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Effect of Sodium chloride (NaCl) on germination, growth and yield of Pea plant (*Pisum sativum* L.)

[1]*Fiza khan, [2]Gulafshan, [3]Farha Rahman, [4]Hira khan, [5]Ruhi khan & [6]Noor Fiza

[1]Department of Botany

[1]Mohammad Ali Jauhar University

Rampur U.P. India

ABSTRACT

In the present study germination, photosynthetic pigments growth and yield in P*isum sativum* var. P.Arkel was investigated under salinity stress. Salinity caused by NaCl is an abiotic stress inducing morphological and metabolic disorders. The pot experiment was carried out in department of Botany at Mohammad Ali Jauhar University, Rampur and the plants were exposed to different doses of NaCl and the data was recorded at 65 and 75 days after sowing (DAS). The impact of NaCl concentration (4mmhos/cm, 8mmhos/cm and 12mmhos/cm) is reported on morphological and physiological traits on Pea . A significant reduction of shoot and root biomass, root nodules, plant length and leaf area affected by salinity was observed in 12 mmhos/cm of NaCl. Higher salinity induces a reduction, a delay and even a complete inhibition of germination caused due to osmotic effect or /and ion toxicity . All growth attributes such as plant length leaf number and leaf area decrease in all the salinity levels. Salt (NaCl) stress through enhancement of osmotic pressure leads to the reduction of germination, growth and effect the yield of the plant. Saline soil induces physiological and metabolic disturbances in plants, effecting growth development, and quality of yield in plants. In general, salt stress decreases the chlorophyll content and carotenoids of plants but increased the level of proline.

Keyword: NaCl, salt stress, toxicity, *Pisum sativum*, Proline stomatal index

INTRODUCTION

Salinity is a serious problem in worldwide agriculture areas because it limits plant growth and productivity **(Yildirim, *et al*.,2009 and Qin *et al*.,2010)** salinity is an important abiotic stress which effect strongly on crop productivity by accumulation Na^+ and Cl^- ions and imbalance of nutrient **(Munns, *et al*., 2008)**. About 800 million hectares of land, equivalent to more than 6% of total global area of Earth affected by soil salinity **(FAO, *et al*., 2008)**. In the arid and semiarid regions including Egypt soil salinization caused by many factors such as,poor drainage, low rainfall , poor irrigation water which contain amount of salts that accumulate in the surface layer, high evaporation and nearness of the sea **(Rady,*et al*.,2013)**. Soil salinity mitigate growth and productivity of plants due to increased in water use efficiency and in plants metabolism **(Munns, *et al*., 2002)**. Plants grown under salinity conditions are basically stressed in three ways; phytotoxicity of Na^+ and Cl^- ions , decreased water potential in the rhizophere which caused water deficit and nutrient imbalance by the reduction in the uptake and/or shoot transport **(Marschner.*et al*., 1995).** The negative effect on plant water relations was

induced by an increase in soluble salts which decelerate the uptake of water and nutrients causing osmotic effects and toxicity **(Yang *et al*. 2009; Jiang *et al*. 2014).**

Pea (*Pisum sativum*) is an important legume vegetable. It is consumed both as grain legume and as a vegetable. It is most extensively grown in temperate zone and to a limited extent on the cooler altitudes in the tropics and winter season in the sub tropical regions. ***Pisum sativum***. belongs to fabaceae family and it is an annual plant with a life cycle of one year. The objective of this study was therefore to investigate the effect of salinity on germination growth yield and biochemical activity of *Pisum sativum* (var. P.Arkel) and determine the extent of its tolerance of salt stress.

MATERIAL AND METHOD

The seeds of *Pisum sativum* (Var.P.Arkel) were surface sterilized with 20% Sodium hypochlorite for 20 minutes, rinsed and soaked overnight in sterile water for 1h at 4˚C for uniform germination. The seeds were transferred to 12 cm plastic pots filled with soil 2.0 kg pot^{-1} of reconstituted soil in the Agriculture Farm, Mohammad Ali jauhar University, Rampur, India, under semi-controlled condition. NaCl at the concentration of 4mmhos/cm ,8mmhos/cm and 12mmhos/cm was added to the soil. Sodium chloride (NaCl) were used to prepare different salinity levels **(Richard,1954).** The treatments were arranged in a complete randomized design, and each treatment was replicated three times. Sodium chloride (NaCl) determined by atomic absorption spectroscopy. Sampling of Plant growth and other parameters was done after 65 DAS and 75 DAS. Plant length were measured using a ruler. Fresh and dry mass of plants were weighed individually using electronic balance, and leaf area were determined according to **Gabal *et al*. (1984).** Fruit yield was calculated as number of fruits/ plant at 105 days. Chlorophyll and carotenoid content were estimated by the method of **Arnon (1949).** Stomatal density was studied using clear nail polish impressions on leaf epidermis following the method of **Teare *et al*.,(1971)**. The proline content in control and NaCl treated plants was estimated following the method of **Bates *et al.* (1973).** The number of stomata were counted under the light microscope on both adaxial and abaxial surface in a cm^2 area of eye piece (= 0.41 mm^2 of leaf surface).

RESULT AND DISCUSSIONS:-

Germination is the process by which an organism grows from a seed. The data shows, that 95% seed germination was found in control in the selected var, P.Arkel. There was a progressive decrease at the germination stage under the increased salinity level of sodium chloride, it was noted that high dose of sodium chloride (12mmhos/cm salinity level) cause more reduction in seed germination . Salinity can affect germination by affecting the osmotic component, which is the ionic component, i.e., Na and Cl accumulation **(ZIVKOVIC *et al*.,2007)**. In plants, salt stress is a critical factor that severely affects plant growth and metabolism. Excessive salt also attracts water and blocks its absorption to plants may exhibit signs of drought even when the soil is wet or waterlogged. In **(fig.a and fig.k)** the plant length of pea plants treated with 4mmhos/cm - 8mmhos/cm of NaCl did not vary significantly from control plants. However, higher dose (12mmhos/cm) reduced the plant length of pea plant significantly. Shoot and root lengths are the most important parameters for salt stress because roots absorb water due to direct contact with soil and then shoots enable its supply in whole plant. For this reason, shoot and root lengths provide important indications of plants response to salt stress. **Jamil *et al*.2004**. The (**fig. c & d)** represents the fresh and dry weight of plant,indicate the fresh and dry weight as affected by salt treatment at different intervals. The data indicates that control had more fresh and dry weight of plant in comparison to salinity level of 4mmhos/cm.,8mmhos/cm and 12mmhos/cm of sodium chloride irrespective to var. P. Arkel. The findings of the present results confirm some researchers' reports which claimed that plant weight decreased as increscent with NaCl salt levels for some plants **(Kaya *et al*., 2005).** Fresh and dry biomass showed decline with increasing salt stress, which was observed by **Badr-uz-Zaman *et al*., 2006 and 2010.**

There was a consistent decrease in leaf number with the increase in the dose of NaCl. the most significant impact of of NaCl was recorded in the leaf size (area). In **fig.b and fig.l** the leaf area reduced with increase in the concentration of NaCl. **Ulrich *et al.* (1980)** mentioned that it could be due to the accumulation of Na ion in the leaf, but **Hoffman (1981)** attributed leaf injury in salt affected crops due to specific action of Cl ion.

The chlorophyll content **(mg g $^{-1}$ FW)** decrease in response to NaCl treatments at higher concentration. The reduction of chlorophyll content was found at both the growth stages 65 DAS **(fig. no.e)** and at 75 DAS **(fig. no.o)** respectively. The reduction of leaf chlorophyll content was more at 75 DAS as compared to 65 DAS. Several studies suggested that, the chlorophyll content is a biochemical marker of salt tolerance in plants. It is observed that the salt tolerant plants showed increased or unchanged in chlorophyll levels under salinity conditions whereas chlorophyll contents decreased in salt-sensitive plants **(Stepien and Johonson, 2009; Ashraf and Harris, 2013).**

The stomatal frequency in both the leaf surfaces decreased in proportion to different doses of NaCl . The amphistomatic lamina has a larger number of stomata on the abaxial side. The effect of salt in number of stomata increased in the presence of NaCl on both abaxial and adaxial surface **(fig.h and fig.r)**. Osmotic pressure induced stomatal closure and accumulation of toxic levels of Na+ in the cell's cytosol under saline conditions which reduce a plant's capacity to fully utilize light absorbed by the photosynthetic pigments and leads to the formation of various reactive oxygen species **(E. Tavakkoli, et al., 2011).**

NaCl showed reduction in the level of carotenoids (mg g $^{-1}$ FM) at different concentrations i.e; 4mmhos/cm , 8mmhos/cm, and 12mmhos/cm. The maximum reduction reported at 12mmhos/cm level **(fig.f)** as compared to 4mmhos/cm and 8mmhos/cm **(fig.p)** respectively. Carotenoids play a vital role in preventing the chlorophyll-photosensitized formation of O2 by intercepting chlorophyll triplet states **(Demmig-Adams and Adams, 1996).**

Proline (µ mol g $^{-1}$ FM) is an important parameter to measure the stress tolerance capacity of the plants. It is evident from **fig.g and fig.q** that soil amended with NaCl caused a significant increase in the proline content in a concentration (0, 4mmhos/cm, 8mmhos/cm and 12mmhos/cm) dependent manner at the different growth stages. The highest concentration (12mmhos/cm) of NaCl caused maximum accumulation of proline, as compared to the control plant, respectively. Plants also have been shown proline accumulation under environmental stress **(Ahmad and Jhon, ;2005 Ahmad *et al*.,2006 ; Ahmad *et al*.,2008)**.Increase in proline content may be either due to de novo synthesis or decreased degredation or both **(Kasai *et al*.,1998).**

The data given in **fig.j** represents the nodule number as affected by duration and treatments. Generally, all salinity level of sodium chloride in significant decreased the number of root nodules in *Pisum sativum* (var. P Arkel) at 65 days after sowing. The salinity level of 12mmhos/cm salt treatment significantly reduced the number of root nodules per plant in the selected cultivar at 65 to 75 DAS when compared to respective control. In this study, decline in nodule number and nodule fresh dry mass under salt (NaCl) stress is in confirmation with the reported findings **(Ashraf and Bashir,2003).**

In **(fig.t)** indicates the yield parameters of the plant as affected by salt treatments The data indicates that control had more number of pod per plant in comparision to salinity level of 4 mmhos/cm , 8 mmhos/cm and 12mmhos/cm of salt treatment .This, together with identification of the molecular components in crop species that can be modified to increase the amount of energy available for harvestable yield **(Amthor *et al*., 2019),** may provide a new approach to increase yield on saline soils. Changes in growth rate or yield are often the only visual response to salt tolerance at moderate to low salinities **(Shannon, 1985).**

CONCLUSION:-

Pisum sativum var. P arkel tolerate stress at lower concentration as it is evident from the enhancement of photosynthetic attributes at lower concentration of salt (NaCl). The selected cultivar of Pea (*Pisum sativum* var. P arkel) is sensitive to higher concentration of Nacl. The flowering and fruit setting both are sensitive to higher doses at the rate of 12mmhos/cm. it is concluded that Sodium Chloride treatment imposed significant negative effects on shoot and root length, fresh and dry weights, number of leaves plant-1, leaf area , root nodules, photosynthetic pigmens, and stomata numbers as compared to control in pea (*pisum sativum* L.) The increased level of proline showed plant faces stress exposed to different doses of salinity ie;. 4mmhos/cm, 8mmhos/cm and 12mmhos/cm.

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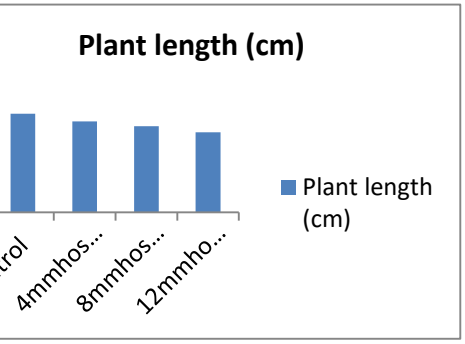
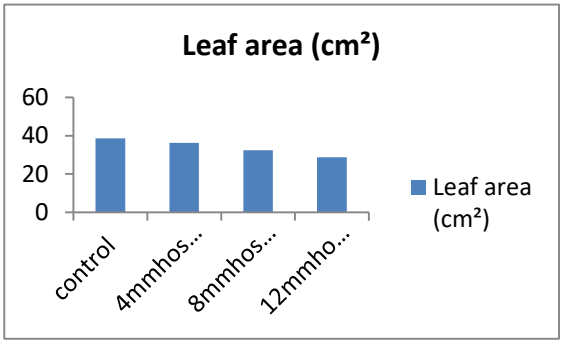


(fig.a)



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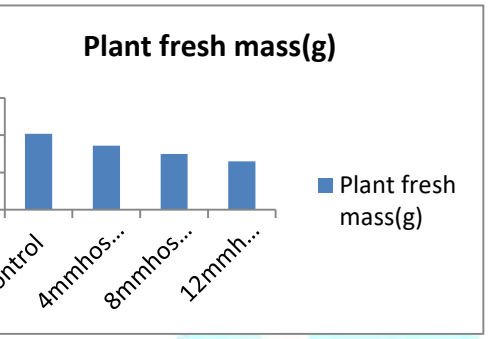

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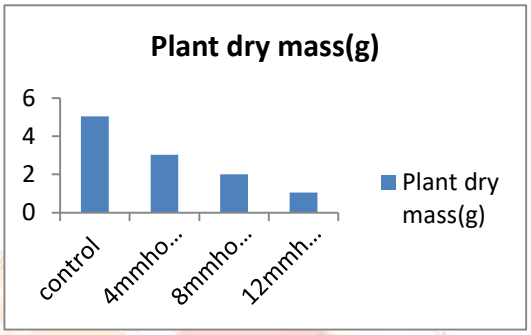

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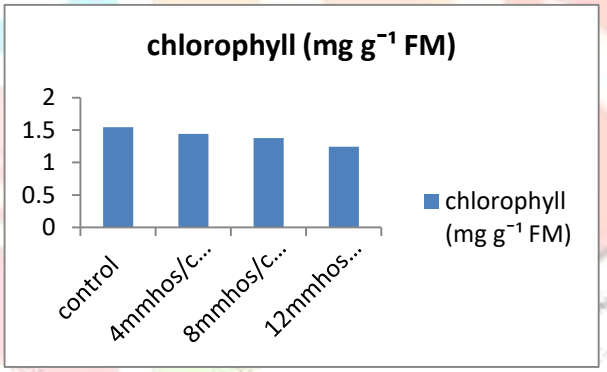
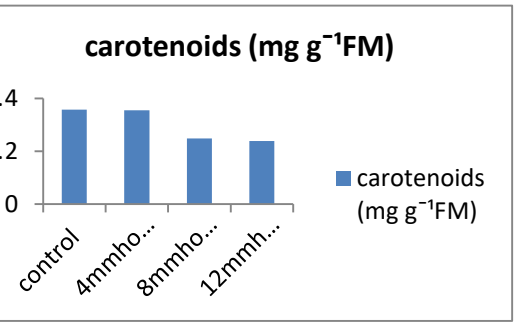
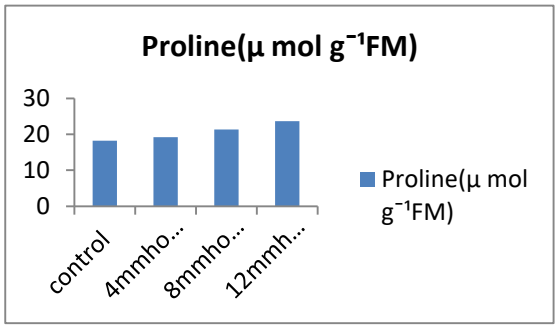


(fig.e)

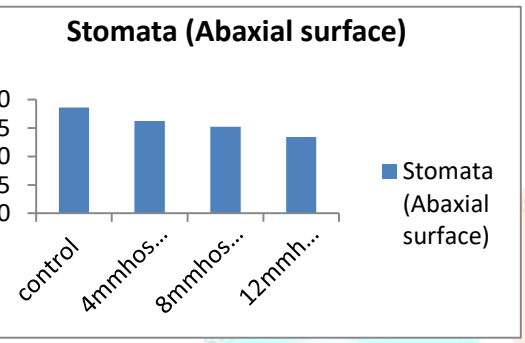
(fig: a to e) shows effect of NaCl treatment (0,4mmhos/cm,8mmhos/cm and 12 mmhos/cm) on plant length(cm),leaf area (cm^2),plant fresh and dry mass (g) and chlorophyll (mg g^{-1} FM) of *Pisum sativum* (var.Arkel) at 65 DAS.



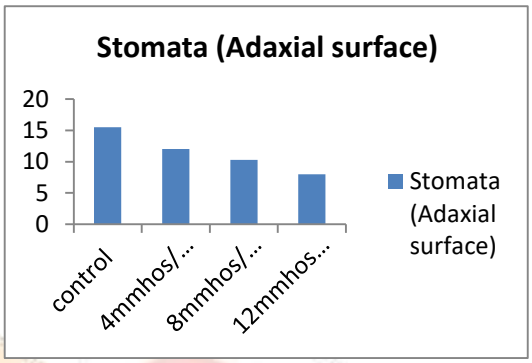
(fig.f)



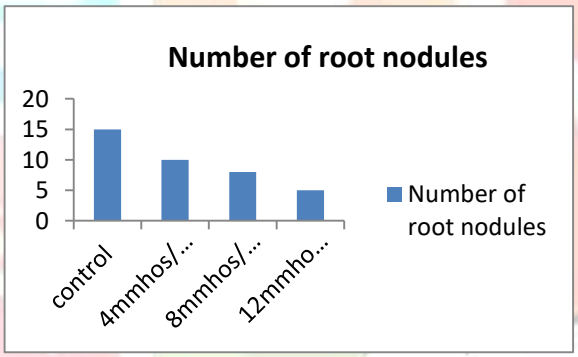
(fig.g)



(fig.h)

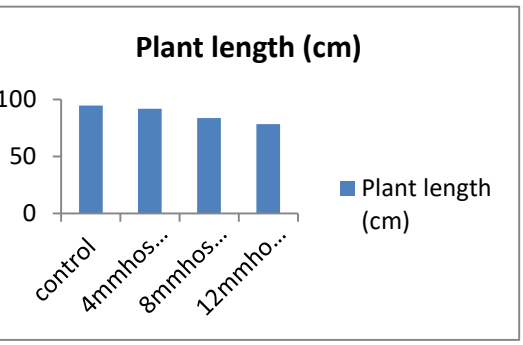


(fig.i)



(fig.j)

(fig: f to j) shows effect of NaCl treatment (0,4mmhos/cm,8mmhos/cm and 12 mmhos/cm) on carotenoids (mg g^{-1} FM), proline(µ mol g^{-1}FM),stomata (Abaxial and Adaxial surface), number of root nodules of *Pisum sativum* (var.Arkel) at 65 DAS.



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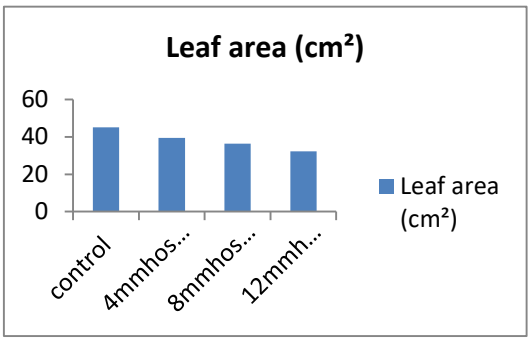

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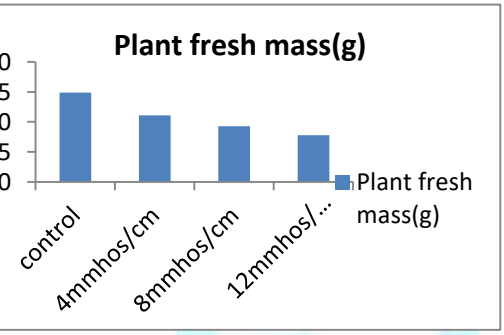

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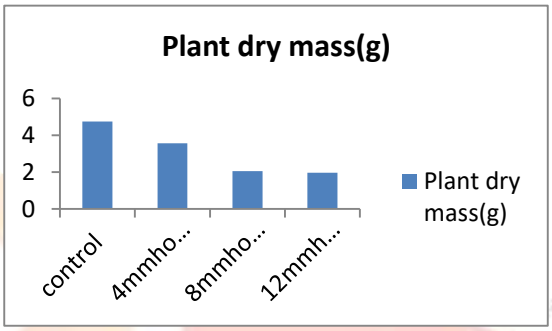

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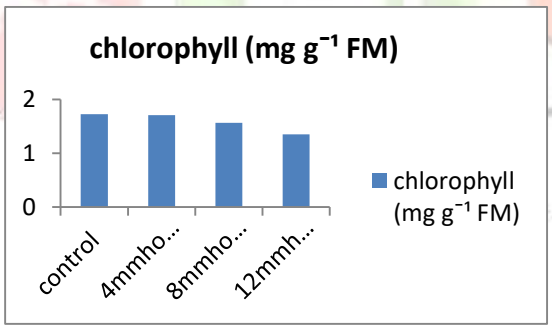
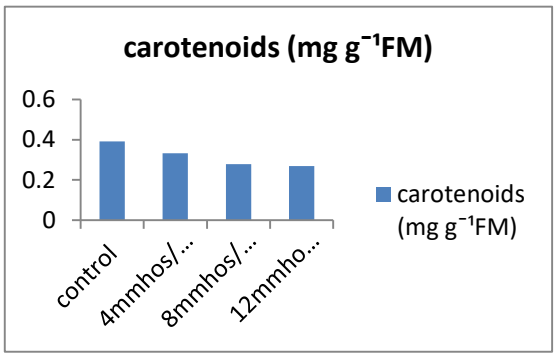
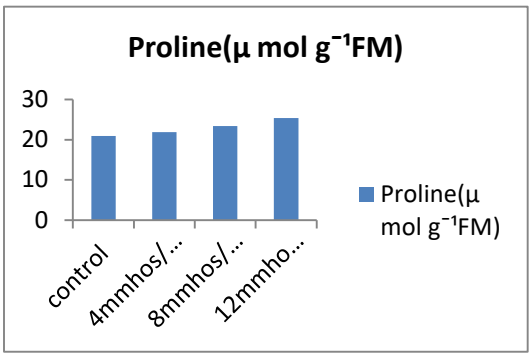


(fig.o)

(fig: k to o) shows effect of NaCl treatment (0,4mmhos/cm,8mmhos/cm and 12 mmhos/cm) on plant length(cm),leaf area (cm^2),plant fresh and dry weight.



(fig.p)



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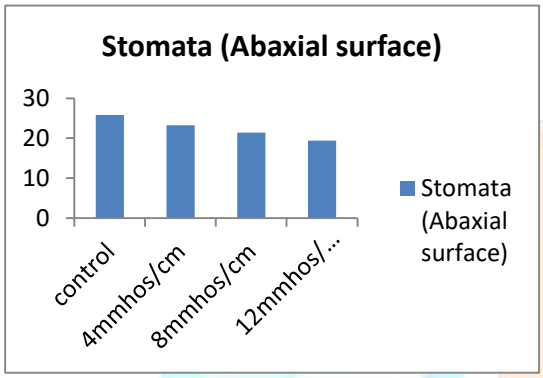
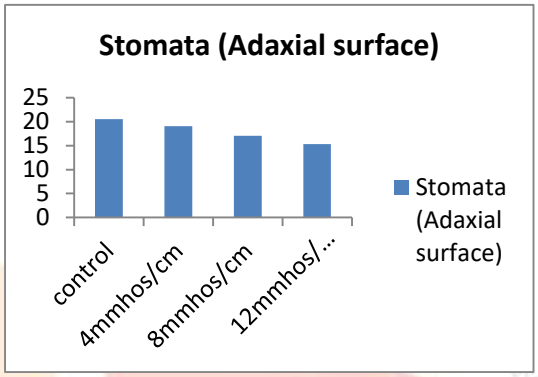
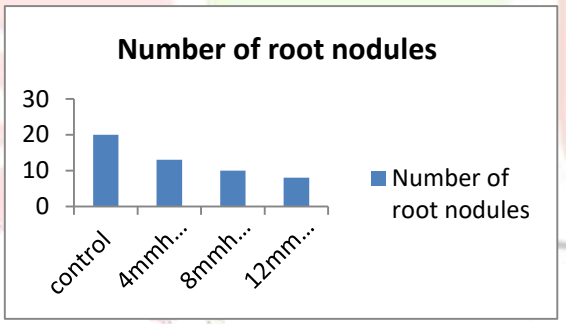


(fig.r)



(fig.s)

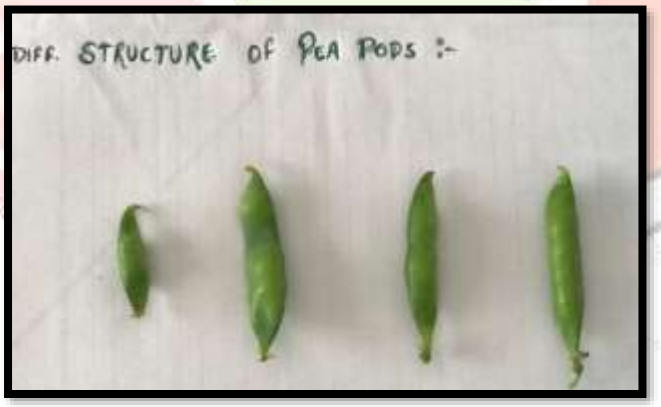


(fig.t)

(fig: p to t) shows effect of NaCl treatment (0,4mmhos/cm,8mmhos/cm and 12 mmhos/cm) on carotenoids (mg g^{-1} FM), proline(µ mol g^{-1}FM),stomata (Abaxial and Adaxial surface), number of root nodules of *Pisum sativum* (var.Arkel) at 75 DAS.

12mmhos/cm 8mmhos/cm 4mmhos/cm CONTROL

(Pic.1) Effect of NaCl treatment (0,4mmhos/cm,8mmhos/cm and 12 mmhos/cm) on plant length(cm) of *Pisum sativum* (var.Arkel).



12mmhos/cm 8mmhos/cm 4mmhos/cm CONTROL

(Pic.2) Effect of NaCl treatment (0,4mmhos/cm,8mmhos/cm and 12 mmhos/cm) on different structure of pea pods of *Pisum sativum* (var.Arkel).

12mmhos/cm **8mmhos/cm** **4mmhos/cm** **CONTROL**

(Pic.3) Effect of NaCl treatment (0,4mmhos/cm,8mmhos/cm and 12 mmhos/cm) on stomatal number of *Pisum sativum* (var.Arkel).