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Toxic Effects of Aluminum on Certain Protein Metabolic Parameters in Two Rice Varieties during Leaf Senescence.

M Muthukumaran*and A Vijaya Bhaskara Rao

Department of Ecology and Environmental Sciences, Pondicherry University, Puducherry.

ABSTRACT

Rice (Oryza sativa) is an important food crop in India as well as in other Asian countries Aluminum is an element causing toxic effects in plants particularly in plants growing in marshy and acidic soils. In our study, four leaf bits of two rice varieties were placed in petridishes, containing different concentrations of aluminum solutions (100µM, 200µM, 300µM) and control and exposed at different periods. On exposure, certain protein metabolic parameters were estimated. The total protein content declined with a progressive increase in accumulation of aminoacidsin both varieties. The decreased protein content can be explained by decrease in protein synthesis or enhanced protein degradation. The elevated activity of protease also correlated to enhanced activities of AAT and AlAT in both varieties. However there was a greater degree of increase was noticed in the activity of AAT and AlAT in the varietiey of ADT 43. In addition to that the accumulation of ammonia was further strongly supported by arising glutamate dehydrogenase activity (GDH). Relatively greater increase in activities of AAT and AlAT, coupled with a lesser magnification of increase in ammonia were observed in ADT 43 indicates the aluminum tolerance nature.

Key words: Rice, Aluminum, Protein, AlAT and AAT, GDH

Corresponding author

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INTRODUCTION

Aluminum is widely distributed in plants, particularly in vegetation in marshy places and acid soils [1].Cruz et al.,demonstrated that total soluble proteins and amino acids were significantly reduced in sorghum leaf on exposure to aluminum at different concentrations [2].Rafiaobserved that total proteins and amino acids increased on exposure to aluminum in *Lens culinaris* cultivars [3]. Zatta et al.,reported that GDH was inhibited by aluminum [4]. Physiologically relevant pH values, the activity of GDH enzyme was strongly dependent on the concentration of aluminum [5]. Aluminum inhibited total soluble proteins and amino acids [6].Aluminum enhanced the activities of aminating-GDH, AlAT, AAT at 160µm in nutrient solution[7].Since ADT-43 and PA-6129 are an important cultivars in Puducherry, a comparative analysis was made to identify the aluminum resistance in these varieties.

MATERIALS AND METHODS

Rice (*Oryza sativa* .L) seeds of varieties ADT 43 and PA 6129 were procured from PKKVK, PondicherryIndia, and plants were grown in field conditions. Leaves from 8 weeks old rice plants were used for further studies. 7cm leaf bits from fully expanded and matured leaves were washed in distilled water. Four leaf bits were placed in petridishes of 20 cm diameter. The petridishes were kept in light and dark periods (48, 96, 144, 192 hrs) as per the standard protocols. The biochemical analysis was carried after at different periods of exposures and at different concentrations (100µM, 200µM, 300 µM). Petri dishes were kept under light intensity of approximately 150wm^{-2} and temperature of 27°±3°C of incubation. The exposed leaf materials were taken for further biochemical studies.

The protein content was estimated by the Folin-phenol method according to Lowry et al., [8], and the results were expressed as mg gm^{-1} fresh wt.The extraction and estimation of amino acids was done according to Moore and Steinandthe results were expressed as mg gm^{-1} fresh wt [9].Estimation of protease activitywas assayed following the method described by Snell and Snelland modified by Biswas and Choudhuri [10, 11].The results were expressed as mg BSA hydrolysed mg^{-1} protein h^{-1}.The enzymes of alanine aminotransferase (ALAT) and aspartate aminotransferase (AAT) were extracted and estimated by Reitman and Frankeland Hedley and Stoddart respectively [12, 13]. The results of AAT and ALAT were expressed as µg keto acids formed mg^{-1} protein h^{-1}. NADH and NADPH are Specific glutamate dehydrogenase activities were determined of Sadler and Scott [14].The results were expressed as mg gm^{-1} fresh wt. The estimation of ammonia was done according to Bergmeyer [15]. The results were expressed as µg gm^{-1} dry wt.All the data obtained per each parameter was statistically analyzed for their significance according to the method of Duncans's Multiple Range Test [16]. The significance was calculated at 5% level ($P<0.05$).

RESULTS AND DISCUSSION

Effect of aluminum on some protein parameters were studied in two rice varieties viz. ADT-43 PA-6129 were studied during leaf senescence. It is observed that relative to controls, the total protein content (Fig.1) significantly decreased in the ADT-43 and PA-6129 and the results indicated that the leaf protein content in both varieties decreased and the magnitude of decrease was found to be dependent on concentration and period of

exposure. It was also observed that there exists a marked difference between the ADT-43 and PA-6129 varieties.The free amino acids levels (Fig.2), protease activity (Fig.3) significantly increased in the leaves of rice exposed to aluminum. A significant elevation was also observed in the free amino acids levels and protease activity in both PA-6129and ADT-43 at all concentrations and at all exposure periods. Further, the results indicated that the free amino acids levels and protease activities increased in both ADT-43 and PA-6129 varieties and the magnitude of increase was dependent on the concentration and period of exposure. In general the degree of increase was more in magnitude in ADT-43 than in PA-6129. The amino acid pool enlargement in the stressed plants can be attributed to a decreased protein synthesis and enhanced proteolysis [17, 18]. Relative to control, both alanine amino transferase (AlAT) (Fig.4), and aspartate amino transferase (AAT) (Fig.5) activities significantly increased in the leaves of rice treated with aluminum. A significant elevation in the activities was noticed in both ADT-43 and PA-6129. The increase in AlAT and AAT was greater in magnitude in the variety PA-6129 than ADT-43. Further the degree of elevation in AlAT and AAT was found to be dependent on concentration and duration of exposure. The increased activities of AAT and AlAT observed in the present study further support the accumulation of Amino acids. The amino transferases (AlAT and AAT) serve as strategic link between carbohydrate and protein metabolism under altered pathophysiological conditions and under environmental stress [19]. Ketoacids are important intermediary metabolites and provide carbon skeletons for the synthesis of amino acids thus help in detoxifying the accumulated ammonia. AAT and AlAT mediated the utilization of excessive ammonia by converting Keto acids into amino acids under water stress conditions [20, 21]. Relatively better activities of AAT and AlAT in the variety ADT-43, supported a greater accumulation of amino acids, compared to PA-6129. The elevated activities of AAT and AlAT on the one hand reduces the accumulated ammonia content and contributed for increase in amino acid pool on the other. GDH activity (Fig. 6) increased in the leaves of aluminum treated rice. GDH activity increased significantly in both ADT-43and PA-6129. Glutamate dehydrogenase activity increased significantly in both cultivars at all concentrations and at all exposure periods. However, the percent increase in the activity, in the variety, was more in PA-6129 than in variety ADT-43. The direct and indirect evidence from the tolerance of accumulation of ammonia and nitrogen containing compounds (amino acids, amides and poly amides) during various environmental stress conditions, support the hypothesis that it is a mechanism of sequestering toxic levels of free cellular ammonia [22]. In addition to that the accumulation of ammonia was further strongly supported by arising glutamate dehydrogenase activity (GDH). Increasing GDH activity was also observed by Pallavi and Dubey in aluminum treated in rice seedlings [23]. Ammonia levels increased significantly in both varieties at all concentrations of aluminum exposure relative to controls (Fig.7). The ammonia accumulation was increased with increasing period of exposure, within ADT-43 and PA-6129, the degree of increase in ammonia levels was more in PA-6129 than in variety ADT-43. The results suggest the suppression of protein synthesis and / or utilization of proteins for energy purposes. Protein depletion might have may contributed a physiological mechanism and may play a key role in compensatory mechanism under aluminum stress to enhance osmolarity, by retaining free amino acidscontent in the cytoplasm and to compensate osmo-regulatory processes. Similarly in the present study it was observed that a variation in the magnitude of amino acids accumulation was observed between the varieties and being greater accumulation in the variety ADT-43 whereas compared to PA-6129. A comparison was made between the protease activity and free

amino acid levels in two varieties of rice on exposure to aluminum. In the present study decrease in total protein content is associated with the sharp elevation in protease activity and free amino acids levels in the leaves of both varieties on exposure to aluminum with different concentrations. There was no marked difference between the varieties in the percent elevation in protease activity. The elevated protease activity under aluminum stress points to enhanced proteolysis in both varieties. Dubeyreported that high protease activity in the water stressed plants appears to be of adaptive significance [24]. Because it leads to the accumulation of free amino acids. Hence, it is clear that aluminum induced protease activity, which leads to the formation of more free amino acids content causing cellular toxicity.The effect of aluminum on protease activity, however, is dose and time dependant.

Thus aluminum toxicity in variety ADT-43 might be attributed at least in part to the ability to shift the metabolic rate and greater accumulation of amino acids coupled with lesser accumulation of ammonia, largely by reassimilation as evidenced by relatively greater activities of AAT and AlAT in the tissues. It is well known that hydrolysis of protein is quite common and proteases split proteins stepwise into polypeptides and amino acids. The products of these degradative processes are utilized for anabolic processes and energy sources.On the whole,the rice varieties ADT-43and PA-6129 exhibitedalterations in the parameters like proteins, amino acids, protease, AlAT, AAT, GDH, and ammoniaon exposure to aluminum at different concentrations and different periods during leaf senescence.However, it is suggested that ADT-43 is resistant variety and PA-6129 is sensitive variety under aluminum stress.

Figure1 : Percent decrease over the control in the protein (mg gm-1) in leaf of two rice varieties (PA 6129, ADT-43) of control on exposure to different concentrations of aluminum at 24, 48, 72, and 96 hours.

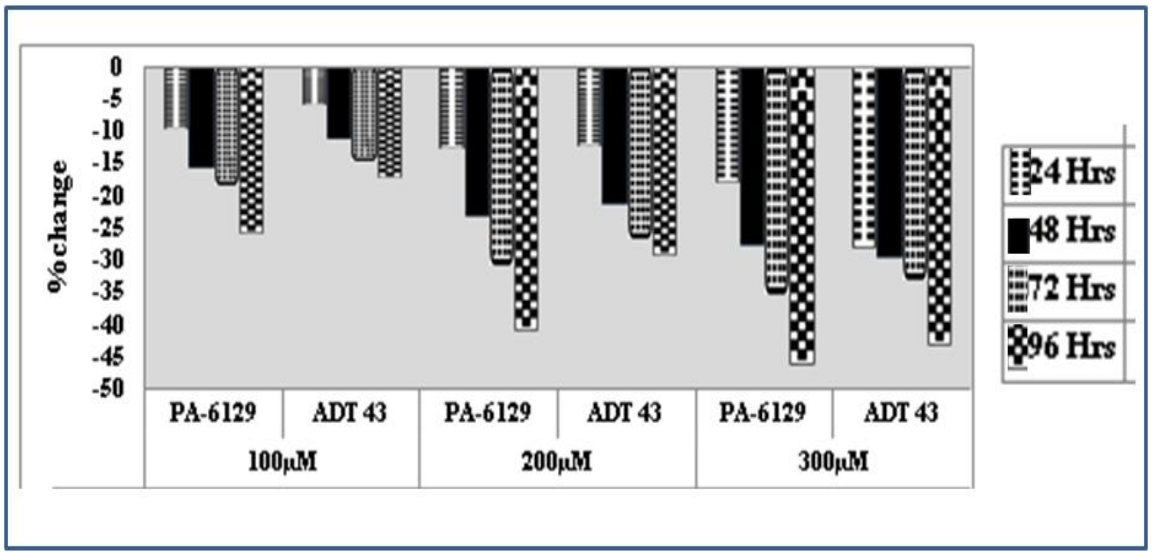

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Figure 2 : Percent increase over the control in the amino acids (mg gm-1) in leaf of two rice varieties (ADT-43, PA 6129) of control on exposure to different concentrations of aluminum at 24, 48, 72, and 96 hours.

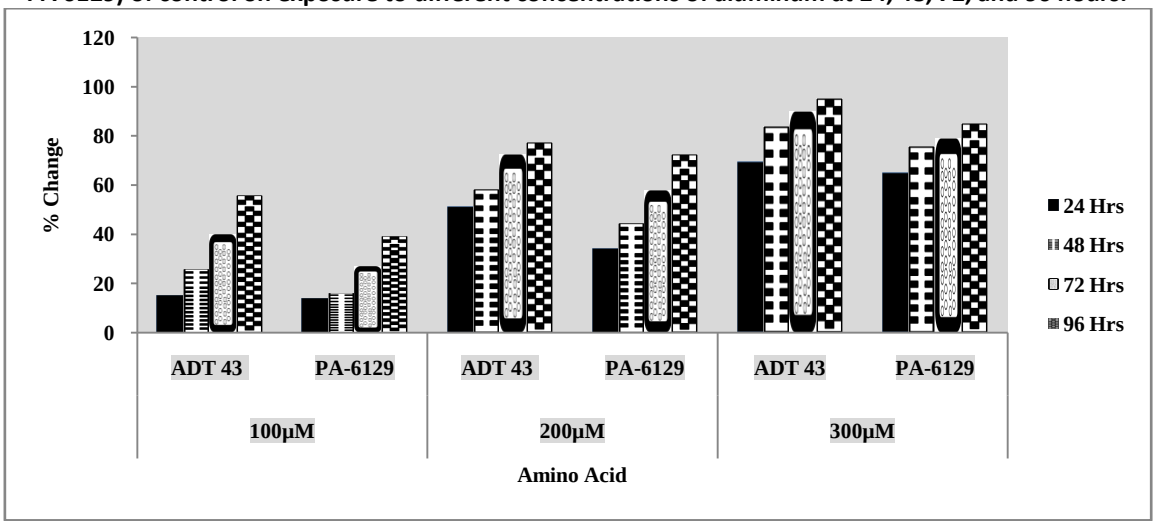

Figure 3 : Percent increase over the control in the protease (mg-1 protein h-1) activities in leaf of two rice varieties (ADT-43, PA 6129) of control on exposure to different concentrations of aluminum at 24, 48, 72, and 96 hours.

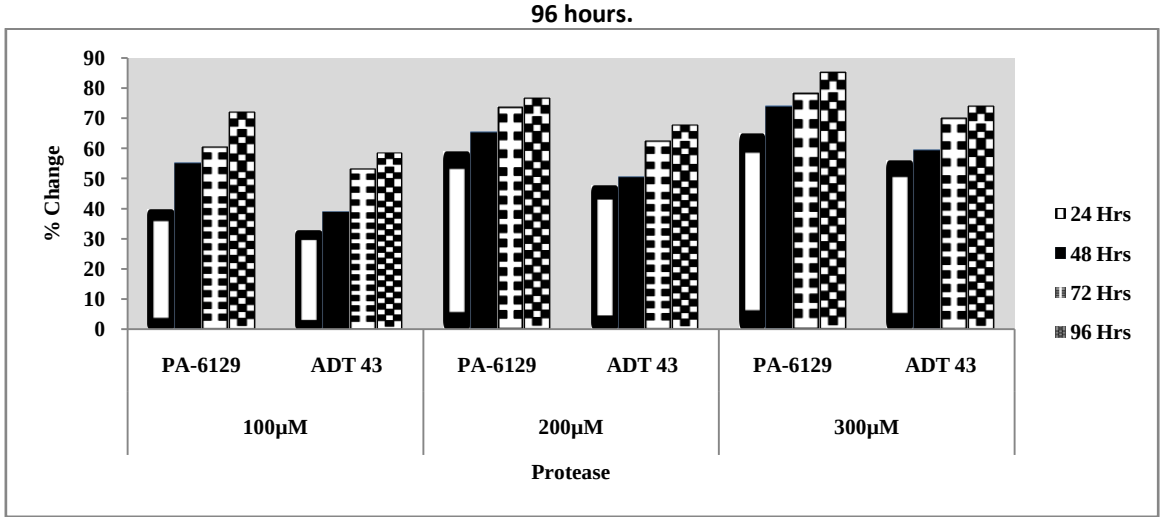

Figure 4 : Percent increase over the control in the Alanine amino transferase (AlAT - mg-1 protein h-1) activities in leaf of two rice varieties (ADT-43, PA 6129) of control on exposure to different concentrations of aluminum at 24, 48, 72, and 96 hours.

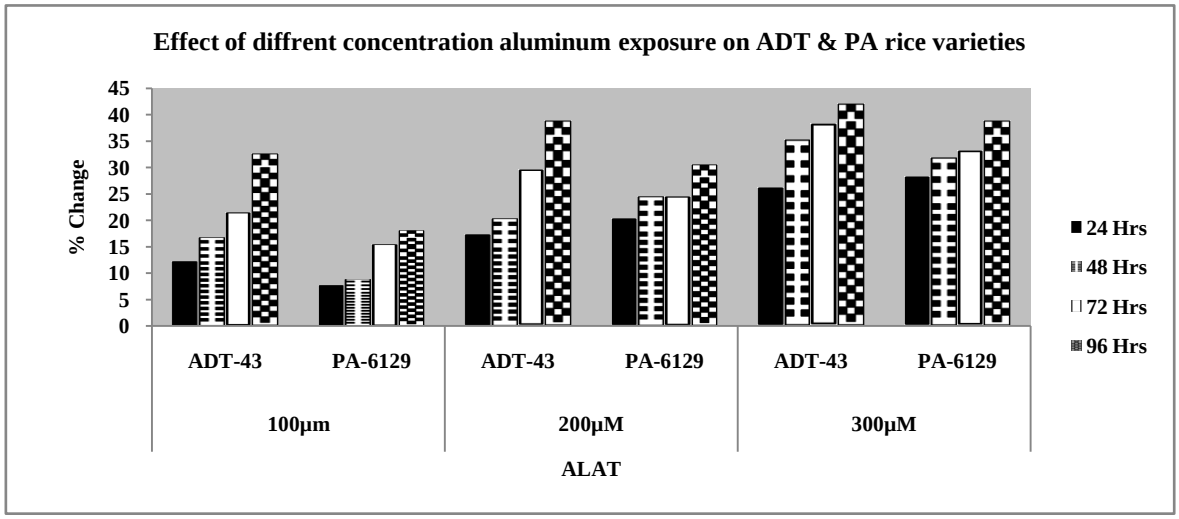

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Figure 5 : Percent increase over the control in the Aspertate amino transferase (AAT - mg-1 protein h-1) activities in leaf of two rice varieties (ADT-43, PA 6129) of control on exposure to different concentrations of aluminum at 24, 48, 72, and 96 hours.

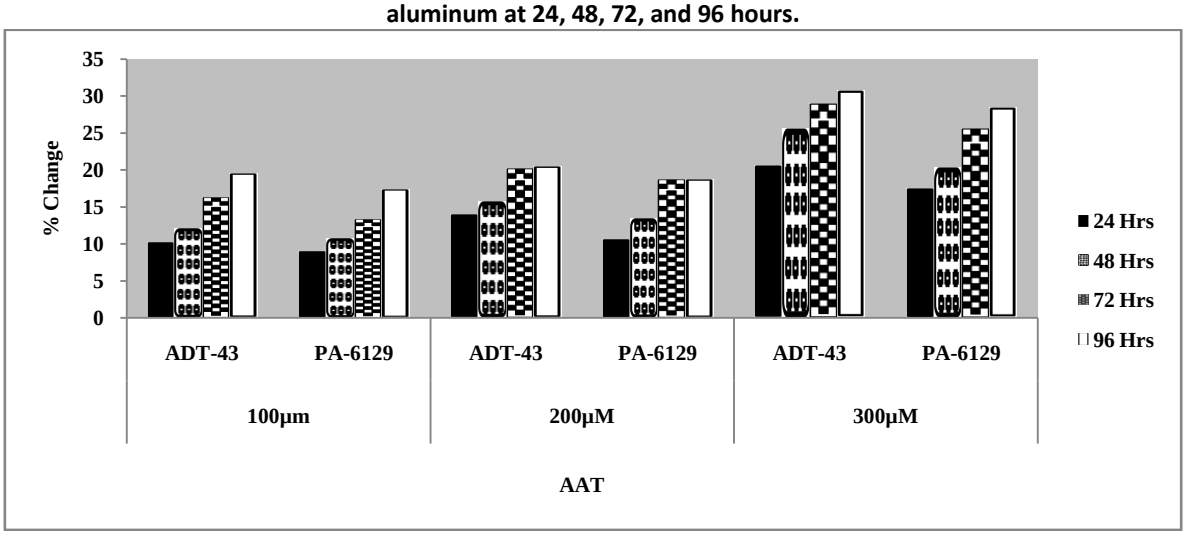


Figure 6 : Percent increase over the control in the Glutamate de hydrogenise (GDH - mg gm-1) activities in leaf of two rice varieties (ADT-43, PA 6129) of control on exposure to different concentrations of aluminum at 24, 48, 72, and 96 hours.

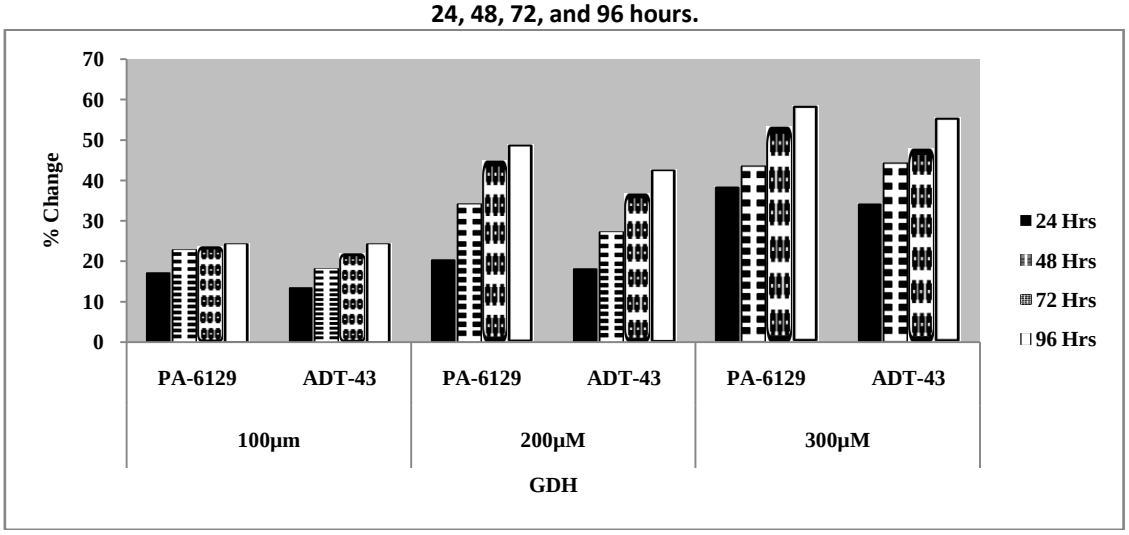
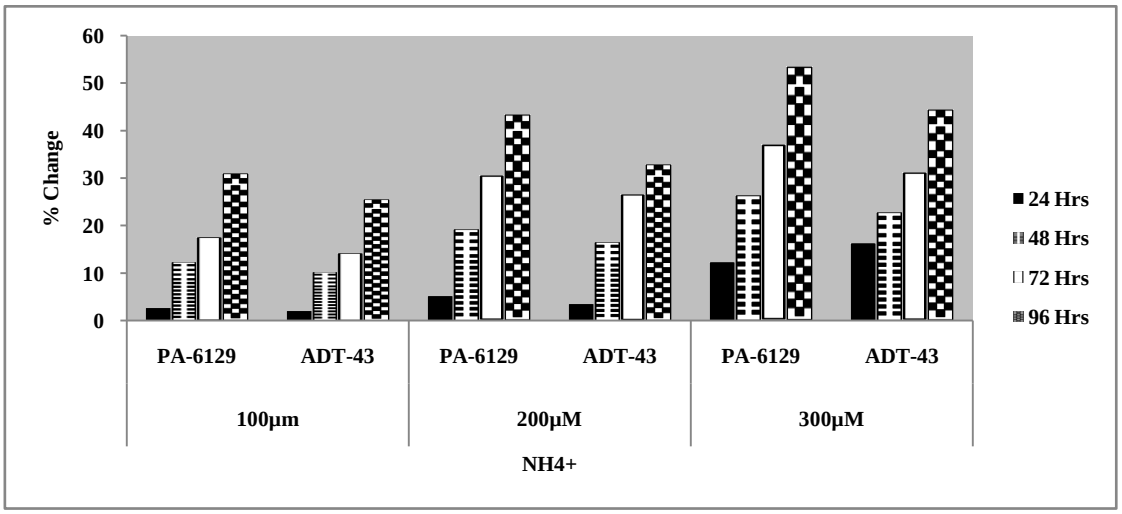


Figure 7 : Percent increase over the control in the NH4+ (µg gm-1)activities in leaf of two rice varieties (ADT-43, PA 6129) of control on exposure to different concentrations of aluminum at 24, 48, 72, and 96 hours.



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