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Salinity induced changes in photosynthetic pigments and polyphenols of *Cymbopogon Nardus* (L.) Rendle

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ABSTRACT
Soil is a valuable resource with a key productive role in agriculture. Abiotic stress is the most harmful factor concerning the growth and productivity of crops worldwide. Physiologically and genetically salt tolerance is complex among the variety of plants with a wide range of adaptations in halophytes and less tolerant plants. Present investigation was carried out to study the effect of NaCl salinity on photosynthetic pigments and polyphenols of the leaves of Cymbopogon nardus (L.) Rendle. It is observed that the total chlorophyll content of the mature leaves was increased considerably due to increasing concentrations of NaCl upto 200 mM in Cymbopogon nardus. Maximum increase in carotenoid content was observed as 6.05% (50 mM) in Cymbopogon nardus. Polyphenol content was increased upto 300 mM NaCl concentration and showed a perfect positive correlation with the increasing levels of salinity stress. The details of results obtained are discussed in the present paper.

Key words: *Cymbopogon nardus*, Photosynthetic pigments, Polyphenols, Salinity.

INTRODUCTION

Environmental pollution is one of the most significant problems that the world faces today. Pollution directly affects the quality of the receiving medium that may be soil, air or water. Soil is a valuable resource with a key productive role in agriculture and forestry, since it is needed to produce crops, vegetables, fruit, timber and other economically important items. Overuse of pesticides, inorganic fertilisers, increased liquid and solid waste disposals, improper irrigation practices, landfill leachates are some of the broad reasons behind the degradation of lands. It is well established that abiotic stress is the most harmful factor concerning the growth and productivity of crops worldwide. The most basic stressors include high winds, extreme temperatures, salinity, drought, flood and other natural disasters, such as tornado and wild fire. Plants under stressful conditions adapt very differently from one another, even from a plant living in the same area. If the soil holding the plant is healthy and biologically diverse, the plant will have a higher chance of surviving under stressful conditions. Even a low concentration of the contaminants typically alter plant metabolism, most commonly to reduce crop yields.

Soil salinity might not be as dramatic as earthquake or large-scale landslides, but certainly, a severe environmental hazard. Salinity is one of the major abiotic stresses that adversely affect crop productivity and quality [1,2] with increasing impact on the socio-economic fabric and health, especially of the farming communities. Statistics about the extent of salt affected areas vary according to authors, but estimates are in general close to one billion hectares, representing about 6% of the earth's continental extent. In addition to these naturally salt affected areas, about 77 mha have been salinised by human activities [3] but Oldeman [4] estimates it to be 76 mha, with 58% of these concentrating in irrigated areas. Based on the FAO [5] soil map of the world, the total area of saline soils is 397 mha and of sodic soils is 434 mha at global level. Of the current 230 mha of irrigated land, 45 mha are salt-affected soils (19.5%) and of the almost 1500 mha of dryland agriculture, 32 mha are salt-affected soils (2.1%) to varying degrees by human-induced processes. On an average, 20% of the world's cultivated area and nearly half of the world's irrigated lands are affected by salinity, but this figure increases to more than 30% [6] in countries such as Egypt, Iran and Argentina.

High concentrations of soluble salts in the soil moisture of the root zone are always associated with the saline soils. All plants are subject to the influence of high osmotic pressure of soluble salts, but sensitivity to high osmotic pressure, varies widely among plant species and it puts various problems to the plants either at the population, organism or even at the molecular level. Physiologically and genetically salt tolerance is complex among the variety of plants with a wide range of adaptations in halophytes and less tolerant plants [7]. Tolerance to high soil (Na^+) involves processes in many different parts of the plant and is manifested in a wide range of specialisations at disparate levels of organisation, such as gross morphology, membrane transport, biochemistry and gene transcription. Along with this, multiple adaptations to high (Na^+) operate concurrently within a particular plant and mechanisms of tolerance show a large taxonomic variation [8].

Photosynthesis is one of the most important biochemical pathways by which plants prepare their own food material and grows. As a matter of fact, there has been knowledge on increase of chlorophylls content in saline environment depending on salt levels [9]. The total chlorophylls content decreases under NaCl salinity stress [10-15] in salt stressed sorghum and maize plants. Salinity stress causes changes in chloroplast ultrastructure [16,17]. There is also a decrease in rate of photosynthesis under saline conditions [18] in different species and clones of genus *Populus*. Photosystem II is a relatively sensitive component of the photosynthetic system with respect to salt stress [19]. A considerable decrease in the efficiency of PS II, electron-transport chain (ETC) and assimilation rate of CO_2 occurs under the influence of salinity [20]. In contrast to this, Mathangi *et al.* [21] observed no change in photosynthetic potential of the plants by the treatment or with increase in time. Levels of polyphenols also increases under increasing levels of salinity, which shows that the induction of secondary metabolism is one of the defence mechanisms adapted by the plants to face saline environment [12,13]. The present investigation was carried out to estimate the effect of Sodium chloride salinity on the photosynthetic pigments and polyphenols of the leaves of *Cymbopogon nardus* (L.) Rendle grown in pot soil culture.

EXPERIMENTAL SECTION

The seedlings of *Cymbopogon nardus* (L.) Rendle were collected from Government nursery, Kagal (Dist. Kolhapur, Maharashtra). The seedlings were uniformly cut to a minimum height required for their growth and were transplanted into the earthen pots (30 cm height with a narrow base) to grow and establish under normal conditions with proper irrigation. After four weeks of their normal growth and stabilisation salinity treatments were commenced. The plants were treated with increasing concentrations of Sodium chloride i.e. 25, 50, 100, 200 and 300 mM. Every alternate day, they were watered with a double amount of water to maintain the uniform salt concentration in the pots and to cope up with the loss of water by evaporation from the soil surface and by transpiration from the plant surface. The chlorophylls of the mature leaves were estimated following the method suggested by Arnon [22]. The carotenoids content of the leaves was determined from the same extract used for chlorophyll estimation and were calculated by using the formula suggested by Kirk and Allen [23]. The polyphenols content of the leaves was estimated following the method suggested by Folin and Denis [24].

Statistical analysis

Statistical analysis of the data was carried out by using GraphPad software. Mean, Standard Deviation and Percent variation was calculated. 'One Way Analysis of Variance' (ANOVA) was tested in order to see the statistical difference among the means. Tukey-Kramer multiple comparison test of significance was carried out which suggested the variation among the column means is significant or not at different levels of significance. The data was analysed for three different levels of significance based on the 'p' values as

*	Significant	(p = 0.01 to 0.05),
**	Very Significant	(p = 0.001 to 0.01)
***	Extremely Significant	($p < 0.001$)

RESULTS AND DISCUSSION

1.1 Photosynthetic Pigments:

A) Chlorophylls:

The effect of NaCl salinity on chlorophyll content of mature leaves of *Cymbopogon nardus* is shown in Table 1 and Fig. 1. It is evident from the results that chlorophyll content of the experimental grass was not much affected by the salinity upto 200 mM salt concentration but drastic changes in it were observed at 300 mM NaCl concentration. It is observed that the total chlorophyll content of the mature leaves was increased considerably due to increasing concentrations of NaCl upto 200 mM in *Cymbopogon nardus.* It is also evident that chl. 'a':chl.'b' ratio was increased considerably in the leaves of *Cymbopogon nardus* grown upto 100 mM NaCl level. It is quite clear that the highest salt concentration (300 mM) is certainly negatively influential on chl. ‘a’: ’b’ ratio, in the experimental grass species. However, chl. ‘a’ appears to be more sensitive to salinity than chl. ‘b’.

Chlorophyll content in plants correlates directly to the healthiness of plant [25,26]. The resistance of photosynthetic systems to salinity is associated with the capacity of the plant species to effectively compartmentalise the ions in the vacuole, cytoplasm and chloroplast [27]. Al-Sobhi [28] noticed that the high levels of salinisation induces a significant decrease in the content of pigment fractions and consequently of the total chlorophyll content as compared with control plants of *Calotropis procera*.

Usually there is dominance of chlorophyll ‘a’ over chlorophyll ‘b’ in plants but their values become closer with increasing salinity. Similar case was observed in *Arachis hypogaea* with a reduction in the chlorophyll ‘a’:’b’ ratio due to salinity (especially from 40 mM NaCl and up) [29]. Khan *et al*. [30] reported that three tolerant genotypes of wheat viz. Lu-26s, Sarsabz and KTDH-22 had less chlorophyll degradation under the influence of NaCl. Tantawy *et al*. [31] also observed the decrease in total chlorophyll content in tomato with the increasing level of salinity. Jarunee [32] observed a decrease in total chlorophyll content with increasing NaCl concentration in *Phaseolus vulgaris* which showed that both chlorophyll ‘a’ and ‘b’ were decreased. The decrease in chlorophyll content under stress is a commonly reported phenomenon and in various studies, this may be due to different reasons, one of them is related to membrane deterioration [33].

Table 1 Effect of Sodium Chloride Salinity on Chlorophyll Content of the Leaves of *Cymbopogon nardus*

Sr. No.	Chlorophyll	Sodium Chloride (mM)					
		Control	**25**	**50**	**100**	**200**	**300**
1.	**Chl. 'a'**	177.57 (±1.34)	194.52*** (±1.85) +9.55	234.66*** (±0.81) +32.15	259.01*** (±1.86) +45.86	134.55*** (±1.39) -24.23	118.64*** (±2.88) -33.19
2.	**Chl. 'b'**	103.99 (±3.63)	107.33 (±1.42) +3.21	114.50* (±3.27) +10.11	122.33*** (±2.93) +17.63	148.91*** (±2.60) +43.20	143.67*** (±5.89) +38.16
3.	**Total Chl.**	281.49 (±4.03)	316.86*** (±2.71) +12.57	349*** (±3.54) +24.01	381.24*** (±4.73) +35.44	283.37*** (±2.05) +0.67	262.23*** (±3.84) -6.84
4.	**Chl. 'a' : Chl. 'b' ratio**	1.71 (±0.06)	1.81 (±0.01)	2.05** (±0.06)	2.12** (±0.04)	0.904*** (±0.023)	0.83*** (±0.05)

Each value is expressed as mg 100^{-1} g fresh tissue
Each value is a mean of three determinations

Djanaguiraman *et al*. [34] also observed a decrease in chlorophyll content in rice under saline conditions. They observed that chlorophyll ‘b’ was degraded at a higher rate than chlorophyll ‘a’ in the leaves exposed to NaCl and as a result of which chlorophyll ‘a’:’b’ ratio was increased. This can be explained by the fact that the first step in chlorophyll ‘b’ degradation involves its conversion to chlorophyll ‘a’ [35]. The decrease in chlorophyll content of sorghum leaves as a result of salinity, especially in the salt-sensitive genotype [36] could be a result of oxidative stress. Some studies have shown that salt stress inhibits PS II activity [37,38] . Whereas, other studies have indicated that salt stress has no effect on PS II [39,40]. Photosynthesis is inhibited especially in carbon fixation and photophosphorylation phases when Na^+ and/or Cl^- are densely found in the chloroplasts [41]. Mayber and Gale [42] stated that salinity causes a destruction of chlorophyll, which is correlated with the lowering of photosynthetic rate.

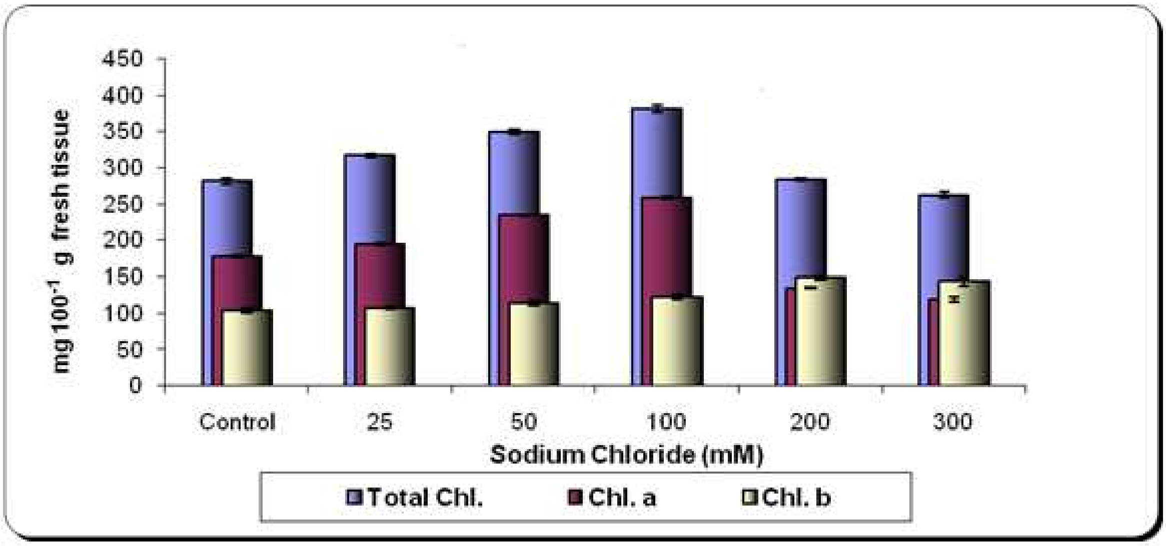

Fig. 1 Effect of Sodium Chloride on Chlorophyll Content of the Leaves of *Cymbopogon nardus*

Suriyan *et al*. [43] observed a decrease in chlorophyll 'a', chlorophyll 'b', total chlorophyll and total carotenoid concentrations of salt-stressed Thai jasmine rice seedlings with increasing exposure time to salt-stressed conditions. Total chlorophyll concentrations were reduced by 68, 82 and 95% relative to control seedlings, after exposure to salt stress for 2, 4 and 8 days respectively. Parvaiz and Riffat [44] also noticed a decrease in chlorophyll content in *Pisum sativum* as the concentration of salt increased from 50 to 200 mM which could be associated with the accumulation of Na^+ in the leaves. Such a reduction in chlorophyll content coupled with increased salt concentration was also reported by Croser *et al*. [45] in *Picea mariana*, *Picea glauca* and *Pinus banksiana*.

Jarunee *et al*. [32] also observed that the NaCl apparently reduced the total chlorophyll content in *Sesbania rostrata* (L.) (Brem and Oberm). The loss of chl. 'a' was minor in *S. rostrata*, but loss of chlorophyll 'b' was evident, resulting in enhancement of the chlorophyll a:b ratio. They concluded that the strong salinity stress severely reduces the potential of electron transport in PS II, which results in growth inhibition. Decrease in total chlorophyll content was also reported by Khan *et al*. [30] in six different genotypes of wheat where decrease was significant in sensitive genotypes in comparison to tolerant genotypes. Yeonghoo *et al*. [46] noticed that the photosynthetic rate in the three gramineous plants decreased after NaCl treatment and was greatly inhibited in *Oryza sativa* more than that of *Echinochloa oryzicola* and *Setaria viridis*. They also suggested that salinity induced an over-production of activated oxygen species in chloroplasts of *O. sativa*.

Jaleel *et al*. [47] noticed a decrease in photosynthetic pigment content of *Catharanthus roseus* under salt stress, which caused a decrease of 11 and 38% in chlorophyll 'a' respectively at 50 and 100 mM NaCl treatment. While the chlorophyll 'b' showed 16 and 33% decrease respectively at 50 and 100 mM NaCl treatment. Total chlorophyll content was reduced by 14 and 34% under low and high salinity, respectively. Similar findings are obtained by of Azooz *et al*. [48] for sorghum and Dager *et al*. [49] for salvadora.

From the present investigation, it is clear that the total chlorophyll content in the leaves of grass is increased at lower levels of salinity. Such an increase in the chlorophyll content might be due to the osmotic adjustment mechanism developed by the experimental grass while a decrease at higher levels might be associated with disruption in cellular functions, membrane deterioration, damage to photosynthetic electron transport chain due to accumulated ions and the instability of the pigment protein complex with increased activity of chlorophyllase enzyme. The drastic reduction in chl. 'a':'b' ratio at higher levels of salinity indicates that chl. 'a' might have been replaced by chl. 'b'.

B) Carotenoids:

The carotenoid content of the leaves of *Cymbopogon nardus* under the salinity stress is recorded in Table 2 and depicted in Fig. 2. From the results it is evident that the carotenoid content of the leaves in the experimental grass at lower levels of salinity was increased. Maximum increase in carotenoid content was observed as 6.05% (50 mM) in *Cymbopogon nardus*.

Table 2 Effect of Sodium Chloride Salinity on Carotenoid and Polyphenol Content of the Leaves of *Cymbopogon nardus*

Sr. No.	Name of the Parameter	Sodium Chloride (mM)					
		Control	25	50	100	200	300
1.	Carotenoid	13.23 (±0.98)	13.97*** (±0.76) +5.65	14.03* (±0.98) +6.05	9.12*** (±0.80) -31.05	8.37*** (±0.72) -36.69	5.92*** (±0.73) -55.24
2.	Polyphenol	686.24 17.72	501.56*** (±17.50) -26.91	552.49*** (±21.04) -19.49	666.80 (±12.14) -2.83	900.08*** (±29.35) +31.16	1082.82*** (±20.48) +57.79

Values in parenthesis indicate standard deviation
Each value is expressed as mg 100^{-1} g fresh tissue
Each value is a mean of three determinations

Carotenoids are organic pigments that are naturally occurring in chromoplasts of plants and some other photosynthetic organisms like algae, some types of fungi and some bacteria. There are over 600 known carotenoids and are split into two classes, xanthophylls and carotenes. Carotenoids with molecules containing oxygen, such as lutein and zeaxanthin, are known as xanthophylls. Carotenoids in all higher plants are synthesized and located in the chloroplast along with the chlorophyll.

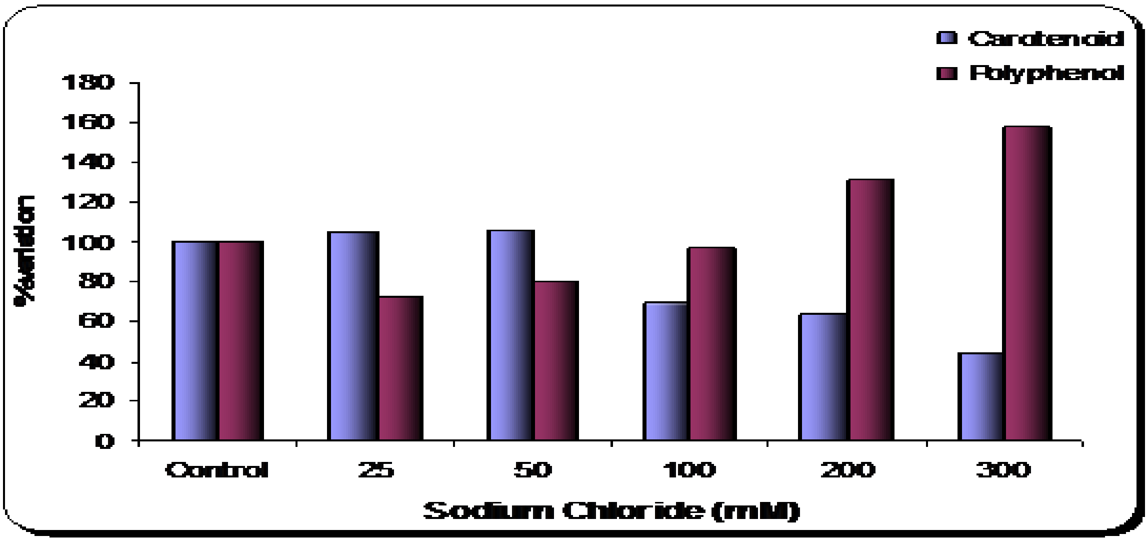

Fig. 2 Percent variation in Carotenoid and Polyphenol Content of *Cymbopogon nardus*

According to Armstrong [50] carotenoids have two major functions in photosynthesis. They protect chloroplast from photo-oxidative damage and they also act as accessory light harvesting pigments because they absorb the light energy in the range of 400-500nm (blue), which is not accessible by the chlorophylls and pass this excitation energy to chlorophyll molecules. They are also one of the non-enzymatic antioxidants [51] alongwith vitamin C, vitamin E and lipoic acid and play an important role in the protection against oxidative stress [52].

The response shown by the plants with respect to accumulation of carotenoids under the salinity stress varies from plant to plant. Nedret and Bengu [51] recorded the highest values of carotenoids in the control group of the Akhisar culture and in the 120 mM NaCl treatment group of the Kaya culture, while the lowest values were observed in the 240 mM NaCl treatment groups of both barley cultures. A reduction in carotenoids content due to salinity stress has been observed by Ali *et al*. [53] in *Brassica juncea*, Agastian *et al*. [54] in mulberry and Parida *et al*. [55] in *Aegiceros corniculatum*. According to Hamada and El-Enany [56] the concentration of carotenoids was increased in most cases of broad bean leaves, while they remained more or less unaffected upto 80 mM NaCl and there above declined significantly in pea plants. On the other hand, an increase in carotenoid content in lupine plants [57] and in wheat [58] has been reported under saline conditions.

From the present investigation it appears that the total carotenoid content in the leaves of *Cymbopogon nardus* was reduced due to higher NaCl salinity and increased at lower levels. It indicates that the higher concentration of salt did not show much inhibitory effect on the carotenoids which might be due to the protective role of carotenoids for chloroplast from photo-oxidative damage by acting as accessory pigments.

Polyphenols:
The influence of NaCl salinity on polyphenol content of the leaves of *Cymbopogon nardus* is shown in Table 2 and depicted in Fig 2. It is evident that polyphenol content of the leaves

decreases at lower levels of salinity i.e. 25 and 50 mM but again shows an increasing trend. Polyphenol content was increased upto 300 mM NaCl concentration and showed a perfect positive correlation with the increasing levels of salinity stress. The maximum increase in polyphenols was observed as 57.79% (300 mM) in *Cymbopogon.*

Polyphenols are a group of chemical substances found in plants, characterised by the presence of more than one phenol units or building blocks per molecule. Polyphenols are generally divided into hydrolysable tannins and phenylpropanoids, such as lignins, flavonoids and condensed tannins. The largest and the best studied polyphenols are the flavonoids, which include several thousand compounds, among them are the flavonols, flavones, catechins, flavanones, anthocyanidins and isoflavonoids. The most abundant polyphenols are the condensed tannins found in virtually all families of plants and comprising upto 50% of the dry weight of the leaves.

Very little attention has been paid towards the influence of salinity on the polyphenol metabolism in plants. Karadge [59] observed a linear decrease in polyphenol content of the leaves of *Portulaca oleracea* with increasing concentrations of NaCl in the rooting medium. Parida *et al*. [60] observed an accumulation of polyphenols in *Bruguiera parviflora* with increasing levels of salinity. According to them such an accumulation of polyphenols played a key role in the plants towards stress. A considerable increase in polyphenol content of the leaves under NaCl salinities has been recorded by Karadge and Chavan [61] in Groundnut var. TMV-10, Parida *et al*. [62] in *Aegiceros corniculatum* and Singh and Kumari [63] in *Brassica campastris*.

From the present investigation it is clear that the increased levels of polyphenols at elevated levels of salinity induce accumulation of secondary metabolites in the experimental species in order to tolerate higher levels of salinity stress and adverse conditions aroused.

CONCLUSION

From the present investigation, it is clear that the total chlorophyll content in the leaves of all the *Cymbopogon nardus* is increased at lower levels of salinity. Such an increase in the chlorophyll content might be due to the osmotic adjustment mechanism developed by the plants. The drastic reduction in chl. 'a':'b' ratio at higher levels of salinity indicates that chl. 'a' might have been replaced by chl. 'b'. Similarly the higher concentration of salt did not show much inhibitory effect on the carotenoids which might be due to the protective role of carotenoids for chloroplast from photo-oxidative damage by acting as accessory pigments. The increased levels of polyphenols at elevated levels of salinity induce accumulation of secondary metabolites in the experimental species in order to tolerate higher levels of salinity stress and adverse conditions aroused.

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