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Mitigating Salt-Induced Damages in Wheat with Foliar-Applied Nigella sativa Seed Extract: A Comprehensive Study

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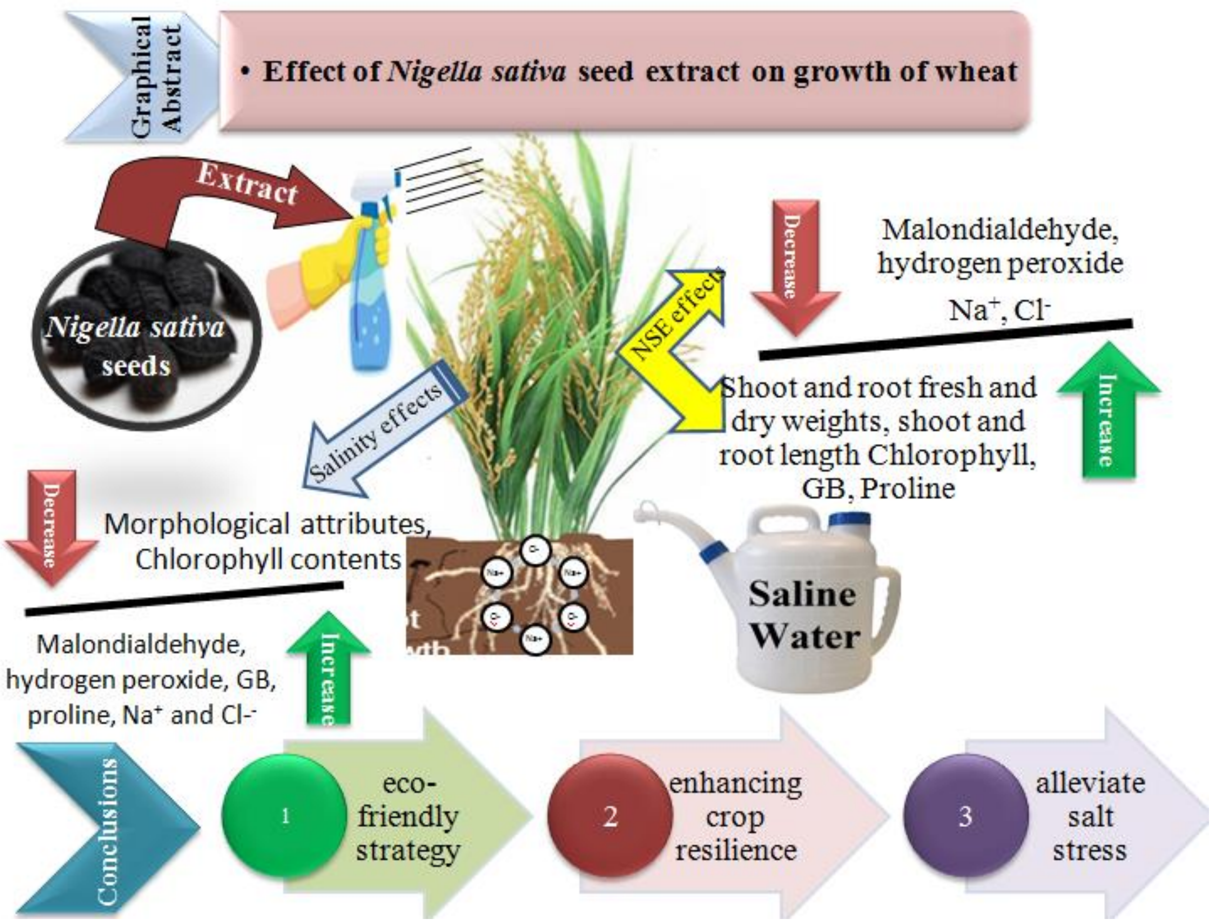
Abstract

Nigella sativa, a medicinal plant, known for its diverse bioactive compounds, including antioxidants and phytohormones, have shown potential in mitigating salt stress in various plant species. Amongst naturally occurring plant growth stimulants, it has attained enormous attention being rich in thymoquinineand carvacrol in seeds for scavenging free radicals. This research aimed to investigate the effect of *Nigella sativa* seed extract (NSE) as foliar spray (0, 50, 100 and 150g seeds per liter each) on wheat growth under salt stress (0mM, 75mM and 150mM). Results revealed that salinity decreased growth attributes and accumulation of photosynthetic pigments. On the other hand, salinity stress boosted the contents of malondialdehyde, hydrogen peroxide, glycinebetaine, leaf free proline, Na^+ and Cl^-. Foliar application of NSEameliorated the negative effects of salinity to considerable extent by enhancing growth traits, chlorophyll contents, glycinebetaine and prolineand decreased Na^+, Cl^-,malondialdehyde andhydrogen peroxide. This research provides valuable insights into the potential use of NSE as natural and sustainable solution to alleviate salt stress in wheat crop. These findings contribute to the development of eco-friendly strategies for enhancing crop resilience in saline environments, ultimately addressing the global challenge of food security in the face of increasing soil salinity.

Key words: Wheat; salinity; Nigella sativa; H_2O_2; chlorophyll pigments; thymoquinine



Introduction

Environmental constraints like salt stress severely affects yield and growth attributes of crops(1). Soil salinity is one of the foremostbarricade which diminishes the productivityof the crops (2). In Pakistan, soil salinity increased by human-induced soil erosion and long-term mismanagement of irrigation (3).Though it is difficult to approximate accurately, salt affected soil area increased, and this fact is particularly severe in irrigated lands(4).It was anticipated that about 45 Mha.of cultivated soilonly 20%, is producing foodall over the world, is damaged by salt (5).

Wheat is a grass, broadly grown to get its seed, used as staple foodin most of the countries (6). Wheat is cultivated on land area (220.4 m hectares)and its trade is higher which is more than all other crops(7).But due to increase in salinization wheat yields still lag behind world's average, leads to sharp downturn in the global economy (8). With increased population and demand for the feed, consumption of wheat is rising due to the novel adhesive properties and visco-elasticity of gluten proteins. Global demand of wheat is growing at a rapid pace in all developing countries and to meet this requirement, dramatic yield improvement is required (9).

Application of plant's extract has been reported to reinforce the growth of crop plants, however; this stimulatory effect is concentration dependent (Tomar and Agarwal 2013; Tomaret al., 2015). *Nigella sativa*leaves and seeds are well known to have broad range of activities like antihypertensive, bronchodilator, diuretic, immuno-modulatory, analgesic, antioxidant properties(10). Themost vital active componentsare thymoquinine (30%-48%),dithymoquinone, thymohydroquinonep-cymene (7%-15%), carvacrol (6%-12%)4-terpineol (2%-7%), sesquiterpenelongifolene (1%-8%) t-anethol (1%-4%), α-pinene and thymol(11,12). But scanty reports are available discussing the ameliorative effects of salt stress by nigella seed extracts. Application of herbal extracts to plants can be beneficial from the stress perspectives that mostly hamper the synthesis of important metabolites. So the aim of the present work is to study the possible ameliorative role of *Nigella stiva* seed extract(NSE) in NaCl stressed wheat plants.

Methods

To assess the effect of *Nigella sativa*seed extract (NSE) an independent experiment was conducted in the field area of The University of Lahore, Sargodha campusin winter 2017-2018. The seeds of four wheat varieties(Ujala 2016, Hashim 2008, Aari 2011 and Aas 2011) were obtained from NARC, Pakistan.The value of average humidity was 63.15% and temperature was 21.3 °C.The average pH of soil measured was 7.8, and ECe was 4.16 dS/m at depth 0-6cm, 6-12cm, 12-18cm, 8-24cm. Seeds were sown in rows of sandy loam soil under completely randomize design. After germination thinning was done with 10 plants in each row havingdistance of 10 cm between each plant. After 22days of sowing, the plants were gradually treated with varying levels of salt stress (0, 75and 150mM NaCl) through root growing medium and then foliar applied NSE (0, 50, 100and 150g seeds per liter each) at evening. After 21 days of treatment, plants were uprooted for morpho-physiological analysis. Threereplicates were taken from each treatment and thoroughly washed with tap water. Fresh weight of shoots and roots were measured with the help of physical balance. Leaves were counted and leaf area was calculated by using meter rod. After thatsamples were kept in oven (60-65^{o}C)for one week and dry weights were recorded with physical balance. The following biochemical attributes were analyzed.

- The contents of chlorophyll *a* and*b*werecalculatedafter placing leaf tissues in 80% acetone overnight at 4^{o}C. With the help of spectrophotometer optical densities of supernatants were noted at wave length 663,645 and 480 nm(13).
- Prolinecontents werecalculated by using spectrophotometerat wavelength of 520nm. Standard curve was drawn for final estimation of leaf free proline contents (14).
- Glycinebetaine(GB) contents were measured by measuring the absorbance of organic layer with the help of standard spectrophotometer at 365 nm. Final values of GB were recorded against standard curve (15).
- Malondialdehyde (MDA) contents of fresh leaf tissues were determined at two wavelengths (532 and 600nm) with the help of spectrophotometer to determine the level of lipid per-oxidation (16).
- Hydrogen peroxide (H_2O_2) was calculatedbyusing standard curves. OD was calculated at 390nm with the help of spectrophotometer following Velikovaprocedure (17).
- The shoot samples were digested with sulphuric acid. After digestion, supernatant was used in flame photometer for measuring K^+, Na^+ and Ca^{2+}ions after optimizing flame photometer with extra pure A grade series of standards. $Cl^-$was determined with the help of chloride meter.
- The experiment was carried out in completely randomized design (CRD) with three replicates. Analysis of variance of all samples werecalculated through Co-STAT computer software.

Results

Salinity (0, 75 and 150mM NaCl) applied through root growing medium caused a huge reduction in all growth attributes of all wheat varieties. Reduction in shoot and rootfresh and dry weights, shoot and root length, number of leaves and leaf area wasobserved.Under foliar treatment of *Nigella sativa*seeds extract (0, 50, 100 and 150mM NSE)all growth parameters were significantly increased in all wheat varieties particularly in Ujala2016 (Table 01; Fig.01).
With the higher level of NaClcontents of chlorophyll *a*and*b*substantially (P < 0.001) decreased in all wheat varieties. The foliar application of NSE considerably increased both photosynthetic pigments (*a*, *b*)in all varieties of wheat. All varieties exhibit differential sensitivity to salt stress in terms of chlorophyll synthesis with grater reduction observed in Hashim 2008 and least in Ujala 2016 (Table 01; Fig.03).
Salt stress considerably (P< 0.001) decreased the shoot calcium and potassium while increased in sodium and chloride ions in all wheat varieties. Exogenous application of NSE posedsignificant effects onabove mentioned ionic contents. Foliar application of NSEslightly improved shoot calcium and potassium in all wheat varieties. Significant reduction was observed in shoot sodium and chloride ionsin all wheat varieties (Table 01; Fig.02). Of all wheat varieties,higher reduction of toxic ions was observed in Hashim 2008 as compared to other wheat varieties.
Salt stress elevated the accumulation of glycinebetaine (GB), proline,malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) increased(P< 0.05)with the increase in severity of salts in all wheat varieties. However the pattern of mean values for NSE treated plants under control and salt stress was different. Foliar application of NSEincreased the level of osmolytes (GB and Proline) and reduced reactive oxygen species (MDA and H_2O_2) in all wheat varieties (Table 01; Fig.03). Maximum resistance was observed in Ujala 2016 as compared to all other wheat varieties, as it showed remarkable decrease in oxidative stress.

Discussion

Salinity is one of the most significant environmental challenges, limiting plant productivity in arid and semiarid areas(1).However foliar application of herbal extracts improved morphology and physiology of plants (18). In this experimental study, salinity suppressed wheat performance as indicated by decreased growth and related traits. Such types of growth changes were observed in *Hordiumvulgare (*19),mungbeen (20) and moth bean plant (21).In saline conditions level of sodium ions increased upto toxic level leading to nutritional imbalance, oxidative stress, osmotic toxicity and low water potential.All these biochemical changes decreased the photosynthesis and ultimately reduced biomass production (21,22).The decreased photosynthetic capacity of the plant under salt stress, decreased leaf growth (23).Foliar spray of NSE enhanced the growth attributes of all four wheat varieties, because seeds of *N. sativa*chemically composed of TQ and thymoquinone that exhibited the ability to scavenge free radicals, regulate the mechanism of source to sink, release of osmotic stress andwater potential regulation(24).

The decrease in chlorophyll contents was projected due to salt stress because of direct contact with toxic ions (25). According to literature reduction in the contents of chlorophyll under salt stress was observed in *P. vulgaris* and *V.subterranran*(26,27). In hyper saline conditions (sodium toxicity), decreased chlorophyll contents can be the result of reduced stomatal conductivity, owing to destruction in thylakoid membranes(28). Another clue for the reduced photosynthetic activity was reported due to over-reduction of electron transport in cellular organelles (21). But this reduction was improved by foliar applied NSE. It has been reported thatNSE is the rich source of active antioxidants like thymoquinine and thymolthat scavenge free radicals and lowered the ion toxicity hence increasing the biosynthesis of photosynthetic pigments(29,30,31).

In the present study increased concentration of Na^+ and $Cl^-$was observed in wheat varieties while decreased concentration of $Ca^{2+}$and K^+ was observed in all wheat varieties under saline and non-saline conditions. It has been identified that increased salt concentration in root zone lead to increased accumulation of Na^+ and Cl^- in shoot tissues and reduced Ca^{2+}, K^+ and Mg^{2+} levels in different plants (32).According to earlier studies salts accumulation in the shoot increased osmotic stress and disturbs ionic homeostasis (33).But the foliar treatment of NSEenhanced the concentration of k^+ and Ca^{2+} and reduced the level of Na^+ and Cl^-. Foliar treatment of NSE reports significant results against ions imbalance due toincreasing salinity.Previous studies reported that NSEis a good source to increase the concentration of K^+ and Ca^{+2} by reducing salinity effects(34,35).

Increased concentration of MDA and $H_2O_2$was observed in all wheat varieties under saline and non-saline conditions. Due to saline regimes increased H_2O_2 production has been reported in rice (36) and wheat(37). It has been reported that salinity increased the production of reactive oxygen species(ROS) due to high osmotic potential, which can lead to growth reduction (38,39).Salt stress induced photosynthetic electron transport chain and production of ROS leads to oxidative stress. Lipid membranes and other indispensable macromolecules including proteins and nucleic acids, pigments are severely damaged by ROS and eventually caused cell death(21,40).Foliar treatment of NSE has reduced MDA and H_2O_2 concentration. Decreasing pattern of ROSand then oxidative stress was reported as a result of thymoquinine (TQ),thymohydroquinone, dithymoquinone and carvacrol(11,41). Badaryreported that TQ is ROS scavenger and possess antioxidant property (42).

In our experiment increased concentration of NaCl decreased the biosynthesis of Proline and GB in all wheat varieties. The Proline accumulation in plants in salt stress is interrelated either with expression of genes (P5CR, P5CS) responsible for biosynthesis of Proline or by the suppression of the genes(PDH silencing) for its degradation (43).As ROSare responsible for oxidative stress (44,45) causing lipid per-oxidation (46,47,48). This oxidative burst confirms the direct attack on lipid membranes (38) and osmolytesdegradation causing disturbance in metabolic processes (49). But foliar applications of NSE show somewhat significant results. The increased*P5CS* expression, increases the Proline accumulation. Foliar treatment of NSE increase the formation of osmolyte might be due to the improvement in its gene expressionand so for GB (50,51,52).

Conclusion

One of the sustainable and economically viable solution for increasing crop stability and productivity is the use of herbal extract for higher tolerance to a biotic stresses. In this field experiment, overall salt stress drastically affected vegetative growth, as well as physiology of wheat (*Triticumaestivum* L.). *Nigella sativa*seed extract has shown good results to over-come the drastic loss caused by salinity. In this experiment

Ujala-2016 wheat variety showed high potential to grow under salt stress. So the use of black cumin seed extract can potentially be a viable option to increase wheat grain yield.

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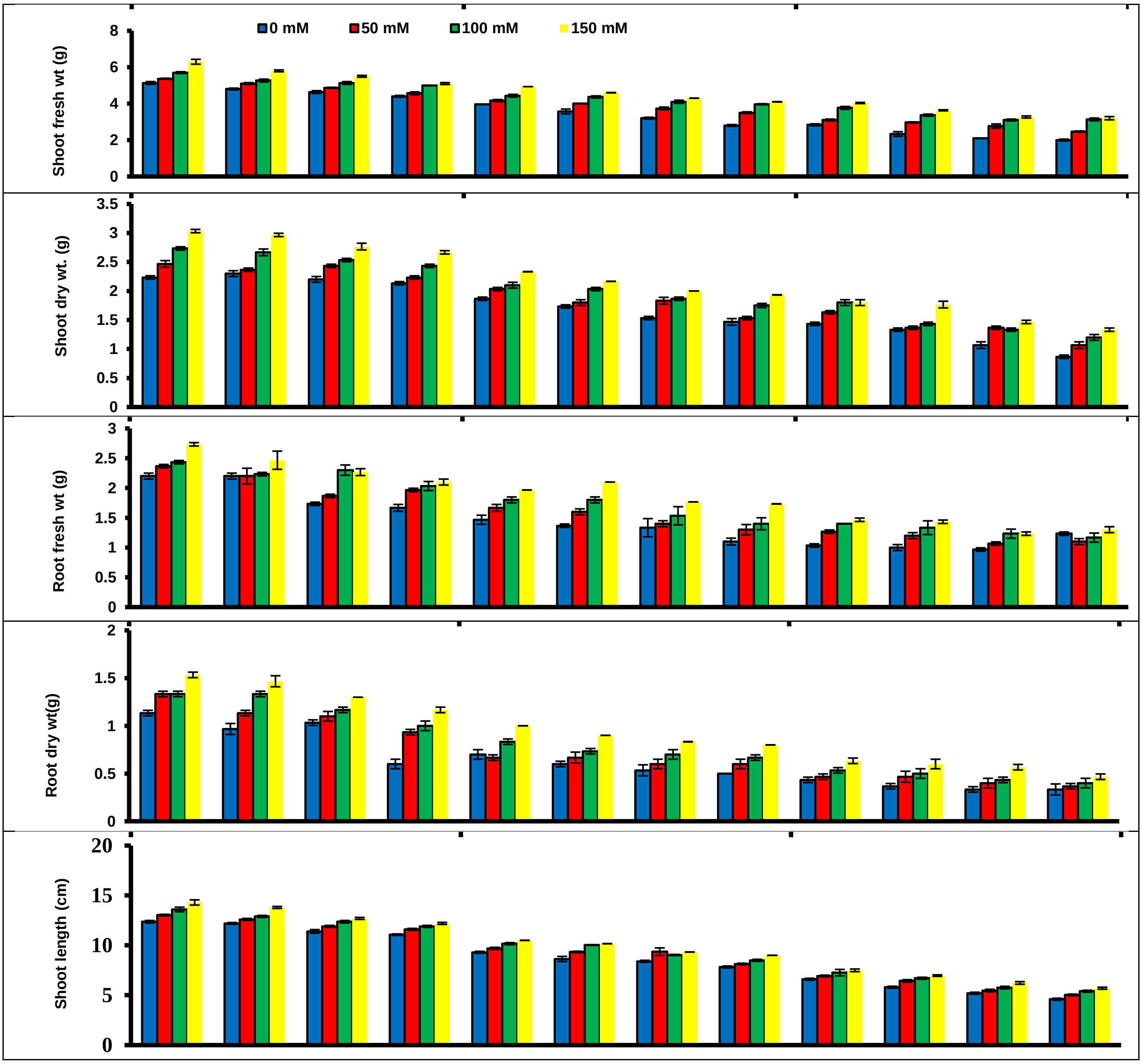
Disclosure statement

No potential conflict of interest was reported by the authors.



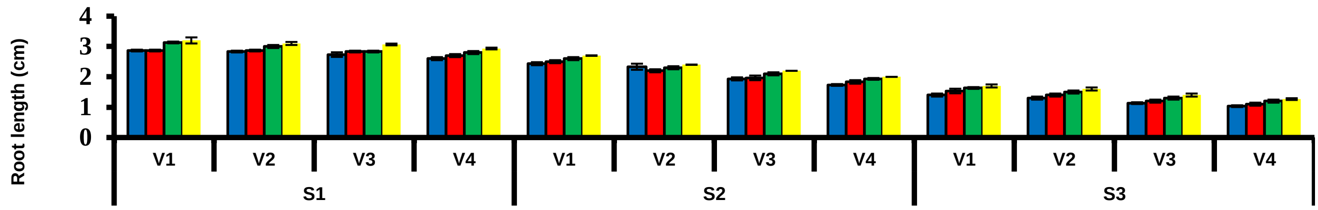
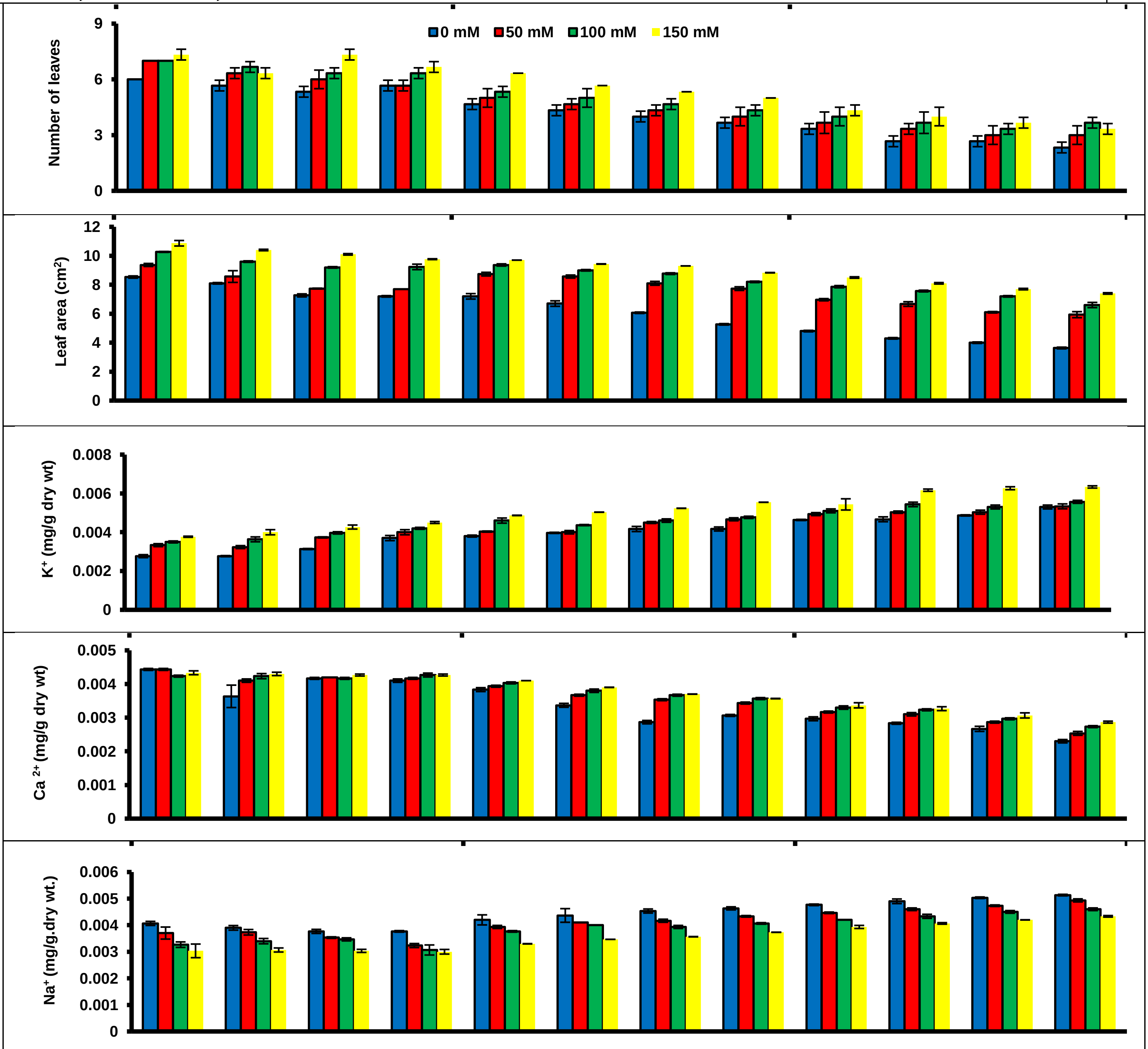


Fig.01 Growth attributes of four wheat varieties when subjected to different levels of NSEunder normal and saline condition
V1=Ujala 2016, V2=Aari, V3=Aas, V4=Hashim2008
S1=0mM, S2=100mM , S3=150Mm NaCl



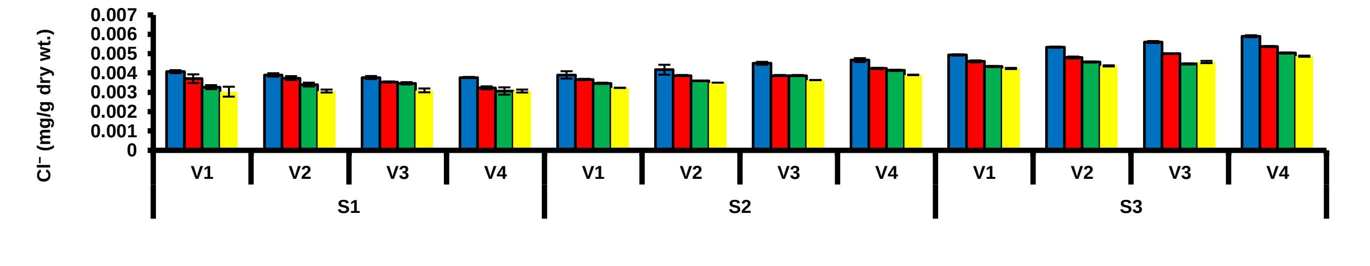
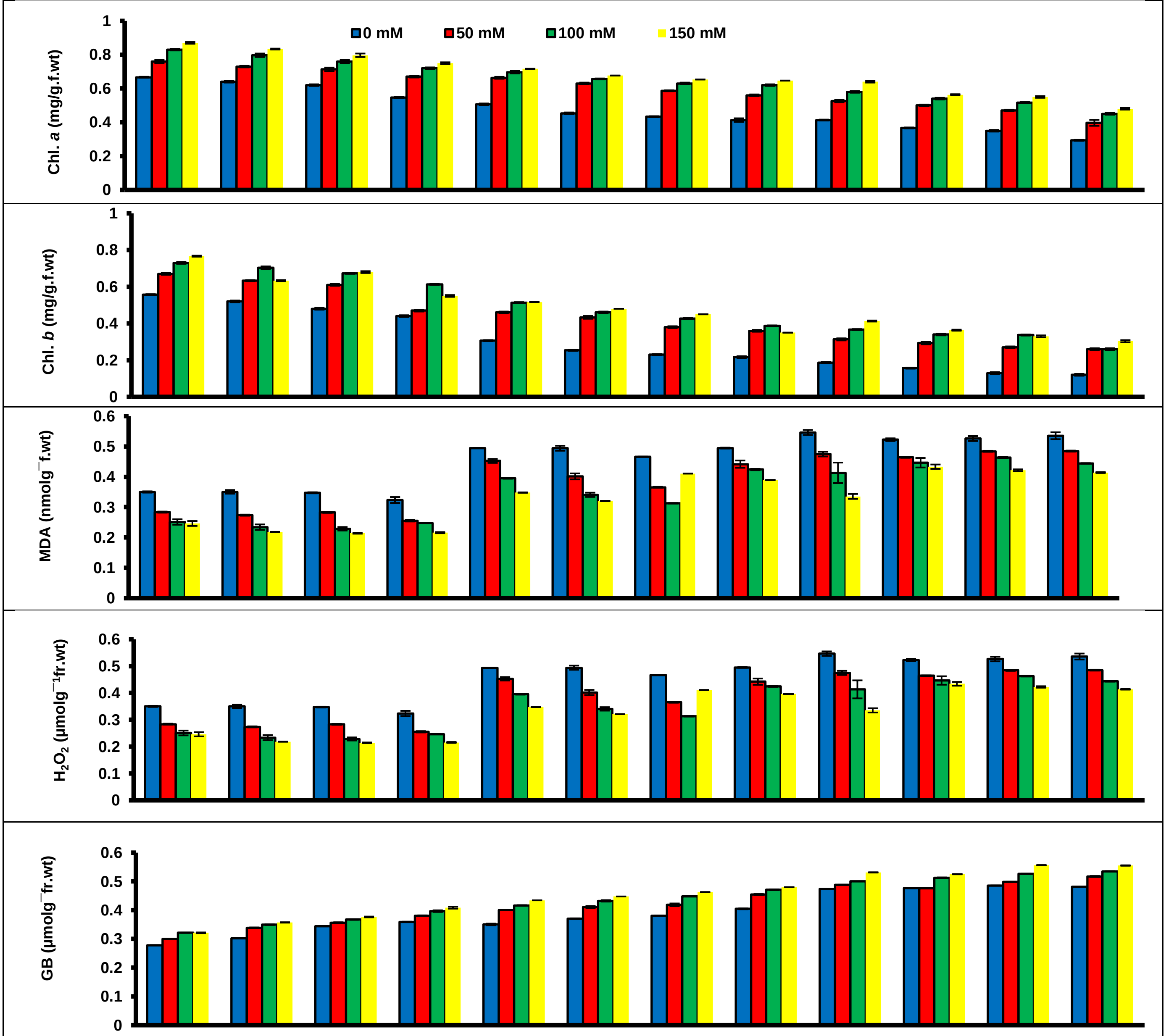


Fig.02 Inorganic ions (K^+, Ca^{+2}, Na^+andCl^-,) of four wheat varieties when subjected to different levels of NSE under normal and saline condition
V1=Ujala 2016, V2=Aari, V3=Aas, V4= Hashim2008
S1=0mM, S2=100mM , S3=150mMNaCl



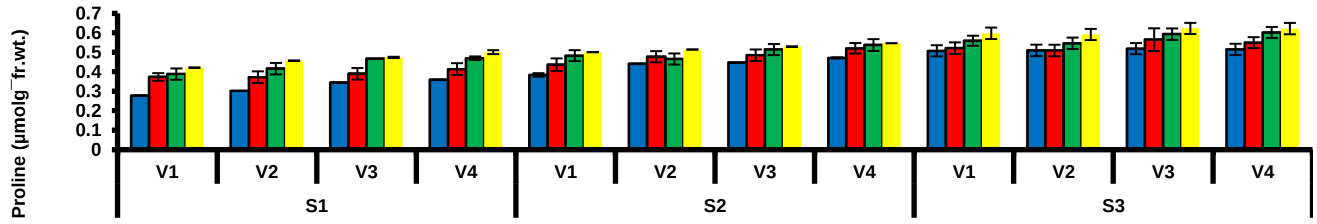


Fig.03: Contents of Proline, hydrogen peroxide (H_2O_2), glycine betaine (GB) and malondialdehyde (MDA)of four wheat varieties when subjected to different levels of NSE under normal and saline condition
V1=Ujala 2016, V2=Aari, V3=Aas, V4= Hashim2008
S1=0mM, S2=100mM, S3=150mM NaCl

Table 01: Analysis of variance of four wheat (*TriticumaestivumL.*) varieties subjected to different levels of foliar-applied seed extract of *N.sativa*grown under salt stress and non-stress conditions

SOV	df	Shoot FW	Shoot DW	Root FW	Root DW	Shoot L	Root L
Salinity (S)	**2**	56.598***	18.074***	10.047***	4.6467***	49.099***	29.177***
NSE	**3**	8.3267***	1.475***	1.2924***	0.6122***	10.114***	0.516***
Varities (V)	**3**	4.3023***	1.2191***	0.7028***	0.2687***	20.11 ***	1.477***
SxNSE	**6**	0.1440ns	0.0401ns	0.0574*	0.267***	0.130ns	8.101ns
SxV	**6**	0.0246***	0.0326ns	0.0905 ns	0.0287 ns	0.1668 ns	0.0823ns
NSExV	**9**	0.0426 ns	0.0047ns	0.0128***	0.0053 ns	0.0748 ns	5.864ns
SxNSExV	**18**	0.0218 ns	0.0075 ns	0.0175ns	0.0081 ns	0.1078 ns	9.336ns
Error	**96**	0.0356	0.025	0.0199	0.0029	0.0759	0.0116
SOV	**df**	**No of leaves**	**Leaf area**	**Chl a**	**Chl b**	**Shoot Na+**	**Shoot Ca^{2+}**
Salinity (S)	**2**	100.67***	82.938***	0.920***	1.2473***	1.972***	3.427***
NSE	**3**	10.703***	65.338***	0.3258***	0.434***	5.530***	3.834***
Varities (V)	**3**	6.796***	10.168***	0.0458ns	0.0682***	1.181***	3.676***
SxNSE	**6**	0.210ns	1.807***	0.011ns	0.0078*	4.094***	1.083ns
SxV	**6**	0.1643ns	0.2020***	0.0166ns	0.0055ns	9.097ns	1.477ns
NSExV	**9**	0.1049ns	4.012*	0.0137ns	0.0015ns	2.253ns	2.050***
SxNSExV	**18**	0.1581ns	0.081*	0.0191ns	0.0028ns	6253 ns	2.769ns
Error	**96**	0.6527	0.0415	0.0197	0.0027	7.291	4.948
SOV	**df**	**Shoot K+**	**Shoot Cl-**	**GB**	**Proline**	**H_2O_2**	**MDA**
Salinity (S)	**2**	4.587***	3.427***	0.8795***	0.308 ***	0.471***	0.4718***
NSE	**3**	5.135***	3.834***	0.289***	0.041***	0.0031*	0.117***
Varities (V)	**3**	4.016ns	3.676***	0.0816***	0.0245***	0.1172***	0.0031*
SxNSE	**6**	5.5132*	1.083ns	9.877ns	1.512ns	0.0061***	0.0012ns
SxV	**6**	2.337ns	1.477ns	0.0021ns	.0010ns	0.0012ns	0.0061***
NSExV	**9**	1.253ns	2.050ns	8.843ns	1.0413ns	9.984ns	9.904ns
SxNSExV	**18**	5.670ns	2.769ns	0.001 ns	2.0106ns	0.0011ns	0.001ns
Error	**96**	2.207	4.948	0.00168	0.0010ns	0.0010	0.0010

ns= non –significant; *, ** and *** significant at 0.05, 0.01 and 0.001 levels, respectively
W=Fresh weight, DW=Dry weight, L=Length, Chl.=Chlorophyll, GB=Glycine betaine, MDA=Malondialdehyde and H_2O_2=Hydrogen peroxide

FW=Fresh weight, DW=Dry weight, L=Length, Chl.=Chlorophyll, GB=Glycine betaine, MDA=Malondialdehyde and H_2O_2=Hydrogen peroxide
NSE= *Nigella sativa* seed extract

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