International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Mitigation impacts of brassinolide, gamma radiation and endophytic fungi on *Calendula officinalis* grown under salinity stress

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Abstract

In order to evaluate the impact of brassinolide, gamma radiation and endophytic fungi on *Calendula officinalis* subjected to salinity stress, Pots experiment was carried out at the Experimental Nursery of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, during the two successive seasons of 2020/2021 and 2021/2022. Salinity stress was created using saline water at concentrations of 0, 1500 and 3000 ppm. Brassinolide was sprayed monthly at 0, 25 and 50 ppm. The seeds were irradiated with gamma rays at 0, 20, and 40 Gy. Two Endophytic Fungus *Alternari alternata* (Fungi 1) and *Chaetomium subaffine* (Fungi 2). The results indicated that, within each level of the tested treatments (brassinolide, radiation and endophytic fungi), increasing salinity to 1500 ppm decreased the vegetative growth, while 3000 ppm decreased the flowering parameters. Increasing salinity from 0 to 3000 ppm caused reduction in most of chemical constituents while caused increase in total phenols, proline, CAT and SOD, compared to control. Under the same level of salinity, the plants treated brassinolide, radiation and endophytic fungi treatments had significantly higher values of tested vegetative growth and flowering parameters and chemical constituents than those of control plants, with superiority of radiation or brassinolide, especially the highest level. Based on the outcomes, it could be suggested that for mitigating the harmful impact of salinity stress on *Calendula officinalis* plants irrigated with salinity up to 3000 ppm, the seeds could be irradiated with gamma rays at 40 Gy or the plants foliar sprayed with brassinolide at 50 ppm.

Key words: Pot-Marigold, brassinolide, gamma rays

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1. Introduction

Pot-Marigold (*Calendula officinalis*), is annual plant utilized as a medicinal and ornamental plants belonged to Asteraceae, native to the Mediterranean region from the Canary Islands to Iran. It is an upright herbaceous plant with yellow flowers that can reach a height of 80 cm. The flower is composite and measures 5 to 7 cm. Along with using the plants for landscaping activities it has medicinal properties, marigold's essential lipids and pigments are utilized as components in pharmaceutical goods, the flowers are used traditionally as antispasmodic and stimulant. Marigold has anti-inflammatory and diaphoretic properties for skin, heal burns, inflammation, and skin irritation. Calendula flowers' main ingredients include polysaccharides, essential oil, triterpenoids, and flavonoids [1,2].

Salinity is one of the most important environmental problems affecting plant growth at current times, which, along with drought, remains one of the most significant environmental issues facing agriculture globally [3]. In dry and semi-arid regions of the world, saline irrigation waters have the potential to significantly increase soil salinity levels above threshold values, which could be harmful for the plants. Plants under salt stress suffer a drop in photosynthesis and respiration rate; salinity not only effect negatively on carbohydrate, fatty acid, and protein content, but also raised the level of amino acids, especially proline. In comparison to plants produced under normal conditions, plants grown under salt stress have a much higher amount of certain secondary plant products. Plants' ability to tolerate salinity depends on how salinity interacts with other environmental factors [4]. It is necessary to use different approaches to improve the tolerance of plants to salt stressed conditions.

Alleviating salinity stress by using chemical, physical and biological strategies such as brassinolide BRs, gamma radiation and endophytic fungi are successfully used on

seeds, seedlings, or plants that are grown in salinity stress [5]. BRs have received great attention from plant scientists in the last years owing to their flexible ability to alleviate various environmental stresses.it plays a vital role to mitigate different stresses, such as salinity via improving biomass and photosynthesis, enhancing antioxidant enzymes, improving detoxifying capability, and promoting the expression of related genes [6] Gamma radiation is recognized to have a number of possible uses in agriculture in agriculture, including plant improvement, depending on the exposure level. Under salt stress, gamma radiation led to a suitable changes in shoot–root partitioning of recently fixed carbon (^{14}C), enhanced glycine betaine content, decreased protease activity, decreased the partitioning of Na+, and enhanced K+accumulation. It has been reported that irradiation of seeds increased plant tolerance of salinity compared to control [5]. Endophytic fungi is one strategy of sustainable solutions to mitigate adverse effects of salinity stress, that create a symbiotic relationship with the host plant by preventing plant diseases and producing nutrients which provide suitable conditions to tolerate salinity by promoting systemic resistance, raising the levels of useful metabolites, activating antioxidant mechanisms to scavenge reactive oxygen species (ROS), and adjusting phytohormones that influence plant growth. By controlling ion accumulation, endophytic fungi enhance nutrient absorption and preserve ionic homeostasis. This limits the flow of Na+ to leaves thereby the plants have a low cytosolic Na^+: K^+ ratio [7].

The aim of this study was to evaluate the potential use of brassinolide, gamma radiation and endophytic fungi to alleviate the negative effects of salinity on *Calendula officinalis.*

2. Material and Methods

This study was carried out at the Nursery of the National Center for Radiation Research and Technology, Egyptian Atomic Energy Authority, Cairo Egypt, during the two successive seasons of 2020/2021 and 2021/2022. The aim of this study was investigate the effect of bio stimulants (brassinolide), gamma radiation and endophytic fungi on vegetative growth and chemical constituents and essential oil of marigold (*Calendula officinalis* L.) grown under salinity stress.

2.1. Plant material

Marigold (*Calendula officinalis* L.) seeds were obtained from Floranova creative plant breeding company.

2.2. Experimental procedures

On 15th August in both seasons, the seeds of *Calendula officinalis* L were sown in plastic trays and located inside the greenhouse. After 45 days old from sowing (on the 1th of October, 2013/2014 and 2014/2015, in both seasons, respectively), uniform seedlings were transplanted to 20 cm diameter plastic pots filled with a mixture of clay + sand+ compost (1:1:1: v/v), each pot had one seedling. The physical and chemical characteristics of soil mixture were determined according to Jackson [8] and the results are presented in Table 1.

Table 1: Some physical and chemical characteristics of soil mixture used in the study during two successive seasons of 2020/2021 and 2021/ 2022

Physical properties							
Field capacity (% V)		Clay (%)	Coarse sand (%)	Fine sand (%)	Silt(%)	WHC	Soil texture
8.45		8.1	73.4	10.6	7.9	22.3	loamy sand
Chemical properties							
Macro-nutrients (ppm)							
N	P	K	Mg	PH	OM (%)	EC (dS/m)	Total carbonate (%)
21.75	9.15	20.5	4.55	7.96	0.21	2	0.29

WHC: Water holding capacity; N: Nitrogen; P: phosphorous; K: potassium; Mg: magnesium; pH: soil acidity; OM: organic matter; EC: Electrical conductivity.

2.2.1. Salinity treatments

The treatments were initiated after 15 days from the transplanting. On 15 th of October the plants were irrigated twice/week with saline water at concentration of 1500 and 3000 ppm in addition to the control (tap water, 280 ppm). Salinity concentrations water was prepared by mixing NaCl and $CaCl_2$ at the ratio of 1:1 (w/w).

2.2.2. Brassinolide treatment

On 1 th of October The plants were sprayed monthly with brassinolide at the concentrations of 25 and 50 ppm in addition to the control plants which sprayed only with tap water. Brassinolide were obtained from the Union for Agriculture Development Co., UAP, Egypt. The plants' foliage was sprayed with an automatic atomizer until the run-off point (50 ml of bio-solution / plant) was reached after adding Tween 20 as a wetting agent to the bio-solution at a concentration of 1 mL L^{-1}.

2.2.3. Radiation treatments

Seeds were irradiated with (0, 20, and 40 Gy) of gamma rays. The irradiation facility was carried out at the National Center for Radiation Research and Technology (NCRRT), Nasr city, Cairo Egypt using Cesium 137 in Gamma cell as source of gamma rays. The dose rate was 0.633 rad /sec.

2.2.4. Fungi treatment

Endophytic Fungus *Alternari alternata* (Fries) Keissler (Fungi 1) and *Chaetomium subaffine* Sergeeva (Fungi 2) which were identified in the Culture Collection of "Assiut University Mycological Center, Assiut, Egypt. Endophytic fungus was isolated from *Rosmarinus officinalis* and *Salvia officinali* and identified as *Alternari alternate* and *Chaetomium subaffine*. To purify the fungal strain, it was regularly sub-cultured on potato dextrose agar (PDA) media and then incubated at 25°C for 2 weeks to produce sufficient spores (conidia). Mycelia and spores are

cultivated on PDA media with the use of a sterile blade (No. 21) and placed in a sterile conical tube for spore dispersion (50 ml). The tube was then filled with 20 ml of pure water and vortexed for 5 min. The suspension is then filtered with a filter paper (Whatman filter paper, No. 2), with the help of distilled water, the final concentration of spore suspension (10^6 spores/mL). Seeds were soaked in 20 ml of each fungus and placed in autoclaved petri dishes containing three layers of filter paper and kept in the dark at 25°C for 3 days [9].

2.3. Experimental layout

The layout of the experiment was a split-plot design in randomized complete blocks with 21 treatments [3 salt concentrations (including the control) × 7 tested treatments], each treatment consisting of 9 pots arranged in 3 replicates, each consisting of 63 plants (3 plants from each treatment). Salinity treatments were assigned to the main plots, while tested treatments were assigned to the sub-plots and were assigned randomly under each salinity treatments.

2.4. The data recorded

2.4.1. Vegetative growth and flowering parameters

Vegetative growth parameters were recorded once at about 72 days from transplanting (On 12th December in both seasons respectively). Two samples of plants were randomly taken from each replicate to determine the growth parameters including, plant height (cm), number of leaves /plant, root length (cm), fresh and dry weights of herb (g/plant), fresh and dry weights of the roots (g/plant). Flowering parameters were continuously recorded during the flowering stage (20 th of January, at about 110 days from transplanting). The flowering parameters including, number of flower /plant, diameter of flower (cm) as well as fresh and dry weights of flower (g/plant). The average diameter of five flowers per plant was used to measure the flower's diameter.

2.4.2. Chemical constituents

Total chlorophylls and carotenoids were determined in fresh leaf samples according to the method of [10]. Dried herb samples were digested to extract nutrients and the extract was analyzed to determine concentrations of K, Ca and Na (% of dry herb) which were determined according to [11]. Total soluble phenols were determined in dried herb, (3 g) of herb were crushed and extracted with 80% ethanol at 0^{O}C for 72 hours, the ethanol being changed every 24 hours, as described by Selim *et al*. [12]. The proline content in dried herb (µ moles /g dry matter of herb) was also determined using the method recommended by [13]. Antioxidant enzyme extraction were carried out using fresh herb at 40 °C in a buffer solution (3: 1 buffer: fresh weight v/v) in a pastel. It was mortared with 100 mM potassium phosphate buffer (at pH 7.5) containing 1 mM EDTA, 3 mM DL-dithiothreitol and 5% (w/v) insoluble polyvinyl pyrolidone. The homogenates were centrifuged at 10000 g for 30 min and then the supernatants were stored in separate aliquots at 8 °C. Antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), were assayed as described by [14]. Enzymes activities were expressed as units/min/mg protein.

For determination of essential oil, collected flower air- dried at electric oven temperature (70^{o} C) for three days, then they were combined together to determine the essential oil content for each replicate by steam hydro-distillation according to [15,16]. Oil percentage was calculated as follows:

Oil %= oil volume in the graduated tube /dry weight of flower (500 g) × 100

2.5 Statistical analysis

The mean of all obtained data were subjected to statistical analysis of variance (ANOVA) in split plot design. Combined analysis of the two growing seasons was carried out. Means of data were compared by using Duncan's multiple range tests at 5% level [17].

3. Results and Discussion

3.1. Vegetative growth parameters

The data in Table 2 showed that within each level of the tested treatments (brassinolide, radiation and endophytic fungi) increasing salinity from 0 to 1500 ppm resulted in reduction in the studied vegetative growth (in terms of plant height, number of leaves/ plant, root length, dry weights of herb and roots), then tended to increase (in most cases) with increasing salinity to 3000 ppm compared to control. Similar reduction in vegetative growth parameters due to salinity stress are in harmony with the finding of previous studies [18,25].

Results in Table 2 also revealed that, under the same salinity level, treating the plants with different treatments of brassinolide, radiation and fungi caused significant ($p<0.05$) increase (in most cases) in tested vegetative growth traits compared to control (those exposed to salt stress and not received any treatments). However, radiation was superior in its effect than the others treatments and among the different doses of radiation, the highest on (40 Gy) was the most effective for increasing the tested parameter since recorded the highest values (in most cases). These results confirmed the findings of previous researches which reported increase in vegetative growth parameters due to brassinolide treatments [26-28] or endophytic fungi treatments [29,31], while the marked increase in growth characteristics due to radiation treatments is matched well with those of prior studies [32,34].

Table 2. Plant height, number of leaves, root length, dry weights of herb and roots of *Calendula officinalis* as affected by the interaction between salinity and brassinolide, radiation or endophytic fungi (mean of two seasons)

Salinity	Treatments*	Plant height (cm)	number of leaves/ plant	Root length (cm)	herb dry weight (g/plant)	Roots dry weight (g/plant)
Control	**0**	14.83±0.17l	3.83±0.17e-h	8.68±0.43gh	5.39±0.16j	0.38±0.07i-k
	BRs (1)	23.25±0.45ef	4.17±0.17d-f	8.68±0.43fg	11.24±0.18e	0.91±0.15ef
	BRs (2)	18.13±0.13k	3.9±0.1e-h	9.22±0.06e-g	6.35±0.19i	0.67±0.09f-h
	R(1)	20.53±0.78hi	5.83±0.17c	9.88±0.22de	13.29±0.36d	1.52±0.22ab
	R(2)	32.52±0.4b	6.00±0.29bc	13.12±0.49ab	14.36±0.07c	1.49±0.18b
	F(1)	22.53±0.93fg	4.33±0.33d-f	11.50±0.51c	9.41±0.25f	0.89±0.09e-g
	F(2)	33.75±0.49b	5.67±0.33c	13.38±0.20a	11.52±0.04e	1.00±0.05de
1500 ppm	**0**	14.68±0.39l	3.17±0.17hi	7.47±0.15i	3.82±0.25l	0.28±0.03k
	BRs (1)	19.33±0.22i-k	3.67±0.17f-h	8.60±0.26gh	4.83±0.14k	0.56±0.08h-j
	BRs (2)	18.12±0.32k	3.30±0.17g-i	7.85±0.32i	4.95±0.12jk	0.58±0.05h-j
	R(1)	20.22±0.96ij	5.80±0.35c	9.15±0.10e-g	8.45±0.14h	1.28±0.13bc
	R(2)	27.62±0.18d	5.83±0.33c	9.57±0.26d-f	14.18±0.16c	1.20±0.08cd
	F(1)	21.58±0.32gh	4.17±0.44d-f	8.67±0.48gh	9.14±0.12fg	0.64±0.08f-i
	F(2)	29.82±0.42c	4.50±0.29de	11.07±0.17c	9.24±0.24f	0.56±0.06h-j
3000 ppm	**0**	19.15±0.09jk	2.67±0.17i	7.63±0.29i	3.99±0.11l	0.34±0.03jk
	BRs (1)	20.48±0.43hi	4.00±0.29e-g	8.03±0.22hi	8.69±0.14fg	0.63±0.06g-i
	BRs (2)	24.07±0.26e	4.83±0.17d	8.80±0.19g	9.58±0.21f	0.69±0.06f-h
	R(1)	22.48±0.19fg	6.67±0.44ab	12.50±0.32b	16.39±0.14b	1.32±0.12bc
	R(2)	35.27±0.13a	6.83±0.17a	9.85±0.30de	17.72±0.14a	1.77±0.10a
	F(1)	21.98±0.24fg	4.83±0.33d	8.68±0.18gh	11.12±0.2e	0.79±0.02e-h
	F(2)	30.88±0.74c	6.00±0.29bc	10.30±0.15d	9.27±0.09f	0.63±0.01g-i

* BRs (1) = Brassinolide at 25 ppm, BRs (2) = Brassinolide at 50 ppm, R (1) = Radiation (20 Gy), R (2) = Radiation (40 Gy), F(1) = Fungi1, F(2) = Fungi 2. Each value represents the mean ± standard error of three replicates, means in a column with different letters indicate a significant difference for each variable at 5% level using Duncan multiple rang test

3.2. Flowering parameters

It is clear from data in Fig.1 that within each level of the tested treatments (brassinolide, radiation and endophytic fungi) increasing salinity from 0 to 1500 ppm resulted in increase in the studied flowering parameters (*i.e* number of flowers/ plant, diameter of flowers and dry weight of flowers), then tended to decrease with increasing salinity to 3000 ppm compared to control. The reductions in flowering parameters owing to salinity stress are in conformity with the finding of numerous studies [18,20,25].

Results in Figure 1 also showed that, under the same level of salinity the plants treated with any one of brassinolide, radiation and endophytic fungi had significantly higher values of tested flowering parameters (in most cases) than those of control (the plants exposed to salt stress and not received any treatments). Radiation was more effective than the others treatments especially at the maximum dose (40 Gy) which produced the highest mean values of studied parameter (in most cases).These results are in close conformity with the findings of other authors which indicated increase in flowering parameters due to brassinolide tratments [26] or endophytic fungi tratments [35,36], wherase the valuable increase in flowering traits a result of radiation treatments is matched well with those of prior studies [32,34].

The favorite effect of radiation on enhancing the vegetative growth and flowering characteristics compared to the control may be due to gamma radiation has the potential to enhance the concentration of enzymes and stimulate the endogenous gibberellic acid content, leading to the elongation of the cell wall. Additionally, the radiation dose changed the hormone's effectiveness, which changed production of shoots [37,38].

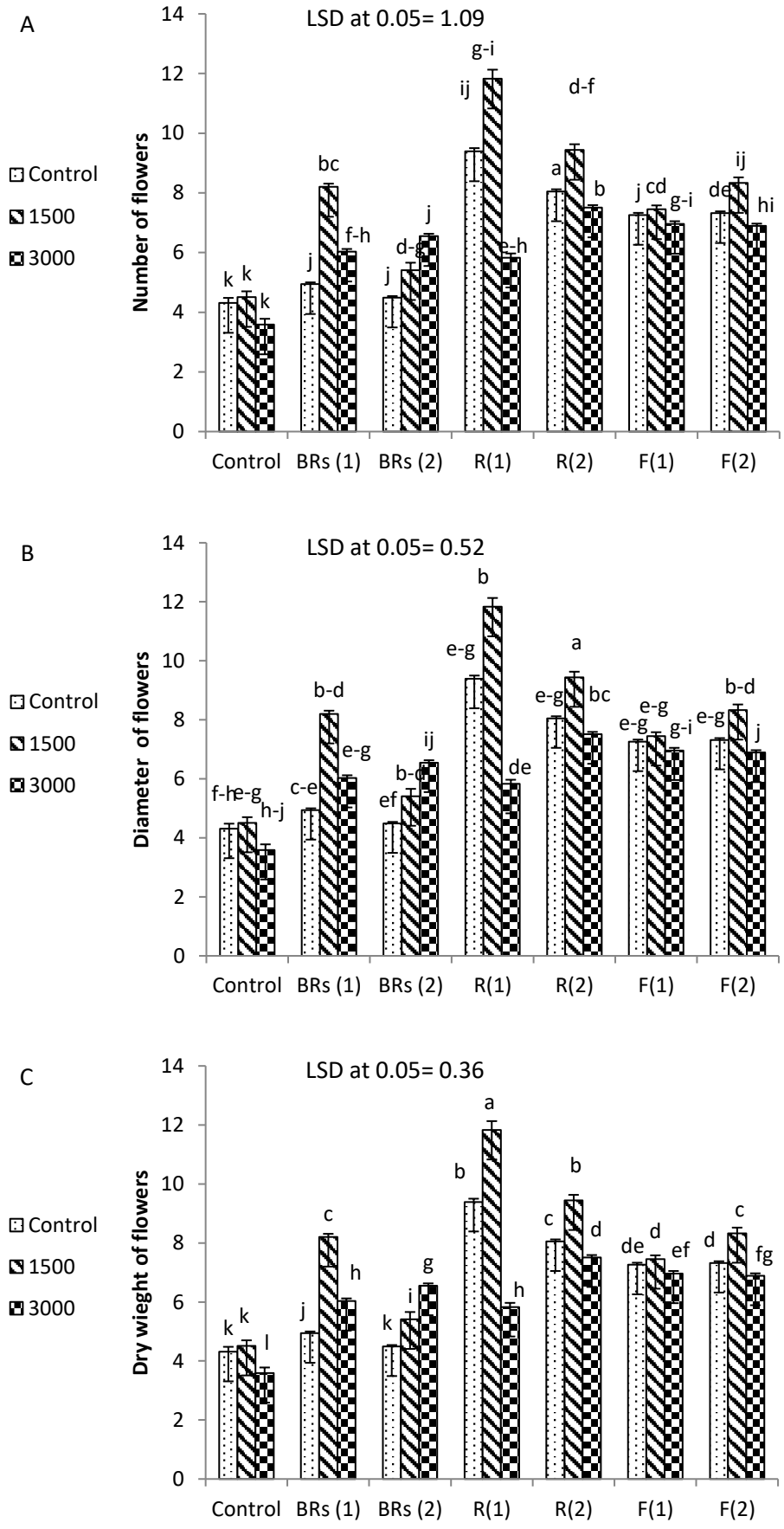

Fig. 1. Number of flowers/ plant (A), diameter of flowers (B) and dry weight of flowers/plant (C) of *Calendula officinalis* as affected by the interaction between salinity and brassinolide, radiation or endophytic fungi (mean of two seasons). Column with different letters indicate a significant difference at 5% level. Vertical bars indicate to standard error (SE) of three replicates

3.3. Chemical constituents

3.3.1. Total chlorophylls, carotenoids content, K^+, Ca^{2+}% and K^+/Na^+ ratio

The data in Table 3 displayed that within each brassinolide, radiation and endophytic fungi treatments increasing salinity from 0 to 3000 ppm resulted in steady reduction (in most cases) in the tested components of total chlorophylls, carotenoids content, K^+, Ca^{2+}% and K^+/Na^+ ratio compared to control. These results are similar to those reported by various studies which reported that salinity stress lead to a reduction in total chlorophylls and carotenoids content [22 and 39], reduction in K^+ and Ca^{2+} % [39- 40] and reduction in K^+/Na^+ ratio [25,40].

The reduction in total chlorophylls may be due to structural harm to the light-harvesting complex, pigment disorders, pigment-protein complex instability, and alterations to the photosynthetic process. Additionally, a high salt concentration damages the chloroplast membrane and causes ROS to be generated in the chloroplasts, which then break the double bonds of unsaturated fatty acids and cause chlorophyll loss from the thylakoids [41]. The potential of Na^+ to compete with K^+ for significant binding sites as well as the physical and chemical resemblance between K^+ and Na^+ may be related to the reduction of K^+ and Ca^{+2}% in herb caused by salt stress. The opposing action of Ca^{2+} and Na^+ ions, which causes membrane integrity and selectivity to deteriorate by dislodging Ca^{2+} linked with the membrane in favor of Na^+, is what causes the suppression of Ca^{+2} uptake [42].

The data in Table 3 also elucidated that, under the same level of salinity the plants treated with any one of brassinolide, radiation and endophytic fungi had significantly higher values of total chlorophylls, carotenoids content, K^+, Ca^{2+} % and K^+/Na^+ ratio than those of control (the plants that are subjected only to salt stress and are not being treated). Radiation appeared to be more effective, especially when used at the maximum dose of 40 Gy, since resulted in the highest mean values of determined components compared to other treatments. These results in agreement with the findings of earlier authors that reported increase in the tested components due to brassinolide treatments [28,43,45], or fungi treatments [35], whereas the valuable increase in the tested components with radiation treatments are coincided with those of previous studies [46].

3.3.2. Essential oil (%)

The data shown in Table 4 disclosed that within each level of brassinolide, radiation and endophytic fungi treatments increasing salinity from 0 to 3000 ppm resulted in decrease in essential oil compared to control. The reductions in essential oil owing to salinity stress are analogy with many studies [47,49].

Results also indicated that under the same level of salinity the plants treated with different treatments of brassinolide, radiation and endophytic fungi had significantly higher values of essential oil (in most cases) than those of control plants with the superiority of radiation particularly the highest on (40 Gy) since recorded the highest mean values. These results are in harmony with the findings of other authors that reported increase in essential oil due to brassinolide treatments [50,51], or endophytic fungi tratments [30,52], while the noticeable increase in essential oil with radiation treatments are analogy with those of numerous researches [53,54].

3.3.3. Total phenols and proline

Evidently data in Table 4 showed that within each level of brassinolide, radiation and endophytic fungi treatments increasing salinity from 0 to 3000 ppm resulted in increase in total phenols and proline, in most cases, compared to control. The results of increasing total phenols or proline content due to salinity stress are the same as the results of various studies [20,40,47,55].

Results also in Table 4 elucidated that at the same salinity level the plants treated with brassinolide, radiation and endophytic fungi treatments had significantly higher values of total phenols and proline (in most cases) than those of control plants. Brassinolide appeared to be more effective, especially the highest on (50 ppm) since registered the highest mean values with salinity threshold (3000 ppm). These results are in conformity with the findings of previous authors that revealed increase in total phenols or proline due to either radiation treatment [46,56], or endophytic fungi treatments [30]. However, the rise in total phenols and proline because of brassinolide treatments are in harmony with those elicited by earlier reports **[**27,28].

3.3.4. Enzyme activity

It is obvious from data in Fig.2 that within each level of the tested treatments (brassinolide, radiation and endophytic fungi), CAT and SOD were increased as salinity concentrations increased from 0 to 1500 ppm compared to control. The present findings are in line with those findings of previous workers [25,41] who reported increase in CAT and SOD due to salinity stress

Increasing antioxidant enzymes of CAT and SOD under salinity stress may be due to defeat the negative impact resulting from oxidative stress. CAT is incredibly effective in eliminating H_2O_2; it is able to transform two H_2O molecules into water and molecular oxygen without the need for reduction equivalent. SOD plays a significant role in oxidative stress by lowering the risk of OH production via metal-catalyzed procedures [57].

Results in Figure 2 also showed that, within the same salinity level the plants treated with any one of brassinolide, radiation and endophytic fungi had significantly higher values of CAT and SOD than those of control (the plants exposed to salt stress and not received any treatments). Radiation was more effective than the others treatments especially at the lowest dose (20 Gy) which produced the highest mean values of CAT and SOD. These results are in harmony with the findings of other authors that reported increase in in CAT and SOD owing to either brassinolide tratments [58], or fungi tratments [30], whil the noticeable increase in CAT or SOD a result of radiation treatments are analogy with those of previous researches **[**59].

Table 3. Total chlorophylls, carotenoids, K, Ca and K^+/Na^+ ratio of *Calendula officinalis* as affected by the interaction between salinity and brassinolide, radiation or endophytic fungi (mean of two seasons)

Salinity	Treatments*	Total chlorophylls content (mg/g FW)	Carotenoids (mg/g FW)	K %	Ca%	K^+/Na^+ ratio
Control	**0**	1.37±0.04kl	1.37±0.04e-g	3.05±0.03g-i	0.88±0.02c	1.04±0.01ij
	BRs (1)	1.56±0.02h-j	1.64±0.08a-d	4.11±0.02b	0.98±0.03b	2.58±0.07cd
	BRs (2)	1.55±0.04h-j	1.71±0.11a-c	4.16±0.04ab	0.98±0.01ab	2.53±0.15c-e
	R(1)	2.03±0.15bc	1.71±0.08a-c	3.16±0.02ef	0.99±0.03ab	3.10±0.07b
	R(2)	2.26±0.02a	1.76±0.06a	4.22±0.03a	1.02±0.01a	3.39±0.06a
	F(1)	1.88±0.03de	1.67±0.03a-d	3.33±0.07c	0.99±0.04ab	2.78±0.03c
	F(2)	2.11±0.01b	1.72±0.13ab	4.09±0.01b	0.97±0.01b	2.70±0.02c
1500 ppm	**0**	1.35±0.03l	1.15±0.02gh	2.06±0.01j	0.76±0.01hi	0.98±0.07j
	BRs (1)	1.54±0.02h-j	1.48±0.09b-f	3.19±0.02ef	0.83±0.01de	2.53±0.07c-e
	BRs (2)	1.51±0.04ij	1.52±0.12a-f	3.19±0.01ef	0.83±0.02de	2.39±0.11d-f
	R(1)	2.03±0.05bc	1.44±0.23d-f	3.03±0.01hi	0.80±0.01e-g	2.21±0.04f
	R(2)	1.97±0.09cd	1.67±0.05a-d	3.22±0.07de	0.82±0.02d-f	2.27±0.08ef
	F(1)	1.74±0.01fg	1.43±0.07d-f	3.12±0.03f-h	0.84±0.02de	2.39±0.06d-f
	F(2)	1.78±0.02ef	1.60±0.09a-e	3.10±0.01f-h	0.84±0.01d	2.41±0.05d-f
3000 ppm	**0**	1.19±0.05m	1.05±0.01h	1.87±0.02k	0.64±0.01j	0.98±0.01j
	BRs (1)	1.50±0.01i-k	1.47±0.03c-f	3.13±0.02fg	0.77±0.01g-i	1.31±0.01hi
	BRs (2)	1.42±0.02j-l	1.35±0.06e-g	3.17±0.03ef	0.78±0.01f-h	1.34±0.01h
	R(1)	1.60±0.03hi	1.31±0.04fg	2.98±0.02i	0.74±0.01i	1.87±0.02g
	R(2)	1.81±0.02ef	1.54±0.10a-f	3.13±0.04e-g	0.78±0.01g-i	2.22±0.06f
	F(1)	1.48±0.02i-l	1.36±0.05e-g	3.30±0.01cd	0.74±0.01i	1.41±0.35h
	F(2)	1.65±0.01gh	1.33±0.04fg	3.13±0.01fg	0.78±0.01f-h	1.31±0.01hi

* BRs (1) = Brassinolide at 25 ppm, BRs (2) = Brassinolide at 50 ppm, R(1)= Radiation (20 Gy), R(2) = Radiation (40 Gy), F(1) = Fungi1, F(2) = Fungi 2. Each value represents the mean ± standard error of three replicates, means in a column with different letters indicate a significant difference for each variable at 5% level using Duncan multiple rang test

Table 4. Essential oil (%), Total phenols and proline content of *Calendula officinalis* as affected by the interaction between salinity and brassinolide, radiation or endophytic fungi (mean of two seasons)

Salinity	Treatments*	Essential oil (%)	Total phenols) (mg/g100 FW)	proline (µ moles /g dry matter
Control	**0**	0.18±0.03f-h	3.04±0.10i	2.75±0.32k
	BRs (1)	0.23±0.02c-f	5.30±0.09g	3.39±0.07j
	BRs (2)	0.24±0.01b-e	4.85±0.16g	4.59±0.03h
	R(1)	0.31±0.03a	3.79±0.10h	4.73±0.15gh
	R(2)	0.27±0.03a-c	5.95±0.10ef	5.60±0.12ef
	F(1)	0.29±0.01ab	5.18±0.18g	4.25±0.18hi
	F(2)	0.29±0.01ab	6.25±0.17d-f	3.83±0.03ij
1500 ppm	**0**	0.14±0.02hi	5.90±0.25f	3.94±0.55i
	BRs (1)	0.19±0.02d-h	6.53±0.29b-d	4.62±0.11h
	BRs (2)	0.19±0.03d-h	6.38±0.08de	4.66±0.16h
	R(1)	0.19±0.02d-h	7.29±0.04a	5.37±0.05f
	R(2)	0.24±0.01b-e	7.09±0.05a	5.92±0.13c-e
	F(1)	0.21±0.02c-g	7.18±0.11a	4.70±0.21h
	F(2)	0.24±0.02b-d	7.18±0.06a	4.62±0.23h
3000 ppm	**0**	0.10±0.01i	6.52±0.08cd	4.68±0.29h
	BRs (1)	0.15±0.01hi	6.98±0.40ab	8.49±0.08b
	BRs (2)	0.15±0.02hi	7.34±0.13a	10.99±0.36a
	R(1)	0.18±0.02e-h	7.17±0.03a	5.71±0.11d-f
	R(2)	0.23±0.02c-f	7.08±0.08a	6.24±0.01cd
	F(1)	0.16±0.02gh	6.96±0.18a-c	5.25±0.02fg
	F(2)	0.17±0.01gh	7.11±0.28a	6.35±0.08c

* BRs (1) = Brassinolide at 25 ppm, BRs (2) = Brassinolide at 50 ppm, R(1)= Radiation (20 Gy), R(2) = Radiation (40 Gy), F(1) = Fungi1, F(2) = Fungi 2. Each value represents the mean ± standard error of three replicates , Means in a column with different letters indicate a significant difference for each variable at 5% level using Duncan multiple rang test.

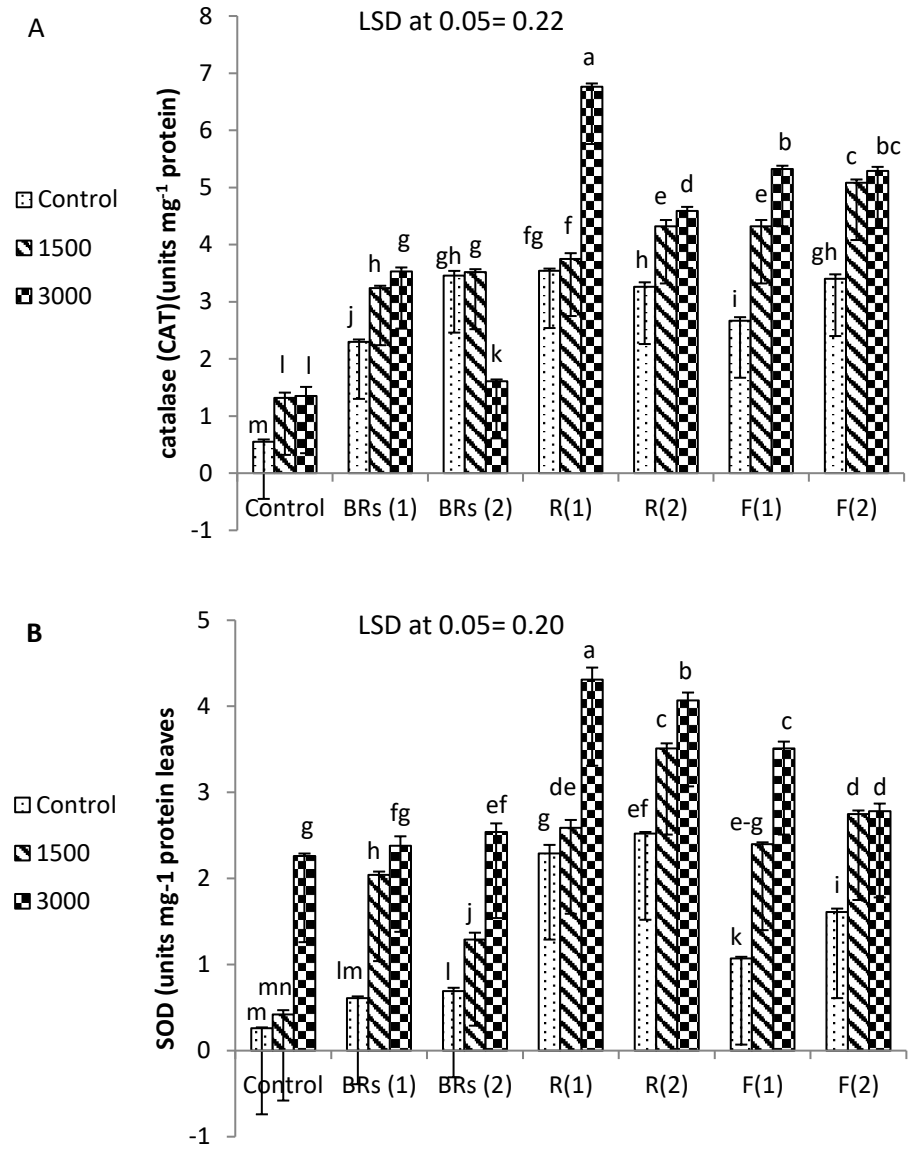

Fig. 2. Catalase (A), superoxide dismutase (B) of *Calendula officinalis*as affected by the interaction between salinity and brassinolide, radiation or endophytic fungi (mean of two seasons). Column with different letters indicate a significant difference at 5% level. Vertical bars indicate to standard error (SE) of three replicates

4. Conclusions

Based on the outcomes, it could be suggested that for mitigating the harmful impact of salinity stress on *Calendula officinalis* plants irrigated with salinity up to 3000 ppm, the seeds could be irradiated with gamma rays at 40 Gy or the plants foliar sprayed with brassinolide at 50 ppm.

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