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ORIGINAL ARTICLE

The Effects of Aluminum and Phosphorous on some of Physiological Characteristics of *Brassica napus*

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The environmental toxicity of Aluminum causes nervous, lung and kidney disease in human body. Aluminum toxicity depends on different factors and these factors cause penetration of toxic element to environment soil. The acidity of environment and the root of plants cause the lack of tolerance in absorption of nutrients and makes abnormality in the growth of plants. In this study the effect of Aluminum (40 µmol) and different levels of Phosphorous (0, 40, 80, 320 µmol) in one cultivar of *Brassica napus* were investigated. Results showed in plants that treated with Aluminum, Shoot length, Root length and Chlorophyll a, b content significantly decreased but Malondialdehyde (MDA) and Reduced Sugars content significantly increased. On the other hand, in plants that treated with Phosphorous and Aluminum decreased in growth parameters and Chlorophylls content moderated against plants that treated just with Al and MDA content and reduced sugar content decreased. From these results we suggested that Phosphorous treatment reduced the harmful effect of Aluminum toxicity in this cultivar of canola.

Key words: Aluminum , Phosphorous , Brassica napus

Aluminum is of those elements which its toxic effect on living creatures has been proved vividly, though its components such as Aluminum Soleplate used to purify water (as sedimentary) but food is the main source for Aluminum to be exposed to human (Aniol,1990). Aluminum exists in soil, water and air naturally and it enters the environment through nature and human activities (Wilkens *et al.,* 1997). Aluminum particles are freed form plants, herbal metabolism and other metabolism processes. Aluminum can mix with very tiny particles of creatures in soil and air (Tangen *et al.,* 2002). Human might be exposed to absorb extra Aluminum of the environment through food, drinking water, transpiration and digesting of drugs like anti-acid drugs. Dermal contact with substances containing Aluminum also could poison human (Luo, Bi, 2003). Also environmental toxicity of Aluminum causes various neural diseases such as Alzheimer, nephric and pulmonary diseases (Aniol, 1990). Aluminum is of those non-heavy important elements which show its toxicity in low acidity environments. Therefore, Aluminum toxicity exists in most of acidic soils. Soil acidity prevents growth of most living creatures in the soil. As it is observed in plants, the negative effect of soil acidity includes Calcium shortage or Aluminum or Magnesium toxicity (Delhaize *et al.,* 1995). In farming, Aluminum toxicity causes decrease in production and growth of the plants. Moreover, Aluminum concentration in plants leads to transfer into and concentration in human body and may cause an increase of living problems in human environments (Amangel *et al.,* 1993). In most of acidic soils around the world and maybe in 70% of farming lands which have potential of producing food and herbal substances, Aluminum is the most important factor of growth limitation. Consequently, scientific references which are about Al toxicity and plants tolerance exceptionally focused on farm plants. Acidic rains and doing some of farming functions causes farming lands to be acidic. Acidic soil leads to increase in solubility of some elements such as Aluminum and Magnesium and finally their toxicity will be observed in plants (Delhaize *et al.,* 1995). Acidity environment of plants root causes imbalance in absorption of many nutrients and leads to plants abnormal growth (Amangel *et al.,* 1993; Acreduany, 1997). That is, some of nutrients absorbed lesser and some like Aluminum and etc. absorbed more than natural. Therefore, acidity environment around the root may cause secondary toxicity of some elements (Amangel *et al.,* 1993). Most important researches done in toxic and heavy metals field are about Aluminum. Though, many aspects of Aluminum toxicity on plants remain unknown. Aluminum can become ionic in acidic environments and causes toxicity. Plants grow under the stress of Aluminum and heavy metals in nature, along with the permanent existence of toxic ions and experience damage caused by concentrative toxicity. Different intoxication symptoms are sign of various levels and degrees of adaptation to stressful environment. Since acidic soils occupy 50% of all soils of arid regions of the world; this limits the growth of farming plants in such areas. Factors like toxic concentrations of Aluminum, Magnesium, Iron and also lack of necessary elements such as Phosphorus, Nitrogen, Potassium, Calcium

and Magnesium are growth limitation factors in such regions. Among them, Aluminum toxicity and lack of phosphorus have much more effects on biochemical and physiological growth of plants. Many researches done in relation to the effect of Aluminum toxicity on growth of farming plants like cultivars of wheat, bean, Soya and etc (Amami, 1996). Researches which done few years ago about the issue were mostly related to the study of Aluminum effect on the above mentioned plants; but today, most of the researches are about the study of mutual effect of Phosphorus and Aluminum to decrease Aluminum toxicity effects. On the other hand, considering the importance of Brassica napus cultivation in Iran, the mutual effect of Aluminum and Phosphorus on the marker cultivar of Brassica napus was studied in this research.

MATERIALS AND METHODS

The test plant in this research was *Brassica napus*. It belongs to the Brassicaceae family and it is economically important due to its oil seeds. At first, the selected seeds were disinfected by Sodium Hypochlorite 0.1%. Then they were washed by distilled water and were put in it for an hour. To grow the plant in a flower pot, seeding bed was chosen of prelate. This bed is almost lack of any ion and its action exchange capacity is ignorable. To be assure that prelate as seeding bed is lack of Al ion, it was washed with distilled water of pH=5.5, finally sterilized by autoclave. The pots of seeds were moved to greenhouse. Greenhouse conditions: light mixture of sodium halide lamp and halogen lamp with 800 Lux, 16 hours light period, 8 hours darkness, 23±1°C. During the first week after seeding in flower pot, irrigation was done by distilled water. Irrigation was twice a day and 80ml in each one. After a week of irrigation with distilled water, irrigation was started with Long Ashton perfect nutritional solution once a day for two weeks. Preparing the solution repeated for two weeks and used. PH of the prepared solution was regulated about 5.5 by Potash solution of 0.1 molar and sulphoric acid of 0.1 molar. Two weeks after irrigation with the nutrition solution lacking Al ion, treatment of the plants started as following: some of pots irrigated with nutrition solution contains 0, 20, 40 and 60 micro molar of $AlCl_3$ and some other pots irrigated with nutrition solution contains 0, 40, 80 and 320 micro molar of KH_2PO_4 with $AlCl_3$ fix concentration of 40 micro molar. This irrigation method was done daily for three weeks. After 3 weeks different parts of the plant removed and were kept in freeze liquid nitrogen of -16ºC in a fridge until the time of experiment. The following cases were studied in order to study the effect of Al on Brassica napus plantlet: Evaluation of chlorophyll and carotenoid (Lichtenthaler, 1987), Evaluation of flavonoids by spectrophotometer (-Krizek *et al.,* 1981), Evaluation of peroxidation of membrane fats such as MDA (Heath *et al.,*

1969), And also other Aldehydes of the membrane (Meirs *et al.,* 1992), Evaluation of phenol compounds (Sonald *et al.,* 1999), Evaluation of reducing sugar (Somogy, 1952), Evaluation of solution carbohydrate (Fales, 1951), Evaluation of protein concentration (Bradford, 1976) and Evaluation of proline (Bates *et al.,* 1973).

RESULTS

Treatment by both Al and P causes significant increase in carotenoid of the plant that this increase is significant (P≤0.05) in 80 micro molar concentration of P. Also treatment by Al causes decrease in carotenoid of the leaf which is not significant statistically (Fig. 1). Treatment by both Al and P causes significant increase of chlorophyll *a* just with 320-micromolar concentration of P. Treatment by Al also causes significant decrease (P≤0.05) of chlorophyll *a* (Fig. 2). Treatment by Al causes significant decrease (P≤0.05) of chlorophyll *b* in the plant leaf; this decrease is highly significant in 60-micromolar concentration. Treatment by both Al and P also causes an increase in chlorophyll *b* which is not a significant increase statistically (Fig. 3). Results of the effect of treatments on MDA are in Fig. 7. Treatment by Al causes significant increase (P≤0.05) of membrane MDA concentration; treatment by both Al and P also causes significant decrease (P≤0.05) of membrane MDA concentration. Results of the effect of treatment by Al and treatment by both Al and P on other membrane Aldehydes delineated in Fig. 8. In marker plant the treatment by Al causes significant increase (P≤0.05) in concentration of other membrane MDAs. Treatment by both Al and P also causes significant decrease (P≤0.05) in concentration of other membrane lipids. Treatment by Al causes significant decrease (P≤0.05) in absorption of flavonoids in wavelength of 270nm (Fig. 4); treatment by both Al and P causes an increase in absorption of flavonoids in wavelength of 270nm. Treatment by both Al and P causes significant increase (P≤0.05) in absorption of flavonoids in wavelength of 300nm (Fig. 5), just in 320-micromolar concentration of P. Treatment by Al causes decrease in absorption of flavonoids in wavelength of 300nm which is not a significant decrease statistically. Treatment by both Al and P causes significant increase (P≤0.05) in absorption of flavonoids in wavelength of 330nm (Fig. 6), just in 320-micromolar concentration of P. Treatment by Al also causes a bit decrease in absorption of flavonoids in wavelength of 330nm which is not a significant decrease statistically. Treatment by both Al and P causes significant increase (P≤0.05) in phenol compounds of the plant in proportion to the marker plant, and the most

influence observed in 320-micromolar concentration of P. Treatment by Al also causes decrease in phenol compounds of the plant which is not a significant decrease statistically. Treatment by Al causes significant increase (P≤0.05) in plant proline which is a very significant increase in 40- and 60-micromolar concentration of Al. Treatment by both Al and P also causes decrease in plant proline which is not a significant decrease statistically. Treatment by both Al and P causes a very significant decrease (P≤0.05) in plant protein. Treatment by Al also causes significant increase (P≤0.05) in plant protein which its most influence is in 60-micromolar concentration of Al. Treatment by Al causes a very significant increase (P≤0.05) in reducing sugars of the plant which its most influence observed in 40- and 60-micromolar concentration of the Al. Treatment by both Al and P also causes a very significant decrease (P≤0.05) in reducing sugars which its most influence observed in 80- and 320-micromolar concentration of P. Treatment by Al causes significant decrease (P≤0.05) in soluble carbohydrates of the plant which its most influence observed in 40- and 60-micromolar concentration of Al. Treatment by both Al and P also causes a very significant increase (P≤0.05) in soluble carbohydrates of the plant which its most influence observed in 80- and 320-micromolar concentration of P.

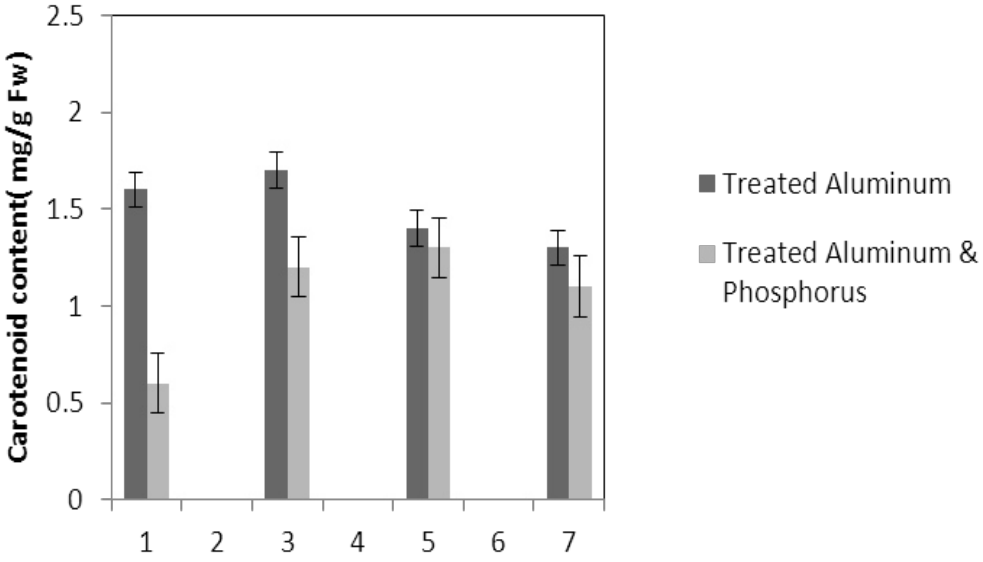

Figure 1. The effect of Aluminum and Phosphorus concentration on carotenoids content

Al: **1**- 0; **3**- 20; **5**- 40; **7**- 60 µM $AlCl_3$

Al and P: **1**- 0; **3**- 40; **5**- 80; **7**- 320 µM of KH_2PO_4 with fix concentration of 40 µM $AlCl_3$

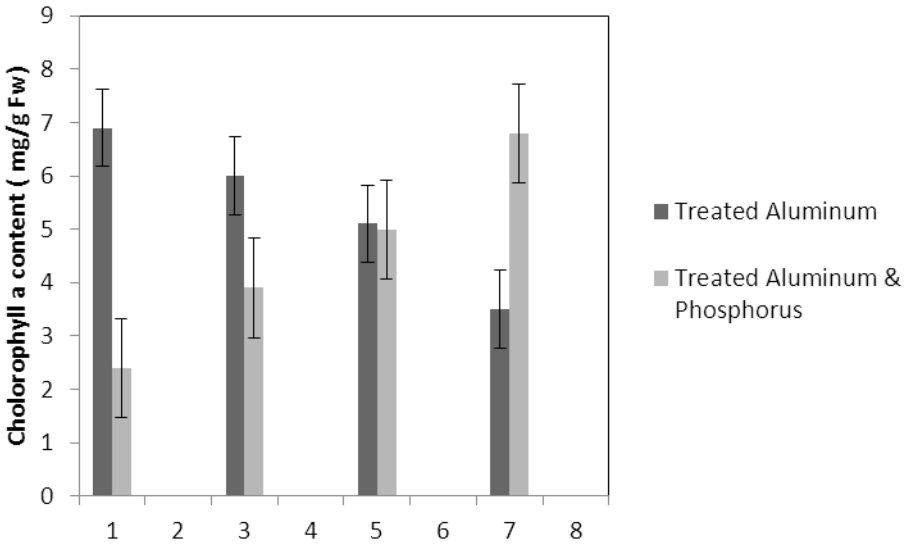

Figure 2. The effect of Aluminum and Phosphorus concentration on chlorophyll a content

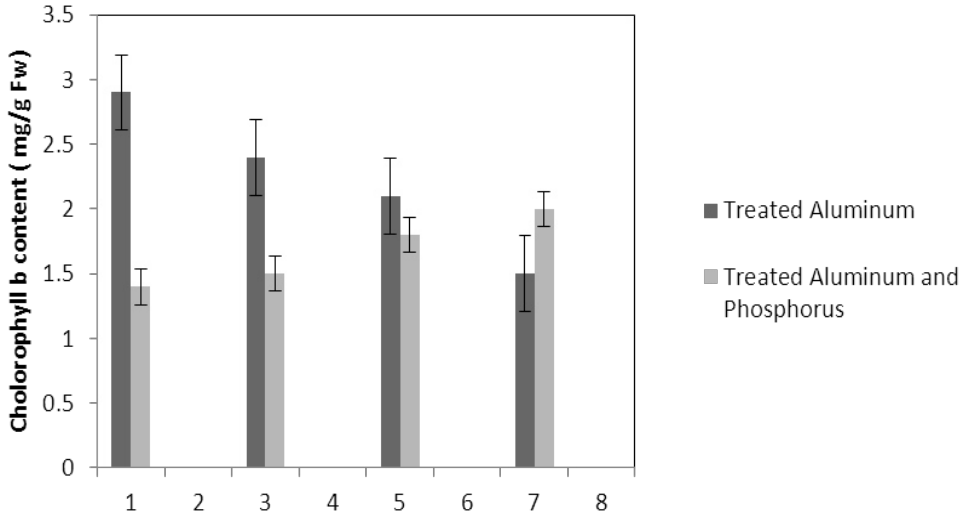

Figure 3. The effect of Aluminum and Phosphorus on chlorophyll b content

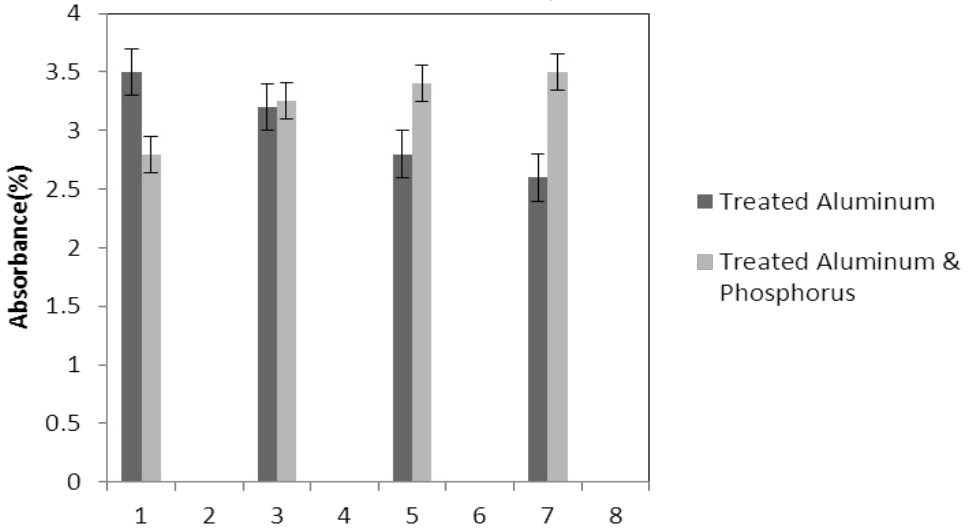

Figure 4. The effect of treatments on flavonoids absorb (270=λ)

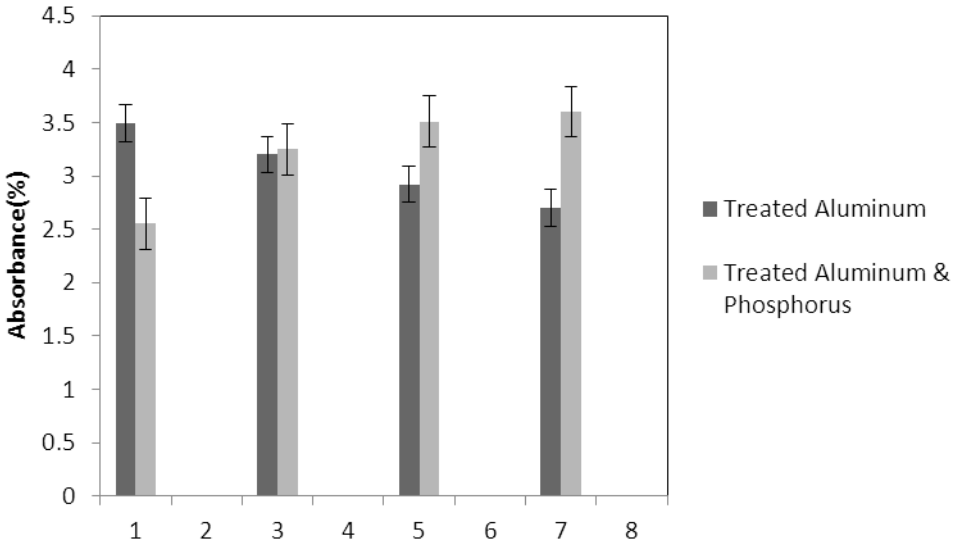

Figure 5. The effect of treatments on flavonoids absorb (300=λ)

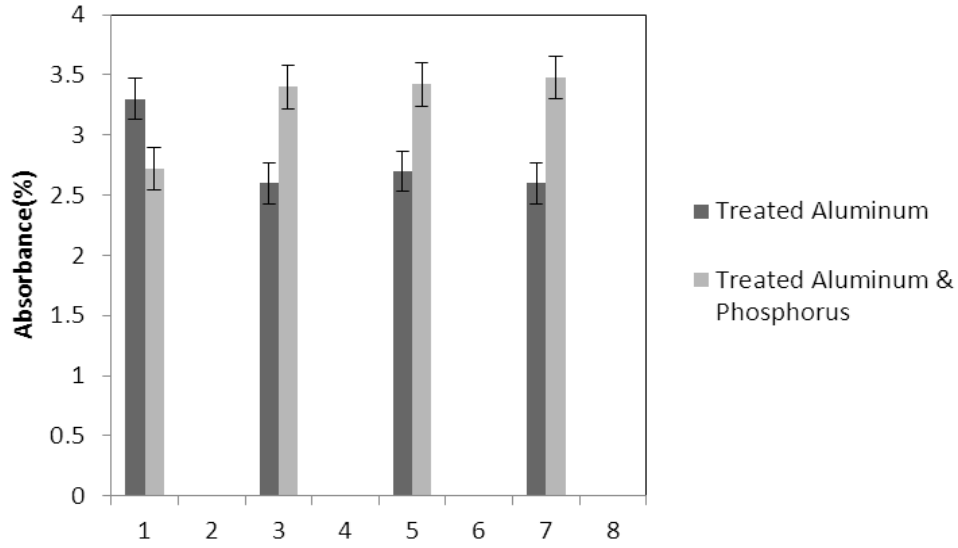

Figure 6. The effect of treatments on flavonoids absorb (330=λ)

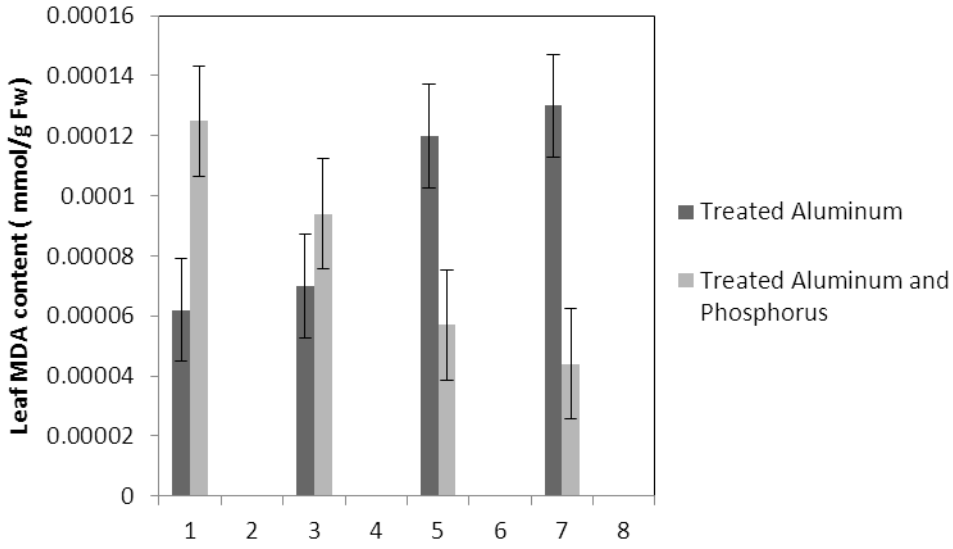

Figure 7. The effect of treatments on membrane MDA content

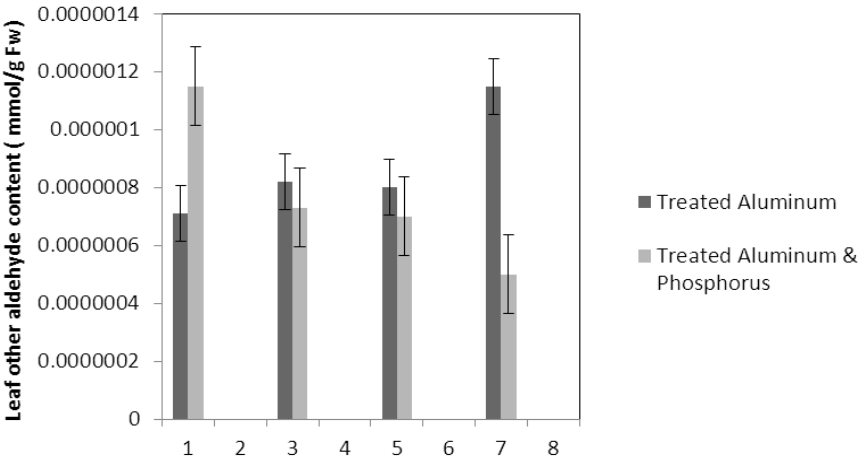

Figure 8: The effect of treatments on the aldehyde content

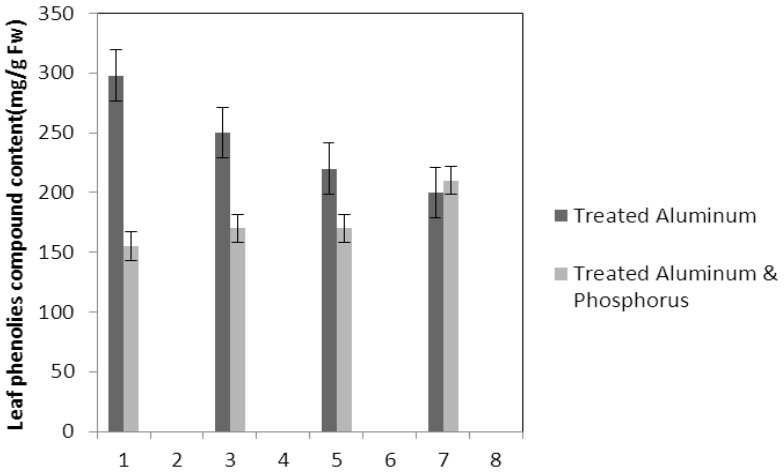

Figure 9: The effect of treatments on the phenolies compound content

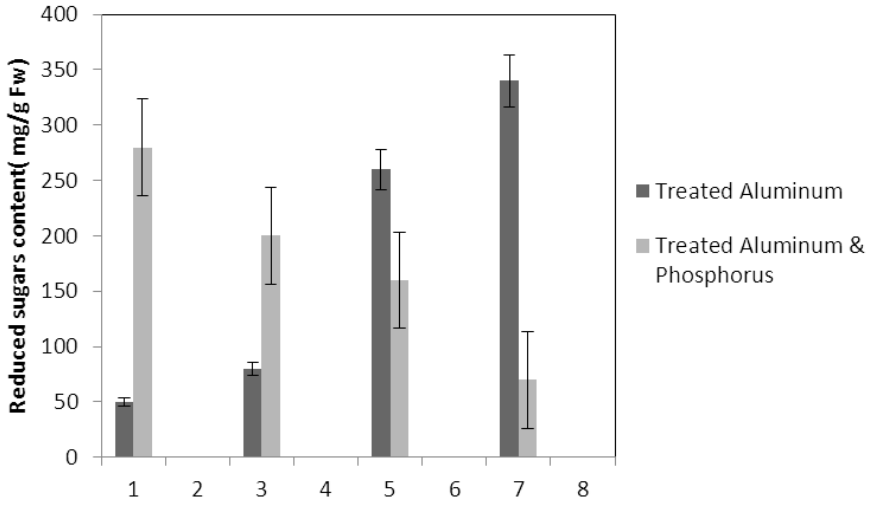

Figure 10: The effect of treatments on reduced sugars content

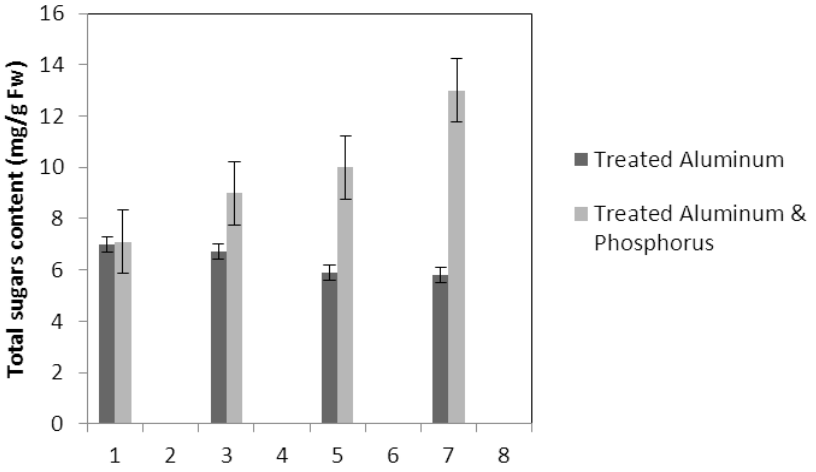

Figure 11: The effect of Treatments on Total sugars content

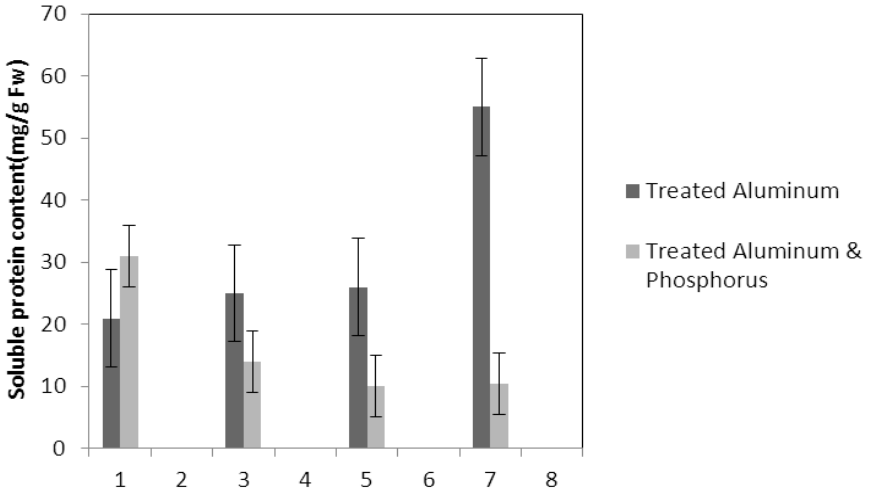

Figure 12: The effect of Treatments on Soluble protein content

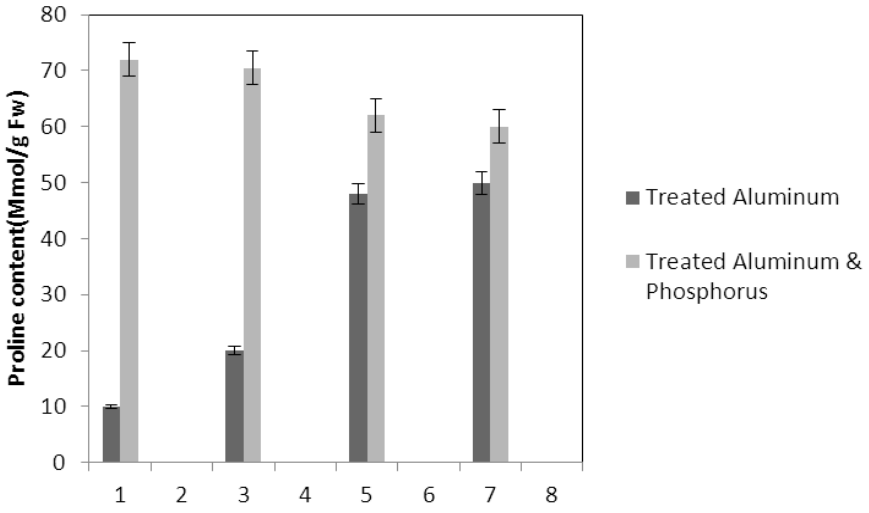

Figure 13: The effect of Treatments on Proline content

DISCUSSION

There are many reports about the effect of Al on carotenoids content of the plants (Greger *et al*., 1991). The results of this research also delineated that Al has significant effect on chlorophyll and carotenoids content of *Brassica napus* plantlet in its all concentrations. Intense effects of Al on photosynthesis have some aspects that one of them is defection in chlorophyll biosynthesis (Haag-kerwer *et al.,* 1999). Al can control chlorophyll biosynthesis by means of controlling protochlorophyllide reductase enzyme or by controlling enzymes exist in water-break complex at place of photo-system II oxidation, it controls photosynthesis electron transmission and through this it prevents the chlorophyll stimulation effect and consequently prevents the transmission of electron reactions chain (Lagriffoul *et al.,* 1998). Decrease in chlorophyll content in presence of heavy metals is probably due to oxidative induction. It was reported that decrease in carotenoid content can be due to non-photochemical collapse of carotenoids-stimulated chlorophylls and lead to break of carotenoid structure. Flavonoids are introduced as secondary metabolites of the plant based on phenyl benzopropen structure and also as bio-flavonoid. Biosynthesis of the flavonoids is complex and causes involvement of several enzyme phases. In initial phases of flavonoids synthesis, phenyl alanine which is derived from shikmic path, changes into coenzyme A by means of enzymes of methylpropanoid path: PAL, C_4H and 4CL. CH_S is the first enzyme of synthesizing flavonoids and causes the combination of coenzyme A and three molecules of Malonyl-CoA (made from Acetyl-CoA) which is necessary for formation of Naringenin. Then Naringenin change into other types by, Glycolysation and Metilation of its three rings. In this research also treatment by Al decreases the rate of flavonoids absorption in three wavelengths of 300, 270 and 330. Probably Al affecting on enzymes existing in flavonoids path such as Chalkone synthetize and phenyl ammonia leas cause decrease in aggregation of this pigment in plant. In treatment by both Al and P, Phosphorus also prevents the effect of Al on this group of enzymes and therefore this pigment is increasing in the plant (Jenkins, 1999). Peroxidation of fats is a free radicals-based process. Free radicals of oxygen, oxygen, radicals of hydroxyl and protonized superoxide anions attack unsaturated fat acids (i.e. linoleic acid). The result of this process is formation of analyzed products like Aldehydes. This process is used as an index to choose tolerant and sensitive plants against environmental stresses (Frankel, 1985). Increase of fats peroxidation usually considered as index of oxidative stress increase. Fats peroxidation Increase in treatment by Al shows insufficiency of not tolerance mechanisms emerged in plant against oxidative stress. Phenolic compounds especially hydroxyl-cinamic acids exist too much in epidermis. Existence of these compounds in leaf makes it thicker, removing free radicals and changing the amount of chlorophyll and carotenoid. Aluminum effects on enzymes like phenyl alanine ammonia leas, CH_S and other enzymes in phenyl propanoid path. The phenyl alanine ammonia leas enzyme causes phenyl alanine changing into trans-cinamic acid and formation of

phenolic compounds like flavonoids, tannins and lignin (Gitz *et al.,* 2004). Probably Al causes decrease of phenolic compounds by affecting on this enzyme (Gitz *et al.,* 2004). Existence of phenolic compounds like esters of Hydroxy-Cinamic acid in Epidermis cells increases tolerance of plant cells against damages of environmental stresses. Therefore, in Brassica napus, formation of Phenolic compounds under the treatment of both Al and P increases the plant tolerance against Al and increasing the amount of theses compounds in Phosphorus-treated plants is probably due to effect of thus element on synthesizing enzymes of the compounds in Phenyl Propanone direction (Sonald *et al.,* 1999).

Many of the environmental conditions effect on sugars metabolism and distribution of photosynthesis in growing plants. In this research also, Al effects significantly on contents of reducing sugars in Brassica napus and increase it; this because of decrease in water transmission to the leafs and therefore leads to disorder in leaf transpiration rate and change in behavior of key enzymes in some metabolic paths such as of sugars. Decreasing in water transmission to the leafs and following that the aggregation of Al in cells, the content of plant reducing sugars increases, this phenomena is probably mechanism of plant adjustment to maintain suitable osmosis potential in toxicity conditions. In addition to the role of sugars in regulation of osmosis, it seems that by increasing soluble sugars, plant can keep its carbohydrate reserve at desirable level for metabolic processes and maintenance of cell-base metabolism in stress conditions (Verma *et al.,* 2001). The role of Al in Brassica napus causes increase in protein content. Content of proteins in plantlet leafs also increase in presence of heavy metals. It seems that Al stimulates mRNA synthesis in cell and through this causes increase in total protein. It is reported that decrease in protein content of high ionic concentration can due to decrease in synthesizing of some proteins or maybe due to increase in activity of proteolytic enzymes (Khudsar *et al.,* 2001). In most of plants, the role of carbohydrate is accepted as a factor of regulating osmosis in environmental stresses. The effect of Al on carbohydrates is descending and decreasing (Verma *et al.,* 2001). Among soluble sugars, sucrose and fructans play important role in adjustment with environmental stresses. Sucrose plays an important role in protection of membrane cialite and in phospholipids structure, and preventing any changes in the structure of water soluble proteins. One of the reasons of decrease in carbohydrates of plants which are tolerant against Al stress is probably the effect of Al on membrane, Tilacoids, amount of photosynthetic pigments and as a result the amount of photosynthesis. The amount of Trehalose non-reducing sugar causes increase of plant tolerance against non-environmental stresses. Phosphorous may cause delay in decreasing the amount of photosynthesizing pigments in environmental stresses. Therefore, due to balance in amount of photosynthesizing pigments and maintaining Rubisco enzyme, they prevent harmful effects of Al (Koster *et al.,* 1988). One of the most abundant changes induced in plants due to the effects of environmental stresses is accumulation of Proline which is interfering

in tolerance mechanisms against these stresses. Proline Accumulation in cells cytoplasm plays role in osmosis regulation of cell (Demir, 2000). The role of Proline in protecting enzymes and cellular structures was proved that is act as a sweeper of free radicals. Proline increase causes decrease in amount of Malon Dealdehyde and Proline accumulation is sign of stresses. Aluminum causes the accumulation of Proline amino acid in plant. Proline is of those organic elements with low molecular weight and act as osmosis protector of cellular proteins. When cell exposed to Al stress, Proline accumulation will increase in plant regarding the Proline biosynthesizing path (Demir 2000). Probably P causes decrease in Proline concentration of plant through impacting on enzymes activities within Proline biosynthesis path like R5CS and P5CR enzymes (Yokoi *et al.,* 2002). In absence of Phosphorus, Al causes the production of cloning factors like LOS, COS, HOS and SOS_3. These factors stimulates the enzyme proteins (R5CS & P5CR) form the related genes. Consequently Proline biosynthesis path activated and causes accumulation of Proline in stress conditions (Yokoi *et al.,* 2002).

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