Salt Stress Dynamics in Monarda citriodora Cerv.ex Lag; An Integrative Analysis of Growth, Biochemical responses and essential oil modulations

Ruby Singh
CSIR-Indian Institute of Integrative Medicine: Council of Scientific & Industrial Research Indian Institute of Integrative Medicine

Mehnaz Gull
CSIR-Indian Institute of Integrative Medicine: Council of Scientific & Industrial Research Indian Institute of Integrative Medicine

Sumit G. Gandhi
CSIR-Indian Institute of Integrative Medicine: Council of Scientific & Industrial Research Indian Institute of Integrative Medicine

Rajendra Bhanwaria
rbhanwaria.iiim@csir.res.in

CSIR-Indian Institute of Integrative Medicine: Council of Scientific & Industrial Research Indian Institute of Integrative Medicine https://orcid.org/0000-0002-1896-5519

Research Article

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Abstract

Monarda citriodora, a highly valued plant with essential oil and medicinal properties, is being exclusively cultivated at CSIR-IIIM Jammu, India. Salt stress poses significant challenges to plant growth, development, and productivity, impacting agricultural output worldwide. In this study, the impact of four salts NaCl, KCl, $MgSO_4$ and $CaCl_2$ at concentrations (0, 25, 50, 100, and 200 mM) on the growth, biomass, nutrient, relative water content, proline content, antioxidant activity, and essential oil constituents of *M. citriodora*. A completely randomized design was used in a pot experiment and plants were irrigated with different salinity levels for 60 days, followed by two months of transplanting. The results showed that higher salt concentrations decreased nutrients, growth, biomass, chlorophyll and relative water content. Proline concentration and antioxidant activity were enhanced with increasing salt concentrations. Thymol is the main constituent in essential oil (39.26–91.36%). The thymol content was elevated at 50 mM salt concentrations, with the highest content (91.36%) found in the 50 mM KCl treatment, followed by 50 mM NaCl (91.07%) and 100 mM KCl (88.58%). The lowest thymol content (39.26%) was observed with 200 mM $CaCl_2$. These findings suggest that a 50 mM concentration of salts enhances thymol content. Salt treatments significantly influenced the chemical composition of *M. citriodora*, with the emergence of novel compounds in the essential oil under salt stress. The study highlights the impact of environmental stressors on metabolite profiles and suggests that adjusting salt concentrations could optimize essential oil quality in *M. citriodora*.

Introduction

During their natural life, plants experience a wide spectrum of biotic and abiotic stresses (Dixit et al. 2024). These stresses have significant potential to alter both the quality and quantity of crop production in the agricultural system. Among these stresses, salt stress is the most prevalent abiotic component having global ramifications. In plants, salt stress is responsible for osmotic stress, ionic stress and nutrient imbalance, which reduces plant growth and development, resulting reduction in crop yields (Munns and Tester 2008; Isayenkov and Maathuis 2019; Wu et al. 2023; Alabdallah et al. 2024). In medicinal plants, salt stress alters the synthesis of secondary metabolites by impairing plants' physiological and metabolic activities (Koocheki et al. 2008; Kavian et al. 2022). In response to salt stress, plants change several aspects of their growth and development, and also modify the regulation and synthesis of primary and secondary metabolites. Subsequently, for the reduction in ionic and osmotic stress, plants activate adaptive mechanisms that result in increased synthesis of bioactive secondary metabolites. Due to their strong biological characteristics, these metabolites are essential for plants to survive in hostile environments(Azad et al. 2021). Additionally, salinity stress influences the biosynthesis of terpenoids and alkaloids, which are known for their therapeutic properties, including anti-inflammatory, antimicrobial, anticancer, analgesic, antispasmodic, and antibacterial effects (Liu et al. 2021; Nazari et al. 2023).

Salinity affects about 20% of agricultural irrigated land and 6% of all land globally (Kumar and Sharma 2020). Salinization of soil is caused by natural processes such as weathering of rocks, arid climates, high rates of evaporation and human actions like using brackish water for irrigation and poor tillage practices. In agricultural system, these factors have major issues (Hussain et al. 2019; Alabdallah et al. 2024). In soil salt

stress is mainly caused by accumulation of soluble salts within the root zone of plants which leads to a decreases in rates of seed germination, plant growth, yield and availability of essential nutrients (N, P, K, Ca, Mg, Zn, Cu and Fe), with the extent of these effects varying based on the type and amount of the salts (Shrivastava and Kumar 2015). Saline soil contains soluble mineral salts such as cations like sodium, calcium, magnesium, potassium, and anions such as chlorine, sulfate, bicarbonate, carbonate, and nitrate. Additional elements, including boron, selenium, strontium, lithium, silica, rubidium, fluorine, molybdenum, manganese, barium, and aluminium may also be found in an extremely salinized environment (Kumar and Sharma 2020). Elevated sodium concentrations in sodic soils can directly damage plants and simultaneously degrade soil structure, reducing its porosity and impairing water permeability (Taiz and Zeiger 2002; Martínez-Alvarez et al. 2018).

There are significant time and financial resources being utilized for the amelioration of salt-affected soil. Therefore, the emergence of salt-tolerating plant species is more efficient in successful agricultural outcomes in these regions. For a productive agricultural system in salt-affected areas, this is necessary to identify and select the appropriate salt-tolerating plant species (Sharma et al. 2015). It is necessary to focus on the cultivation of plants having a desirable chemical profile and high economic value to maximize the economic return. As secondary metabolites obtained from aromatic and medicinal plants have recently acquired commercial value, there has been a focus on increasing their synthesis. It has been reported that different stress factors often stimulate the biosynthesis of secondary metabolites in aromatic and medicinal plants (Neffati and Marzouk 2010; Baâtour et al. 2011; Haider et al. 2023; Rahman et al. 2024).

M. citriodora is an annual medicinal and aromatic plant in the family Lamiaceae. It is cultivated as an ornamental species (Gocher et al. 2022). *Monarda* consists of approximately 30 species and is indigenous to Eastern North America (Mazza and Marshall 1992; Ciurușniuc et al. 2012). This plant is particularly cultivated in the outer Himalayas, which is the Shivalik Hill region and also found in various parts of Asia and Europe and since 1990 (Katoch and Pull 2017; Charak et al. 2023; Luxmi et al. 2023; Yadav et al. 2024). It consists of well-developed roots and upright, branched stems. The leaves are petiolated or oblong-lanceolate, arranged oppositely and have a lemon scent. The pink to purple colour flowers are present in clusters. This plant can withstand cold weather and adapt to different soil conditions, making it suitable for urban landscaping (Juhanoja and Tuhkanen 2011). The *M. citriodora* essential oil is abundant in volatile compounds. Notably, the dominant components include thymol, α-phellandrene, β-cymene and 1,8-cineole (Lu et al. 2011). This oil has significant medicinal and aromatic properties; a tea made from its leaves can alleviate symptoms such as coughs, colds, high fevers, and respiratory issues. The fragrant oil is also used as an insect repellent and in the perfume industry. Its essential oil exhibits considerable antibacterial and antioxidant activities against food-borne pathogens (Lu et al. 2011). Thymol, which constitutes more than 70.6% of the essential oil (Dorman and Deans 2004), is incorporated into commercial mouthwash products due to its antiseptic properties.

According to the current study, there is limited research available on the effects of various salinity treatments with different salt compounds on *M. citriodora*. A study by Niu et al. (2012) and (2020) determined that *M. citriodora*, a wildflower, showed little tissue tolerance to Na^+ concentrations, with overall mortality occurring at salinity (EC) 2.8 dSm^{-1}. In contrast, the purpose of the study is to evaluate the effect of different salt

compounds such as NaCl, KCl, $MgSO_4$, and $CaCl_2$ at different concentrations (0, 25, 50, 100, and 200 mM) on growth characteristics (plant height, number of branches, and inflorescence count), biomass, chlorophyll contents, relative water content, proline accumulation, antioxidant activity, nutrients such as nitrogen, phosphorous, potassium content, and essential oil composition of *M. citriodora*.

Materials and methods

Plant transplantation and salt treatments

The current research was carried out in the greenhouse facilities at CSIR-IIIM, Jammu, in the years 2022 and 2023 and soil samples were taken from the experimental field. The authentication of the planting material was confirmed by a plant taxonomist. The verified plant specimen has been deposited in the J. A. Herbarium (IIIM-Herbarium), under voucher number RRL-27053. A completely randomized design was used for experiment with 17 treatments, each with three replications. The seeds were sown in November within a shaded glasshouse to establish the nursery. The 9 kg capacity of plastic pots having dimensions 31 cm × 31 cm × 19.5 cm (top diameter × height× base diameter) were filled with a 3:1 ratio of soil to manure. The physico-chemical properties of soil such as its pH, electrical conductivity, texture, organic carbon percentage, as well as availability of N, P and K were analyzed (Table 1). Three *M. citriodora* seedlings were transplanted into each pot, which was then placed in a greenhouse. After two months of transplantation, the plants were subjected to various salt stress conditions by irrigation for the next two-months. The salts used in the experiment were NaCl, KCl, $MgSO_4$, and $CaCl_2$, applied at concentrations of 25, 50, 100, and 200 mM, along with control (0 mM).

Table 1
Physico-chemical properties of experimental soil

Sand (%)	**49.45**	**Organic carbon (%)**	**0.98**
Silt (%)	30	Available nitrogen (kg/h)	314.5
Clay (%)	20.54	Available phosphorous(kg/h)	20.6
pH	6.89	Available potassium (kg/h)	256.14
EC ($dS\ m^{-1}$)	1.6	Water holding capacity (%)	54.32
Bulk density (Mgm^3)	1.35		

Table 2
No. of inflorescence and biomass of *M. citriodora* in various salt treatments

		No. of Inflorescence	Shoot fresh weight	Shoot dry weight	Root fresh weight	Root dry weight
	Control	8.67 ± 0.77 ab	99.98 ± 7.20 a	23.08 ± 1.56 a	7.17 ± 0.35 a	2.12 ± 0.15 a
NaCl	25mM	7.67 ± 0.77 a-d	83.74 ± 4.81 bc	20.19 ± 1.11 bc	5.90 ± 0.46 b	1.24 ± 0.18 d
	50mM	7.00 ± 0.50 cd	69.84 ± 12.38 de	16.22 ± 1.42 d-f	3.80 ± 0.56 de	0.98 ± 0.24 ef
	100mM	4.50 ± 1.00 e	57.01 ± 6.63 g	13.3 ± 0.96 gh	3.64 ± 0.56 d-f	0.98 ± 0.13 ef
	200mM	3.50 ± 1.00 ef	38.08 ± 5.93 h	11.02 ± 0.49 hi	3.01 ± 0.41 fg	0.88 ± 0.06 f
KCl	25mM	8.17 ± 0.58 a-c	90.17 ± 8.62 ab	22.51 ± 2.29 ab	6.94 ± 0.27 a	2.04 ± 0.11 ab
	50mM	6.50 ± 0.10 d	74.36 ± 5.40 c-e	18.08 ± 0.97 cd	4.71 ± 0.81 c	1.15 ± 0.16 de
	100mM	4.50 ± 1.00 e	68.32 ± 3.76 d-f	14.72 ± 0.84 fg	3.56 ± 0.34 ef	0.61 ± 0.04 g
	200mM	2.17 ± 0.58 f	42.32 ± 5.48 h	8.70 ± 0.54 i	2.58 ± 0.06 g	0.54 ± 0.05 g
$MgSO_4$	25mM	8.84 ± 0.58 a	93.56 ± 3.72 ab	23.00 ± 1.68 a	4.70 ± 0.05 c	1.59 ± 0.02 c
	50mM	8.50 ± 1.00 abc	93.35 ± 2.71 ab	21.28 ± 0.97 ab	4.29 ± 0.1 cd	1.00 ± 0.03 ef
	100mM	7.50 ± 1.20 a-d	72.49 ± 3.63 de	17.59 ± 0.75 de	4.24 ± 0.13 cd	1.05 ± 0.05 d-f
	200mM	7.17 ± 1.16 b-d	64.22 ± 7.84 e-g	17.51 ± 2.07 de	3.1 ± 0.32 fg	0.85 ± 0.02 f
$CaCl_2$	25mM	8.84 ± 1.53 a	89.64 ± 3.54 ab	22.90 ± 2.43 a	5.68 ± 0.06 b	1.93 ± 0.02 ab
	50mM	7.17 ± 1.53 b-d	77.35 ± 5.08 cd	17.58 ± 2.08 de	5.91 ± 0.13 b	1.93 ± 0.08 ab
	100mM	6.50 ± 1.31 d	67.11 ± 10.56 d-g	16.16 ± 1.91 d-f	5.51 ± 0.85 b	1.83 ± 0.34 b
	200mM	3.83 ± 1.16 e	58.367 ± 1.40 fg	15.314 ± 0.61 e-g	4.71 ± 0.05 c	1.56 ± 0.06 c
	LSD	1.5436	10.7996	2.4461	0.6567	0.2149

Data are presented as mean ± standard deviation. Values in the same column with the same letter are not significantly different (α = 0.05). LSD represents the least significant difference.

Soil analysis

The physical and chemical parameters of the initial soil were analyzed and presented in Table 1. The potentiometric method was used to determine the concentration of H^+ ion in soil (Jackson 1958). Electrical conductivity (EC) was determined using a conductivity meter(Jackson 1958). Organic carbon was determined by the rapid titration method as described by Walkley and Black (1934). A combination of 1N $K_2Cr_2O_7$ (10ml) and concentrated H_2SO_4 (20ml) was used to digest 0.1 gm of soil. Titration with standard ammonium ferrous sulphate solution in presence of orthophosphoric acid and DPA as an indicator was used to assess the organic carbon until a blue colour appears.

The determination of available N using the Alkaline $KMnO_4$ method as given by (Subbiah and Asija 1956), with the help of a Kjeldahl apparatus. N extraction was performed using a solution of 0.32% potassium permanganate ($KMnO_4$) and 2.5% sodium hydroxide (NaOH) for sample distillation. A 4% boric acid with a combination of methyl red and bromocresol green and was used to absorb ammonia. The absorbed ammonia was then quantified by titration with 0.02N (H_2SO_4) until the color changed from green to pink.

The available P was determined using the Ascorbic Acid method as described by (Jackson 1973). To prepare the extract, 5.0 g of soil were combined with 100 mL of 0.5 N sodium carbonate solution and a small quantity of activated charcoal. The mixture was then shaken for half an hour and then filtered. Using a simple Biospectrometer (Eppendorf AG, 22331 Hamburg), the P content was calculated using a standard curve of known P values.

The available K content in the soil was analysed by extraction with 1N ammonium acetate as given by Jackson (1967). 5.0 g of soil was placed into a 150ml conical flask and extracted using 25ml of neutral 1N ammonium acetate solution. After that filtrate was aspirated into the atomizer of a calibrated flame photometer (VSI-604, Sr. No. 0302020), and the K concentration was recorded.

Measurement of growth attributes and biomass

The number of branches and plant height were evaluated at 60, 90, and 120 days after transplanting, until the harvesting stage. By using a ruler, the height of the salt-treated and control plants was measured in cm from the soil surface to each plant's apex. After 120 days of transplantation, the plants were harvested. The number of branches per plant was counted during harvesting. After harvesting, plant samples were taken to the laboratory for further analysis. The shoots and roots were separated and then cleaned with regular water and distilled water to remove surface dirt. Excess moisture was carefully wiped away using tissue paper and samples were left to air dry at room temperature for 30 minutes. Fresh weights of roots and shoots were measured by an electronic weighing balance (Intel DC-9-12 V). Samples were dried for 24hours at 60^0c in an oven to determine dry weight, until a constant weight was attained.

Measurement of leaf relative water content (RWC) and Chlorophyll content

The relative water content (RWC) of *M. citriodora* leaves was determined using the procedures described by (Henson et al. 1981). Leaves were collected from each replicate after the salt treatments. The fresh weight of the leaves was measured first, then the leaves were subsequently soaked in distilled water in sealed test tubes for an entire night at room temperature to reach full turgidity, after which the turgid weight was recorded. Lastly, the dry weight of the leaves was measured after they were dried for 24 hours at 80^{o}c in an oven.

The method described by Arnon (1949) was used to determine the content of chlorophyll. 20 ml of 80% acetone was used to grind 100 mg of fresh leaves. After that, the leaf extract was centrifuged for 8 minutes at 8000rpm. The supernatant was transferred, and the process was repeated until the residue became colorless. To protect the chlorophyll pigments, the extract was stored in the dark until absorbance measurements were taken. Absorbance was measured at wavelengths of 645 nm and 663 nm against an 80% acetone blank.

Proline content and Antioxidant activity

To determine proline content, the procedure outlined by Bates et al. (1973) was followed. 10 ml of 3% sulphosalicylic acid was used to homogenize 0.5g of leaf tissue and Whatman No. 40 filter paper was used to filter the resultant extract. For the assay, 2 ml of the filtered extract was mixed with 2 ml of reagents (ninhydrin solution, 6 M phosphoric acid, and glacial acetic acid) in a separate test tube. After an hour of boiling in a water bath, this mixture was allowed to cool at room temperature. For the assay, 2 ml of the filtered extract was mixed with 2 ml of reagents (ninhydrin solution, 6 M phosphoric acid, and glacial acetic acid) in a separate test tube. After an hour of boiling in a water bath, the mixture was allowed to cool at room temperature. After cooling, 4 ml of toluene was added and thoroughly mixed. At 520nm, the optical density (OD)of toluene was measured. The concentration of proline in M. citriodora was calculated by comparing the OD values to a proline standard curve, with toluene used as a blank.

The DPPH (1, 1-diphenyl-2-picrylhydrazyl) test was used to measure the antioxidant activity (Brand-Williams et al. 1995). In brief, 100 mL of 99.5% ethanol was mixed with 7.89 mg of DPPH to prepare a DPPH solution, which was then left to stand in the dark for an hour. Using ascorbic acid dissolved in methanol (1 mg/mL), a standard curve was created. In the test, 800 µL of Tris-HCl buffer (pH 7.4) was incubated at room temperature for 30 minutes after 200 µL of the extract sample was combined with 1000 µL of DPPH solution. Next, the absorbance at 517 nm was determined. 1200 µL of ethanol and 800 µL of Tris-HCl buffer (pH 7.4) were used for the blank. The positive control was made with 200 µL of ethanol.

Nutrient analysis

The nutrient profile of *M. citriodora* plants, particularly N, P, and K was comprehensively evaluated among various parts of plants such as leaves, stems, roots, and inflorescences. To prepare the samples, they were first washed with a 0.2% liquid detergent solution and then treated with 0.1N dilute hydrochloric acid and finally rinsed with distilled water to eliminate any dirt and extraneous materials. The cleaned samples were oven dried at 60°C, then ground and sieved (1mm mesh) to ensure uniformity, before analysis.

The modified Kjeldahl method was used to determine the total N content. as outlined by (Jackson 1973) and further adapted by (Srivastava et al. 2020). N distillation was performed using a KEL PLUS Classic-DX VATSE (NSIC (C) GP/17 (5336)/2003) apparatus. In this procedure, 0.2 g of the finely crushed plant sample was digested by using 10 mL of concentrated H_2SO_4 and 3 g of a catalyst mixture for 120 minutes, or until the solution became colourless. Following digestion, the extract was distilled and subsequently titrated with 0.1 N HCl using methyl red as the indicator.

For the analysis of total P and K, 0.2 g of plant powder was digested with a triacid mixture consisting of HNO_3, H_2SO_4, and $HClO_4$ (10:1:4) for 3 hours. The resulting extract was then filtered using Whatman No. 1 filter paper, and the final volume was adjusted to 100 ml in a volumetric flask. Total P content was assessed using the ammonium molybdate-ammonium vanadate method as outlined by (Jackson 1973). P concentrations were determined by comparing the sample absorbance to a standard phosphorus curve using a Biospectrometer (Eppendorf AG, 22331 Hamburg) at a wavelength of 470 nm. Total K content was measured following the protocol of (Bhargava and Raghupathi 1993), employing a Microcontroller Flame Photometer (Model VSI-604, Serial No. 0302020) with appropriate standardization.

Essential oil extraction and essential oil composition analysis by GC-MS

Fresh plant materials (100 g) were hydro-distilled for four hours using a 500 mL Clevenger apparatus, with a volume of 250 mL of water to extract the essential oil fraction. Each replicate's pooled sample undergoes hydro-distillation, resulting in essential oil extraction after four hours. The collected oil was dehydrated using anhydrous sodium sulfate and a refrigerator at 4°C for preservation.

The components of essential oils were examined using an Agilent 7890A gas chromatograph connected to a 5875C mass spectrometer detector (XL MSD), which is run by Mass Hunter Workstation software and has a triple-axis detector (Agilent Technologies, USA). For chromatographic separation, a DB-5 column (30 m × 0.25 mm i.d. × 0.25 µm film thickness) was used. The carrier gas, helium, was used and flowed at a rate of 0.50 mL/min. The oven temperature was set to increase up to 280°C (held for 20 minutes) from 200°C (held for 2 minutes) at a rate of 10°C/min. A 1 µL sample was split at a ratio of 1:50 during injection with an ionization energy of 70 eV, and mass spectra were obtained in electron impact mode, scanning from 50 to 600 amu at a rate of 0.5 seconds per scan. The temperature of the transfer line and the inlet was fixed at 250°C. The NIST and Wiley libraries were used to identify the components. Without using correction factors, the percentages of the Peak areas were electronically measured using the total ion chromatogram (TIC) response.

Statistical analysis

The data are presented as means ± standard deviation (SD), and graphical representations are generated using Microsoft Excel. Statistical significance among groups was estimated by the least significant difference (LSD) test, using R-studio. At $p < 0.05$, differences in means were considered statistically significant. Principal component analysis and correlation mapping were carried out using Origin Pro 2021 software.

Results

The effect of various salts, including NaCl, KCl, $MgSO_4$, and $CaCl_2$, at different concentrations (25, 50, 100, and 200 mM), on plant growth parameters, physicochemical properties and essential oil composition was systematically investigated. The findings indicate that concentrations exceeding 100 mM of these salts significantly impair the growth and development of *M. citriodora*.

Plant's height and number of branches

The impact of salts (NaCl, KCl, $MgSO_4$, and $CaCl_2$) at concentrations of 25, 50, 100, and 200 mM on plant height and number of branches were determined at 60, 90, and 120 days with respect to growth parameters. At 60 days nonsignificant effects were observed on these parameters. While with increasing salt concentration, plant height decreased at 90 days. However, plant height significantly decreases at 120 days by 12.75%, 24.30%, and 20.72% at 200 mM concentration of NaCl, KCl, and $CaCl_2$, respectively, compared to the control plants (Fig. 1). Moreover, at 90 days the number of branches reduced with 200 mM concentrations of NaCl, KCl, and $CaCl_2$. In contrast, lower salt concentrations and $MgSO_4$ did not significantly affect number of branches compared to the control. At 120 days, there was a significant decrease in the number of branches 43.18%, 42.04%, and 39.77% with 200 mM concentrations of NaCl, KCl, and $CaCl_2$, respectively, compared to the control (Fig. 2). Notably, the number of branches slightly increased with $MgSO_4$ salt treatment.

Number of inflorescence and root shoot biomass

The number of inflorescences as well as the fresh and dry weight of the shoots and roots was measured at the harvesting stage. The number of inflorescences in *M. citriodora* was extensively impacted by various salt treatments. The number of inflorescences increased by 1.92% with 25 mM concentrations of $MgSO_4$ and $CaCl_2$, but decreased with increasing salt concentrations. Salt concentrations of 25, 50, 100, and 200 mM decreased shoot fresh weight by 16.25%, 30.14%, 42.98%, and 61.91% with NaCl; 9.81%, 25.62%, 31.67%, and 57.67% with KCl; 6.42%, 6.63%, 27.49%, and 30.77% with $MgSO_4$; and 10.34%, 22.64%, 32.88%, and 41.62% with $CaCl_2$, respectively, compared to control plants. Similarly, shoot dry weight decreased by 12.50%, 29.70%, 42.39%, and 52.24% with NaCl; and by 2.44%, 21.66%, 36.31%, and 62.31% with KCl at concentrations of 25, 50, 100, and 200 mM, respectively. Additionally, shoot dry weight decreased by 7.80%, 23.79%, and 24.14% with $MgSO_4$; and by 23.82%, 29.98%, and 33.63% with $CaCl_2$ at concentrations of 50, 100, and 200 mM, in comparison to the control. On the other hand, there was no significant difference between $MgSO_4$ and $CaCl_2$ at 25 mM concentrations. Concentrations of 25, 50, 100, and 200 mM decreased root fresh weight by 17.64%, 47.00%, 49.39%, and 58.00% with NaCl; 3.21%, 34.34%, 50.00%, and 64.04% with KCl; 34.44%, 40.16%, 40.91%, and 56.78% with $MgSO_4$; and 20.75%, 17.54%, 23.13%, and 34.21% with $CaCl_2$, respectively, compared to control plants. Root dry weight decreased by 41.82%, 54.08%, 54.08%, and 58.80% with NaCl; 4.08%, 46.06%, 71.22%, and 74.68% with KCl; 25.31%, 52.98%, 50.47%, and 59.90% with $MgSO_4$; and by 9.27%, 8.96%, 13.67%, and 26.41% with $CaCl_2$ at concentrations of 25, 50, 100, and 200 mM, respectively, compared to the control.

Relative water content

The effect of salt treatments on the RWC of *M. Citriodora* leaves was significantly affected. (Fig. 3). Compared to the control, RWC decreased by 9.10%, 13.11%, 19.87%, and 23.17% under NaCl treatment and by 9.29%, 22.16%, 17.65%, and 27.24% under $CaCl_2$ treatment at concentrations of 25, 50, 100, and 200 mM, respectively. Under KCl treatment, there was no significant difference at 25 mM; however, RWC decreased by 6.26%, 6.66%, and 21.74% at 50, 100, and 200 mM, respectively. A 25 mM $MgSO_4$ treatment improved RWC by 6.52%, but further increases in $MgSO_4$ concentration led to a decrease in RWC.

Proline content and Antioxidant activity

Proline concentrations were determined using leaves from all salt-treated *M. citriodora* plants (Fig. 4). The unknown proline concentration of the plant samples were calculated by referencing standard proline curve, represented in mg/g dry weight. Proline concentrations increased with increasing concentrations of 25, 50, 100, and 200 mM, showing increases of 4.62%, 31.47%, 148.12%, and 169.07% with NaCl; 30.18%, 53.60%, 122.13%, and 166.17% with KCl; 16.00%, 16.65%, 52.63%, and 116.00% with $MgSO_4$; and 9.02%, 77.01%, 164.45%, and 151.13% with $CaCl_2$, respectively, compared to control plants. The maximum proline accumulation (83.50 mg/g) was recorded with the 200 mM NaCl treatment.

Antioxidant activity was analyzed by determining the DPPH inhibition percentage (Fig. 5). The DPPH radical scavenging activity was quantified by referencing a standard curve of ascorbic acid. The antioxidant activity of *M. citriodora* increased with NaCl by 0.07%, 7.43%, 7.9%, and 5.93% at 25, 50, 100, and 200 mM concentration respectively. While KCl treatment increases antioxidant activity by 6.31%, 9.85%, 13.56%, and 8.00% respectively at same concentration. However, $MgSO_4$ treatment increases antioxidant activity by 2.72%, 6.75%, 8.49%, and 14.10% respectively at same concentration. Moreover, plants treated with 25 mM CaCl2 showed a 4% reduction in antioxidant activity, which subsequently increased with higher concentrations. The maximum DPPH inhibition percentage (43.24%) was recorded with 200 mM $MgSO_4$ treatment.

Chlorophyll contents

In plants chlorophyll a and b are essential photosynthetic pigments have an important role in light absorption and energy conversion process. With increasing concentration total chlorophyll content in *M. citriodora* decreases (Fig. 6). Among the four salts studied, $MgSO_4$ exhibited the least toxic effect on total chlorophyll. At salt concentrations of 25, 50, 100, and 200 mM, the reduction in Chl a was as follows: 33.70%, 43.42%, 48.21%, and 46.93% with NaCl; 11.28%, 44.29%, 48.69%, and 48.40% with KCl; 5.12%, 25.10%, 35.74%, and 43.10% with $MgSO_4$; and 34.83%, 44.31%, 49.10%, and 48.31% with $CaCl_2$, respectively, compared to control plants. Interestingly, at a lower concentration of 25 mM, there was an increase in Chl b by 25.60%, 15.39%, 8.80%, and 20.57% with NaCl, KCl, $MgSO_4$, and $CaCl_2$, respectively, compared to control plants. However, at higher salt concentrations of 50, 100, and 200 mM, Chl b decreased by 12.70%, 19.53%, and 39.95% with NaCl; 21.90%, 35.24%, and 39.29% with KCl; and 21.69%, 28.39%, and 40.79% with $CaCl_2$, respectively, compared to control. Notably, Chl b increase by 32.85% and 11.47% at 50 and 100 mM concentrations of

$MgSO_4$ respectively, and decrease by 3.22% at 200 mM $MgSO_4$ compared to control. Additionally, total chlorophyll (Chl a + Chl b) content decreased with salt concentrations of 25, 50, 100, and 200 mM by 13.61%, 33.00%, 38.48%, and 44.56% with NaCl; 2.25%, 36.70%, 44.13%, and 45.31% with KCl; 0.40%, 5.40%, 19.74%, and 29.58% with $MgSO_4$; and 16.06%, 36.64%, 42.08%, and 45.76% with $CaCl_2$, respectively, compared to control plants.

Nutrients (N, P and K contents)

N, P and K are essential for growth and development of roots, shoots and reproduction. Leaf N content decreases with increasing salt concentrations of 25, 50, 100, and 200 mM by 4.61%, 8.46%, 8.85%, and 10.38% with NaCl, and 7.69%, 9.23%, 12.69%, and 24.23% with $CaCl_2$, respectively, compared to control plants. While, leaf N content increases by 1.15%, 4.23%, and 5.39% at 25 mM KCl, 25 and 50 mM $MgSO_4$ respectively, but further decreases with increasing salt concentration. Inflorescence N shows a significant reduction at 200 mM salt concentrations, decreasing by 13.21%, 28.19%, 12.33%, and 30.84% with NaCl, KCl, $MgSO_4$, and $CaCl_2$, respectively. Similarly, stem N content also decreases significantly at 200 mM salt concentrations, by 33.80%, 25.35%, 25.35%, and 42.25% with NaCl, KCl, $MgSO_4$, and $CaCl_2$, respectively. Furthermore, NaCl and $MgSO_4$ reduce root N content at 100 and 200 mM by 4.66%, 6.67% and 8.89%, 2.22%, respectively. In contrast, KCl and $CaCl_2$ reduce stem N content at 50, 100, and 200 mM by 2.22%, 2.22%, 11.11% and 6.67%, 6.67%, 11.11%, respectively, compared to control. Root N percentage improves at lower salt concentrations (Fig. 7).

The maximum reduction in leaf P was observed at salt concentrations of 100 and 200 mM, with decreases of 28.24% and 33.07% for NaCl, 10.77% and 32.04% for KCl, 25.00% and 39.07% for $MgSO_4$, and 6.36% and 336.39% for $CaCl_2$, respectively. Additionally, the inflorescence P decreased at salt concentrations of 25, 50, 100, and 200 mM by 16.03%, 16.38%, 26.67%, and 42.64% for NaCl; 10.31%, 14.80%, 15.34%, and 28.82% for KCl; 4.80%, 31.00%, 33.18%, and 33.20% for $MgSO_4$; and 20.39%, 26.50%, 35.88%, and 40.85% for $CaCl_2$. Stem P decreases by 50.83%, 29.42%, 24.02% and 23.97% at 200 mM NaCl, KCl, $MgSO_4$, and $CaCl_2$ respectively compared to control. Root P also decreased with increasing salt concentration but not in a concentration-dependent manner (Fig. 8).

Leaf K content decreases by 4.51%, 33.25%, 40.74%, and 40.04% with NaCl at concentrations of 25, 50, 100, and 200 mM, respectively, and by 7.11%, 34.14%, 51.60%, and 50.98% with $CaCl_2$ at the same concentrations. In contrast, KCl treatment increases leaf K content by 36.06%, 69.41%, 20.23%, and 3.59% at these concentrations, respectively. MgSO4, at 25 mM, does not significantly alter leaf K and subsequently decreases K content by 22.12%, 44.30%, and 59.97% at 50, 100, and 200 mM, respectively. In inflorescences, K content with NaCl at 25 mM does not show a significant difference, but decreases by 12.22%, 15.07%, and 30.39% at 50, 100, and 200 mM, respectively. Inflorescence K content decreases by 1.78%, 2.81%, 25.27%, and 26.34% with $MgSO_4$ at 25, 50, 100, and 200 mM concentrations, respectively, and by 2.64%, 2.64%, 13.79%, and 30.72% with $CaCl_2$ at these concentrations. However, KCl treatment increases inflorescence K content by 20.31%, 54.75%, 54.75%, and 51.28% at the same concentrations. In stem, the maximum reduction of K content occurs by14.64% at 200 mM $CaCl_2$. While, KCl treatment increases stem K by 38.30%, 64.39%, 97.32%, and 90.22% at 25, 50, 100, and 200 mM concentrations, respectively. However, in root K

decreases with increasing salt concentration, but not in a concentration-dependent manner. Moreover, the maximum reduction in root K observed at 200 mM NaCl treatment (79.58%). **(**Fig. 9**)**.

Essential oil constituents

Essential oil composition in response to various concentrations of different salt compounds were analysed by GC-MS technique and results provided in Table 3**(a-d)**. In essential oil obtain from control plant groups, forty-seven components were identified, with a focus on assessing the major compounds. The main component in *M. Citriodora* essential oil were thymol (39.26–91.36%); γ- terpinene (2.62–19.41%); benzene, methyl(1-methylethyl) (1.10-17.79%); α- terpinene (0.43–6.53%); phenol, 2-methyl-5-(1-methylethyl) (1.72–4.15%); 1–6 octadiene, 7-methyl-3-methylene (0.07 to 3.76%); caryophyllene (0.05–0.91%); β- myrcene (0.02 to 0.67%);cyclohexene 1-methyl-4-(1-methylethenyl) (0.09–1.09%); bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)- (0.07–0.51%); isopropyl-1-methyl-5-methylene-1,6- cyclodecadiene (0.04–0.47%); bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl), (1.alpha.,2.beta.,5.alpha.) (0.03–0.04%). The GC-MS chromatogram of control group essential oil was shown in Fig. 10. Different salt concentrations influenced the biosynthesis of thymol, while, the thymol % did not exhibit a linear relationship with increasing salts concentration. The highest thymol content (91.36%) was observed with 50 mM KCl treatment, followed closely by 50 mM NaCl (91.07%) and 100 mM KCl (88.58%). NaCl treatment increased thymol content by 5.14%, 14.93%, 9.83%, and 7.13% at 25, 50, 100, and 200 mM concentrations, respectively. KCl treatment resulted in thymol increases of 13.6%, 15.3%, and 11.79% at 25, 50, and 100 mM, respectively, with a subsequent decrease at 200 mM. $MgSO_4$ treatment reduced thymol content by 3.09% and 13.68% at 25 and 200 mM, respectively, while it increased thymol content by 3.62% and 0.31% at 50 and 100 mM. At 200 mM $CaCl_2$ treatment, thymol content decreased drastically by 50.45%, and decreased by 1.15% and 8.60% at 25 and 100 mM, respectively. No significant change in thymol content was observed with 50 mM $CaCl_2$ compared to the control **(**Fig. 11**)**.

Table 3

(a). The essential oil composition of *M. citriodora* under various concentrations of NaCl treatment.

S.No.	Compounds name	RT	Control	NaCl 25 mM	NaCl 50 mM	NaCl 100 mM	NaCl 200 mM	LSD
1	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	10.0816	0.17 ± 0.03 h	0.16 ± 0.01 h	0.08 ± 0.02 i	0.11 ± 0.01 i	0.11 ± 0.02 i	0.0381
2	1,6-Octadiene, 7-methyl-3-methylene-	12.1232	0.35 ± 0.30 b-d	0.33 ± 0.29 b-d	0.07 ± 0.12 cd	0.14 ± 0.23 b-d	0.09 ± 0.16 b-d	0.5345
3	Beta-Myrcene	12.1233	0.14 ± 0.24 c-e	0.03 ± 0.05 de	0.17 ± 0.15 b-e	0.24 ± 0.21 b-e	0.18 ± 0.16 b-e	0.3434
4	Alpha. Terpinene	12.9246	1.10 ± 0.17 d-f	0.83 ± 0.56 d-i	0.46 ± 0.08 hi	0.70 ± 0.06 e-i	0.44 ± 0.02 i	0.5833
5	Benzene, methyl(1-methylethyl)-	13.1798	2.77 ± 0.48 ef	2.64 ± 1.66 e-g	1.98 ± 0.38 f-h	4.15 ± 0.41 cd	6.71 ± 0.22 b	1.2342
6	Cyclohexene, 1-methyl-4-(1-methylethenyl)-	13.2926	0.17 ± 0.01 d-f	0.15 ± 0.08 ef	0.13 ± 0.02 ef	0.21 ± 0.02 c-e	0.21 ± 0.01 c-e	0.0849
7	Gamma-Terpinene	14.2185	10.76 ± 1.46 bc	8.34 ± 5.49 b-d	2.63 ± 0.54 g	4.21 ± 0.43 fg	3.04 ± 0.08 g	3.4836
8	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-	14.4500	0.16 ± 0.02 b-d	0.12 ± 0.10 b-d	0.14 ± 0.08 b-d	0.22 ± 0.01 b	0.14 ± 0.07 b-d	0.1557
9	Thymol	20.4743	79.25 ± 3.58 e-g	83.32 ± 8.76 c-f	91.08 ± 1.39 ab	87.03 ± 1.04 a-d	84.9 ± 0.34 b-e	6.2066
10	Phenol, 2-methyl-5-(1-methylethyl)-	20.6286	4.1 ± 1.25 ab	3.09 ± 0.46 ab	2.09 ± 0.30 bc	1.73 ± 0.10 c	2.62 ± 0.15 bc	1.2136
11	Caryophyllene	23.2579	0.22 ± 0.05 d-	0.23 ± 0.04	0.15 ± 0.01	0.19 ± 0.01	0.16 ±	0.1073

Data are presented as mean ± standard deviation. Values in the same row with the same letter are not significantly different (α = 0.05). RT; retention time, and LSD; least significant difference.

S.No.	Compounds name	RT	Control	NaCl 25 mM	NaCl 50 mM	NaCl 100 mM	NaCl 200 mM	LSD
			f	d-f	f-h	e-g	0.01 fg	
12	Isopropyl-1-methyl-5-methylene-1,6-cyclodecadiene	24.5400	0.16 ± 0.03 d-g	0.20 ± 0.06 b-e	0.12 ± 0.01 f-j	0.06 ± 0.04 ij	0.04 ± 0.01 j	0.0804

Data are presented as mean ± standard deviation. Values in the same row with the same letter are not significantly different (α = 0.05). RT; retention time, and LSD; least significant difference.

Table 3

(b). The essential oil composition of *M. citriodora* under various concentrations of KCl treatment.

S.No.	Compounds name	RT	Control	KCl 25 mM	KCl 50 mM	KCl 100 mM	KCl 200 mM	LSD
1	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	10.0816	0.17 ± 0.03 h	0.08 ± 0.03 i	0.12 ± 0.08 i	0.19 ± 0.03 gh	0.28 ± 0.01 cd	0.0381
2	Beta-Myrcene	12.1233	0.14 ± 0.24 c-e	0.13 ± 0.11 e	0.27 ± 0.13 b-e	0.24 ± 0.21 b-e	0 ± 0 e	0.3434
3	1,6-Octadiene, 7-methyl-3-methylene-	12.1232	0.35 ± 0.3 b-d	0.1 ± 0.16 b-d	0 ± 0 d	0.15 ± 0.26 b-d	0.58 ± 0.01 b-d	0.5345
4	Alpha Terpinene	12.9246	1.1 ± 0.17 def	0.49 ± 0.14 g-i	0.55 ± 0.26 f-i	0.77 ± 0.16 e-i	1.08 ± 0.01 d-g	0.5833
5	Benzene, methyl(1-methylethyl)-	13.1798	2.77 ± 0.48 ef	1.11 ± 0.33 h	1.46 ± 0.51 gh	2.28 ± 0.54 e-h	7.46 ± 0.07 b	1.2342
6	Cyclohexene, 1-methyl-4-(1-methylethenyl)-	13.2926	0.17 ± 0.01 d-f	0.1 ± 0.01 f	0.17 ± 0.1 d-f	0.17 ± 0.02 d-f	0.25 ± 0.01 b-d	0.0849
7	Gamma-Terpinene	14.2185	10.76 ± 1.46 bc	4.95 ± 1.75 d-g	3.27 ± 0.96 g	4.69 ± 1.27 e-g	7.7 ± 0.06 c-e	3.4836
8	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-	14.4500	0.16 ± 0.02 b-d	0.12 ± 0.07 b-d	0.17 ± 0.06 bc	0.08 ± 0.07 b-d	0.1 ± 0.01 b-d	0.1557
9	Thymol	20.4743	79.25 ± 3.58 e-g	90.03 ± 2.91 ab	91.37 ± 2.02 a	88.59 ± 2.59 a-c	78.89 ± 0.09 e-g	6.2066
10	Phenol, 2-methyl-5-(1-methylethyl)-	20.6286	4.1 ± 1.25 ab	2.16 ± 0.5 bc	1.92 ± 0.57 bc	1.97 ± 0.43 bc	2.54 ± 0.04 bc	1.2136
11	Caryophyllene	23.2579	0.22 ± 0.05 d-f	0.18 ± 0.02 e-g	0.06 ± 0.09 h	0.16 ± 0.01 f-h	0.3 ± 0.01 cd	0.1073
12	Isopropyl-1-methyl-5-methylene-1,6-cyclodecadiene	24.5400	0.16 ± 0.03 d-g	0.15 ± 0.03 d-h	0.08 ± 0.07 h-j	0.12 ± 0.01 e-i	0.15 ± 0.01 d-h	0.0804

Data are presented as mean ± standard deviation. Values in the same row with the same letter are not significantly different (α = 0.05). RT; retention time and LSD; least significant difference.

Table 3

(b). The essential oil composition of *M. citriodora* under various concentrations of KCl treatment.

S.No.	Compounds name	RT	Control	KCl 25 mM	KCl 50 mM	KCl 100 mM	KCl 200 mM	LSD
1	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	10.0816	0.17 ± 0.03 h	0.08 ± 0.03 i	0.12 ± 0.08 i	0.19 ± 0.03 gh	0.28 ± 0.01 cd	0.0381
2	Beta-Myrcene	12.1233	0.14 ± 0.24 c-e	0.13 ± 0.11 e	0.27 ± 0.13 b-e	0.24 ± 0.21 b-e	0 ± 0 e	0.3434
3	1,6-Octadiene, 7-methyl-3-methylene-	12.1232	0.35 ± 0.3 b-d	0.1 ± 0.16 b-d	0 ± 0 d	0.15 ± 0.26 b-d	0.58 ± 0.01 b-d	0.5345
4	Alpha Terpinene	12.9246	1.1 ± 0.17 def	0.49 ± 0.14 g-i	0.55 ± 0.26 f-i	0.77 ± 0.16 e-i	1.08 ± 0.01 d-g	0.5833
5	Benzene, methyl(1-methylethyl)-	13.1798	2.77 ± 0.48 ef	1.11 ± 0.33 h	1.46 ± 0.51 gh	2.28 ± 0.54 e-h	7.46 ± 0.07 b	1.2342
6	Cyclohexene, 1-methyl-4-(1-methylethenyl)-	13.2926	0.17 ± 0.01 d-f	0.1 ± 0.01 f	0.17 ± 0.1 d-f	0.17 ± 0.02 d-f	0.25 ± 0.01 b-d	0.0849
7	Gamma-Terpinene	14.2185	10.76 ± 1.46 bc	4.95 ± 1.75 d-g	3.27 ± 0.96 g	4.69 ± 1.27 e-g	7.7 ± 0.06 c-e	3.4836
8	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-	14.4500	0.16 ± 0.02 b-d	0.12 ± 0.07 b-d	0.17 ± 0.06 bc	0.08 ± 0.07 b-d	0.1 ± 0.01 b-d	0.1557
9	Thymol	20.4743	79.25 ± 3.58 e-g	90.03 ± 2.91 ab	91.37 ± 2.02 a	88.59 ± 2.59 a-c	78.89 ± 0.09 e-g	6.2066
10	Phenol, 2-methyl-5-(1-methylethyl)-	20.6286	4.1 ± 1.25 ab	2.16 ± 0.5 bc	1.92 ± 0.57 bc	1.97 ± 0.43 bc	2.54 ± 0.04 bc	1.2136
11	Caryophyllene	23.2579	0.22 ± 0.05 d-f	0.18 ± 0.02 e-g	0.06 ± 0.09 h	0.16 ± 0.01 f-h	0.3 ± 0.01 cd	0.1073
12	Isopropyl-1-methyl-5-methylene-1,6-cyclodecadiene	24.5400	0.16 ± 0.03 d-g	0.15 ± 0.03 d-h	0.08 ± 0.07 h-j	0.12 ± 0.01 e-i	0.15 ± 0.01 d-h	0.0804

Data are presented as mean ± standard deviation. Values in the same row with the same letter are not significantly different (α = 0.05). RT; retention time and LSD; least significant difference.

Table 3

(b). The essential oil composition of *M. citriodora* under various concentrations of KCl treatment.

S.No.	Compounds name	RT	Control	KCl 25 mM	KCl 50 mM	KCl 100 mM	KCl 200 mM	LSD
1	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	10.0816	0.17 ± 0.03 h	0.08 ± 0.03 i	0.12 ± 0.08 i	0.19 ± 0.03 gh	0.28 ± 0.01 cd	0.0381
2	Beta-Myrcene	12.1233	0.14 ± 0.24 c-e	0.13 ± 0.11 e	0.27 ± 0.13 b-e	0.24 ± 0.21 b-e	0 ± 0 e	0.3434
3	1,6-Octadiene, 7-methyl-3-methylene-	12.1232	0.35 ± 0.3 b-d	0.1 ± 0.16 b-d	0 ± 0 d	0.15 ± 0.26 b-d	0.58 ± 0.01 b-d	0.5345
4	Alpha Terpinene	12.9246	1.1 ± 0.17 def	0.49 ± 0.14 g-i	0.55 ± 0.26 f-i	0.77 ± 0.16 e-i	1.08 ± 0.01 d-g	0.5833
5	Benzene, methyl(1-methylethyl)-	13.1798	2.77 ± 0.48 ef	1.11 ± 0.33 h	1.46 ± 0.51 gh	2.28 ± 0.54 e-h	7.46 ± 0.07 b	1.2342
6	Cyclohexene, 1-methyl-4-(1-methylethenyl)-	13.2926	0.17 ± 0.01 d-f	0.1 ± 0.01 f	0.17 ± 0.1 d-f	0.17 ± 0.02 d-f	0.25 ± 0.01 b-d	0.0849
7	Gamma-Terpinene	14.2185	10.76 ± 1.46 bc	4.95 ± 1.75 d-g	3.27 ± 0.96 g	4.69 ± 1.27 e-g	7.7 ± 0.06 c-e	3.4836
8	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-	14.4500	0.16 ± 0.02 b-d	0.12 ± 0.07 b-d	0.17 ± 0.06 bc	0.08 ± 0.07 b-d	0.1 ± 0.01 b-d	0.1557
9	Thymol	20.4743	79.25 ± 3.58 e-g	90.03 ± 2.91 ab	91.37 ± 2.02 a	88.59 ± 2.59 a-c	78.89 ± 0.09 e-g	6.2066
10	Phenol, 2-methyl-5-(1-methylethyl)-	20.6286	4.1 ± 1.25 ab	2.16 ± 0.5 bc	1.92 ± 0.57 bc	1.97 ± 0.43 bc	2.54 ± 0.04 bc	1.2136
11	Caryophyllene	23.2579	0.22 ± 0.05 d-f	0.18 ± 0.02 e-g	0.06 ± 0.09 h	0.16 ± 0.01 f-h	0.3 ± 0.01 cd	0.1073
12	Isopropyl-1-methyl-5-methylene-1,6-cyclodecadiene	24.5400	0.16 ± 0.03 d-g	0.15 ± 0.03 d-h	0.08 ± 0.07 h-j	0.12 ± 0.01 e-i	0.15 ± 0.01 d-h	0.0804

Data are presented as mean ± standard deviation. Values in the same row with the same letter are not significantly different (α = 0.05). RT; retention time and LSD; least significant difference.

Various salt treatments significantly influence the percentage of γ-terpinene. All salt concentrations decrease γ-terpinene content except for 200 mM $MgSO_4$ and 25 and 200 mM $CaCl_2$. NaCl and KCl reduce γ-terpinene content by 22.52%, 75.55%, 60.85%, and 71.80% at 25, 50, 100, and 200 mM, respectively, and by 54.02%, 69.59%, 56.42%, and 28.45% at the same concentrations, respectively. Conversely, $MgSO_4$ reduces γ-terpinene content by 7.92%, 15.41%, and 10.53% at 25, 50, and 100 mM, respectively, while $CaCl_2$ reduces γ-terpinene content by 17.21% and 30.55% at 50 and 100 mM, respectively. At 200 mM, $MgSO_4$ increases γ-terpinene content by 63.04%, and 25 and 200 mM $CaCl_2$ increase γ-terpinene content by 4.26% and 80.59%, respectively, compared to the control.

A quantitative change in the benzene, methyl(1-methylethyl) content was observed following salt stress treatments. The percentage area of benzene, methyl(1-methylethyl) improved with various salt treatments, except for 25 and 50 mM NaCl and 25, 50, and 100 mM $MgSO_4$, which decreased by 4.77%, 28.07%, 47.17%, and 17.79%, respectively. Notably, a 200 mM concentration of all salts resulted in better improvement in benzene, methyl(1-methylethyl) percentage, with 200 mM $CaCl_2$ showing the highest percentage area compared to all other treatments. The α-terpinene percentage increased at 25 and 200 mM concentrations of $MgSO_4$ by 27.72% and 92.62%, respectively. Additionally, $CaCl_2$ improved α-terpinene at 25, 50, 100, and 200 mM by 24.89%, 15.73%, 76.62%, and 499.03%, respectively, while other salts generally decreased α-terpinene percentage, with the greatest reduction observed at 200 mM NaCl. Salt treatments decreased the phenol, 2-methyl-5-(1-methylethyl) percentage area, except for 100 mM $CaCl_2$ (4.15%) compared to the control. The minimum percentage of phenol, 2-methyl-5-(1-methylethyl) was recorded with 100 mM NaCl (1.72%), with no significant changes observed between 25, 50, and 100 mM KCl, $MgSO_4$, 50 mM NaCl, 200 mM $MgSO_4$, and 25 and 50 mM $CaCl_2$. The maximum percentage area of 7-methyl-3-methylene, 1, 6-octadiene was noted with 100 mM $CaCl_2$ (3.76%). The percentage of 7-methyl-3-methylene, 1,6-octadiene increased with 200 mM KCl, $MgSO_4$, and all concentrations of $CaCl_2$, although this compound was not detected in essential oils treated with 50 mM KCl, 25, 50, and 100 mM salt stress. Cyclohexene 1-methyl-4-(1-methylethenyl) decreased by 14.57% and 23.68% at 25 and 50 mM NaCl, by 41.62%, 2.56%, and 1.67% with KCl, and by 20.15% with 50 mM $MgSO_4$, while other salt treatments increased cyclohexene 1-methyl-4-(1-methylethenyl) percentage. The maximum increase was observed with 200 mM $CaCl_2$ (1.08%), representing a 541.55% increase compared to the control. Other compounds, such as caryophyllene, β-myrcene, bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl); isopropyl-1-methyl-5-methylene-1,6-cyclodecadiene, and bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl), (1.alpha.,2.beta.,5.alpha.) exhibited major variations in percentage area with different salt treatments; however, their % area in essential oil remained below 1% in every treatments.

Correlation analysis

The study examined the Pearson correlation between several plant characteristics in *M. citriodora*, including growth attributes (plant height, number of branches, number of inflorescences), plant biomass (fresh and dry weight of shoots and roots), DPPH inhibition percentage, proline content, and relative water content, under varying salt stress treatments (Fig. 12). In the correlation plot, blue colour boxes indicate a positive correlation, while orange colour boxes represent a negative correlation. Shoot fresh and dry weights exhibited a strong positive correlation (r = + 0.96). Growth attributes and plant biomass were positively correlated with each other. Relative water content and chlorophyll content were positively correlated with each other, as well

as with growth attributes and plant biomass. Shoot fresh weight showed a strong negative correlation (r = -0.89) with proline content. Conversely, proline content and DPPH inhibition percentage exhibited a positive correlation with each other, but a negative correlation with growth attributes and plant biomass.

A separate Pearson correlation analysis was carried out between the different chemical components *M. Citriodora* essential oil subjected to different salt stress treatments, as illustrated in Fig. 13. The constituents analyzed include thymol; γ-terpinene; methylbenzene (1-methylethyl); α-terpinene; 2-methyl-5-(1-methylethyl)phenol; 7-methyl-3-methylene-1,6-octadiene; 1-methyl-4-(1-methylethenyl)cyclohexene; caryophyllene; β-myrcene; 2-methyl-5-(1-methylethyl)bicyclo[3.1.0]hex-2-ene; isopropyl-1-methyl-5-methylene-1,6-cyclodecadiene; and 2-methyl-5-(1-methylethyl)bicyclo[3.1.0]hexan-2-ol (1α,2β,5α). According to analysis α-terpinene and cyclohexene 1-methyl-4-(1-methylethenyl) have a strong positive correlation (r = + 0.98). While, Thymol and α-terpinene showed a strong negative correlation (r = -0.97). Additionally, thymol and β-myrcene demonstrated negative correlations with all other compounds, although thymol and β-myrcene themselves showed a positive correlation with each other (r = + 0.14).

Principal component (PCA) analysis

Principal component analysis was used to examine the dependent variables of M. citriodora, such as growth parameters (plant height, number of branches and number of inflorescences), plant biomass (fresh and dry weight of shoot and root), DPPH inhibition percentage, proline, relative water content, and total chlorophyll content. As per the biplot (score and loading) attained through PCA analysis, PC1 and PC2 contributed 70.64% and 13.97% of the variance, respectively, and accounted about 84.01% of the dataset **(**Fig. 14**)**. Furthermore, another PCA was carried out on the essential oil's components under several salt treatments (NaCl, KCl, $MgSO_4$, and $CaCl_2$) at distinct concentrations of 25, 50, 100, and 200 mM. The biplot from this PCA exhibited approximately 76.33% of the dataset and was described by PC1 and PC2, with PC1 and PC2 contributing 62.61% and 13.72% of the variance, respectively **(**Fig. 15**)**.

Discussion

Inappropriate irrigation water management containing high salt concentrations, coupled with insufficient agronomic practices such as poor fertilization and climate change, is gradually increasing soil salinity in arable lands globally (Atta et al. 2023). Salt stress hampers plant growth due to osmotic stress, ionic stress and nutritional imbalances (Shrivastava and Kumar 2015). Osmotic stress caused by presence of high salts in soil disturbs water absorption and transport. This disturbance initiates hormonal responses that can reduce stomatal opening, CO_2 assimilation, and photosynthetic rate.(Ma et al. 2020).

The present study investigated the morphological, biochemical and physiological responses of *M. Citriodora* to four distinct salts (NaCl, $CaCl_2$, KCl, and $MgSO_4$) at various concentrations (25, 50, 100, and 200 mM) applied to soil. Salt stress adversely affected on the physiological and morphological characteristics of plants. According to results NaCl, $CaCl_2$, and KCl shown adverse effects at concentrations increasing 100 mM, with NaCl, KCl, and $CaCl_2$ have more toxic effect compared to $MgSO_4$. Number of inflorescences slightly increased with 25 mM $MgSO_4$ and $CaCl_2$ treatments. However, the number of branches increased with $MgSO_4$. Conversely, plant growth, root and shoot biomass decreases by other salts treatments. Previous

studies revealed that NaCl and KCl have more pronounced harmful effects on growth of *Salvia officinalis* and *Mentha longifolia* compared to $CaCl_2$ and $MgSO_4$ (Kulak et al. 2020; Singh et al. 2023).

NaCl, usually known as table salt, can significantly affect plants growth and soils properties when it presents in high concentration. Earlier it was reported that increased NaCl concentrations have adverse impact on plant morphology. For instance, NaCl salt treatment have negative effects on *Glycine max* L. (Jabeen et al. 2021), *Dracocephalum moldavica* L. (Mohammadi et al. 2021), *Oenanthe javanica* (Kumar et al. 2021), *Ocimum basilicum* L. (Lazarević et al. 2021), *Lavandula angustifolia* (Chrysargyris et al. 2018), *Launaea sarmentosa* (Tran et al. 2024), and *Solanum nigrum* L. (Ben Abdallah et al. 2016). NaCl have negative impact on plant growth primarily through osmotic stress and then it causes ionic toxicity (Atta et al. 2023). High NaCl concentrations create osmotic pressure, which decreases water uptake and causes dehydration (Lu and Fricke 2023). Increasing NaCl concentration increases Na^+ concentration in medium and these Na^+ competes with K^+ uptake, disrupting enzyme functions and protein synthesis (Raddatz et al. 2020). Cl^- ions are involved in regulating turgor pressure, pH balance, enzyme stability, membrane potential, charge balance, volume control, stomatal conductance and osmoregulation, thereby influencing water retention, water consumption, and photosynthetic processes (Shelke et al. 2019). Both Na^+ and Cl^- ions disturb cellular homeostasis, resulting in reduced plants growth and yield. In *M. citriodora* increasing concentrations of NaCl have been shown to enhance proline content and total antioxidant activity, while decreasing leaf relative water content, chlorophyll levels, and nutrient content. Similar results have been reported in reduction of photosynthetic activities, nutrient uptake and flowering process in *Foeniculum vulgare*, (Beyk-Khormizi et al. 2023; Lamsaadi et al. 2024). Recent studies also indicate that NaCl treatment increases proline content and antioxidant activity in *Moringa oleifera* (Azeem et al. 2023) and *Origanum vulgare* (Azimzadeh et al. 2024).

Potassium is animportant macronutrientfor plant growth and development; however, both its deficiency and toxicity can impairseveral physiological functions in plants (Johnson et al. 2022). Asthe seventh most rich element in the earth, K is readily available in soils, primarily through weathering of K-rich minerals and excessive fertilizer application.(Pantha et al. 2023). Although KCl shares a similar salty taste with NaCl, it is distinguished by more pronounced offensive tastes such as bitterness, metallicity, and acridity (Rasheed et al. 2020). The results indicate that KCl significantly reduces growth attributes and biomass in *M. citriodora*. However, a 25 mM KCl concentration does not significantly affect plant height and improves no of branches. Due to the high external K^+ concentration, extra K^+ is absorbed into the cytoplasm under KCl stress by an electrochemical gradient, disrupting the Na^+/K^+ balance within the cytoplasm, leading to ion toxicity (Park et al. 2016; Li et al. 2021). Furthermore, high concentrations of K + in the soil cause osmotic stress, reduce the water potential of the soil and restrict plant uptake of water (Yang and Guo 2018). Previous research has indicated that KCl salt stress negatively affects plant growth in species such as *Chenopodium album* (Yao et al. 2010), *Triticum aestivum* L. (Rasheed et al. 2020), and *Carthamus tinctorius* L. (Leblebici et al. 2021). Recently by Raneenga et al. (2024) evaluated the impact of KCl salt stress on *Bacopa monnieri*, revealing that at 100 mM KCl treatment, plants exhibited up to a 1.8-fold increase in bacoside-A content, alongside significant improvements in plant height and biomass. Additionally, chlorophyll content and antioxidant enzyme activities were highest at this concentration. However, higher KCl levels (200 mM) did not benefit plant growth or physiological parameters. Furthermore, increased KCl concentrations trigger physiological drought response in plants, inducing osmotic stress that subsequently leads to a decrease in nutrient

content (N and P), chlorophyll, and relative water content but an increase in antioxidant activity and proline content, in *M. citriodora*. Moreover, KCl treatment significantly enhances K accumulation in different parts of *M. citriodora*.

Mg^{2+} is an essential cofactor for enzymes involved in chlorophyll biosynthesis and carbon fixation (Ahmed et al. 2023). It is also essential for the activity of several cellular enzymes, such as carboxylases, adenosine triphosphatases, RNA polymerases, protein kinases and glutathione synthase (Hauer-Jákli and Tränkner 2019; Xie et al. 2021). However, elevated Mg^{2+} levels can adversely affect plant growth, as excess Mg^{2+} may replace Ca^{2+} and K^{+}, leading to disruptions in cell wall stability and membrane permeability (Guo et al. 2015; Lamichhane et al. 2023). Mg^{2+} in soils primarily originates from source rock materials with different silicate types, such as muscovite, biotite, hornblende, augite, and olivine, due to the substitution of Al^{3+} for Mg^{2+}. Additionally, carbonates such as magnetite, dolomite, and calcite play a significant role in contributing to the Mg content in soil (Gransee and Führs 2013). The treatment of *M. citriodora* with $MgSO_4$ salt resulted in decreased growth attributes, biomass, chlorophyll content, leaf relative water content, and nutrient levels. However, a concentration of 25 mM $MgSO_4$ improved these parameters. All concentrations of $MgSO_4$ enhanced the number of branches. Similarly, Mg concentrations of ≥ 8.5 mM (207 mg L^{-1}) in the soil solution have been shown to affect the growth of *Arabidopsis thaliana* (Canham et al. 2020). Guo et al. (2015) determined that a concentration of 1.5 mM Mg^{2+} is optimal for *Arabidopsis* growth, with deviations from this concentration disrupting processes such as ion uptake, starch production, degradation, and plant morphology. In *Echinochloa species*, high $MgCl_2$ or $MgSO_4$ (30 mM) decreased growth by 33–42% and caused calcium deficiency symptoms, whereas in rice, growth was reduced by 54–67% without such symptoms. In *E. oryzicola* and rice, high Mg significantly decreased the calcium content (Kobayashi et al. 2005). Recent studies have assessed the impact of varying Mg^{2+} levels and sources on the rice cultivar 'XP 753'. Excess magnesium salts led to reduced plant growth, biomass, and yield, accompanied by increased oxidative stress and altered mineral uptake, highlighting Mg^{2+} toxicity in rice (Lamichhane et al. 2023).

The results showed that *M. Citriodora* plants treated with $CaCl_2$ exhibit a significant reduction in growth parameters, biomass production, chlorophyll content and leaf relative water content. However, a 25 mM $CaCl_2$ treatment enhances the number of inflorescences. Additionally, proline content and antioxidant activity in *M. Citriodora* also increased in response to $CaCl_2$ treatments. Plant cells take calcium through Ca^{2+}-permeable ion channels in the plasma membranes. Due to the cytotoxic nature of elevated intracellular calcium concentrations $[Ca^{2+}]_{(}cyt_{)}$, Ca^{2+}-ATPases and H^{+}/Ca^{2+}-antiporters keep submicromolar $[Ca^{2+}]_{(}cyt_{)}$ in unstimulated cells (White and Broadley 2003). Additionally, calcium influences the uptake of other elements, exhibiting both antagonistic and synergistic interactions with these elements (Weng et al. 2022). Previous studies have demonstrated that irrigation with high $CaCl_2$ containing water led to structural damage and decreased photosynthetic performance in *C. Citrinus* leaves, as assessed through digital image analysis (De Micco et al. 2021). Voutsinos-Frantzis et al. (2023) reported that NaCl had a lesser impact on the agronomic traits of corn salad compared to $CaCl_2$ at isosmotic levels, with reduced nitrate concentrations and unchanged total N. For hydroponic cultivation of corn salad, water with up to 20 mM NaCl is preferable, as NaCl is less harmful than $CaCl_2$. Additionally, $CaCl_2$ also altered mineral elements, proline, and total protein levels in *Oryza sativa* (Orcan and Orcan 2024).

The variation in the quantity of essential oil compounds is influenced by several factors, including climate, edaphic factors, plant species, fertilizers, growth factors, harvest time, storage procedures, cultivation methods, and oil extraction processes (Preedy 2015). Alterations in the composition of essential oil constituents have been observed in various aromatic plants subjected to stress, with variations differing by stress type and plant characteristics (Aziz et al. 2008). When there is a salt stress condition, excess salt in the protoplasm disrupts ionic balance and enzymatic function. These disturbances lead to reduced energy production through phosphorylation and photorespiration, impaired nitrogen assimilation, and disruption of various metabolic pathways. Typically, saline stress extensively alters terpenoid metabolism (Sarmoum et al. 2019). Our results indicate that the percentage of thymol in *M. Citriodora* essential oil increases at 50 mM concentrations of NaCl, KCl, $MgSO_4$, and $CaCl_2$ salts, but decreases with further increases in salt concentration. Additionally, γ-terpinene decreases with increasing salt concentration, except at 200 mM $MgSO_4$ and $CaCl_2$, where its percentage improves. Previous studies have shown that cinnamic acid and thymol levels increase under salinity stress in *Thymus vulgaris* and *Thymus daenensis* (Bistgani et al. 2019). Other compounds in *M. citriodora* essential oil detected by GC-MS was γ- terpinene; benzene, methyl(1-methylethyl); α- terpinene; cyclohexene 1-methyl-4-(1-methylethenyl); 1–6 octadiene, 7-methyl-3-methylene; phenol, 2-methyl-5-(1-methylethyl); caryophyllene; β- myrcene; bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl); isopropyl-1-methyl-5-methylene-1,6- cyclodecadiene and bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl), (1.alpha.,2.beta.,5.alpha.). Previous studies have identified that the main constituents of *M. citriodora* essential oil include thymol, γ-terpinene, cymene, and cyclohexene (Katoch and Pull 2017; Lawson et al. 2021; Markovska et al. 2020). Valifard et al. (2014) reported that in *Salvia mirzayanii*, the synthesis of total phenolics and several important volatile components was improved by moderate salinity (6.8 dS m − 1 NaCl).

Conclusions

The present study analysed the influence of four different salts (NaCl, $CaCl_2$, KCl, and $MgSO_4$) at various concentrations (25, 50, 100, and 200 mM) on *M. citriodora*. The results determined that growth attributes, biomass, pigment content, and relative water content were adversely affected, whereas in *M. Longifolia*, proline content and antioxidant activity improved upon exposure to salt treatments. Additionally, novel chemotypes emerged in the essential oil with salt treatments. The essential oils' quality, particularly thymol content, increased with mild to moderate (25 mM and 50 mM) salt concentrations. Future studies should investigate the biological or industrial potential of these findings, as the efficacy and usage of plants depend on their metabolites and herbs, enhancing *M. citriodora* cultivation and production of essential oil.

Declarations

Author Contributions

All the authors have made their contribution to the article as follows; **RS** conceived the idea and performed the experiment. **SGG** and **RB** supervised the work. **MG** helped **RS** to draft manuscript preparation. All authors revised the manuscript.

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Declarations of Competing Interest

All the authors have reported no potential conflict of interest.

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Figures

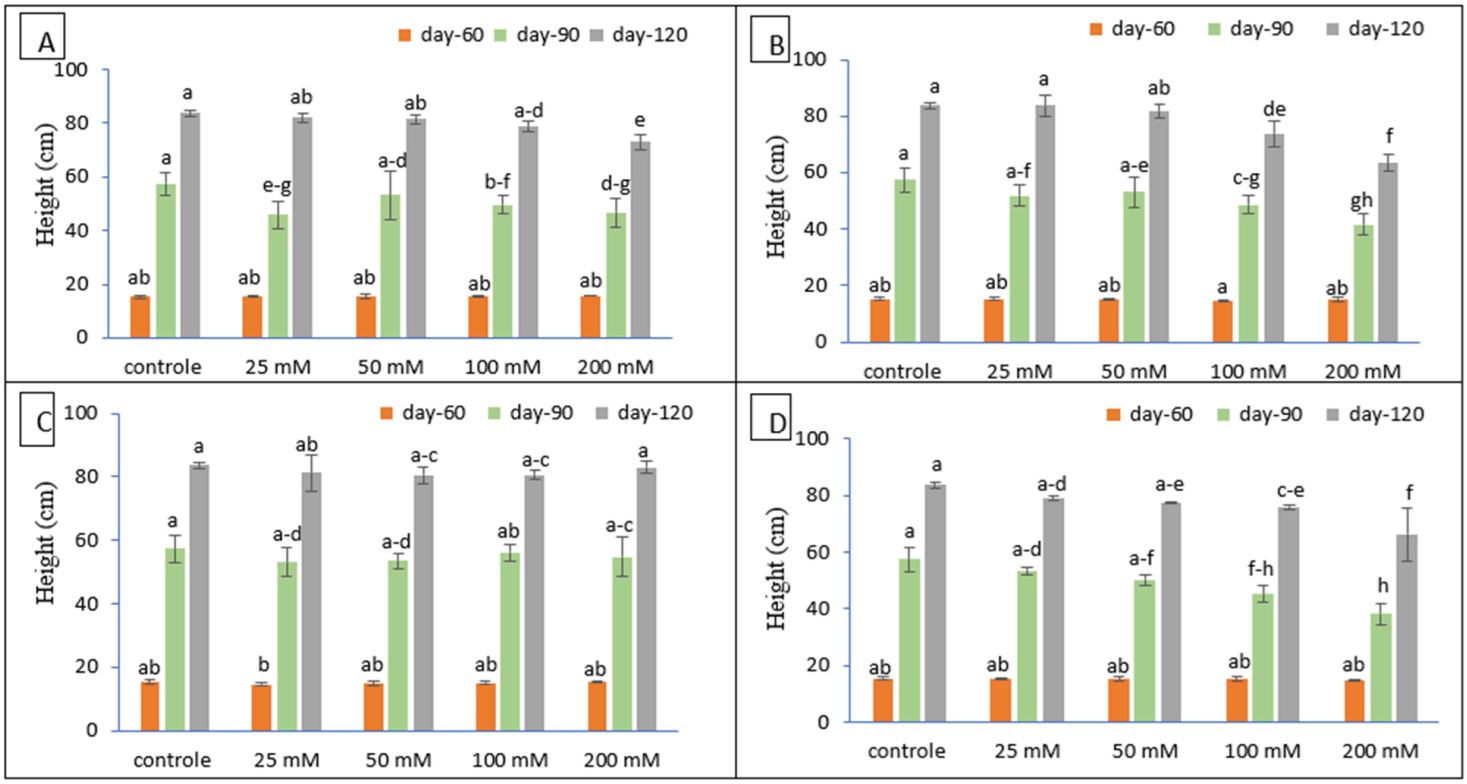

Figure 1

The heights of *M. citriodora* at 60, 90, and 120 days were measured under various concentrations of salts: (A) NaCl, (B) KCl, (C) $MgSO_4$, and (D) $CaCl_2$. The data are presented as mean ± standard deviation (n=3). Same colour bar with the same letter is not significantly different (α = 0.05)

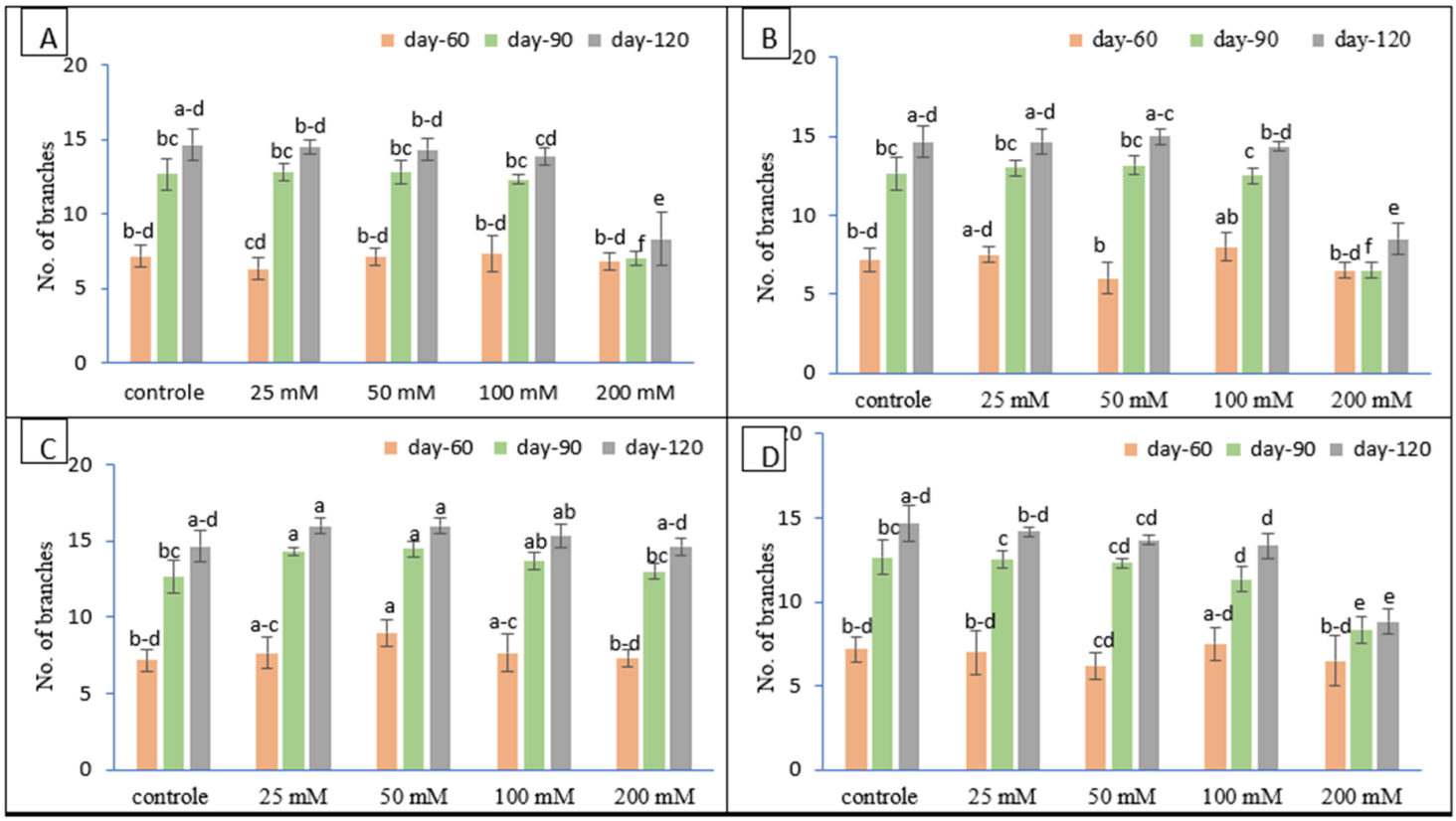

Figure 2

N0. Of branches of *M. citriodora* at 60, 90, and 120 days were measured under various concentrations of salts: (A) NaCl, (B) KCl, (C) $MgSO_4$, and (D) $CaCl_2$. The data are presented as mean ± standard deviation (n=3). Same colour bar with the same letter is not significantly different (α = 0.05)

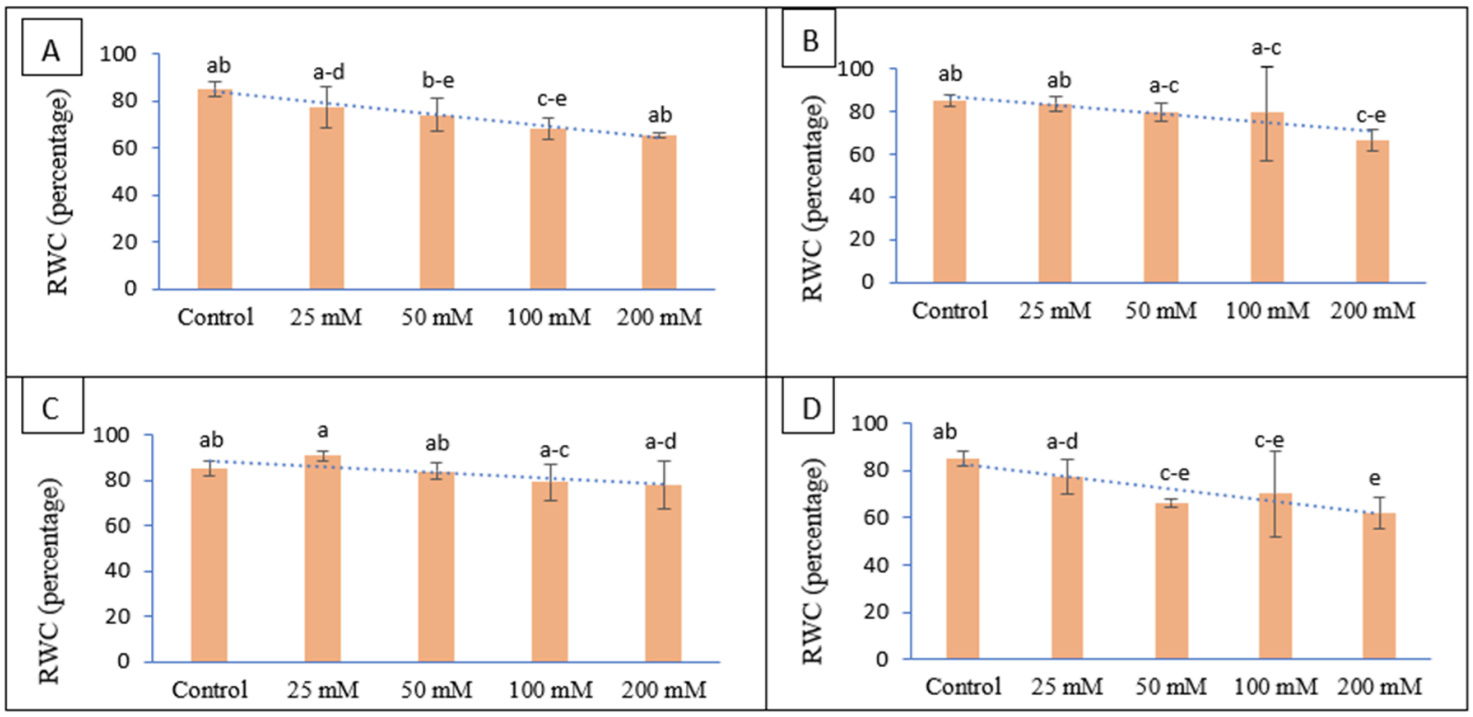

Figure 3

Leaf relative water contents of *M. citriodora* were measured under various concentrations of salts: (A) NaCl, (B) KCl, (C) $MgSO_4$, and (D) $CaCl_2$. The data are presented as mean ± standard deviation (n=3). Bar graph with the same letter is not significantly different (α = 0.05)

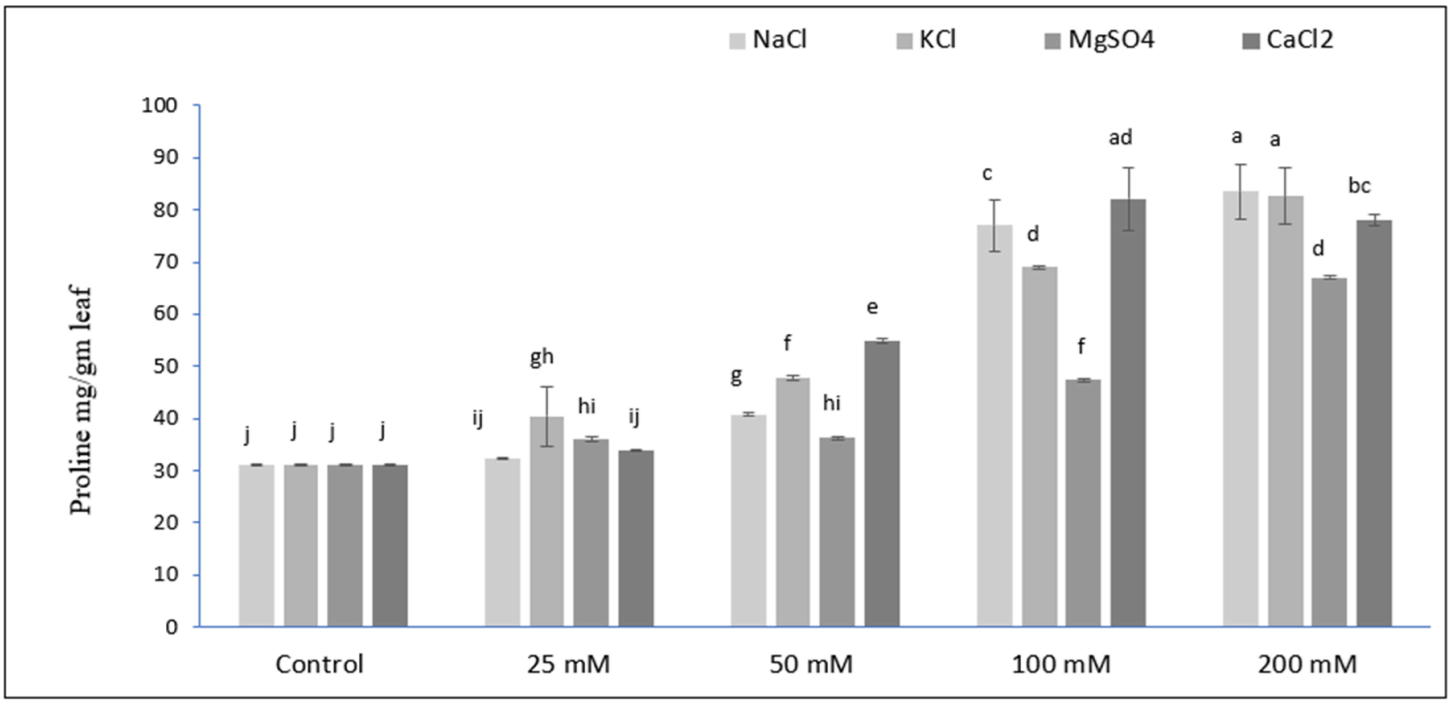

Figure 4

Proline content in leaf of *M. citriodora* were measured under various concentrations of salts (NaCl, KCl, $MgSO_4$ and $CaCl_2$). The data are presented as mean ± standard deviation (n=3). Same colour bar with the same letter is not significantly different (α = 0.05)

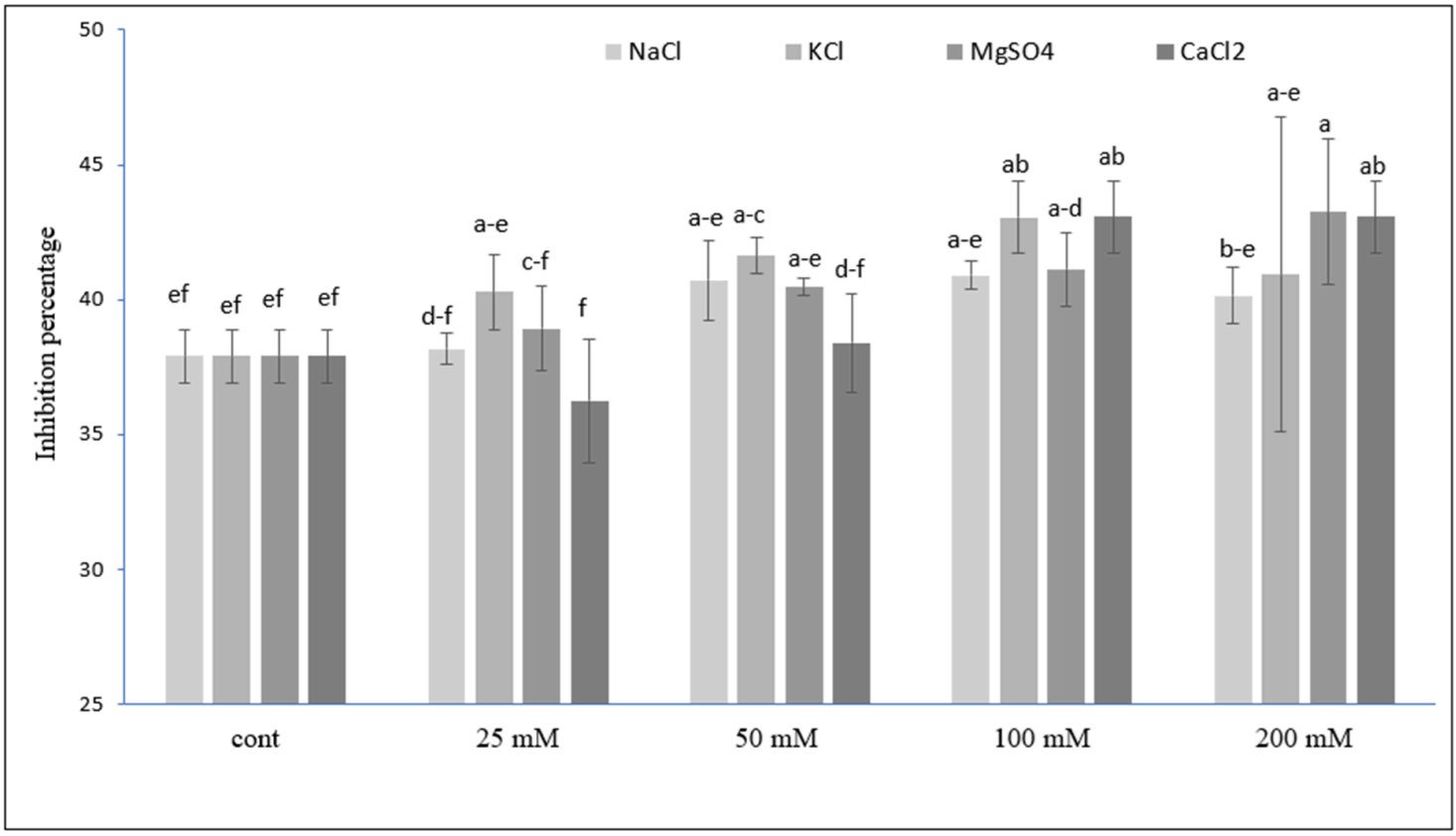

Figure 5

DPPH free radical scavenging activity, percent inhibition in leaf extract of *M. citriodora* were measured under various concentrations of salts (NaCl, KCl, $MgSO_4$ and $CaCl_2$). The data are presented as mean ± standard deviation (n=3). Same colour bar with the same letter is not significantly different (α = 0.05)

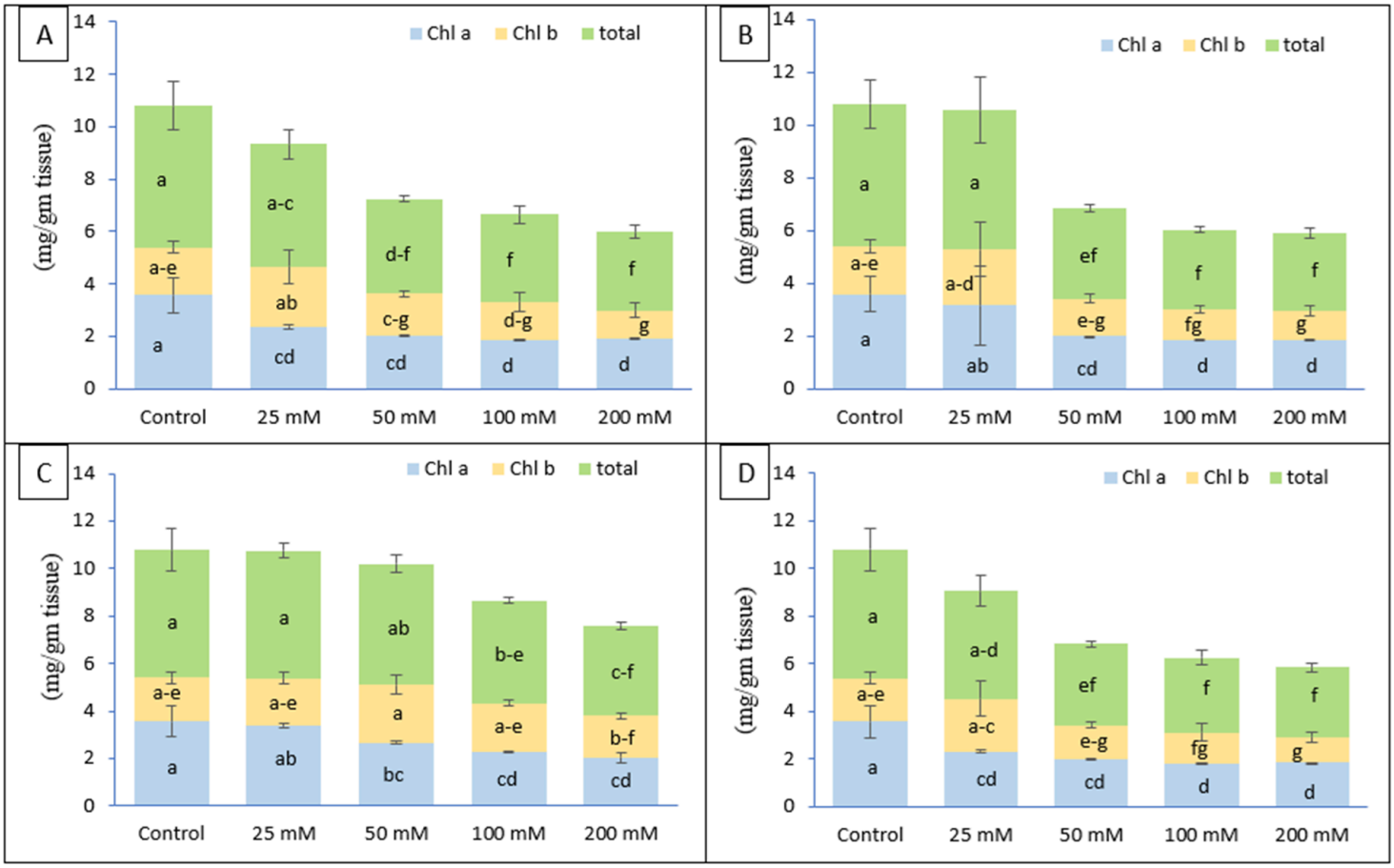

Figure 6

Photosynthetic pigments such as; chl a, chl b and total (chl a + chl b) in leaf of *M. citriodora* were measured under various concentrations of salts: (A) NaCl, (B) KCl, (C) $MgSO_4$, and (D) $CaCl_2$. The data are presented as mean ± standard deviation (n=3). Same colour bar with the same letter is not significantly different (α = 0.05)

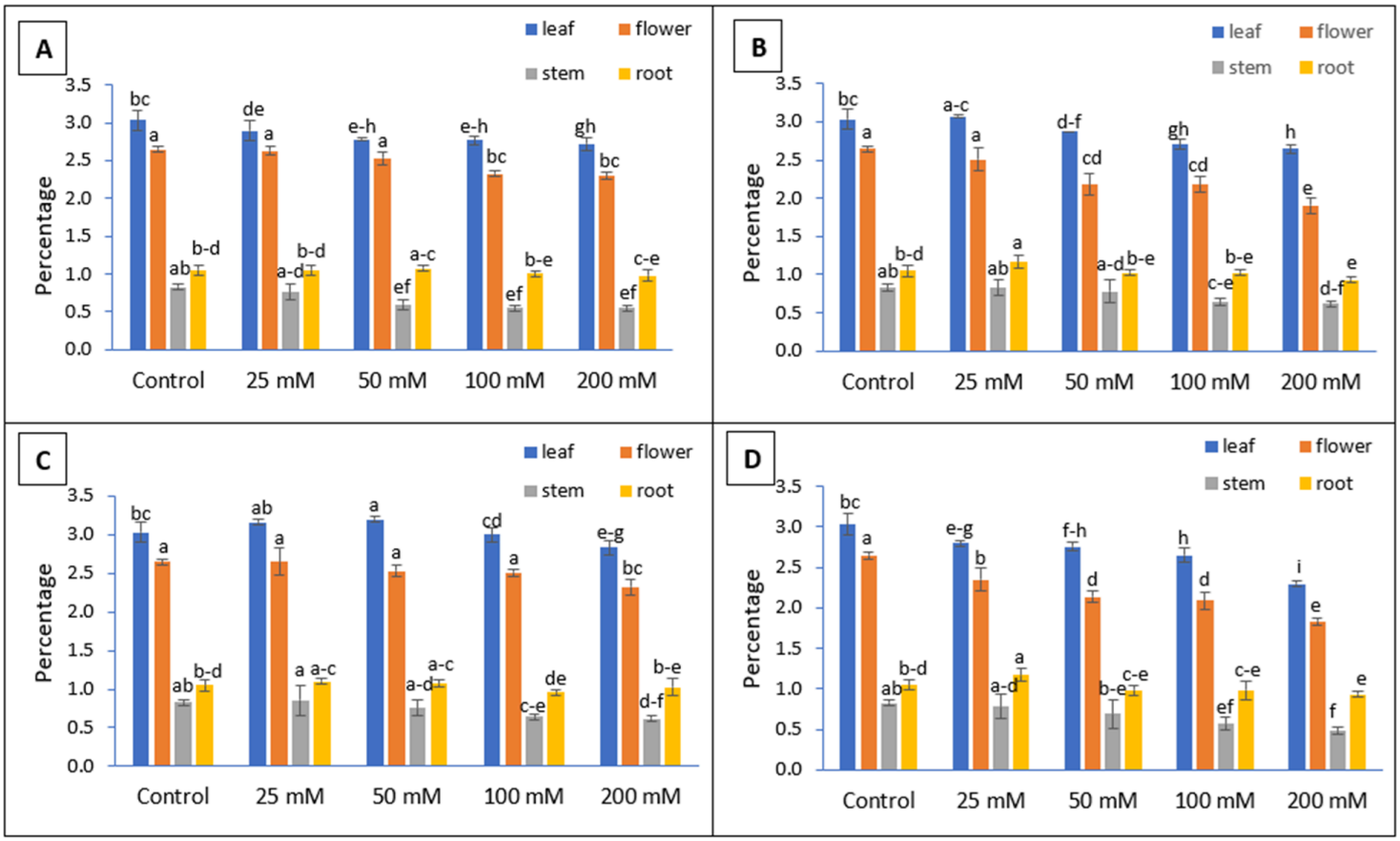

Figure 7

Total nitrogen percentage in leaf, stem, root and flower of *M. citriodora* treated were measured at various concentration of salts; (A) NaCl, (B) KCl, (C) $MgSO_4$, (D) $CaCl_2$. The data are represented as mean ± standard deviation (n=3). Values represented by same colour bar with the same letter is not significantly different (α = 0.05)

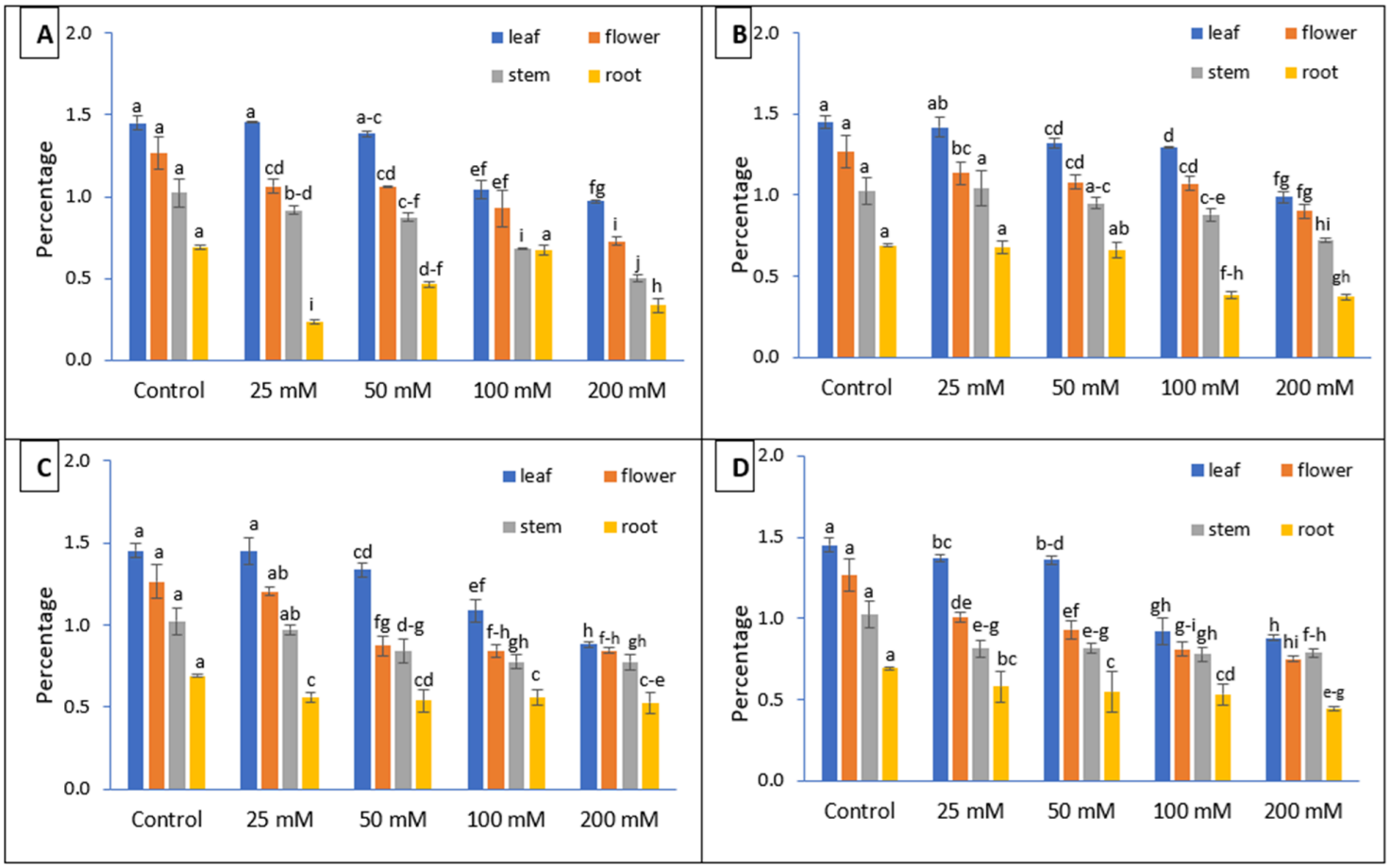

Figure 8

Total phasphorous percentage in leaf, stem, root and flower of *M. citriodora* treated were measured at various concentration of salts; (A) NaCl, (B) KCl, (C) $MgSO_4$, (D) $CaCl_2$. The data are represented as mean ± standard deviation (n=3). Values represented by same colour bar with the same letter is not significantly different (α = 0.05)

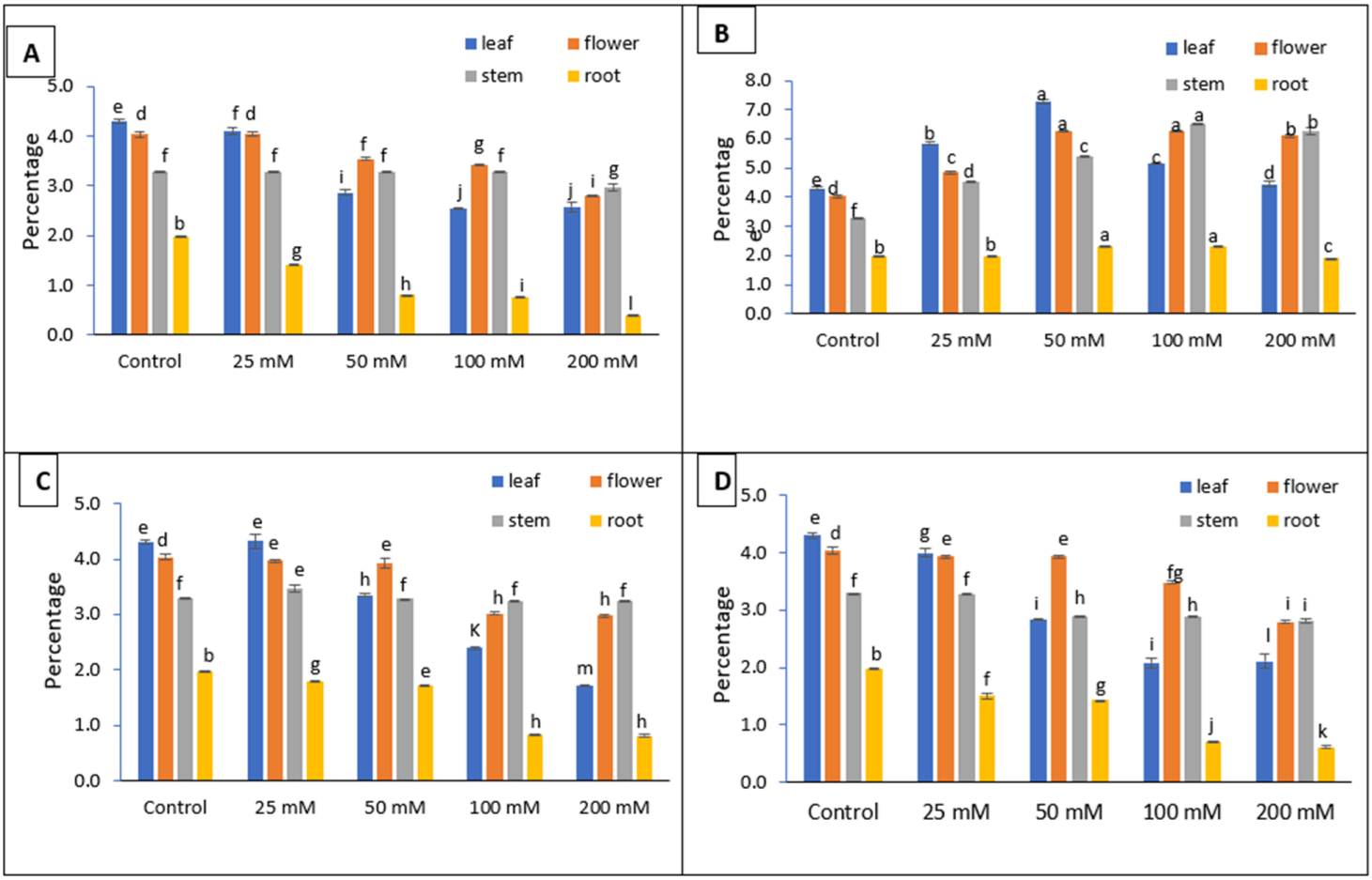

Figure 9

Total potassium percentage in leaf, stem, root and flower of *M. citriodora* treated were measured at various concentration of salts; (A) NaCl, (B) KCl, (C) $MgSO_4$, (D) $CaCl_2$. The data are represented as mean ± standard deviation (n=3). Values represented by same colour bar with the same letter is not significantly different (α = 0.05)

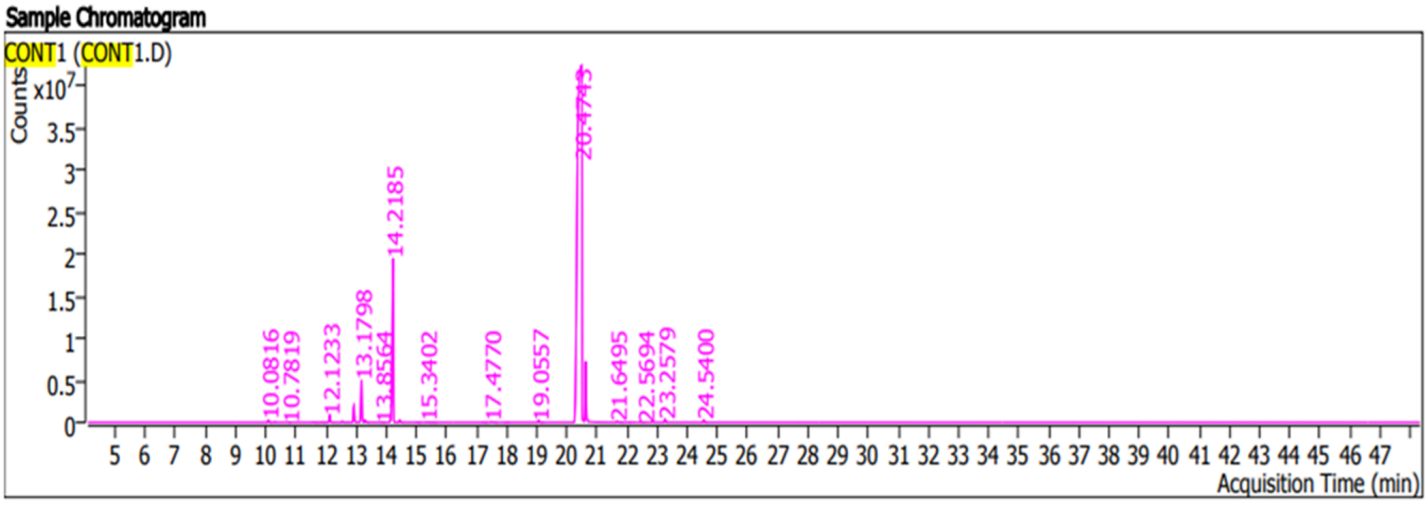

Figure 10

Chromatogram of control sample obtain from Gas Chromatography - Mass spectrometry in essential oil of *M. citriodora*. Peak at retation time 20.474 shows the compound thymo and peak at retation time 14.218 shows the compound Gamma-Terpinene

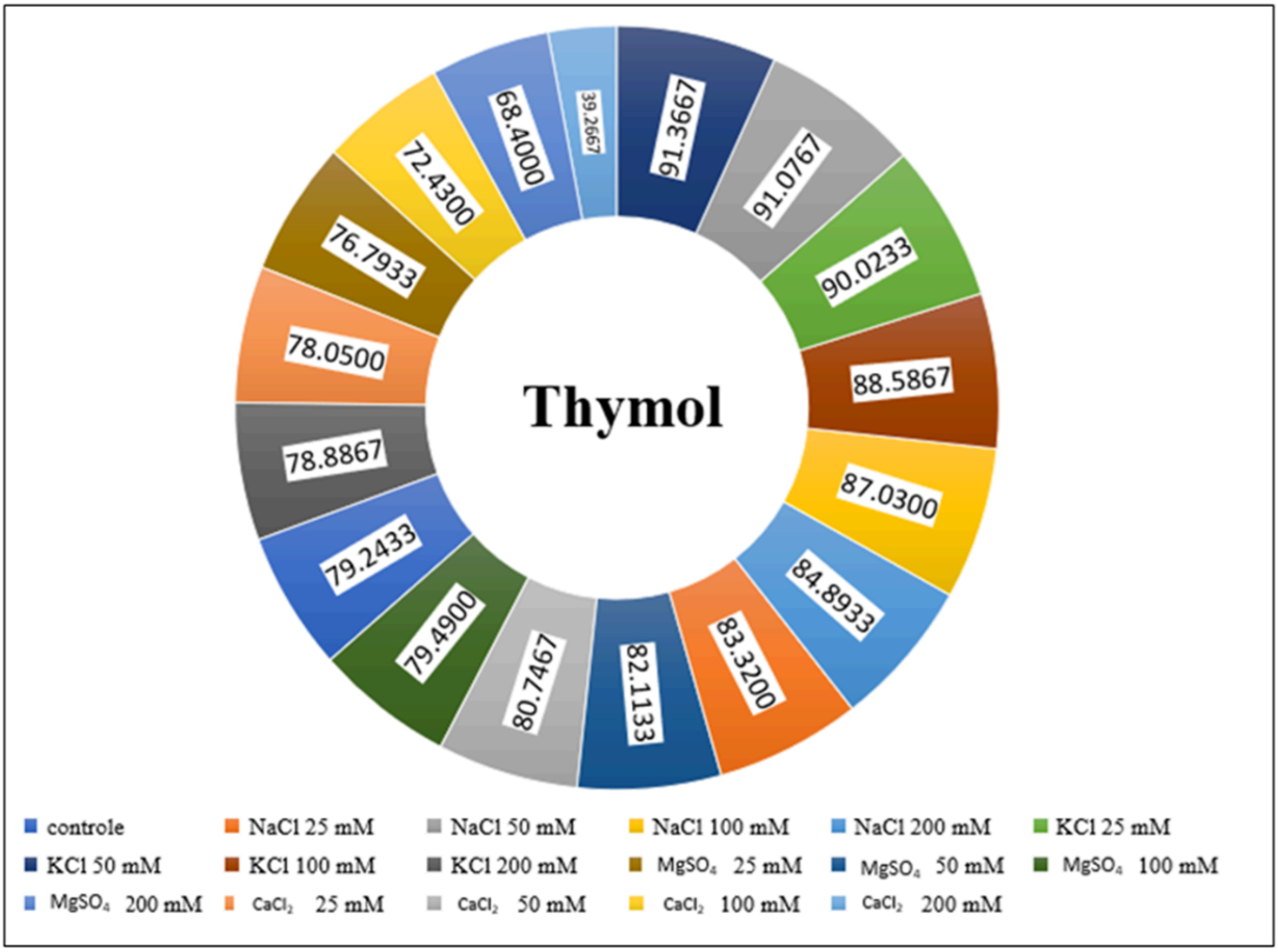

Figure 11

Percent area of thymol obtain from Gas Chromatography and Mass spectrometry in essential oil of *M. citriodora* were analysed at various concentrations of salts (NaCl, KCl, $MgSO_4$ and $CaCl_2$).

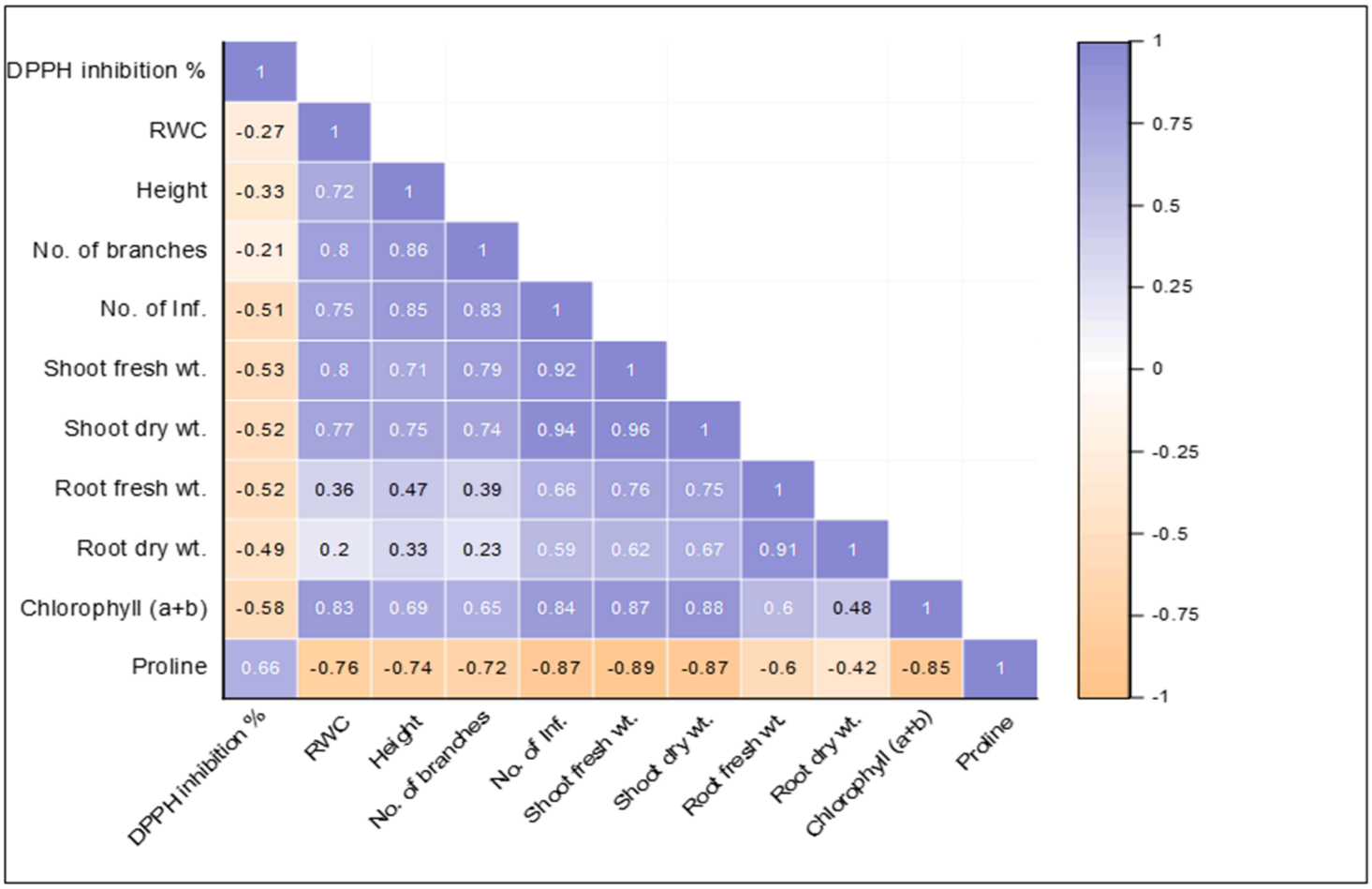

Figure 12

Correlation graph; plotted between DPPH inhibition percentage, relative water content, plant height, number of branches, number of inflorescences, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, chlorophyll (a+b) content, and proline content was analyzed. The blue boxes indicate strong correlations, while the orange boxes indicate weak correlations. The values within the boxes represent the correlation coefficients.

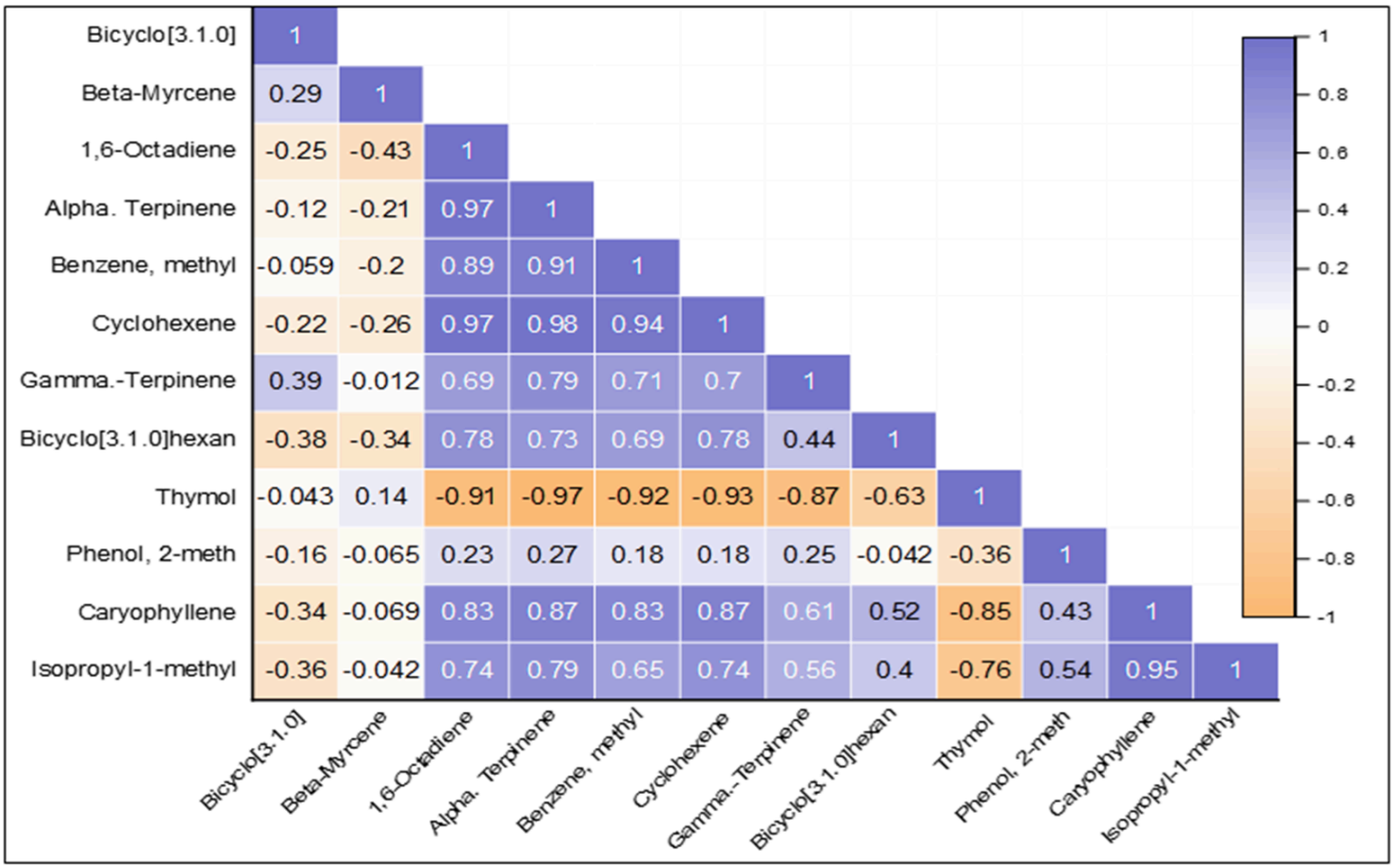

Figure 13

Correlation graph; plottedbetween essential oil component such as bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl); β- myrcene; 1-6 octadiene, 7-methyl-3-methylene; alpha terpinene; ; benzene, methyl(1-methylethyl); cyclohexene 1-methyl-4-(1-methylethenyl); gama- terpinene; bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl); thymol; phenol, 2-methyl-5-(1-methylethyl); caryophyllene and isopropyl-1-methyl-5-methylene-1,6- cyclodecadiene, (1.alpha.,2.beta.,5.alpha.)was analyzed. The blue boxes indicate strong correlations, while the orange boxes indicate weak correlations. The values within the boxes represent the correlation coefficients.

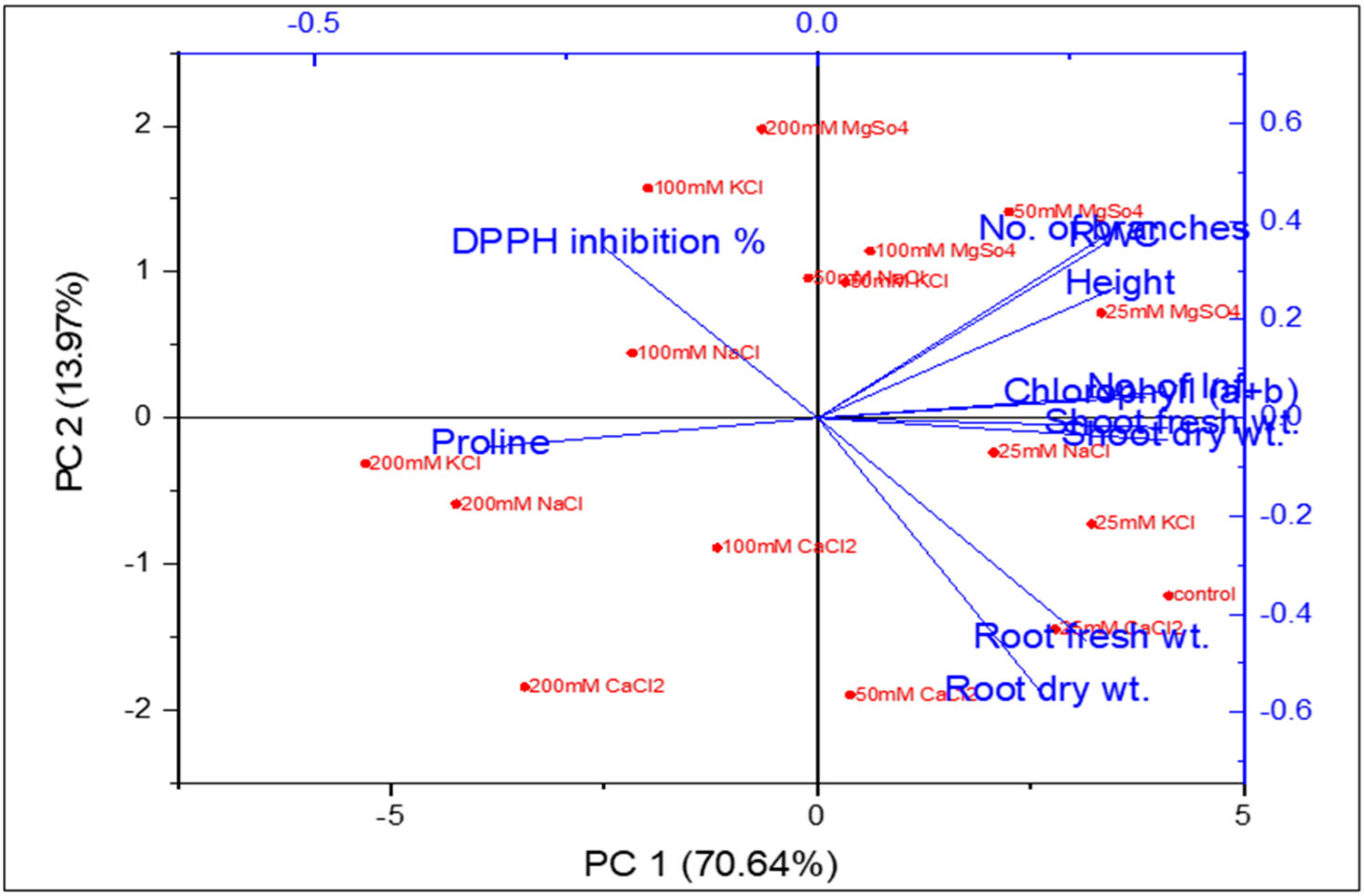

Figure 14

Principal component analysis (PCA) biplots among DPPH inhibition percentage, relative water content, plant height, number of branches, number of inflorescences, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, chlorophyll (a+b) content, and proline content in M. longifolia under different concentrations (25 mM, 50 mM, 100 mM, and 200 mM) of NaCl, KCl, $MgSO_4$, and $CaCl_2$ salts, compared to the control.

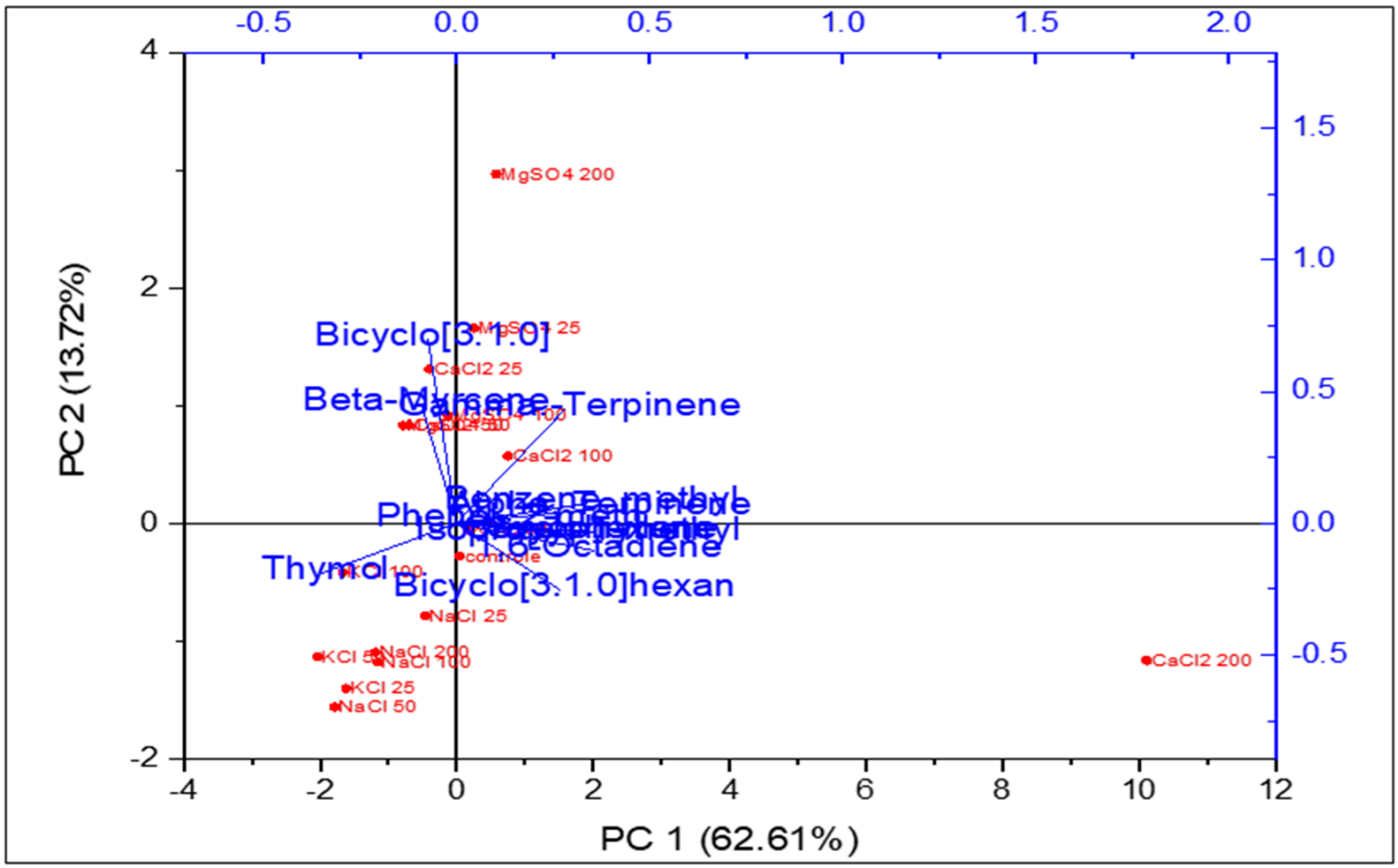

Figure 15

Principal component analysis (PCA) biplots among essential oil component such as bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl); β- myrcene; 1-6 octadiene, 7-methyl-3-methylene; alpha terpinene; ; benzene, methyl(1-methylethyl); cyclohexene 1-methyl-4-(1-methylethenyl); gama- terpinene; bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl); thymol; phenol, 2-methyl-5-(1-methylethyl); caryophyllene and isopropyl-1-methyl-5-methylene-1,6- cyclodecadiene, (1.alpha.,2.beta.,5.alpha.)in M. longifolia under different concentrations (25 mM, 50 mM, 100 mM, and 200 mM) of NaCl, KCl, $MgSO_4$, and $CaCl_2$ salts, compared to the control.

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