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Application of *Spirulina platensis* for developing stress tolerant and biochemical activity of micropropagated *Moringa oleifera* plant

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Abstract

In recent years, the efforts to develop a stress tolerant of the plant are of great importance for increasing crop yield using tissue culture as a feasible and cost-effective tool for this purpose. This work aimed firstly to establish an *in vitro* culture of *Moringa oleifera* using two different types of culture media (MS and WPM) at two strengths (full or half) and three concentrations of benzyladenine (0.1, 0.2 or 0.3 mg l^{-1}) in combination with 0.05 mg l^{-1} of IBA. Secondly; the Application of *Spirulina platensis* extract at 1 g l^{-1} as an effective stimulant on the micropropagated plants on various levels of saline media (0, 1000, 2000, 4000, and 6000 ppm of NaCl) to mitigate the effect of salinity stress. The results showed that full strength of MS culture medium supplemented with 0.3 BA mg/l + 0.05 mg/l IBA +1g charcoal /l caused the highest survived and proliferated shootlets with the highest number of roots. Alleviation of salinity stress on the *in vitro* grown plants at all tested levels using the extract of *S. platensis* in the culture medium had noticed while, increasing salinity level to 6000 ppm caused a noticeable decline of micropropagation ability. The highest content of total flavonoid was recorded at 4000 ppm of salinity in the presence or absence of *S. platensis* in the culture media which also led to a decrease in the content of H_2O_2 radicals in shootlets. The further improvement effect of *S. platensis* was noticed by superoxide dismutase and glutathione peroxidase activities in shootlets grown under saline condition.

Keywords; Moringa oleifera, in vitro, salinity, Spirulina platensis, and superoxide dismutase activity

1. Introduction

Moringa oleifera tree belongs to the family Moringaceae. It is found mostly in tropical, subtropical and semi-arid areas in Africa and tropical Asia to the westward in Egypt. It grows in all the phytogeographical regions, but its native region is India. It is a fast-growing, and drought-resistant tree [1,2]. Moringa trees are known as “miracle trees” due to their numerous uses. It is a tall, evergreen tree with a corky, whitish bark and straight trunk. The tree has a tubular tap root system. Leaves are compound, tri-pinnate with several small leaflets and the color is palish green. The flowers are yellowish-white [3]. Humans have consumed all parts of *M. oleifera* in various ways. The high concentration of minerals, vitamins, proteins, carbohydrates, fiber, free proline, phenolics and phytohormones was found in moringa leaves extract [4].

The growth of *M. oleifera in vitro* is faster than in the greenhouse [5]. Morphogenesis and growth of *in vitro* cultures are extremely influenced by several factors as culture medium composition [6]. MS medium [7] consists of all the nutrients essential for plant growth *in vitro*, So it is the most used one, While Woody plant Medium (WPM- medium) was established by [8] and it is used in the propagation of many woody plants. Also, the plant growth regulators

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selection is one of the factors that affect *in vitro* culture explants activity [9].

Salinity of the most important problem in Egypt and it is one of the environmental factors which inhibit plant productivity, particularly in arid and semiarid areas [10]. Salinity leads to ion toxicity, plant growth change, membrane instability as a result of replacing sodium instead of calcium and an increase in respiratory rate [11]. It also reduces the rate of photosynthesis [12], decreases protein synthesis and enzymatic activities [13]. The tissue culture technique helps us to study the influence of salinity on plants under controlled conditions. This technique has been successfully used by a wide range of plant species in the world [14].

The currently available strategies to alleviate the abiotic stresses which strongly affect the productivity of the plant are few [15]. The external addition of some fertilizers, plant growth regulators and osmoprotectants decreases the damages caused by the salt stress [16], [17]. Biostimulants are products derived from organic matter which used in small quantities that are capable to encourage the growth and development of different plants under stressful and optimal conditions [18], [19]. It can alleviate the harmful effects of abiotic stress by improving the beneficial phytochemicals content of plant, as well as inducing stress-related genes and antioxidant molecules [20]. Among the naturally occurring plant growth enhancers, microalgae and macroalgae that have received much attention due to the presence of cytokinins such as zeatin in addition to antioxidants such ascorbic acid, flavonoids, phenols, carotenoids, amino acids, and macro and micronutrients in their cells. Green and blue-green algae showed many biostimulants activities, including increments in seed germination and seedling growth, crop yield, nutrient utilization efficiency and nutrition as well as the functional quality of the plant products, and tolerance to abiotic stress [21]. One of the most important features that make microalgae valuable, it contains a high proportion of macronutrients and a large number of micronutrients and amino acids [22].

Spirulina platensis is a kind of photosynthetic cyanobacteria [23]. These cyanobacteria have a high content of essential amino acids, vitamins, minerals, essential fatty acids, and protein [24]. In addition, *Spirulina platensis* exhibited activities similar to IAA “indole acetic acid" and GA “gibberellin". The influence of *S. platensis* on plant growth rate is positively clear under normal or stress conditions [20]. Like the growing plants under saline stress conditions through its valuable components that remove reactive oxygen species (ROS) and mitigate their negative effects by regulating the plant metabolism [23].

This study aimed to optimize the *in vitro* culture growth of *Moringa oleifera* plant and the application of *Spirulina platensis* as an eco-friendly approach for addressing saline conditions on micropropagated plants.

2. MATERIALS AND METHODS

The present investigation was conducted at the Tissue Culture Technique Laboratory, Department of Ornamental Plants and Woody Trees, National Research Centre (NRC), Egypt, during the years 2022 and 2023.

2.1. Procedure layout

Plant material and surface sterilization

Seeds were harvested from *M. oleifera* tree maintained at National Research Centre, Egypt and prepared by washing under tap water with a few drops of liquid soap for 1h and rinsed three times in sterile demineralized water. Healthy uniform seeds were surface sterilized under aseptic conditions in a laminar air flow hood. Initially, seeds were disinfested by stirring in 70% (v/v) ethanol solution for 30 sec., followed by 15% (v/v) commercial sodium hypochlorite solution (NaOCl 5.25%) with shaking for 7 min. then, rinsed three times with autoclaved distilled water. For 2 min., seeds were soaked in silver nitrate 0.2% (v/v). Afterward, the seed surfaces were immersed in mercuric chloride ($Hg\ Cl_2$) 0.2% for 5min. and finally, the seeds were rinsed three times with autoclaved distilled water. The germination of seeds was on MS [7] basal medium at full strengths supplemented with sucrose at 2.5% (w/v) and solidified with agar at 0.7% (w/v) which was adjusted to 5.7 ± 0.2 pH medium, followed by incubation in the dark until germination.

Culture medium and incubation condition

For the first experiment, four weeks old germinated seeds, the epicotyls were transferred to two different types of culture media {MS and woody plant medium (WPM)} at two strengths (full or half strength) and three benzyladenine (BA) concentrations (0.1, 0.2 or 0.3 mg l^{-1}) in combination with 0.05 mg l^{-1} indol-3-butyric acid (IBA), sucrose at 25g l^{-1}, agar at 7g l^{-1} and activated charcoal at 1g l^{-1}.

On a growth chamber at 25 ±2 °C under photoperiod 16h of fluorescent light with 30µmol m^2/sec, all cultures media were incubated for three months. Survival of explants (%), shootlets number/explant, shootlets length (mm), as well as rooting parameters (rooting percentage (%), roots number/shootlets and root length (mm)) were recorded.

Cultivation and harvesting of algal cells

Spirulina platensis was cultivated at National Research Centre, Egypt in 300 liters of Zarrouk's medium. The purity of the cultures was checked periodically by microscopic observation following classification instructions. All solutions and glassware were sterilized at 121 °C for 15 min before use. The growth rate of *Spirulina platensis* was monitored every three days during the cultivation period by determination of dry weight and optical density at 670 nm by Vonshak methods (1997)[25]. Cells were harvested in the stationary phase by centrifugation at 10,000 (4°C) for 15 min and the biomass was stored in -20 °C.

Application of *S. platensis* cell on micropropagated plants under saline conditions:

The optimized culture medium from the previously tested multiplication media was full strength MS supplemented with 0.3 mg l^{-1} BA and 0.05 mg l^{-1} IBA that was selected as a multiplication culture medium where the maximum growth performance of *M. oleifera* shootlets was obtained and repeated to produce a massive desired number of homogeneous shootlets. This culture medium was supplemented with different five concentrations of NaCl (0, 1, 2, 4, and 6 g l^{-1}).

Spirulina platensis cell was added to the various saline media at 1 g l^{-1} as an effective stimulant to mitigate the effect of salinity stress, where ten elicitation media were prepared and incubated under the same conditions for the first experiment.

In vitro proliferated shootlet parameters were represented in the survived explants (%), shootlets number/ explant, shootlets length (mm), as well as the *in vitro* root growth parameters (rooting percentage %, roots number/shootlets and root length mm), data were recorded after two months.

2.2 Biochemical analysis

Shootlets extraction

Shootlets of *Moringa oleifera* (10g) were soaked in 100 ml 80 % ethanol and shacked at room temperature for 48 h. The extracts were filtered and extracted twice. The final extract was used for the assay of the phenolic compound. Total phenols were assayed using Folin–Ciocalteu's reagent [26]. The tannins content was determined according to Tambe et al. (2014) [27]. Total flavonoid was assayed by the method of Zhishen et al., (1999) [28].

Free radical determination

The hydrogen peroxide content in fresh shootlets of *Moringa oleifera* was evaluated by Shi et al. (2005) [29] method. Fresh samples (0.5 g) were homogenized with 5 ml of (m/v) trichloroacetic acid (TCA, 0.1 %) and centrifuged at 12000 g for 15 min. Then, 1 ml of supernatant was mixed with 1 ml of 100 mM potassium phosphate buffer (pH 7.0) and 2 ml of 1 M potassium iodide. The absorbance was measured at 390 nm and the H_2O_2 was calculated by using a standard curve from different concentrations of H_2O_2.

Antioxidant enzymes extraction and determination of enzyme activities

Fresh shootlets of *Moringa oleifera* (0.1g) were grind and then extracted with 5 ml of ice-cold buffer phosphate (Ph 7.4). The homogenate was centrifuged at 10,000 rpm for 30 min and the supernatant was collected. The resulting supernatant was used for the determination of enzyme activities.

The enzyme Superoxide dismutase activity (SOD) was measured according to Marklund and Marklund (1974) [30].

The glutathione peroxidase activity in the supernatant was determined by the method of Chiu *et al* (1976) [31].

2.3 Statistical analysis

A randomized complete block design was used with three replicates. The treatments' means were compared for significance by Duncan's New Multiple Range test at 0.05% level of probability using COSTATV-63[32].

3. RESULTS

3.1 *In vitro* culture establishment

To establish an *in vitro* culture of *Moringa oleifera*, Table (1) and Fig.1 (a-d) showed the effect of two types of culture media (MS and WPM) at full or half strength applied with BA at different three concentrations (0.1, 0.2 and 0.3 mg/l) on micropropagation ability of shootlet explants. All treatments were combined with 0.05 mg/l of IBA and 1g/l charcoal. The results indicated that the explants could survive at the high percent (91.67-100%) on most treatments of both full and half strengths of MS and WPM culture media. While using ½ strength of WPM supplemented with 0.1 or 0.2 mg/l BA declined this percentage to the lowest ones (66.67 and 85.73%, respectively).

Table 1 Micropropagation ability of *Moringa oleifera* effecting by type and strength of culture media applied with BA (mg/l)at different concentrations, IBA at 0.05mg/l and activated charcoal at 1g/l.

Treatments	Survival (%)	Shootlet number/ explant	Shoot length (mm)	Rooting (%)	Number of roots/ shootlet	Length of roots (mm)
MS free of hormones (Control)	91.68 b	3.08 cd	93.33 a	50.08 h	3.00 de	54.96 i
MS + 0.1 BA	100.00 a	3.32 c	75.86 e	93.33 b	3.00 de	66.67 f
MS + 0.2 BA	99.97 a	4.15 b	84.99 c	91.50 c	4.00 b	55.00 i
MS + 0.3 BA	100.00 a	5.01 a	91.60 b	90.05 d	5.00 a	80.06 c
1/2 MS + 0.1 BA	100.00 a	3.11 cd	62.89 h	100.0 a	1.99 fgh	110.1 a
1/2 MS + 0.2 BA	100.00 a	3.42 c	71.79 fg	74.53 f	2.53 ef	100.1 b
1/2 MS + 0.3 BA	91.67 b	4.86 a	80.84 d	66.65 g	3.67 bc	60.06 h
WPM + 0.1 BA	100.00 a	4.67a	72.77 fg	80.08 e	3.33 cd	78.66 d
WPM + 0.2 BA	100.00 a	2.55 de	75.02 e	66.69 g	3.00 de	27.47 j
WPM + 0.3 BA l	100.00 a	1.58 f	81.26 d	41.67 i	1.67 gh	23.37 k
1/2 WPM + 0.1 BA	66.67 d	2.67 d	54.46 j	100.0 a	3.47 bcd	75.52 e
1/2 WPM + 0.2 BA	85.73 c	2.11 e	58.37 i	88.89 d	2.24 f	66.80 f
1/2 WPM + 0.3 BA	100.00 a	1.34 f	70.26 g	66.69 g	1.54 gh	64.67 g

Means within a column having the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level

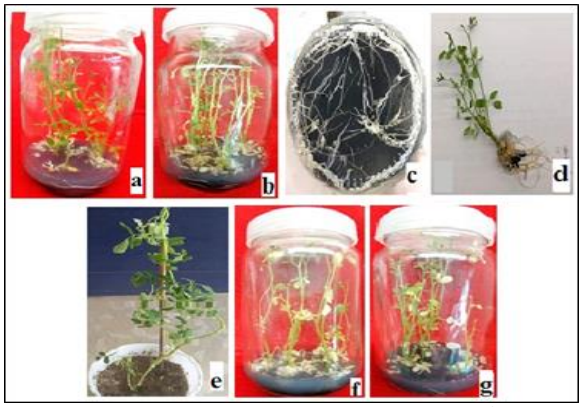

Fig. 1 (a-g): *In vitro* shooting and rooting ability of *Moringa oleifera*, (a) Shootlets development on MS free of hormones (Control), (b) Shootlets development that were produced from using full strength MS supplemented with 0.3 mg l-1 BA and 0.05 mg l-1 IBA (optimized culture medium), (c) Rooting of shootlets cultured on the optimized culture medium, (d) Prepared rooted plantlets for the acclimatization stage, (e) Acclimatized plants to greenhouse, (f) Shootlets cultured on the optimized culture medium + NaCl at 2000 ppm and (**g**) Shootlets cultured on the optimized culture medium + NaCl at 2000 ppm + *Spirulina platensis* at 1 ml/l.

Lowering the strength of both MS and WPM to half strength with a low concentration of BA (0.1 mg/l) resulted in the highest rooting percent (100%) compared with control and other treatments. Also, it could be observed that full strength of MS culture medium supplemented with 0.3 BA mg/l+0.05 IBA+1g charcoal/l caused the highest survived and proliferated shootlets with the highest number of roots formed per shootlet (100%, 5.01 and 5.00 respectively) as compared to control which caused the longest shootlets (93.33 mm).

The *in vitro* survived and rooted plantlet were successfully acclimatized to greenhouse in growth media (1 peat moss: 1 perlite (v: v)). The successfully acclimatized plants were 80%, as shown in Fig.1 (e).

4.2 Effect of *S. platensis* on micropropagated *Moringa oleifera* under salinity stress:

From the recorded results in Table (2), the explants of *Moringa oleifera* could be propagated *in vitro* on the optimized culture medium (MS + 0.3 BA mg/l + 0.05 IBA mg/l +1g charcoal/l) and survived to the highest percent (93.33- 100%) under salinity levels that attained to 4000 ppm, while increasing this level to 6000 ppm caused a noticeable decline of the micropropagation ability of shootlets. Using *S. platensis* in the culture medium had a clear promotion effect to alleviate the salinity stress at all tested levels (2000, 4000 and 6000 ppm) on micropropagated plants. This promotion effect of *S. platensis* was indicated with the highest values of survival % and shootlet number with the longest roots (100%, 5.40 shootlets and 90.00 mm, respectively) for the micropropagated shoots under 2000 ppm of salinity as compared with control which were represented in Fig.1(f and g).

Table 2 Effect of *Spirulina platensis* on micropropagated *Moringa oleifera* under various salinity stress levels

Treatments	Survival %	Shootlet number/ explant	Shootlet length (mm)	Rooting %	Number of roots	Length of roots (mm)
OMS (Control)	100.00 a	4.00 ab	82.00 a	93.30 b	5.0 ab	82.00 b
OMS + *Spirulina platensis* 1 ml /l	100.00 a	3.83 ab	81.00 ab	100.00 a	5.3 a	94.00 a
OMS +1000 ppm NaCl	100.00 a	3.63 abc	70.00 d	93.33 b	4.0 bc	65.30 d
OMS +1000 ppm NaCl + *Spirulina platensis* 1 ml/l	100.00 a	3.67 abc	79.00 bc	100.00 a	4.7 ab	75.00 c
OMS + 2000 ppm NaCl	93.33 b	3.63 abc	65.33 e	80.00 d	3.0 cd	70.00 cd
OMS + 2000 ppm NaCl+ *Spirulina platensis* 1 ml/l	100.00 a	5.40 a	77.00 c	86.00 c	4.0 bc	90.00 a
OMS + 4000 ppm NaCl	93.33 b	3.33 abc	52.00 g	33.00 f	1.3 e	40.00 f
OMS + 4000 ppm NaCl+ *Spirulina platensis* 1 ml/l	100.00 a	3.60 abc	55.67 f	66.00 e	2.7 d	47.00 e
OMS + 6000 ppm NaCl	66.00 d	1.33 c	35.00 h	0.00 h	0.0 f	0.00 h
OMS + 6000 ppm NaCl+ *Spirulina platensis* 1 ml/l	87.00 c	1.77 bc	50.00 g	13.33 g	1.0 e	30.00 g

OMS → Optimum MS media (MS + 0.3 BA mg/l + 0.05 IBA mg/l +1g charcoal / l)

"Means within a column having the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level"

4.3 Effect of *S. platensis* on the chemical composition of *in vitro* grown *Moringa oleifera* under salinity stress:

Table (3) showed that antioxidant compounds such as total phenols and flavonoids were remarkably increased at all salinity stress levels, while tannin contents were decreased even with the highest level of salinity (6000 ppm) as compared with the control treatment. Applying *S. platensis* in the culture media enhanced the antioxidant compound values (total phenols, tannins and flavonoids) in the micropropagated shoots under saline conditions.

Table 3 Effect of *Spirulina platensis* on the chemical composition of micropropagated *Moringa oleifera* explants under salinity stress

Treatments	Phenols(µg/g)	Tannin(µg/g)	Flavonoid (µg/g)
OMS	15.53 d	1.31 ef	2.90 d
OMS+*Spirulina platensis* 1 ml /l	15.11 d	1.26 f	2.68 d
OMS+1000 ppm NaCl	18.16 c	1.34 ef	3.55 d
OMS+1000 ppm NaCl+ *Spirulina platensis* 1 ml/l	19.00 c	1.40 e	3.53 d
OMS+2000 ppm NaCl	19.72 c	2.26 d	10.16 b
OMS+2000 ppm NaCl+ *Spirulina platensis* 1 ml/l	19.30 c	2.9 c	11.23 ab
OMS+4000 ppm NaCl	20.99 b	3.16 b	12.01 a
OMS+4000 ppm NaCl+ *Spirulina platensis* 1 ml/l	21.88 b	4.45 a	11.96 a
OMS+6000 ppm NaCl	25.37 a	0.15 g	8.27 c
OMS+6000 ppm NaCl+ *Spirulina platensis* 1 ml/l	25.13 a	0.11 g	7.98 c

OMS → Optimum MS media (MS + 0.3 BA mg/l + 0.05 IBA mg/l +1g charcoal / l)

"Means within a column having the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level"

4.4 Hydrogen peroxide

As illustrated in Table 4, the salt stress increased the content of H_2O_2 concentration in *M. oleifera* shootlets as compared with the control. The values of H_2O_2 were increased by increasing the salt concentrations, reached to 3.92 µg/g at 6000 ppm of NaCl, however, the addition of *S. platensis* in the culture medium led to decreasing in these values.

4.5 The activity of Antioxidant Enzymes

Data cleared that, salt-stressed moringa showed increased enzyme activities of superoxide dismutase (SOD) and glutathione peroxidase that were detected

as compared to those in the control. The addition of *S. platensis* caused further improvement of enzyme activities in moringa shootlets *in vitro* grown under saline conditions. Moringa shootlets exposed to combined treatment of 4000 ppm NaCl and *S. platensis* recorded the highest value of superoxidase dismutase activity (5.7 U/g), while the maximum activity of glutathione peroxidase (GPx) (90.02) obtained with 6000 ppm of NaCl.

Table 4 Effect of salinity stress and *Spirulina platensis* on hydrogen peroxide and antioxidant enzyme (SOD, GPx) of micropropagated *Moringa oleifera*

Treatments	H_2O_2 (µg/g)	SOD (U/g)	GPx (U/g)
OMS	0.37 b	3.14 b	49.99 h
OMS + *Spirulina platensis* 1 ml /l	0.23 b	3.17 b	51.68 h
OMS +1000 ppm NaCl	1.62 ab	3.22 b	54.16 g
OMS +1000 ppm NaCl + *Spirulina platensis* 1 ml/l	0.64 b	4.30 ab	65.63 f
OMS + 2000 ppm NaCl	2.80 ab	3.79 ab	68.08 e
OMS + 2000 ppm NaCl+ *Spirulina platensis* 1 ml/l	0.53 b	5.11 a	77.53 c
OMS + 4000 ppm NaCl	2.8 ab	3.90 ab	70.49 d
OMS + 4000 ppm NaCl+ *Spirulina platensis* 1 ml/l	0.55 b	5.70 a	80.77 b
OMS + 6000 ppm NaCl	3.92 a	4.03 ab	90.02 a
OMS + 6000 ppm NaCl+ *Spirulina platensis* 1 ml/l	0.90 b	5.40 a	78.69 c

OMS → Optimum MS media (MS + 0.3 BA mg/l + 0.05 IBA mg/l +1g charcoal / l)

"Means within a column having the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5% level"

4. Discussion

The important role of cytokinin for induce shootlet multiplication was explained in Table (1), that the presence of BA at any concentration in the culture medium caused the highest proliferation of shootlets and reduced their heights. Confirmed studies [[33], [34]] stated that the endogenous hormonal content in *Moringa oleifera* nodal segments was adequate to induce shoot formation and the application of exogenous cytokinin improved this process as was observed in other plants. The strong effect of culture medium and cytokinin concentration on *in vitro* propagation ability has been conducted in previous studies. The cultural medium composition has a key important role to induce new proliferated shoots [35]. Taha et al. (2018) [36] observed the highest formed shoots per explants of *Dillenia indica* on MS or WPM supplemented with BA. Youssef et al. (2021[a]) [37] on *Sequoia sempervirens* found that rooting ability was best on half strength of MS medium added with BA. The explants of *Antigonon leptopus* were able to develop on MS culture medium supplemented with cytokinin plus IBA and showed more proliferation [38].

From the results in Table (2), it seemed that the explants of *Moringa oleifera* could be survive under various salinity levels but, increasing this level to 6000 ppm caused a noticeable decline of the proliferation ability of shootlets. The tolerance of *in vitro* propagated moringa to various salinity levels was attributed to the leaves of this tree which are rich in minerals having a high rate of antioxidant activity [39]. Allen (1995) [40] attributed the decrease in many plants' mass production under salt stress to generate of reactive oxygen species (ROS) in the chloroplasts which led to a decrease in plant growth in case of the absence of any protective system. Youssef et al. (2019) [41] observed that the highest proliferated shootlets of *Populus alba* were formed for control (salt-free MS culture medium) and all concentrations of NaCl had inhibited effect on rooting percent. In the present study, the promotion effect of *S. platensis* on the micropropagated shoots under salinity stress may be due to it being a supplement source for vitamins and phytohormones [42].

The enhanced antioxidant compound values (total phenols and flavonoids) confirm that all antioxidant defense parameters have appeared a positive relation by increasing salinity levels (Table 3), these findings were in agreement with those obtained by Azeem et al. (2023)[43] and Elkarmout et al. (2022) [44] on *Moringa oleifera* grown under saline conditions. Abdel-Magied et al. (2023) [45] mentioned that the maximum values of the antioxidant enzymes activity

as well as the total phenolic content were recorded by exposed eucalyptus plants to the abiotic stress.

The effect of salt stress on the content of H_2O_2 radicals in *M. oleifera* shootlets (Table (4)) may be attributed to that the plants exposed to salt stress may undergo osmotic stress, ion toxicity and nutritional imbalance that led to the production of reactive oxygen species (ROS) such as H_2O_2 (hydrogen peroxide), these results were in the agreement with Elkarmout, et al. (2022) [44] on moringa plants grown under oxidative stress (salinity) that were recorded high values of H_2O_2. However, *S. platensis* has an antioxidant activity against H_2O_2 and improved the activity of phenylalanine ammonia lyase (PAL), a key enzyme in activating the phytoalexin pathway that reduces H_2O_2 [46]. So, using *S. platensis* inhibit the harmful impacts of oxidative stress [47]. The enzymatic antioxidants main role as ROS scavengers, which are necessary for abiotic stress alleviation as was mentioned by Hassan, et al. (2021) [48] explained the behavior of enzyme activities in moringa shootlets under saline conditions that was increased by *S. platensis* microalga.

5. Conclusions

Moringa oleifera plant could be proliferated, grown *in vitro* and tolerated a range of salt levels. Using *S. platensis* could mitigate the harmful effects of these stress conditions and impact on the morphological and biochemical changes as well as enzyme activity of the micropropagated plants.

6. Conflicts of interest

There are no conflicts to declare.

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