biochemical processes and their connection to climate change. This study aims to investigate whether the growth and development of *Z. piperitum* can be improved by an exogenous supply of GABA or OA as osmolyte compounds to mitigate the negative impacts of different salt stress levels.

MATERIALS AND METHODS

Plant Material and Practical Procedures

This study was conducted at the Ornamental Nursery, Faculty of Agriculture, Cairo University, Giza, Governorate (latitude 30° 03′ N, longitude 31° 13′ E, altitude 19 m ASL), Egypt, during two seasons of 2023 and 2024. The basal physical and chemical properties (Table 1) of the used pot experimental soil were determined by the Water and Environment Research Institute, Agriculture Research Centre (A.R.C) according to Jackson (1973). The analysis showed that the soil was classified as Clay loam with EC and pH of 1.06 $dS\,m^{-1}$ and 7.68, respectively. In this experiment, the effect of irrigation with different levels of saline water and foliar application of GABA or OA, and their interaction, were investigated on the growth parameters, anatomical, and chemical composition of the *Z. piperitum* plant.

One-month-old, healthy, and uniform in shape, *Zanthoxylum piperitum* transplants (length 20 cm) were purchased from a private nursery in Giza, Egypt. Each single transplant was cultivated on the 15^{th} and 18^{th} of April (in both seasons 2023 and 2024, respectively) in a plastic pot (40 cm diameter) filled with a mixture of clay and sand (1:1 v/v). The experiment was conducted under partial shade conditions in a saran house (50% shade). On the 15^{th} of May, the plants were irrigated twice/ week using control water (tap water, 270 mg L^{-1}), and saltwater application at concentrations of 2000, 4000, and 6000 mg L^{-1} were used, salt waters were prepared using two different salts ($CaCl_2$ and NaCI as 1:1 w/w) and applied at one liter/ pot. In both seasons, plants were irrigated with salinity to apply the foliar applications of exogenous osmolyte compounds to the leaves as foliar applications every month, which of them GABA at two different doses (0.1 and 0.2 g/l), and OA (0.1 and 0.2 g/l) until the plant's leaves were thoroughly wetted by using automatic atomizer (technical grade, Sigma-Aldrich Chemical Co. Washington, USA). The control plants were sprayed only with distilled water without GABA and OA. Tween-20 (0.1%) was added to ensure that gamma-aminobutyric acid and oxalic acid were retained and dispersed evenly on the plant leaves. Additionally, the pot surfaces were covered with plastic to prevent GABA and OA from entering the plant roots. All plants were fertilized monthly by applying a soil drench of Kristalon (NPK 19:19:19) at a rate of 4 g/ pot.

Table 1. Physical and chemical properties of the experimental soil.

Physical properties						
Soil Texture	**Fine Sand %**	**Coarse Sand %**	**Silt %**	**Clay %**	**Ca CO_3 %**	**O.M %**
Clay loam	43.2	4.07	16.9	35.83	4.02	1.85

Chemical properties										
Ph	EC dS/m	Ion concentration (Mmol/l)						Available nutrients (mg/kg)		
		CO^{-3}	SO^{-4}	Na^{+}	Ca^{+2}	Mg^{+2}	K^{+}	N	P	K
7. 68	1.06	0.6	3.48	1.27	6.76	7.44	3.34	68.60	11.10	120.72

Experimental layout

A factorial experiment was conducted using a split-plot arrangement within a randomized complete block design (RCBD) with four replications. The experimental design included two factors: the main plot factor comprised four levels of salinity (three salt concentrations plus a control), the subplot factor consisted of five levels of osmolyte compound doses, including a control. Each replication included 20 treatments, with three pots per treatment.

Data Recorded and Harvest Dates

On the 15th of October in both seasons, the experiment was terminated, morphological measurements were carried out on the grown *Z. piperitum* plants, such as vegetative growth parameters (plant height (cm), stem diameter (mm, at 5 cm above soil surface), branches number/plant, and fresh and dry weights of leaves and stems (g). The soil was kept dry until roots were dug out, and the experiment was terminated to measure the root characteristics, including root length (cm), lateral root number/ plant, and fresh and dry weights of roots per plant (g). The plants were carefully harvested, leaves, stems, and roots were detached and dried in an oven at 70 °C for 48 hours before recording the dry weights. Additionally, it measures chemical composition, including total chlorophylls in fresh leaf samples, was determined by using a chlorophyll meter Model SPAD 502 according to Netto et al. (2005), and total carbohydrate percentage (% of dry matter) was estimated in dried leaf samples as described by Dubois et al. (1956). Free proline content was estimated in the fresh leaves (mg /g fresh matter of leaves) as described by Bates et al. (1973), using a Spectranic 2000 spectrophotometer (Bausch and Lomb; Irvine, CA, USA). The absorbance was measured at 520 nm. In addition, Samples from dried leaves were taken after the experiment was terminated (dried at 60 °C for 72 hours) to estimate N, P, and K as described by A.O.A.C. (2005).

Anatomical studies were also carried out at the Laboratory of Agriculture. Botany Department, Faculty of Agriculture, Cairo University, Egypt, during the second season. Leaf samples were collected from plants treated with different concentrations, including control plants. Then, materials were killed, and fixed in F.A.A. solution (10 ml formalin, 5 ml glacial acetic acid, and 85 ml ethyl alcohol 70%) for at least 48 hours and dehydrated in a normal butyl alcohol series before being embedded in paraffin wax, melting point 56 °C (Sass, 1951). Sections that were cut on a rotary microtome at a thickness of 15-20 microns were stained with crystal violet/erythrosine before being mounted in Canada balsam. Slides were examined microscopically and photomicrographically. Measurements in microns (µm) of certain histological features in transverse sections, such as palisade tissue thickness, spongy tissue thickness, blade tissue thickness, and thickness of midrib.

Statistical Analysis

All data collected from both seasons were subjected to analysis of variance (ANOVA) using a split-plot arrangement within a randomized complete block design (RCBD), following the procedures described by Gomez and Gomez (1984), and analyzed using the MSTAT-C software package (Freed et al., 1989). Treatment means comparisons were performed using a least significant difference (LSD) at the 5% level of probability.

RESULTS AND DISCUSSION

Morphological Attributes

According to the analysis of variance (ANOVA), both salinity levels and the application of GABA or OA had statistically significant effects ($p < 0.05$) on morphological traits across both experimental seasons, as presented in Tables 2, 3, and 4. The effects of these treatments on various growth parameters are discussed below:

As shown in Table 2, higher salinity levels in irrigation water significantly reduced the height, stem diameter, and branch number of *Z. piperitum* plants. In both seasons, plants irrigated with tap water (control) exhibited significantly superior growth, as reflected in the highest plant height, stem diameter, and number of branches. Conversely, progressive increases in salinity levels, reaching up to 6000 ppm, had a pronounced negative effect on growth, consistently leading to substantial reductions in these parameters. These results highlight the detrimental impact of high salinity on plant development. These results align with previous studies reporting the negative effect of salinity stress on plant growth and performance, including *Zanthoxylum piperitum* (Esmail et al., 2021), *Hibiscus rosa-sinensis* and *Mandevilla splendens* (Yu et al., 2021), and *Pittosporum tobira* (Lasheen et al., 2024). The application of GABA and OA significantly improved growth in *Z. piperitum* under salinity stress (Table 2). Specifically, OA at 0.1 g/l resulted in the tallest plants, measuring 34.88 cm and 33.50 cm in the first and second seasons, respectively. It also produced the highest number of branches, with averages of 9.63 and 8.75 in both seasons, respectively. Additionally, plants treated with OA at 0.2 g/l showed thicker stems, with a diameter of 0.65 cm in the first and 0.64 cm in the second season, outperforming the control and other treatments. These findings underscore the effectiveness of OA in promoting plant growth, due to its multifaceted roles in physiological processes. OA plays a role in metal chelation, serves as an electron acceptor in the manganese peroxidase cycle that is essential for plant survival and contributes to both pH regulation and osmoregulation (Chen et al., 2024; Wei et al., 2021).

The interaction between salinity stress and the application of GABA and OA significantly affected plant growth parameters in both seasons. The tallest plants were observed in the control treatments and sprayed with 0.1 g/l OA. In contrast, irrigation with 6000 ppm saline water combined with the same OA dose resulted in significantly reduced plant height, demonstrating the detrimental effects of high salinity on plant development (Shalaby and Ramadan, 2024). Plants treated with 2 g OA and irrigated with 2000 ppm saline water recorded the stem thickness in both seasons. The highest number of branches per plant was observed in plants sprayed with 0.1 OA and irrigated with tap water (control) in the first season, or with plants irrigated with 2000 ppm saline water in the second season. These results align with previous studies on the role of OA in enhancing plant growth under stress by improving photosynthesis, reducing ROS accumulation, and increasing antioxidant and osmoregulatory activity (Chen et al., 2024). Similarly, Wei et al. (2021) demonstrated that low doses of OA promoted growth and improved metal availability in *Suaeda salsa* plants. In addition, Gupta et al. (2024) identified OA as an effective and eco-friendly strategy to alleviate stress in *Zea mays*, noting that its high solubility facilitates the formation of metal complexes, thereby enhancing overall plant growth and performance.

Table 2. Effect of spraying with GABA and OA on plant growth traits, including plant height, stem diameter, and number of branches per plant of *Zanthoxylum piperitum* under different salinity concentrations

Treatments		Plant height (cm)		Stem diameter (cm)		Number of Branches/ plants	
		1st Season	2nd season	1st Season	2nd season	1st season	2nd season
Effect of salinity (S)							
0 ppm		36.70	36.73	0.67	0.64	9.00	8.13
2000 ppm		36.30	34.53	0.64	0.63	8.40	8.73
4000 ppm		34.80	32.67	0.55	0.56	7.30	7.13
6000 ppm		12.00	11.13	0.23	0.23	2.70	2.80
LSD at 5%		2.87	4.42	0.11	0.12	2.51	3.09
Effect of spraying with osmolyte chemicals (C)							
0		27.75	27.08	0.48	0.45	4.50	4.17
0.1g/l GABA		27.38	26.17	0.45	0.45	5.88	5.67
0.2g/l GABA		28.38	26.42	0.46	0.44	7.50	7.25
0.1g/l Oxalic		34.88	33.50	0.58	0.58	9.63	8.75
0.2g/l Oxalic		31.38	30.67	0.65	0.64	6.75	7.67
LSD at 5%		4.08	4.98	0.05	0.07	1.04	1.82
Effect of SXC							
0 ppm	0	36.00	35.00	0.70	0.63	4.50	4.33
	0.1g/l GABA	37.50	38.33	0.65	0.63	8.00	7.00
	0.2g/l GABA	35.00	35.00	0.70	0.67	11.00	10.33
	0.1g/l Oxalic	42.50	40.33	0.60	0.60	12.00	8.67
	0.2g/l Oxalic	32.50	35.00	0.70	0.67	9.50	10.33
2000 ppm	0	38.00	38.67	0.60	0.57	6.50	5.67
	0.1g/l GABA	40.00	35.00	0.65	0.63	10.00	10.00
	0.2g/l GABA	37.50	35.00	0.60	0.60	9.00	9.33
	0.1g/l Oxalic	38.00	35.33	0.50	0.57	11.50	11.67
	0.2g/l Oxalic	28.00	28.67	0.85	0.77	5.00	7.00
4000 ppm	0	37.00	34.67	0.60	0.60	7.00	6.67
	0.1g/l GABA	32.00	31.33	0.50	0.53	5.50	5.67
	0.2g/l GABA	41.00	35.67	0.55	0.50	10.00	9.33
	0.1g/l Oxalic	31.50	31.67	0.60	0.60	6.50	7.00
	0.2g/l Oxalic	32.50	30.00	0.50	0.57	7.50	7.00
6000 ppm	0	0.00	0.00	0.00	0.00	0.00	0.00
	0.1g/l GABA	0.00	0.00	0.00	0.00	0.00	0.00
	0.2g/l GABA	0.00	0.00	0.00	0.00	0.00	0.00
	0.1g/l Oxalic	27.50	26.67	0.60	0.57	8.50	7.67
	0.2g/l Oxalic	32.50	29.00	0.55	0.57	5.00	6.33
LSD at 5%		8.15	9.96	0.09	0.14	2.08	3.64

The data presented in Table 3 indicate that, in both seasons, increasing the salinity level in irrigation water from 0 ppm (control) to 2000, 4000, and 6000 ppm led to consistent and significant reductions in the mean fresh and dry weights of leaves and stems in *Z. piperitum* plants. Similar decreases in the fresh and dry weights of leaves and stems due to salinity stress have been reported in several ornamental plant species, including *Hedysarum scoparium* and *Avena sativa* (Azeem et al., 2025), *Lavandula dentata* (Shala et al., 2024), *Zanthoxylum piperitum* (Esmail et al., 2021), and *Frankenia pulverulenta* (Bueno and Cordovilla, 2021). The adverse effects of saline irrigation on leaf and stem biomass may be attributed to the plant's sensitivity to salinity stress, which impairs overall growth and performance. Salinity stress is known to inhibit shoot elongation and accelerate leaf senescence, increasing the incidence of leaf necrosis and chlorosis (Lacerda et al., 2020). Additionally, it disrupts cell expansion, impairs membrane integrity, and reduces cytosolic metabolism, which disrupts collectively diminishing photosynthetic efficiency, leading to a significant reduction in biomass accumulation (Pereira et al., 2020). Due to salinity-induced ion toxicity, osmotic stress, and nutrient imbalances, plants experience impaired water uptake and essential minerals (Oliveira et al., 2024). Leaf and stem development are more sensitive to salinity than root growth, possibly as an adaptive mechanism to reduce transpiration and water consumption (Hanin et al., 2016; Mansour and Hassan, 2022). The application of GABA and OA treatments resulted in a significant increase in the biomass of both leaves and stems, demonstrating their pronounced efficacy in promoting plant growth. The data presented in Table 3 indicate that, in both seasons, the highest means of fresh and dry weights of leaves and stems were observed in plants treated with any concentration of OA. It can contribute to a more vigorous and resilient plant structure because OA may function as a chelating agent by elevating pH levels and enhancing the accumulation of key macronutrients in leaves and stems, thereby promoting biomass production and supporting overall plant growth (Zhang et al., 2024).

The findings from both seasons (Table 3) demonstrate that varying concentrations of GABA or OA applied under salinity stress significantly affected the fresh and dry weights of stems and leaves per plant. In many instances, plants irrigated with tap water (control) and treated with GABA at 0.2 g/l exhibited the highest fresh and dry weights of leaves and stems. Closely following were control plants sprayed with OA at 0.1 g/l. The results from both seasons indicate that, in most cases, plants subjected to different concentrations of saline irrigation and treated with foliar applications of OA at 0.1 g/l or GABA at 0.2 g/l exhibited significantly higher fresh and dry biomass of leaves and stems compared to untreated plants under similar salinity conditions. The beneficial effects of GABA and OA on leaf and stem biomass under salinity stress can be attributed to several key mechanisms. These compounds may act as potential growth regulators, aid in the exclusion or compartmentalization of toxic salt ions such as Na^+ and Cl^- into vacuoles and enhance antioxidant defense systems that mitigate excessive ROS (Ansari et al., 2021; Li et al., 2022). Likewise, Qian et al. (2024) reported that GABA alleviated salinity-induced reductions in stem and leaf biomass in soybean seedlings by lowering Na^+ and Cl^- levels and enhancing photosynthetic pigments, nutrient uptake, osmolytes, and antioxidant enzymes activity, thereby improving growth and biomass. In *Larix olgensis*, OA improved growth under stress conditions by enhancing nutrient mobilization for chlorophyll synthesis, preventing the translocation of free metal ions in the cytosol, and boosting photosynthesis and carbon assimilation, thereby increasing biomass and reducing physiological toxicity (Song et al., 2018).

Table 3. Effect of spraying with GABA and OA on fresh and dry weight of stems and leaves per plant of Zanthoxylum piperitum under different salinity concentrations

Treatments		Leaves fresh weight (g)		Leaves dry weight (g)		Stem fresh weight (g)		Stem dry weight (g)	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Effect of salinity (S)									
0 ppm		29.68	27.35	9.43	9.08	14.36	13.75	8.29	8.13
2000 ppm		20.56	20.54	5.20	5.44	13.02	12.42	7.01	6.70
4000 ppm		10.08	10.15	2.08	2.17	7.92	7.78	3.80	3.82
6000 ppm		3.93	4.02	0.90	0.92	2.94	3.07	1.61	1.71
LSD at 5%		7.1	8.21	2.11	2.28	1.61	2.05	1.08	1.25
Effect of spraying with osmolyte chemicals (C)									
0		12.71	12.52	4.19	4.40	8.73	8.61	4.54	4.54
0.1g/l GABA		12.55	13.03	3.73	3.82	9.17	8.67	4.91	4.69
0.2g/l GABA		17.35	14.89	5.43	4.50	9.42	8.50	4.95	4.45
0.1g/l Oxalic		19.13	19.51	4.70	5.11	10.58	11.07	5.56	5.99
0.2g/l Oxalic		18.57	17.61	3.95	4.20	9.90	9.43	5.92	5.77
LSD at 5%		3.28	4.52	1.14	1.22	1.3	2.2	0.91	1.49
Effect of SXC									
0 ppm	0	21.14	20.94	8.94	9.42	14.93	14.50	9.07	8.90
	0.1g/l GABA	24.88	26.05	8.36	8.49	11.98	12.09	6.79	6.66
	0.2g/l GABA	39.35	33.43	14.38	11.93	19.33	16.17	10.54	8.72
	0.1g/l Oxalic	35.08	31.88	8.40	8.05	15.73	16.02	7.91	8.69
	0.2g/l Oxalic	27.98	24.43	7.09	7.51	9.85	9.96	7.15	7.68
2000 ppm	0	21.88	22.48	5.92	6.11	14.48	14.52	6.44	6.58
	0.1g/l GABA	16.19	16.18	4.56	5.02	12.63	12.54	7.48	7.77
	0.2g/l GABA	19.13	16.35	5.45	4.43	11.58	10.94	6.72	6.17
	0.1g/l Oxalic	23.80	28.20	6.39	8.04	11.50	12.47	5.89	6.53
	0.2g/l Oxalic	21.80	19.47	3.68	3.58	14.93	11.65	8.52	6.43
4000 ppm	0	7.83	6.67	1.90	2.05	5.53	5.42	2.64	2.66
	0.1g/l GABA	9.15	9.89	1.99	1.76	12.08	10.05	5.39	4.35
	0.2g/l GABA	10.90	9.78	1.91	1.64	6.77	6.89	2.56	2.92
	0.1g/l Oxalic	9.55	11.03	1.80	2.48	8.43	9.28	4.87	5.32
	0.2g/l Oxalic	12.95	13.37	2.79	2.94	6.80	7.27	3.53	3.84
6000 ppm	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.1g/l GABA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.2g/l GABA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.1g/l Oxalic	8.08	6.92	2.23	1.86	6.66	6.52	3.59	3.42
	0.2g/l Oxalic	11.55	13.18	2.25	2.75	8.04	8.83	4.48	5.13
LSD at 5%		6.56	9.03	2.94	3.76	2.61	4.4	1.83	2.98

Table 4 shows that, in both seasons, the root characteristics of *Z. piperitum* plants were significantly influenced by salinity levels. In both seasons, the highest biomass of root weights per plant was recorded in control plants, which also exhibited the longest roots, followed by plants grown under 2000 ppm salinity, with no significant difference between the two. Interestingly, the highest number of lateral roots per plant was observed at a 4000-ppm salinity level (5.20 and 5.00 roots/plant in the first and second seasons, respectively). In contrast, the lowest root trait values were found under the highest salinity level (6000 ppm). These findings align with previous studies reporting salinity-induced reductions in root characteristics across various species, including Brassica napus (Arif et al., 2020) and Pittosporum tobira (Lasheen et al., 2024). The increase in lateral root number at 4000 ppm suggests that moderate salinity may stimulate root development, potentially as an adaptive response to low phosphorus availability, thereby enhancing nutrient acquisition, which is consistent with the findings of Robin et al. (2016) and other studies, which also indicate that excessive salt concentrations exert inhibitory effects on root growth. The application of GABA and OA had a significant effect on the root characteristics of *Z. piperitum* plants. As shown in the recorded data (Table 4), OA treatment generally produced more favorable root development parameters than either GABA-treated or untreated control plants. Notably, plants treated with OA at a concentration of 0.2 g/l exhibited a significantly higher number of lateral roots per plant across both growing seasons compared to the control and other treatment groups. Furthermore, OA applications at both 0.1 and 0.2 g/l resulted in statistically similar outcomes, with both concentrations promoting greater root length and increased fresh and dry root biomass relative to untreated plants. These findings align with those of Pan et al. (2016), who demonstrated that OA enhances nutrient acquisition by promoting root biomass, length, and lateral root development, thereby supporting improved plant growth and productivity.

The combined effects of salinity stress and GABA or OA treatments on root characteristics are presented in Table 4 for both growing seasons. Notably, the highest number of lateral roots was observed in plants irrigated with 6000 ppm saline water and treated with OA at either 0.1 or 0.2 g/l, with no significant differences between these concentrations. In contrast, the greatest root lengths (62.50 cm) in the first season and (55.00 cm) in the second, were recorded in plants treated with 0.2 g/l OA under 4000 ppm salinity, followed by those receiving 0.1 g/l OA under 2000 ppm salinity. Additionally, the highest fresh and dry root biomass in both seasons was found in plants irrigated with tap water and treated with 0.2 g/l GABA. These findings highlight the potential of targeted irrigation and treatment strategies to enhance root development under saline conditions. The beneficial effect of OA on root development under salinity stress is well-documented in the literature. OA, a simple dicarboxylic acid, exhibits strong chelating properties with multivalent cations and possesses notable antioxidant capacity. These attributes contribute to its potential in mitigating the adverse effects of salinity and promoting plant growth under stress conditions (Çoban and Aras, 2023; El-Shabrawi et al., 2015). Thus, the application of OA represents a promising strategy for enhancing agricultural resilience in saline environments.

Table 4. Effect of spraying with GABA and OA on root characters of Zanthoxylum piperitum plant under different salinity concentrations

Treatments		Lateral root numbers/Plant		Root Length(cm)		Root fresh weight (g)		Root dry weight(g)	
		1st season	2nd Season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Effect of salinity (S)									
0 ppm		4.40	4.27	44.50	46.33	23.69	21.91	10.12	9.38
2000 ppm		4.70	4.80	46.10	46.40	16.14	15.93	8.89	8.75
4000 ppm		5.20	5.00	41.45	40.97	11.09	10.25	5.44	5.06
6000 ppm		2.90	2.53	14.80	14.33	4.98	4.75	2.39	2.42
LSD at 5%		0.62	1.06	2.63	4.59	0.91	2.71	1.52	1.91
Effect of spraying with osmolyte chemicals (C)									
0		4.00	3.75	29.50	32.58	11.99	11.29	5.63	5.57
0.1g/l GABA		3.25	3.67	31.06	30.71	12.20	11.38	5.94	5.41
0.2g/l GABA		4.00	3.92	31.88	31.25	13.84	12.27	6.69	5.83
0.1g/l Oxalic		4.88	4.42	45.25	45.33	16.57	15.55	7.39	7.01
0.2g/l Oxalic		5.38	5.00	45.88	45.17	15.27	15.55	7.90	8.18
LSD at 5%		0.68	0.97	2.12	5.78	1.41	2.44	0.87	1.42
Effect of SXC									
0 ppm	0	3.50	3.00	42.50	43.33	22.84	21.35	10.15	10.36
	0.1g/l GABA	5.00	5.67	42.50	43.33	18.87	17.05	9.12	8.05
	0.2g/l GABA	6.00	6.00	50.00	46.67	33.03	27.46	14.87	12.07
	0.1g/l Oxalic	3.50	3.00	47.50	50.00	24.41	24.02	6.52	6.75
	0.2g/l Oxalic	4.00	3.67	40.00	48.33	19.31	19.67	9.96	9.67
2000 ppm	0	5.00	5.00	42.50	50.00	16.26	14.69	7.77	7.33
	0.1g/l GABA	4.00	4.67	42.50	40.00	15.08	15.01	6.95	6.94
	0.2g/l GABA	5.00	5.00	42.50	43.33	13.86	13.77	8.33	7.57
	0.1g/l Oxalic	4.00	4.33	59.50	56.33	16.23	15.49	10.29	9.98
	0.2g/l Oxalic	5.50	5.00	43.50	42.33	19.27	20.68	11.11	11.90
4000 ppm	0	7.50	7.00	33.00	37.00	8.85	9.13	4.61	4.57
	0.1g/l GABA	4.00	4.33	39.25	39.50	14.86	13.47	7.70	6.65
	0.2g/l GABA	5.00	4.67	35.00	35.00	8.46	7.84	3.56	3.68
	0.1g/l Oxalic	4.50	4.00	37.50	38.33	12.26	9.91	5.62	4.51
	0.2g/l Oxalic	5.00	5.00	62.50	55.00	11.01	10.89	5.71	5.88
6000 ppm	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.1g/l GABA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.2g/l GABA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	0.1g/l Oxalic	7.50	6.33	36.50	36.67	13.40	12.77	7.13	6.80
	0.2g/l Oxalic	7.00	6.33	37.50	35.00	11.51	10.97	4.82	5.28
LSD at5%		1.35	1.94	4.24	11.55	2.82	4.88	1.73	2.84

Physiological and Biochemical Indicators

Ion Concentrations

As shown in Table 5, saline irrigation significantly affected N%, P%, and K% in plant leaves, with notable differences compared to control conditions. Increasing salinity levels significantly reduced the total phosphorus percentage in *Z. piperitum*. Notably, the highest phosphorus percentage was observed in plants irrigated with tap water (control), while the lowest values were recorded under the highest salinity level (6000 ppm) in both seasons.

Interestingly, plants irrigated with 4000 ppm saline water exhibited the highest leaf nitrogen percentage, reaching 2.33% in the first season and 2.20% in the second. In terms of potassium, the highest K% was recorded in plants exposed to 2000 ppm salinity, with values of 0.75% and 0.74% in the first and second seasons, respectively, surpassing those of plants subjected to other salinity levels or control conditions. The observed decline in leaf phosphorus percentage under increased salinity is primarily due to the physiological effects of salt stress, which reduce water potential, osmotic potential, and turgor pressure, while causing the accumulation of toxic Na^+ ions and disrupting nutrient and water uptake (Aazami et al., 2023). Additionally, maintaining a favorable K^+/Na^+ ratio is essential for salinity tolerance, as elevated potassium levels support the exclusion of Na^+ from plant cells (Arif et al., 2020). Among nutrients, nitrogen also plays a pivotal role in enhancing salinity tolerance, which the increased nitrogen percentage observed at 4000 ppm salinity may reflect the plant's adaptive response to accumulate nitrogen for proline synthesis (Ashraf et al., 2018). The data in Table 5 demonstrate that foliar application of 0.1 g/l OA significantly increased mean leaf N%, P%, and K% across both seasons, outperforming untreated plants and those subjected to other treatments. Notably, OA at 0.2 g/l produced comparable results, with no significant differences from the 0.1 g/l treatment. Additionally, GABA applied at 0.1 g/l also significantly enhanced mean leaf P% in both seasons, exceeding levels recorded in untreated plants. Similar results were reported by Song et al. (2018) and Zhang et al. (2024), who found that foliar application of OA enhances nutrient uptake, likely due to its ability to chelate metal ions and form oxalate complexes.

Table 5. Effect of spraying with GABA and OA on the total nitrogen (N%), phosphorus (P%), and potassium (K%) in leaves of Zanthoxylum piperitum plant under different salinity concentrations

Treatments		N%		P%		K%	
		1st season	2nd Season	1st season	2nd season	1st Season	2nd season
Effect of salinity (S)							
0 ppm		2.30	2.17	0.27	0.27	0.73	0.72
2000 ppm		2.07	1.99	0.22	0.21	0.75	0.74
4000 ppm		2.33	2.20	0.22	0.22	0.70	0.70
6000 ppm		0.91	0.87	0.06	0.05	0.29	0.28
LSD at 5%		0.12	0.19	0.01	0.01	0.01	0.01
Effect of spraying with osmolyte chemicals (C)							
0		1.80	1.73	0.21	0.19	0.55	0.55
0.1g/l GABA		1.54	1.47	0.21	0.23	0.57	0.56
0.2g/l GABA		1.63	1.56	0.16	0.15	0.57	0.56
0.1g/l Oxalic		2.36	2.21	0.20	0.19	0.74	0.74
0.2g/l Oxalic		2.17	2.08	0.19	0.19	0.65	0.65
LSD at 5%		0.08	0.11	0.05	0.06	0.01	0.01
Effect of SXC							
0 ppm	0	2.12	2.04	0.32	0.31	0.74	0.76
	0.1g/l GABA	2.19	2.10	0.34	0.35	0.82	0.80
	0.2g/l GABA	2.40	2.30	0.37	0.36	0.72	0.70
	0.1g/l Oxalic	2.54	2.33	0.20	0.19	0.75	0.76
	0.2g/l Oxalic	2.24	2.10	0.14	0.15	0.61	0.59
2000 ppm	0	2.55	2.46	0.26	0.22	0.76	0.74
	0.1g/l GABA	1.84	1.79	0.24	0.26	0.89	0.87
	0.2g/l GABA	1.99	1.89	0.07	0.09	0.78	0.76
	0.1g/l Oxalic	2.00	1.90	0.27	0.22	0.73	0.72
	0.2g/l Oxalic	1.98	1.93	0.26	0.27	0.59	0.60
4000 ppm	0	2.53	2.40	0.28	0.24	0.72	0.71
	0.1g/l GABA	2.12	2.00	0.25	0.30	0.58	0.56
	0.2g/l GABA	2.14	2.04	0.18	0.16	0.77	0.78
	0.1g/l Oxalic	2.50	2.30	0.21	0.23	0.70	0.72
	0.2g/l Oxalic	2.35	2.26	0.21	0.20	0.71	0.73
6000 ppm	0	0.00	0.00	0.00	0.00	0.00	0.00
	0.1g/l GABA	0.00	0.00	0.00	0.00	0.00	0.00
	0.2g/l GABA	0.00	0.00	0.00	0.00	0.00	0.00
	0.1g/l Oxalic	2.40	2.30	0.13	0.12	0.77	0.75
	0.2g/l Oxalic	2.13	2.05	0.15	0.13	0.69	0.67
LSD at 5%		0.17	0.21	0.12	0.13	0.02	0.02

The findings from both seasons, as presented in Table 5, highlight a significant interaction between salinity stress and treatments with GABA and OA. Control plants grown under non-saline conditions and treated with 0.1 g/l OA exhibited the highest leaf nitrogen (N) percentages. Additionally, plants exposed to 4000 or 6000 ppm salinity and treated with OA at 0.1 g/l also showed elevated N levels. Interestingly, untreated plants under 2000 ppm salinity recorded higher N percentages than all other treatments. These results emphasize the potential of OA to enhance nitrogen accumulation even under saline stress conditions. Similar results were obtained by Javkar and Avhad, (2023) on *Portulacaria afra*, Wei et al. (2021) on *Suaeda salsa*, and Gupta et al. (2024) on *Zea mays*. The substantial increase in leaf phosphorus percentage (0.37% in the first season and 0.36% in the second) following foliar application of 0.2 g/l GABA under normal conditions highlights the effectiveness of this treatment in promoting nutrient accumulation. In contrast, most plants exposed to salinity stress did not reach comparable phosphorus levels. Moreover, plants irrigated with 2000 ppm saline water and treated with 0.1 g/l GABA exhibited the highest potassium concentrations in both seasons, outperforming both the untreated control and all other treatment combinations. These results underscore the potential of GABA to mitigate the adverse effects of salinity and enhance nutrient uptake under both optimal and stress conditions. In this regard, Cheng et al. (2018) demonstrated that GABA enhances salinity tolerance in White Clover by stimulating antioxidant enzyme activity, mitigating ROS accumulation, maintaining C/N balance, regulating osmotic potential, and modulating the uptake of Na^+ and K^+. Additionally, exogenous GABA application enhances plant stress tolerance by regulating osmotic balance, protecting the photosynthetic apparatus, reducing Na^+ and Cl^- accumulation, and increasing Mg^{2+} and K^+ levels in leaves (Al-Khayri et al., 2024). Similarly, Su et al. (2019) reported that elevated GABA levels under normal or stress conditions reduce Na^+ and ROS accumulation, activate H^+-ATPase, and alleviate phosphorus and potassium deficiencies, findings consistent with the present study.

Total Chlorophylls, Carbohydrates, and Proline Contents

As shown in Table 6, saline irrigation significantly reduced total chlorophyll and carbohydrate concentrations in the fresh leaves of *Z. piperitum*, indicating the detrimental effects of salinity on key physiological functions. In both seasons, control plants exhibited the highest chlorophyll and carbohydrate levels, followed by those irrigated with 2000 ppm saline water. Although the difference in chlorophyll content between these was not significant, a significant reduction in carbohydrate concentration was observed. With increasing salinity, both parameters progressively declined, with the lowest values at 6000 ppm. These findings play a critical role in optimal conditions by supporting chlorophyll production, carbohydrate metabolism, and plant health. The results are consistent with those reported by Othman et al. (2023) and Shalaby and Ramadan (2024). Conversely, the lowest proline concentrations in leaf tissues were observed in plants irrigated with the highest salinity level (6000 ppm), followed by those receiving tap water (control), with significant differences between them. In contrast, proline levels increased progressively with rising salinity, particularly at 2000 ppm and 4000 ppm. Plants exposed to these moderate salinity levels consistently exhibited the highest mean proline accumulation across both growing seasons, highlighting their pivotal role in osmoregulation, detoxifying ROS, and mitigating salinity stress.

Table 6. Effect of spraying with GABA and OA on chlorophylls (SPAD), total carbohydrates%, and Proline contents (µ moles/g FW) in leaves of Zanthoxylum piperitum plant under different salinity concentrations

Treatments		Chlorophylls(SPAD)		Total Carbohydrates%		Proline(µ moles /g FW leaves)	
		1st season	2ndSeason	1st season	2nd season	1stseason	2ndseason
				Effect of salinity (S)			
0 ppm		32.55	33.27	27.98	27.75	0.55	0.54
2000 ppm		36.58	38.24	23.45	24.56	0.68	0.69
4000 ppm		28.83	29.05	22.04	22.50	0.56	0.57
6000 ppm		14.78	15.55	10.02	9.99	0.24	0.24
LSD at 5%		5.33	5.75	2.92	3.34	0.05	0.05
				Effect of spraying with osmolyte chemicals (C)			
0		21.95	22.88	19.54	20.32	0.38	0.39
0.1g/l GABA		26.53	26.78	16.33	17.29	0.45	0.43
0.2g/l GABA		27.38	27.71	18.65	18.41	0.47	0.47
0.1g/l Oxalic		35.49	36.63	25.48	25.89	0.67	0.67
0.2g/l Oxalic		29.59	31.15	24.36	24.10	0.57	0.60
LSD at 5%		3.87	4.63	2.66	3.08	0.07	0.09
				Effect of S X C			
0 ppm	0	36.25	35.00	26.81	27.63	0.33	0.30
	0.1g/l GABA	32.95	36.70	25.08	26.06	0.35	0.31
	0.2g/l GABA	37.85	38.13	34.77	33.81	0.68	0.62
	0.1g/l Oxalic	37.70	36.80	27.54	26.58	0.73	0.75
	0.2g/l Oxalic	18.00	19.73	25.68	24.66	0.66	0.71
2000 ppm	0	24.60	29.27	26.65	27.43	0.72	0.69
	0.1g/l GABA	44.35	42.87	17.70	19.51	0.82	0.84
	0.2g/l GABA	41.10	43.27	23.52	24.56	0.79	0.81
	0.1g/l Oxalic	35.80	37.23	25.76	27.80	0.61	0.56
	0.2g/l Oxalic	37.05	38.57	23.60	23.52	0.47	0.56
4000 ppm	0	26.95	27.23	24.68	26.21	0.48	0.56
	0.1g/l GABA	28.80	27.53	22.55	23.58	0.64	0.55
	0.2g/l GABA	30.55	29.43	16.30	15.27	0.42	0.45
	0.1g/l Oxalic	32.05	34.90	22.82	22.98	0.65	0.63
	0.2g/l Oxalic	25.80	26.13	23.85	24.48	0.63	0.67
6000 ppm	0	0.00	0.00	0.00	0.00	0.00	0.00
	0.1g/l GABA	0.00	0.00	0.00	0.00	0.00	0.00
	0.2g/l GABA	0.00	0.00	0.00	0.00	0.00	0.00
	0.1g/l Oxalic	36.40	37.60	25.82	26.22	0.70	0.76
	0.2g/l Oxalic	37.50	40.17	24.30	23.74	0.51	0.46
LSD at 5%		7.74	9.27	5.32	6.16	0.14	0.19

Also, elevated proline levels in Portulaca oleracea L. under salinity stress indicate enhanced salt tolerance, as reported by Hnilicková et al. (2021). On the contrary, Hannachi and Van Labeke (2018) demonstrated that while proline accumulation serves as a reliable indicator of salinity-induced stress, it does not inherently enhance a plant's tolerance to high salinity conditions. The data presented in Table 6 also demonstrate that foliar applications of GABA and OA significantly enhanced total chlorophyll, carbohydrate, and proline concentrations in the leaves of *Z. piperitum* in both growing seasons. Plants treated with 0.1 g/l OA exhibited the highest mean levels of these physiological parameters, surpassing those of untreated control plants. Low-dose foliar application of OA has been shown to enhance photosynthetic pigment synthesis, osmolyte accumulation, and overall metabolic activity in plants by attributed to OA's role in maintaining membrane integrity, promoting enzymatic activities, serve as a reserve of CO_2 for photosynthesis, contribute to redox homeostasis, and act as a chelating agent, facilitating nutrient availability (Çoban and Aras, 2023). However, excessive accumulation of OA in plant tissues can be detrimental, whereas higher concentrations have been associated with growth inhibition (Li et al., 2022), necessitating precise regulation of its levels.

The data presented in Table 6 revealed a significant interaction between salinity stress and the application of GABA and OA on leaf chlorophyll content in *Z. piperitum*. Notably, plants subjected to 2000 ppm salinity and treated with any concentration of GABA exhibited the highest chlorophyll SPAD values across both seasons. Similarly, under severe salinity stress (6,000 ppm), plants treated with 0.2 g/l OA demonstrated substantial retention of chlorophyll. These compelling findings indicate that the application of GABA during moderate stress, or OA under conditions of high stress, can effectively counteract salinity-induced chlorophyll degradation. This dual approach not only enhances photosynthetic efficiency but also significantly boosts plant resilience in saline environments. These results agree with those obtained by Al-Khayri et al. (2024) and Chen et al. (2024). On the other hand, control plants (under normal conditions), which were treated with 0.2 g/l GABA, achieved a total carbohydrate concentration of 34.77% in the first season and maintained a similar level of 33.81% in the second season. However, under 4000 ppm salinity stress, plants treated with the same GABA concentration exhibited a substantial decline in carbohydrate percentage, 16.30% in the first season and 15.27% in the second. This contrast underlines the significant impact of salinity on carbohydrate metabolism, even with GABA treatment. Because it is a critical regulator of plant and carbohydrate metabolism, modulating physiological responses under both stressed and non-stressed conditions (Su et al., 2019). Under mild stress, GABA supports plant growth, whereas under intense conditions, it facilitates a shift toward defense responses. This includes the root-mediated activation of invertases to mobilize sucrose from leaves, as well as the concurrent enhancement of antioxidant production (Kaur and Zhawar, 2021). The data collected over two consecutive seasons (Table 6) demonstrate a clear trend; plants exposed to salinity stress and treated with OA or GABA consistently exhibited significantly elevated proline concentrations in their leaves compared to untreated control plants irrigated with tap water. The control plants recorded the lowest proline levels, with values of 0.33 µmol/g F.W. in the first season and 0.30 µmol/g F.W. in the second. In contrast, plants subjected to 2000 ppm salinity and treated with GABA at 0.1 or 0.2 g/l displayed the highest proline accumulation across both seasons. These findings underscore the potential role of GABA and OA in enhancing plant resilience to salinity stress by mitigating the deleterious effects of free radicals (Ansari et al., 2021). Moreover, recent investigations (Qian et al., 2024; Ullah et al., 2023; Ramzan et al., 2023) have documented notable increases in proline accumulation in plants under salinity stress following GABA treatments, thereby providing substantial support for our conclusions.

Leaf Anatomical Studies

Data presented in Table 7 indicate that the application of either GABA or OA yields promising results in mitigating the adverse effects of salinity. This finding suggests significant potential for expanding agricultural practices in desert regions where salinization severely limits arable land. Both treatments, as evidenced by improvements in vegetative traits documented in Tables 2, 3, and 4, play a critical role in enhancing plant resilience under salinity stress. Histological characters reflect the effect of the application of both treatments, which stated that treatment with OA was more effective than GABA for enhancing most histological characters under study, as shown in Table 7 and Figure 1. It's also obvious from the data in Table 7 and Figure 1 that both treatments, OA and GABA, exhibited an increase in most characters under study compared to the control. As shown in Table 7 the trait thickness of the blade exhibited an increase for both treatments over control, in addition, treatment with OA exhibited enhanced more than GABA and reached the maximum at plant treated with 0.1g OA under salinity stress at 2000 ppm by 600µ compared with control 500 µ, followed by plants treated with 0.2 g/l OA under salinity at 4000 ppm, those plants treated with 0.1g GABA under salinity stress at 2000 ppm, or treated plants with 0.2 g/l GABA under salinity at 4000 ppm, by 585 µ, 560 µ, and 540 µ compared with control, respectively. The most pronounced decline was observed at 250 µ, where plants treated with 0.2 g/l of OA and exposed to severe salinity stress (6000 ppm) exhibited outcomes significantly inferior to those of the control group. Moreover, under the same salinity conditions (6000 ppm), treatments with GABA resulted in complete plant mortality. The trait thickness of midrib kept the same trend, which recorded an increase at all treatments and reached the maximum at plants treated with 0.1 OA under salinity stress at 2000 ppm by 687 µ over the control 562.5 µ, whereas planted treated with 0.2 OA under salinity stress at 6000 ppm, exhibited the lowest value at this trait by 312.5 µ, below the control. According to mesophyll thickness, which is divided into palisade and spongy tissues. Related to the thickness of palisade tissue, it is obvious from Table 7 and Figure 1 that plants treated with 0.1g OA under salinity stress at 2000 ppm exhibited the highest value compared to all treatments and control, which recorded 225 µ, followed by plants treated with o.2 g OA under salinity stress at 4000 ppm by 200 µ, over the control which recorded 187.5 µ. On the other hand, plants treated with GABA at any concentration under salinity stress in 2000 or 4000 ppm, as well as plants treated with 0.2 g/l OA under salinity stress at 6000 ppm, showed a decrease in performance compared to other treatments or the control group. Specifically, the reductions were 150 µ, 180 µ, and 62.5 µ, respectively. Whereas the trait thickness of spongy tissue increases in all treatments over the control and reaches the maximum at treatment with 0.1g OA under salinity stress at 2000 ppm or 0.1g GABA under salinity stress at 2000 ppm, which recorded the same value by 300 µ over the control 237.5 µ, followed by 0.2g OA under salinity stress at 4000 ppm, or 0.2g GABA under salinity stress at 4000 ppm by 290 µ and 240 µ respectively. The lowest value was recorded at treatment 0.2g OA under salinity stress at 6000 ppm by 131µ.

It is obvious from the data mentioned before that both treatments share to reduce the harmful effect of salinity and that is verified by the harmonious results as shown in tables 2,3, 4, and 7 and that logic where application of OA plays an important role for protecting the plants against the highest concentrate of salinity more than GABA. These results agree with (Amalia and Rachmawati, 2023), who noticed that OA are important intermediaries in carbon metabolism in plant cells and play a role in controlling cell physiology, including signaling messengers that overall increase plant resistance to some extent in less than optimum environmental conditions. Also, Anwar et al. (2018) reported that the increase in leaf area showed an increase in water ingress to plant tissues, which played a positive role in water uptake, or transport of OA to vegetative organs, thus supporting vegetative growth. Additionally, Martínez-Esplá et al. (2014) noted that OA positively affects photosynthesis, as well as water and nutrient uptake, which encourages vegetative growth and helps prevent or delay the degradation of chloroplasts and chlorophyll. Zhang et al. (2024) and Chen et al. (2024) reported that, although the precise mechanism of action of OA remains unclear, its application in plant growth has been associated with the mitigation of ROS accumulation, an increased ratio of unsaturated to saturated fatty acids, and elevated levels of glucose and fructose. These effects are further accompanied by enhanced activity of antioxidant enzymes linked to improved stress resistance. On the other hand, Kaur and Zhawar (2021) reported a positive correlation between γ-aminobutyric acid (GABA) levels and stress tolerance. Moreover, the foliar application of GABA has been found to significantly reduce levels of ROS, maintain membrane stability, and fine-tune the interplay of phytohormones. This powerful combination not only enhances plant resilience but also empowers them to thrive under various moderate stress conditions.

Table 7. Measurements in microns of certain histological features in transverse sections through the blade of the Zanthoxylum piperitum plant affected by both treatments, OA and GABA, under salinity stress

Treatments	Thick. Of Blad. (µ)	Thick. of Midribe. (µ)	Thick. of Mesophyll	
			Thick. of Palisad. (µ)	Thick. of Spongy (µ)
control	500	562.5	178.5	237.5
0.1g/l OA+2000 ppm	600	687	225	300
0.2g/l OA+4000 ppm	585	575	200	290
0.2g/l OA+6000 ppm	250	312.5	62.5	131
0.1g/l GABA+2000 ppm	560	600	150	300
0.2g/l GABA+4000 ppm	540	575	180,5	240

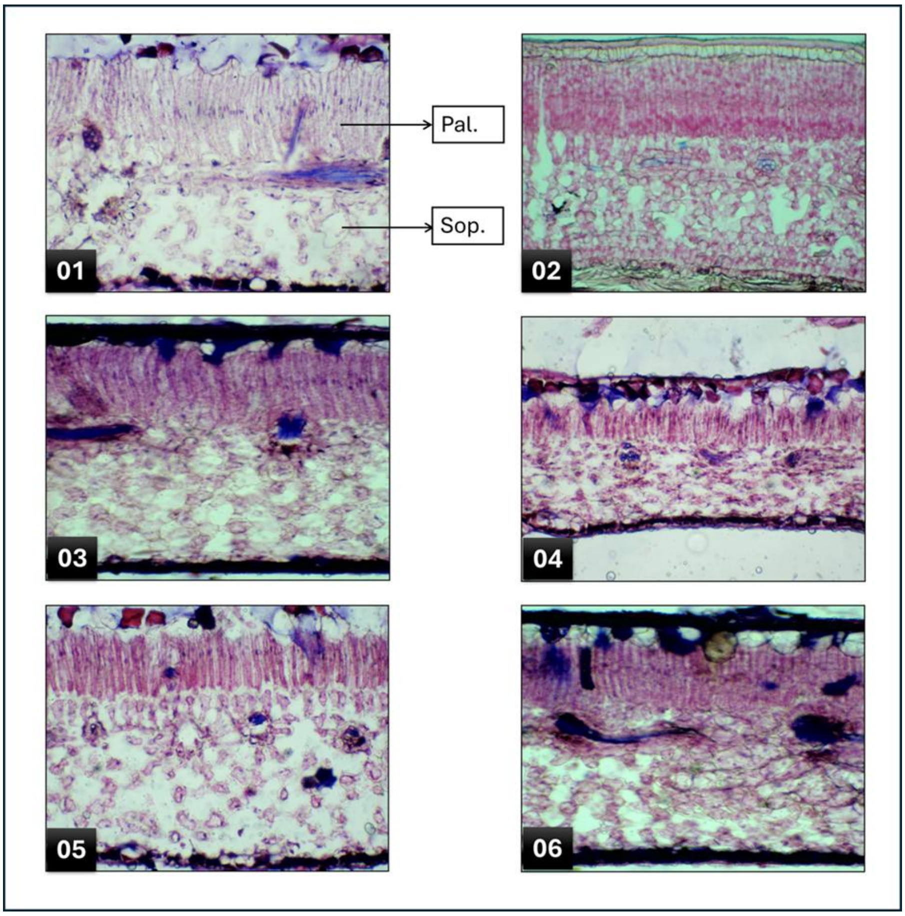

Figure 1. Transverse sections of the leaf blade of *Zanthoxylum piperitum* plants subjected to varying salinity levels and treated with GABA or OA were examined and compared with the control. Pal: palisade; spo: spongy

(01) control; (02) 0.1g/l OA+2000 ppm saline-stress; (03) 0.2g/l OA+4000 ppm saline-stress; (04) 0.2g/l OA+6000 ppm saline-stress; (05) 0.1g/l GABA+2000 ppm saline-stress; (06) 0.2g/l GABA+4000 ppm saline-stress.

CONCLUSION

In summary, salinity stress inhibits plant height, stem diameter, the number of branches per plant, and the biomass of leaves and stems, as well as root development parameters such as root length, the number of lateral roots per plant, and root dry and fresh weight in Japanese pepper (*Zanthoxylum piperitum*) plants. Additionally, salt stress reduced nutrient content and total carbohydrate levels, while inducing chlorophyll degradation. However, the foliar application of GABA and OA effectively modulates morphological, physiological, biochemical, and anatomical parameters, thereby enhancing salt tolerance in *Z. piperitum* plants under salinity stress. Foliar application of OA at a lower concentration of 0.1 g/l enhances nutrient availability, particularly nitrogen, and increases total carbohydrate concentrations, promotes proline accumulation, and mitigates salinity-induced chlorophyll degradation, thereby increasing chlorophyll content and improving photosynthetic efficiency. These effects contribute to delayed plant senescence, sustained growth, and improved histological characteristics of leaves, ultimately reducing physiological toxicity under severe salinity stress conditions. whereas higher concentrations of OA have been associated with growth inhibition, necessitating precise regulation of its levels. Notably, OA proved more effective than GABA in alleviating the effects of severe salinity stress in Japanese pepper plants. While GABA exhibited potential in mitigating salinity-induced damage by enhancing nutrient uptake, particularly phosphorus and potassium, improving photosynthetic efficiency, and regulating carbohydrate metabolism, its effectiveness was primarily observed under moderate salinity conditions. These findings demonstrate that both OA and GABA serve as effective, sustainable, and environmentally friendly strategies for mitigating salinity-induced stress in plant production. This approach presents a promising avenue for addressing the growing challenges associated with climate change and ensuring agricultural resilience.

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تحسين النمو وتحمل الملوحة في نباتات Zanthoxylum piperitum باستخدام حمض الغاما-أمينوبيوتيريك (GABA)وحمض الأكساليك الخارجي: رؤى تشريحية وكيميائية حيوية وفسيولوجية

امال عبدالخالق محمد هيكل ، سناء اسماعيل احمد اسماعيل و إيمان زكى عثمان

قسم بساتين الزينة –كلية الزراعة- جامعة القاهرة –الجيزة – مصر

الملخص

الإجهاد الملحي، وهو أحد العوامل غير الحيوية ، يحد بشكل كبير من إنتاجية النباتات على مستوى العالم. ومع تفاقم هذا التحدي بفعل النمو السكاني، والاحتباس الحراري، وتغير المناخ، فإنه يشكل تهديدًا خطيرًا للزراعة المستدامة. لذلك، أصبح من الضروري إعطاء أولوية قصوى لمعالجتة على الأنظمة النباتية. وفي هذا السياق، هدفت الدراسة الحالية إلى تقييم فعالية التطبيقات الورقية لحمض الغاما-أمينوبيوتيريك (GABA) وحمض الأكساليك (OA) بتركيزات مختلفة في التخفيف من آثار الإجهاد الملحي على ***Zanthoxylum piperitum***. تم تنفيذ التجربة باستخدام تصميم القطع المنشقة ضمن تصميم القطاعات الكاملة العشوائية (RCBD) في مشتل الزينة بكلية الزراعة جامعة القاهرة في مصر، خلال موسمين 2023 و2024. أظهرت النتائج أن الإجهاد الملحي، بأي جرعة، له تأثير سلبي على الخصائص الفسيولوجية والمورفولوجية والتشريحية للنبات. وعلى النقيض من ذلك، أدت إضافات GABA و OA إلى تحسين بعض الخصائص بشكل ملحوظ. وأظهر حمض الأكساليك (OA) بتركيز 0.1 غرام/اللتر فعالية في تعزيز نمو النبات وزيادة تراكم الكتلة الحيوية وتوفر العناصر الغذائية، لا سيما النيتروجين. كما ساهم في رفع تركيز الكربوهيدرات الكلية، والبرولين، والحد من تحلل الكلوروفيل. كذلك، أدى إلى تحسين الخصائص النسيجية للأوراق، مما قلل من السمية الفسيولوجية تحت ظروف الإجهاد الملحي الشديد. علاوة على ذلك، أثبت GABA فعاليته في التخفيف من الآثار السلبية للإجهاد الملحي من خلال تحسين امتصاص العناصر الغذائية، وخاصة الفوسفور والبوتاسيوم، بالإضافة إلى تحسين كفاءة التمثيل الضوئي، وأيض الكربوهيدرات تحت الظروف الملوحة المعتدلة. بشكل عام، أظهرت التطبيقات المنخفضة من OA تحت ظروف الملوحة المعتدلة أو الشديدة قدرة على تعزيز الخصائص المورفولوجية والفسيولوجية والتشريحية، مما يدعم نمو نباتات ***Z. piperitum***وتُبرز هذه النتائج فعالية حمض الأكساليك (OA) كاستراتيجية صديقة للبيئة في التخفيف من آثار الإجهاد.

الكلمات الدالة: *Zanthoxylum piperitum*؛ الاحتباس الحراري؛ إجهاد الملوحة؛ الخصائص التشريحية؛ الصفات المورفولوجية والفسيولوجية.