

## EFFECTS OF ALUMINIUM STRESS ON THE ACCUMULATION OF CATIONS AND ANIONS IN RICE AND CHICKPEA SEEDLINGS GROWN IN SOLUTION CULTURE

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### Abstract

Different concentrations of aluminium (10-150  $\mu\text{M}$ ) inhibited the accumulation of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Fe}^{2+}$  and  $\text{PO}_4^{3-}$  in rice and chickpea seedlings grown in solution culture. In rice and chickpea, the intensity of inhibition of the accumulation of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Fe}^{2+}$  and  $\text{PO}_4^{3-}$  enhanced with the increase in Al concentrations from 10 to 150  $\mu\text{M}$ . On the contrary, application of aluminium caused a dramatic few fold increase in accumulation of  $\text{Al}^{3+}$  in different parts of rice and chickpea seedlings.

### Introduction

A large amount of aluminium (Al) is incorporated into aluminosilicate soil minerals, and very small quantities appear in the soluble form, capable of influencing biological systems (May and Nordstrom 1991). Acid soil having pH below 5.0 increases the solubility of Al, which in subsoil is particularly harmful for plants (Lidon and Barreiro 2002). Nutritional imbalances induced by Al exposure were reported for several plant species (Silva 2012).

Concentration of calcium drastically reduced in presence of aluminium in spinach (Karimaei and Poozesh 2016) and cacao (Ribeiro *et al.* 2013). Aluminium decreased  $\text{Mg}^{2+}$  content in the leaves and roots of physic nut (Steiner *et al.* 2012) and the foliage and root of silver birch (Bojarczuk *et al.* 2006). Toxic Al concentration decreased significantly the concentrations of  $\text{Fe}^{2+}$  in sorghum (Clark *et al.* 1981) and in maize (Lidon *et al.* 1999). Al caused a decrease in Mg and Fe content in the root but an increase in those ions in the stem of *Pinus massoniana* was found (Zhang *et al.* 2014).

Exposure of Al increased  $\text{Al}^{3+}$  concentration in leaves of buckwheat (Ma *et al.* 1998). Concentration of  $\text{Al}^{3+}$  was high in the root and generally low in the tops of honeylocust and loblolly pine seedling (Wagatsuma *et al.* 1987). Phosphate ( $\text{PO}_4^{3-}$ ) uptake was first inhibited and then increased in corn root following aluminium treatment (Facanha and Okorokova-Facanha 2002). Aluminium ions blocked P uptake in soybean plants (Zheng 2010).

### Materials and Methods

Rice (*Oryza sativa* var. BRRI Dhan-53), the main cereal grain of the family Poaceae and chickpea (*Cicer arietinum* var. Bari chhola-7), the third most important pulse of the family Fabaceae; were used as experimental plant materials. Seeds of rice were collected from Bangladesh Rice Research Institute (BRRI) and that of chickpea were obtained from Bangladesh Agricultural Research Institute (BARI), respectively.

The seeds were surface sterilized according to Samad and Karmoker (2013). The sterilized seeds were spread over a cotton gauge placed in a lid having holes (1 cm in diameter) and the lid with seeds was placed on a beaker containing 500 ml of distilled water. The beakers were covered by black plastic sheet to avoid the exposure of light to the roots.

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After germination, the seedlings were transferred to modified half-strength Hoagland solution (Hoagland and Arnon 1950) and the beakers with the seedlings were placed in a light bank. Rice seedlings were grown at a day/night temperature of  $30 \pm 1^\circ\text{C}/25 \pm 1^\circ\text{C}$  and day/night length of 14 hrs/10 hrs. Chickpea seedlings were grown at a day/night temperature of  $25 \pm 1^\circ\text{C}/18 \pm 1^\circ\text{C}$  and day/night length of 10 hrs/14 hrs. Light intensity was  $160 \mu\text{-einstein m}^{-2}\text{s}^{-1}$ . The solution was continuously aerated through bubbler with the help of air compressor (Rockyvac 320). The solution was replenished every 48 hrs. Seven-day-old seedlings were transferred to half strength Hoagland solution as control and 10, 50, 100 and 150  $\mu\text{M}$   $\text{AlCl}_3$  solution made in half strength Hoagland solution were used as treatments. The pH of all solutions including control were adjusted to 4.2 with 0.2N  $\text{H}_2\text{SO}_4$ .

$\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Al}^{3+}$  and  $\text{PO}_4^{3-}$  were extracted by digestion in a mixture of nitric and perchloric acid (4:1) using a hot sand bath. The amount of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Fe}^{2+}$  in the extract were determined by an atomic absorption spectrophotometer (Perkin-Elmer, Model: AAnalyst 200) at wavelengths of 422.67, 285.21 and 248.33 nm, respectively.  $\text{Al}^{3+}$  content was measured by using an atomic absorption spectrophotometer (Shimadzu, Model: AA 7000, Japan). Phosphate was measured by Vanadomolybdate method (Jackson 1967) from the aliquot obtained by digesting the plant material.

In this paper, the effect of aluminium toxicity on the accumulation and distribution of divalent and trivalent cations ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Al}^{3+}$ ) and anion ( $\text{PO}_4^{3-}$ ) is reported.

## Results and Discussion

The highest inhibition of  $\text{Ca}^{2+}$  accumulation in the root was observed at 150  $\mu\text{M}$  Al treatment which ranged from 54.0 to 65.7% from 3 to 96 hrs of application (Fig. 1a). In the shoot of rice the inhibitory effect of Al on  $\text{Ca}^{2+}$  accumulation increased with the increase in Al concentration from 10 to 150  $\mu\text{M}$  from 3 to 96 hrs of Al exposure (Fig. 1b). In chickpea seedlings, Al also caused an inhibition of  $\text{Ca}^{2+}$  accumulation in the root, stem and leaves from 3 to 96 hrs of treatment. (Figs 2a-c). Exposure of chickpea seedlings to 150  $\mu\text{M}$  Al resulted in the maximum 36.0 to 57.8% inhibition of  $\text{Ca}^{2+}$  content in the leaves from 3 to 96 hrs of application (Fig. 2c). This result is in agreement with the work of Zheng *et al.* (2005) who found that  $\text{Ca}^{2+}$  accumulation decreased progressively in the root of buckwheat with the increase in Al concentrations.

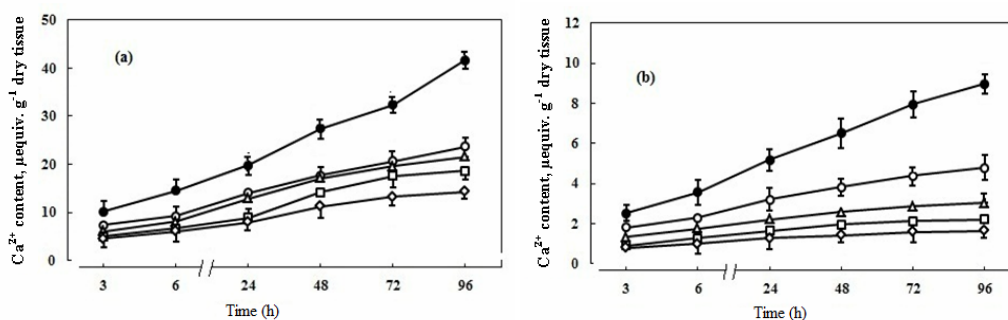


Fig. 1. The effect of different concentrations of aluminium on the accumulation of  $\text{Ca}^{2+}$  in the (a) root and (b) shoot of rice seedlings grown in solution culture. ● represents control; ○ 10  $\mu\text{M}$  Al; Δ 50  $\mu\text{M}$  Al; □ 100  $\mu\text{M}$  Al and ◇ 150  $\mu\text{M}$  Al. Each value is the mean of three replicates  $\pm$  standard error.

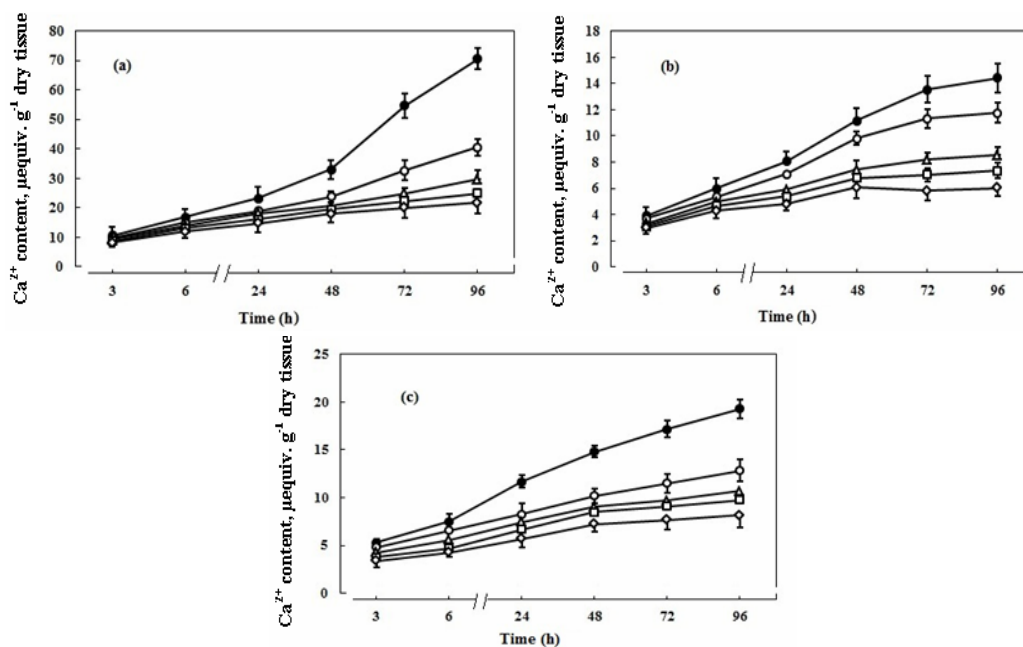


Fig. 2. The effect of different concentrations of aluminium on the accumulation of Ca<sup>2+</sup> in the (a) root, (b) stem and (c) leaves of chickpea seedlings grown in solution culture. Otherwise as Fig. 1.

Exposure of roots of rice seedlings to 10 µM Al caused a 18.0 to 30.0% inhibition of Mg<sup>2+</sup> content from 3 to 96 hrs of treatment (Fig. 3a). Al, at concentrations of 100 and 150 µM, resulted in a 44.6 to 59.0% and 57.8 to 69% inhibition of Mg<sup>2+</sup> content in the shoot respectively, from 3 to 96 hrs of application (Fig. 3b).

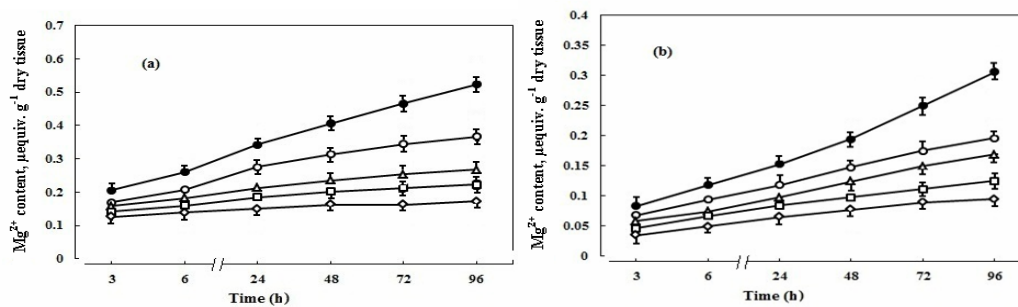


Fig. 3. The effect of different concentrations of aluminium on the accumulation of Mg<sup>2+</sup> in the (a) root and (b) shoot of rice seedlings grown in solution culture. Otherwise as Fig. 1.

In chickpea seedlings, the reduction in Mg<sup>2+</sup> accumulation in the root, stem and leaves gradually increased with the increase in Al concentration from 10 to 150 µM (Figs 4a-c). Similarly, Al decreased Mg<sup>2+</sup> accumulation in cabbage, lettuce and kikuya grass (Huett and Menary 1980).

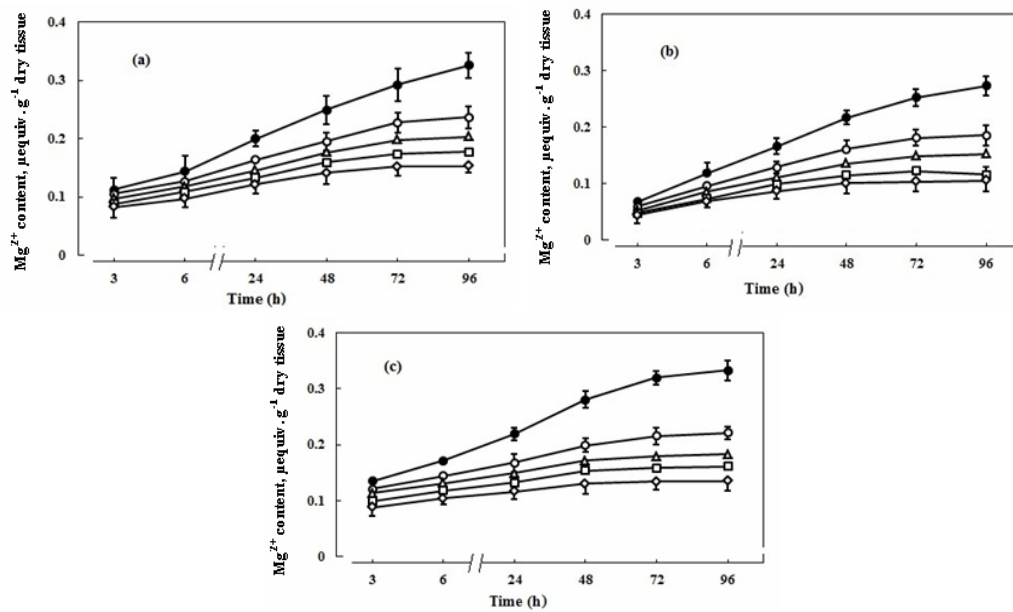


Fig. 4. The effect of different concentrations of aluminium on the accumulation of Mg<sup>2+</sup> in the (a) root, (b) stem and (c) leaves of chickpea seedlings grown in solution culture. Otherwise as Fig. 1.

Al (10-150 µM) decreased Fe<sup>2+</sup> accumulation in the root of rice except an initial stimulation. In the root, 50 µM Al decreased Fe<sup>2+</sup> content by 11.0 to 20.9% from 48 to 96 hrs of treatment (Fig. 5a). 100 µM Al decreased Fe<sup>2+</sup> content in the shoot of rice by 9.9 to 13.0% from 48 to 96 hrs of treatment (Fig. 5b). In chickpea seedlings, 150 µM Al caused a 31.8 to 19.7% stimulation of Fe<sup>2+</sup> content from 3 to 24 hrs followed by a decrease by 9.0 to 32.0% from 48 to 96 hrs of application (Fig. 6a). Al (10 to 150 µM) stimulated Fe<sup>2+</sup> accumulation in the stem of chickpea seedlings from 3 to 48 hrs of treatment. After a stimulation of that period the treatment showed inhibition from 72 to 96 hrs (Fig. 6b). In the leaves of chickpea, Al caused an inhibition of Fe<sup>2+</sup> except an initial stimulation (Fig. 6c). This result is supported by Simon and coworkers (1994) who found that Al decreased Fe<sup>2+</sup> content in the root, stem and leaves of tomato.

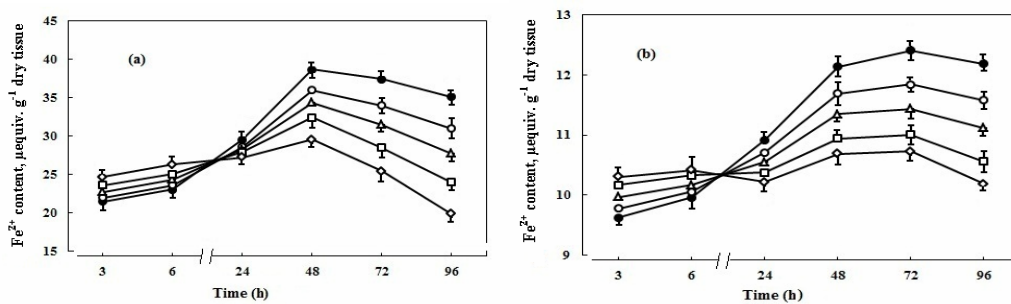


Fig. 5. The effect of different concentrations of aluminium on the accumulation of Fe<sup>2+</sup> in the (a) root and (b) shoot of rice seedlings grown in solution culture. Otherwise as Fig. 1.

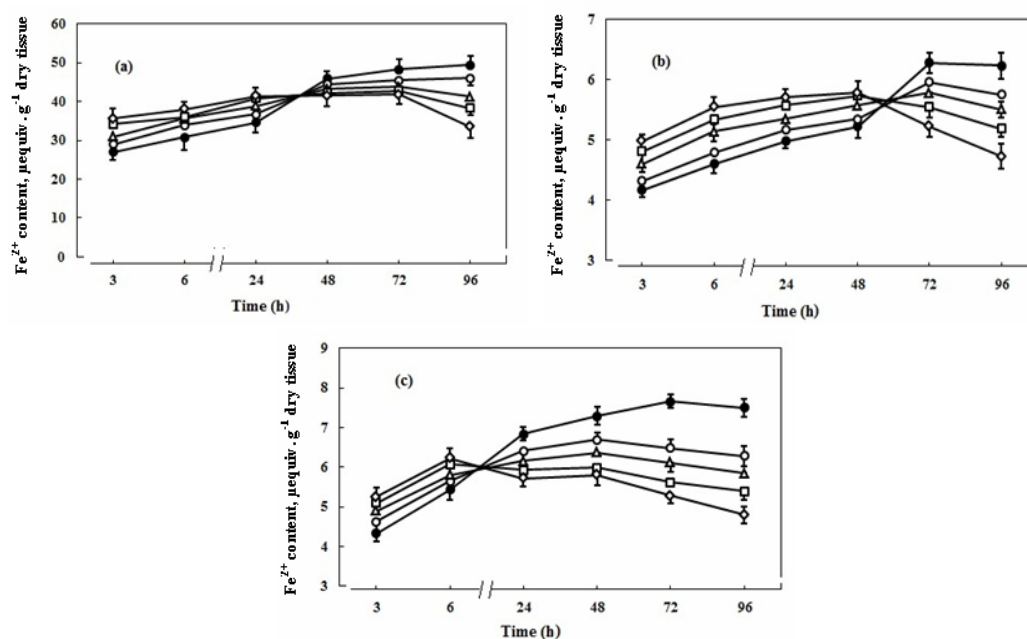


Fig. 6. The effect of different concentrations of aluminium on the accumulation of  $\text{Fe}^{2+}$  in the (a) root, (b) stem and (c) leaves of chickpea seedlings grown in solution culture. Otherwise as Fig. 1.

Aluminium (50  $\mu\text{M}$ ) increased  $\text{Al}^{3+}$  content in the root by 2- to 3.5-fold from 3 to 96 hrs of exposure (Fig. 7a). In the shoot of rice seedlings, 10  $\mu\text{M}$  Al caused a 1.8 to 2-folds increase in  $\text{Al}^{3+}$  content from 3 to 72 hrs of application and the stimulatory effect was sustained up to 96 hrs of treatment (Fig. 7b).

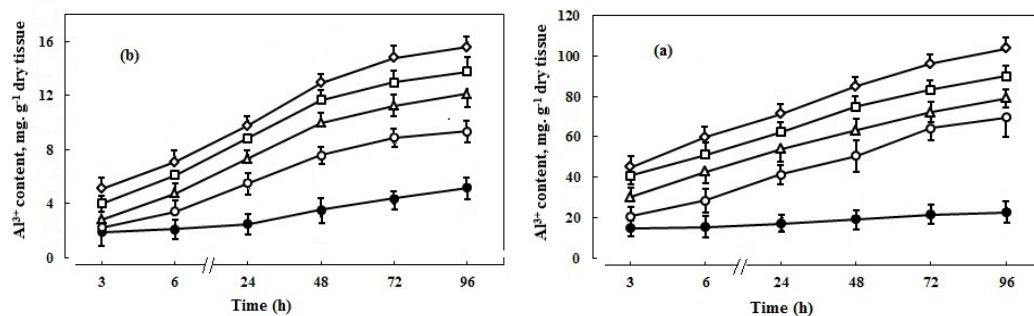


Fig. 7. The effect of different concentrations of aluminium on the accumulation of  $\text{Al}^{3+}$  in the (a) root and (b) shoot of rice seedlings grown in solution culture. Otherwise as Fig. 1.

In chickpea seedlings, 100  $\mu\text{M}$  Al increased the accumulation of  $\text{Al}^{3+}$  in the root by 2.2 to 3-folds from 3 to 96 hrs of treatment (Fig. 8a). The accumulation of  $\text{Al}^{3+}$  in the stem and leaves also increased with the increase in aluminium concentration from 10 to 150  $\mu\text{M}$  (Figs 8b, c). Similarly, Al application caused a 3-folds increase in  $\text{Al}^{3+}$  in the root of maize (Lidon *et al.* 2000). Application of Al increased accumulation of  $\text{Al}^{3+}$  in seedlings of tertiary buckwheat (Wang *et al.* 2015).

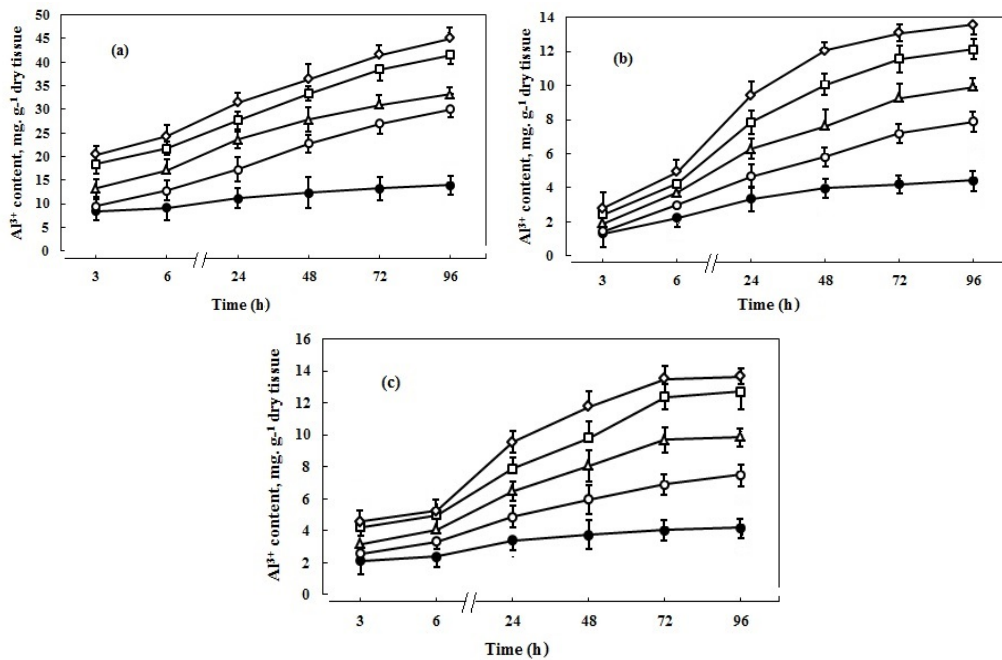


Fig. 8. The effect of different concentrations of aluminium on the accumulation of  $\text{Al}^{3+}$  in the (a) root, (b) stem and (c) leaves of chickpea seedlings grown in solution culture. Otherwise as Fig. 1.

A maximum of 20.0 to 57.0% inhibition of phosphate accumulation in the root of rice was recorded under the influence of 150  $\mu\text{M}$  Al from 3 to 96 hrs of application (Fig. 9a). At a concentration of 50  $\mu\text{M}$ , aluminium decreased phosphate accumulation in the shoot of rice by 8.0 to 22.0% from 3 to 96 h of treatment (Fig. 9b). In the root of chickpea seedlings, 10 to 150  $\mu\text{M}$  Al treatment caused an increase in phosphate accumulation from 3 to 24 hrs followed by an inhibition of that from 48 to 96 hrs of treatment. 100  $\mu\text{M}$  Al caused a 28.5 to 36.0% increase in phosphate accumulation from 3 to 24 hrs of treatment and it gradually decreased that from 12.0 to 26.0% from 48 to 96 hrs of application (Fig. 10a). Al (10  $\mu\text{M}$ ) inhibited phosphate accumulation in the stem of chickpea seedlings from 12.0 to 27.6% from 24 to 96 hrs of treatment except an initial

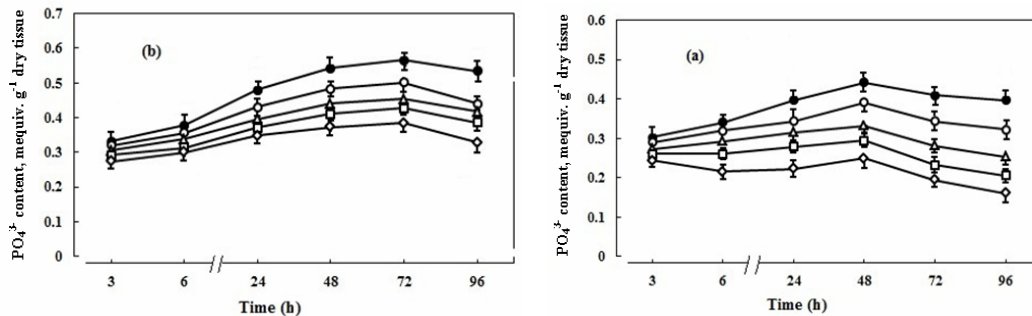


Fig. 9. The effect of different concentrations of aluminium on the accumulation of  $\text{PO}_4^{3-}$  in the (a) root and (b) shoot of rice seedlings grown in solution culture. Otherwise as Fig. 1.

stimulation at 3 to 6 hrs of exposure. This trend of effect exerted by Al was maintained following 50, 100 and 150  $\mu\text{M}$  Al application (Fig. 10b). The maximum inhibition of phosphate was observed in the leaves of chickpea seedlings exposed to 150  $\mu\text{M}$  Al which ranged from 26.8 to 57.5% from 3 to 96 hrs of application (Fig. 10c). Similarly Al inhibited the concentration of phosphate in wheat (Foy and Flemming 1982).

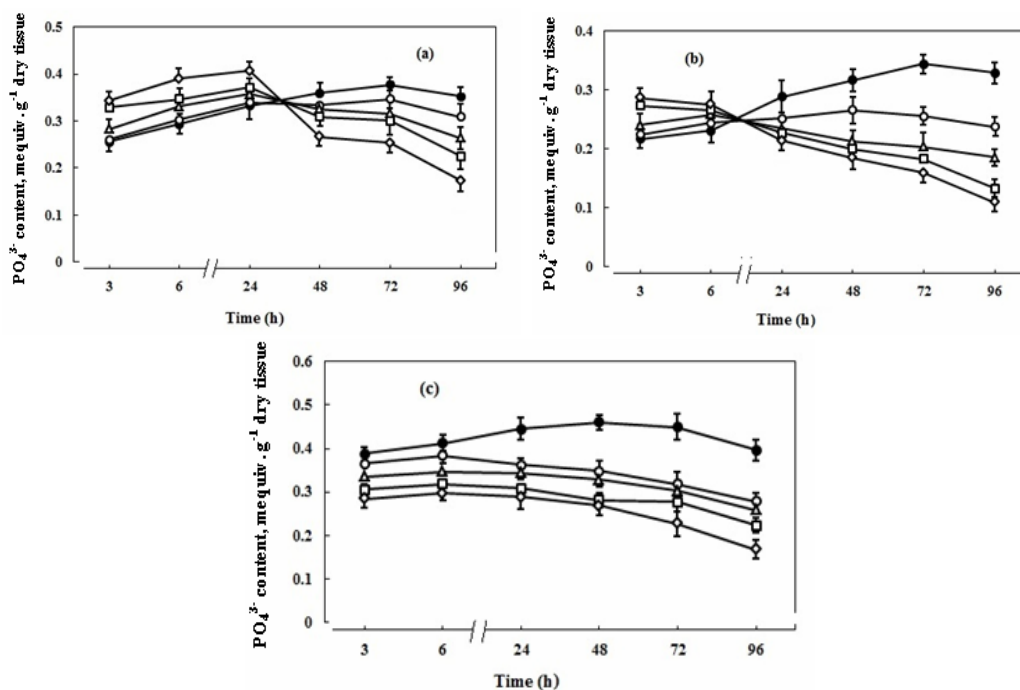


Fig. 10. The effect of different concentrations of aluminium on the accumulation of  $\text{PO}_4^{3-}$  in the (a) root, (b) stem and (c) leaves of chickpea seedlings grown in solution culture. Otherwise as Fig. 1.

Al-induced inhibition of Ca and Mg in rice and chickpea seedlings aggravated the aluminium stress. The inhibition of  $\text{Ca}^{2+}$  accumulation might impair the permeability characteristic of plasmamembrane because  $\text{Ca}^{2+}$  is responsible for maintaining this unique characteristic of plasmamembrane.  $\text{Mg}^{2+}$  is a constituent of chlorophyll. So, Al-induced decrease in  $\text{Mg}^{2+}$  accumulation would decrease chlorophyll synthesis. Al stress caused inhibition in  $\text{Fe}^{2+}$  accumulation would reduce respiration resulting in a decrease in ion transport which is dependent on respiratory energy. The dramatic increase in the concentration of  $\text{Al}^{3+}$  in the root tissue would hinder the absorption of ions.

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