THE INFLUENCE OF TREATMENT WITH SOME PURINIC DERIVATES ON ENZYME ACTIVITY AT *NICOTIANA TABACUM* L. PLANTLETS

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Abstract: This paper present the influence of length treatment with two purinic derivates, on the three antioxidant enzyme activity: catalase(EC 1.11.1.6), peroxidase(EC 1.11.1.7) and superoxide dismutase(EC 1.15.1.1) in *Nicotiana tabacum L* plantlets. The substances using for this treatment was 1,3,7 – trimetilxanthine (teine, caffeine) and 1,3 – dimetilxanthine (theophylline), both with purinic nucleus in their structure. The treatment was applied twenty one days. In this period the plantlets was treated with these substances in following concentrations: 0.025%, 0.05%, 0.1% and 0.25%. In the same time was constituted and a control probe with plantlets treated only with distilled water. The treatment have determined the intensification of peroxidase and catalase activity and decrease of superoxide dismutase activity.

INTRODUCTION

In specialty literature, caffeine and theophylline was described as substance who determine the cytokinesis inhibitions and form binucleated cells (Acatrinei, Acatrinei,1998).

About the effect of this two substances on plantlets know less data. More studies straight on polyploidisation effect who those substances have in certain concentration.

This study purpose the investigation of different concentrations of caffeine and theophylline on catalase, peroxidase and superoxide dismutase activity in *Nicotiana tabacum* L plantlets. The enzymes analyses in this paper are included in oxidoreductase class with strongly antioxidant character.

MATERIALS AND METHODS

The material was constituted by plantlest of *Nicotiana tabacum* L. cropping at 21 days, treated with four concentrations caffeine and theophylline (0,025%, 0,05%, 0,1% and 0,25%) and the control variant treated with distilled water.

The plantlets was treated in each day with the two substances, in Petri box, after this was cropping and usig for the superoxide dismutase, peroxydase and catalase determination.

For enzyme activity analysis at each concentration was accomplish three samples (repetitions). The material vegetal was homogenized in chilled 0,1 M phosphate disodic solution (0,5 : 5 w/v). The homogenate was centrifuged at 3000 rot/min for 15 min at 4° C. In supernanat obtained was determined those three enzymes.

The catalase activity was determined by titrimetric method with sodium thiosulfate (Artenie, Tanase, 1981). A catalase unit was considered this enzyme quantity wich decomposited un micromole of oxigenate water for minute.

The superoxide dismutase activity was determined spectrophotometrically by Winterbourn, Hawkins, Brian and Carell method (Artenie et all., 2008), measuring the percent of the superoxide dismutase-induced inhibition of Nitro Blue Tetrazolium(NBT) reduction by the superoxide radicals resulted in the medium of reaction by riboflavin photoreduction. The NBT reduction was followed at 560 nm using Metertek SP830 spectrophotometer. The rate of NTB reduction in the enzyme absence was taken as the reference value. One unit of superoxide dismutase represents the quantity of enzyme which produces 50% inhibition in the standard conditions.

The peroxydase activity was determined by spectrophotometrical method with o-dianisidine (Gudkova, Degteari, 1968). The peroxydase activity is expresed in enzymatic unities (micromoles of H_2O_2 decomposed in one minute).

Statistical analysis of the enzyme activities was performed using the Student test [Văleanu, Hâncu, 1990], at the 0.05 level of significance.

RESULTS AND DISCUSSIONS

The effect of theophylline on antioxidant enzymes

The effect on superoxide dismutase activity. After ours investigations have found that the treatment with theophylline was determined the decrease of relative enzyme activity in the same time whit the increase of theophylline concentration.. The mean activity values oscillate between 16.2 USOD/g/min at 0.25% concentration and 51.43 USOD/g/min at control(M) variant (Fig.1). The superoxide dismutase activity was strongly inhibited at 0.25% concentration.

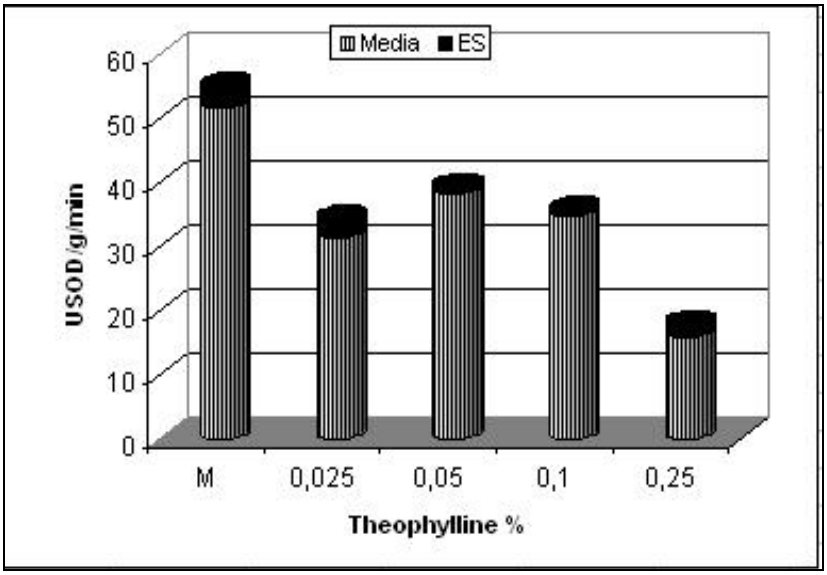

Fig.1. The superoxide dismutase activity after the treatment with theophylline in *Nicotiana tabacum* L. plantlets

The effect on peroxidase activity. Comparatively with superoxide dismutase activity, the peroxidase activity breed after treatment in proportion whit substance concentration increasing. The mean activity values oscillate between 5.14 UE/g/min at control variant(M) and 6,87 UE/g/min at 0.25%. The increases values was obtained and to 0.025% with 6.61 UE/g/min (Fig.2).

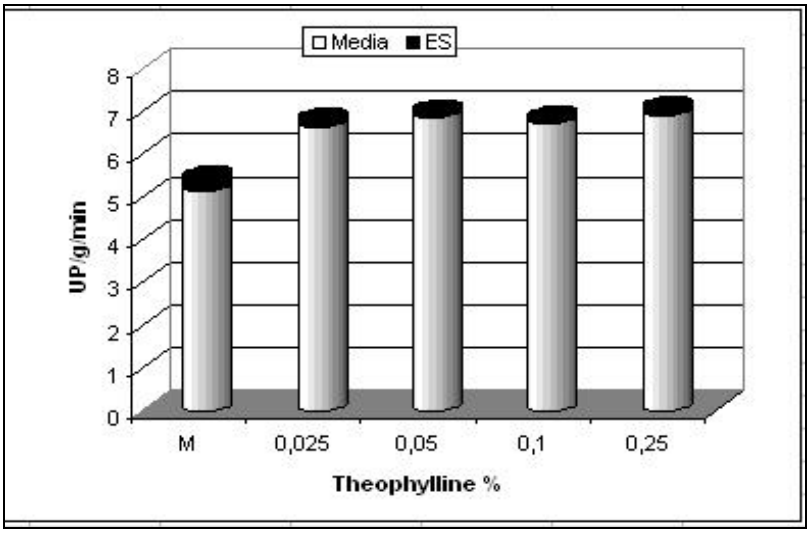

Fig.2. The peroxidase activity after the treatment with theophylline in *Nicotiana tabacum* L. plantlets

The effect on catalase activity. The theophylline have an increasing effect on catalase activity. The enzyme activity increase at minim value 25.5 UE/g/min obtained at control variant(M), until maxim value 156.96 UE/g/min at 0.25% concentration (Fig.3).

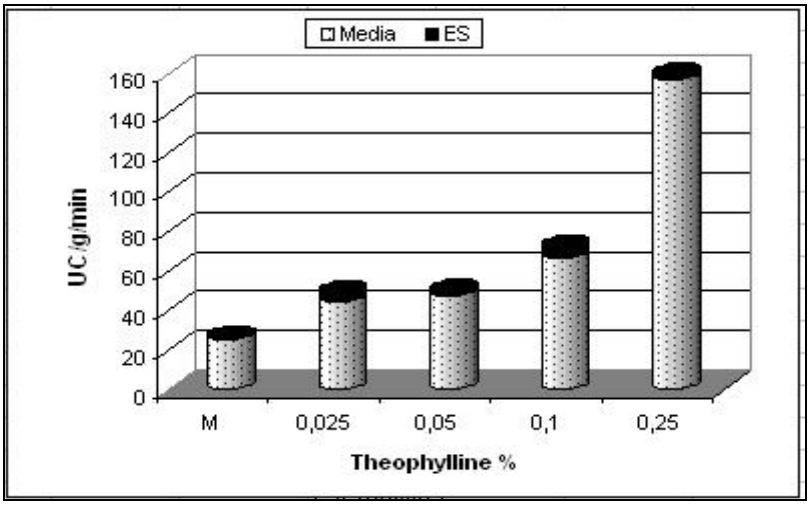

Fig.3. The catalase activity after the treatment with theophylline in *Nicotiana tabacum* L. plantlets

The effect of caffeine on antioxidant enzymes

The effect on superoxide dismutase activity. Like theophylline, the caffeine has un negative impact on superoxide dismutase activity, decreasing their activity with 25% at 0.25% concentration, comparative with control variant(Fig.7). The decreased value was obtained at control (51.43 UE/g/min), at 0.05% concentration (46.91 UE/g/min) and at 0.25% concentration (29.21 UE/g/min)(Fig.4). An easy increase was obtained at 0.025% and 0.1%, the values oscillated between 65.62 UE/g/min and respective 57.78 UE/g/min (Fig.4).

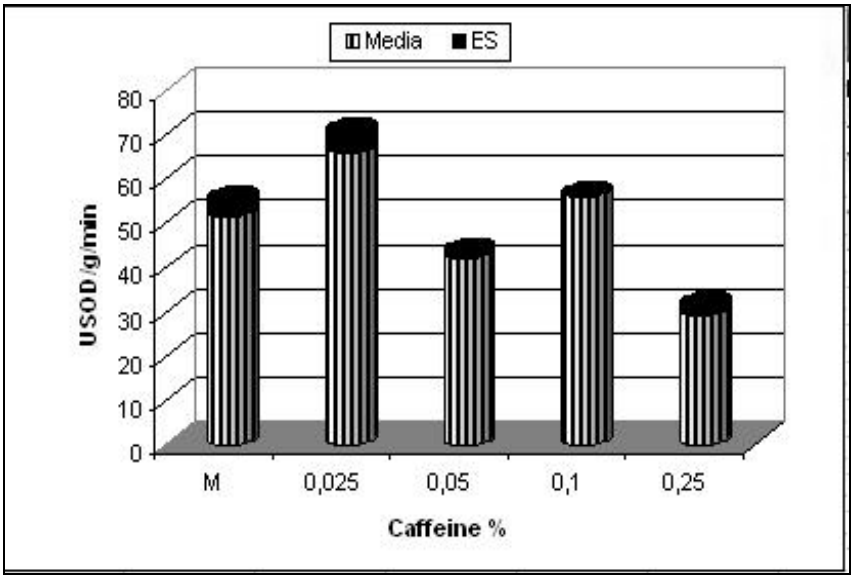

Fig.4. The superoxide dismutase activity after the treatment with caffeine in *Nicotiana tabacum* L. plantlets

The effect on peroxidase activity. The impact of caffeine was positive on peroxidase activity, causing her increase. The increase values was obtained at 0.05% (7.04 UE/g/min) and 0.1% caffeine (7.21 UE/g/min), were increasing was with 140% more strongly comparative with control variant (5.14 UE/g/min) (Fig. 5 and Fig. 8).

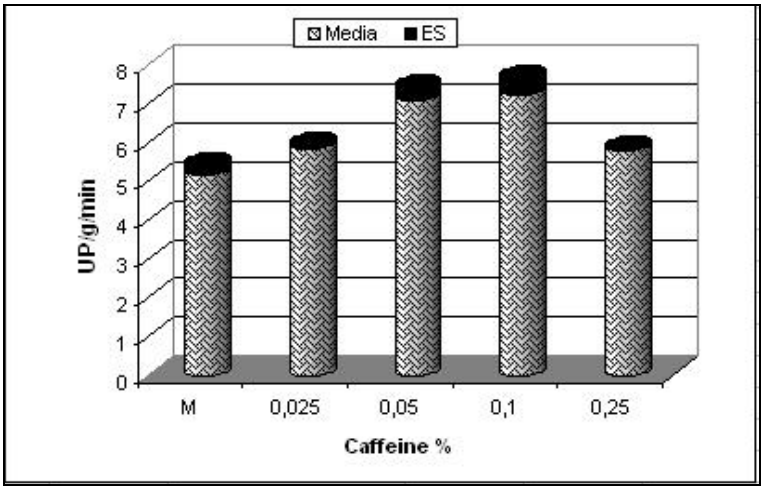

Fig. 5. The peroxidase activity after the treatment with caffeine in *Nicotiana tabacum* L. plantlets

The effect on catalase activity. The enzyme was strongly stimulated by caffeine treatment, theirs values increasing from minim value 25.5 UE/g/min obtained at control, until 161.32 UE/g/min at 0.25%. The increase of enzyme was in proportion with caffeine concentration increase (Fig 6 and Fig.9).

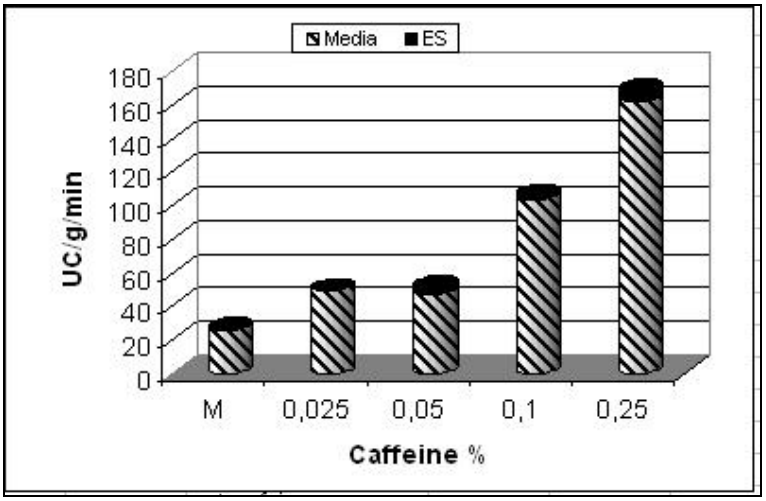

Fig. 6. The catalase activity after the treatment with caffeine in *Nicotiana tabacum* L. plantlets

The theophylline and caffeine stimulate the activity of peroxidase(fig.8) and catalase(fig.9). Comparative with peroxidase and catalase, superoxide dismutase activity was inhibited(fig.7).

The enzymes studied peroxidase and catalase, superoxide dismutase are antioxidant enzymes, involved in defence system of animal and plant organisms against the reactive oxygen species (Olinescu., 1982).

The level of reactive oxygen species in live cells can increase when antioxidant enzyme activity are decrease(Artenie et all, 2005).

With a strongly antioxidant activity, superoxide dismutase was recognized more significant enzyme to aerobe life and first enzyme from enzymatic system involved in protection by oxygen toxicity(Olinescu, 1982).

After the treatment, the superoxide dismutase was strongly inhibit by the caffeine and theophylline. The decrease of superoxide dismutase activity like in this treatment, may demonstrate an intensification of formation of reactive oxygen species mostly superoxide radical who is very toxic.

The decrease of superoxide dismutase activity suggest that theophylline and caffeine causing in *Nicotiana tabacum* plantlets an oxidative stress dependent by theirs substances concentration. This oxidative stress is probable induced by accumulation of superoxide radicals as result of enzyme inhibition.

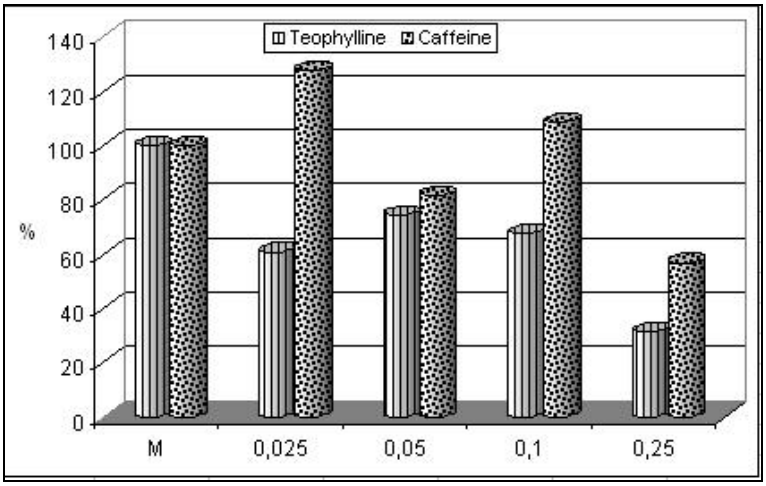

Fig.7. The relative activity of superoxide dismutase after the treatment with theophylline and caffeine in *Nicotiana tabacum* L. plantlets

Through strongly intensification of catalase and peroxidase activity after the treatment, probable the cell compensate the decrease of superoxide dismutase through increase catalase and peroxidase and removed the superoxide anions from plantlets cell.

This antioxidant enzymes are very sensitive at action of different factors. The hard metals in some concentration concern negative the enzyme activity. Cadmium, cooper and lead ions have a toxic effect on *Pistia stratiotes* plants and determined the decrease the catalase activity (Artenie et all, 2005).

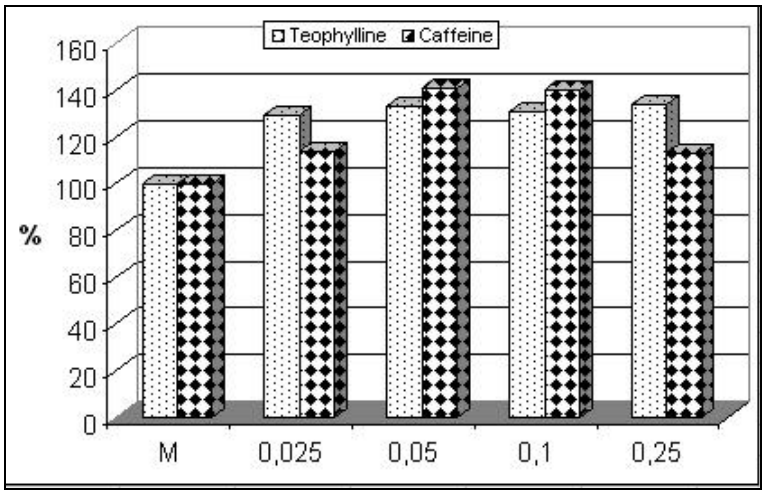

Fig.8. The relative activity of peroxidase after the treatment with theophylline and caffeine in *Nicotiana tabacum* L. plantlets

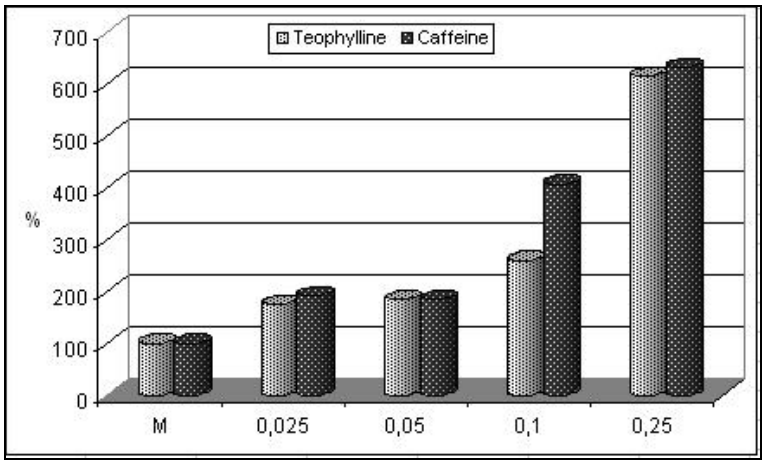

Fig.9. The relative activity of catalase after the treatment with theophylline and caffeine in *Nicotiana tabacum* L. plantlets

CONCLUSIONS

1. Theophylline and caffeine, specially at high concentrations, had an inhibitory effect on superoxide dismutase activity in *Nicotiana tabacum* plantlets. Comparative with caffeine, the negative influence of theophylline is more strongly.

The decrease of superoxide dismutase activity suggest that theophylline and caffeine determinate in *Nicotiana tabacum* plantlets an oxidative stress dependent by these substances concentration.

2. Peroxidase activity in *Nicotiana tabacum* plantlets is stimulated by all theophylline and caffeine concentrations used.

3. Both theophylline and caffeine had an activating effect on catalase activity from *Nicotiana tabacum* plantlets. This effect is proprtional with the concentration in wich the two substances are used.

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