

### SUMMARY

In the present investigation, separation and partial purification of the nucleolytic enzymes from Moth bean (*Phaseolus Aconitifolius*) is attempted. Few properties of these enzymes are also studied.

Purification is achieved with heat treatment, acetone treatment, fractional precipitation with ammonium sulfate and by chromatography on DEAE - Cellulose column.

DEAE Cellulose chromatography of the extract resulted into separation of RNase activity into three peaks, i.e. RNase I, RNase II, RNase III. DNase activity in two peaks, i.e. DNase I and DNase II and a single peak of nuclease activity was obtained. The purification achieved for RNase was 64 fold, for DNase 88 fold and for nuclease-RNase 69 fold and nuclease - DNase was 80 fold.

The enzyme exhibited optimal activity both at acidic and basic pH, the pH optimum being one between pH 4.5 to 4.87 and other between pH 8.0 to 8.2.

The enzymes are fairly heat stable, and even after heat treatment at 70°C for 15 minutes the RNase and DNase activities were retained to about 99% and 87% of the original.

The hydrolysis of both the substrates by nuclease at 24 hrs. is considered 100%. RNase causes only 31.6% hydrolysis of RNA in 24 hours while DNase hydrolyzes DNA to 37.6% during the same period.

Nuclease is strongly inhibited by the cations  $Mg^{2+}$ ,  $Ca^{2+}$  and also by citrate and phosphate.  $Zn^{2+}$ , Cysteine and  $Mn^{2+}$  activate both nuclease — RNase and nuclease-DNase activities. EDTA is inhibitory to nuclease. All cations inhibit the moth bean RNase activity. Phosphate and citrate are also inhibitory. RNase is strongly inhibited by cysteine, suggest that the enzyme is not a sulfhydryl enzyme. The activity is not affected by EDTA.

Moth bean DNase is inhibited by  $Mg^{2+}$ ,  $Mn^{2+}$  and  $Zn^{2+}$  but is not affected by  $Ca^{2+}$  and  $Hg^{2+}$ . Cysteine is also inhibitory, however EDTA causes three fold activation.

All these observations indicate the presence of a DNase in moth bean seedlings which is distinct from RNase and nuclease from the same source.