SUMMARY AND CONCLUSION

The plants growing out of place are generally termed as weeds. Weeds grow very fast and can thrive at any place under any climatic condition. Nitrophilous weeds are found in the soils containing higher levels of nitrogenous compounds and hence are present in the vicinity of human dwelling. High contents of nitrates and other nitrogen compounds are reported in these plants. Nitrate is absorbed by the roots of plants and reduced to ammonia which is further assimilated into organic compounds. Transaminases and nitrate and nitrite reductases are among the important enzymes of nitrogen metabolism.

In the present investigation a few weed species growing in the university campus have been analysed for the activity of NR and aspartate and alanine aminotransferases. These species were collected from roadsides, heaps of manures or garden soils etc. The analysis of nitrate and nitrite content, NO_3-N and NO_2-N from these species is also carried out. From these studies two species of weeds were selected for partial purification of aminotransferases. $(NH_4)_2SO_4$ fractionation and a stepwise purification of AspAT and AlaAT has been carried out. The kinetic behaviour of AspAT is further studied with respect to pH, temperature, protein and substrate concentration.

Results are summarized under different headings as follows :

1. Nitrate reductase and nitrogen fraction analysis :

The NR activity varies greatly in different weed species and in majority of them the activity is more than 1 μ mole NO_2 $g^{-1}h^{-1}$. The soluble nitrate and nitrogen fractions observed in the weeds are higher. A decrease in nitrate content is observed in species exhibiting higher NR activity. A higher amount of nitrite is found in these species. The level of nitrate in Vernonia cineria is found greater than in Amaranthus retroflexus which has been reported to contain a high percentage of nitrate. As the weed species are growing on the places such as piles of manures, garden soil and at roadsides, accumulation of nitrate is natural.

2. Aminotransferases :

A great variation is observed among the activities of aspartate and alanine aminotransferases studied from different weed species. The activity of AspAT is found higher than the activity of AlaAT in most of the species. In a few species both the aminotransferases are at more or less equal levels and in remaining species AspAT is slightly less than AlaAT. The total protein content also varies greatly with the species, the highest being recorded in Euphorbia thymifolia.

3. $(NH_4)_2SO_4$ fractionation :

After screening a number of plants, two species of weeds namely Alternanthera sessilis and Amaranthus viridis are selected for the purification of aminotransferases.

a. Aspartate aminotransferase :

The crude enzyme from the leaves of A. sessilis and A. viridis is treated with increasing concentrations of $(NH_4)_2SO_4$ in order to select the proper range of precipitation of AspAT. The protein content and the specific activity is measured after each and every step. In A. sessilis the specific activity increases 1.5-fold with 50% saturation of $(NH_4)_2SO_4$. It is further increased to 2.5-fold at 70% saturation, with no activity in the supernatant fraction. In A. viridis the 30% fraction enhances the specific activity 3-fold. During 40 and 60% saturation the activity is further increased about 5-fold. A negligible amount of enzyme protein is observed at 70% saturation.

Thus in both the species AspAT has been observed to precipitate between 30 to 70% $(NH_4)_2SO_4$ saturation.

b. Alanine Aminotransferase :

The $(NH_4)_2SO_4$ fractionation of AlaAT has been carried out with the same species of Alternanthera and Amaranthus. The fraction collected at 30% saturation possesses about

the same specific activities as that of the crude enzyme in both the plants. During 50 and 70% saturation the specific activity is slightly increased in A. sessilis. The 50% fraction from A. viridis retains the same specific activity as that of the crude enzyme and it is further reduced during 70% saturation.

The enzyme from both the species is found to precipitate stepwise at 30, 50 and 70% saturation of $(NH_4)_2SO_4$. The supernatant fraction from both the species also exhibits a fairly good amount of enzyme protein. However, most of the enzyme can be precipitated between 30 to 70% saturation of $(NH_4)_2SO_4$.

4. partial purification of aspartate aminotransferase :

The fraction precipitated between 30-70% saturation of $(NH_4)_2SO_4$ is selected for further purification of the enzyme from A. sessilis and A. viridis. The crude enzyme extracted in phosphate buffer from both the plants is passed through a column of Sephadex G-25 to discard the low molecular weight proteins.

In A. sessilis the fraction collected from Sephadex column possesses specific activity 2 times higher than that of the crude enzyme with a loss in the total protein content. During 30-70% saturation with $(NH_4)_2SO_4$ the specific activity becomes about 4-times higher, which is further increased in dialysed fraction. Thus a 5-fold purified enzyme is obtained.

The enzyme from A. viridis has a very negligible response with Sephadex G 25 and a slight reduction in the total protein content is observed at this stage. At 30-70% saturation the specific activity of the enzyme increases about 2-fold and in the final step of dialysis about 4-fold purified enzyme is obtained.

5. Kinetic properties of AspAT :

The partially purified fraction of AspAT from the leaves of A. sessilis and A. viridis is used to study the properties of the enzyme.

a. pH variation :

The highest enzyme activity is observed at an alkaline pH in both the plants. A sharp decline in the enzyme activity is recorded in the acidic range of pH. At pH 5 the enzyme activity is reduced to 25-60% of the maximum in the species studied. The pKa values obtained for A. sessilis and A. viridis are more or less equal.

b. Time variation :

A linear response to the AspAT activity has been observed upto first 20 minutes in both the species. After 30 minutes a gradual fall in the activity is recorded upto 60 minutes. About 70% of the maximum enzyme activity has been achieved during the first 20 minutes in both the plants.

c. Temperature variation :

The optimum temperatures recorded for both the plants are more or less equal. In A. sessilis a gradual decrease in the activity is experienced at higher temperatures with a 25% reduction in the enzyme activity at 60°C. The response of A. viridis to the higher temperature is different from that of Alternanthera. A slow increase in the activity is observed above the optimum temperature upto 60°C, however the rate of reaction is low.

The enzymes extracted from both the plants are thermostable and Q_{10} values obtained for both the plants indicate that the rate of reaction is not affected severely at higher temperatures. The Ea values indicate that the enzyme is more active in the temperature range of 30-40°C.

d. Enzyme variation :

A linear increase in the activity is observed as the protein concentration increases upto about 0.2 mg in A. sessilis and to about 0.6 mg in A. viridis. The rate of reaction decreases thereafter with a slow increase in the activity.

e. Substrate variation :

i) Aspartate variation :

The Michaelis-Menten plots of the enzyme from both the plants exhibit a typical rectangular hyperbola, with

varying concentrations of aspartate. The data obtained for aspartate variatioin is also analysed using Lineweaver-Burk and Eisenthal and Cornish-Bowden plots. The average values of Km and Vmax in A. sessilis are found less than those in A. viridis.

ii) Oxoglutarate variation :

A rapid increase in the enzyme activity has been observed in both the plants upto a concentration of 2 mM oxoglutarate in the assay medium. The data on oxoglutarate variation has been analysed using different plots as in case of aspartate. In general the Km values obtained for 2-oxoglutarate are very less than those recorded for aspartate.

All the findings mentioned above are discussed with the help of relevant literature referred from time to time.