CHAPTER V
DISCUSSION AND CONCLUSIONS

The experimental studies carried out to define cultural requisites for Aspergillus umbrosus, with the objective to understand its physiology of growth and secondary metabolism have provided sufficient information in this respect. The effects of the test parameters were quantitative as well as qualitative upon the growth behaviour of this organism.

Cultural conditions such as the different media, carbon and nitrogen sources, their varying concentrations, pH and temperature of incubation, state of culture, the period of incubation, and co-factors in growth and metabolism like vitamins, plant growth hormones, and metal salts have affected A. umbrosus variously. Some significant quantitative effects have been as under:

1. The culture media containing starch as one of the carbon source proved the best for growth and secondary metabolism of this fungus. In glucose containing media growth and sporulation were adversely affected. It may be a result of rapid utilization of glucose by the fungus which has rather a slow rate of growth. Preference of A. umbrosus for media containing carbohydrates of high energy content showed that such nutrients continue to support biomass production over
long periods of incubation than the glucose or maltose. In one of the most thorough studies of selective exhaustion of nutrients Borrow et al. (1961) have used this approach to analyse the gibberellic acid fermentation to obtain a desired culture biomass of *Gibberella fujikuroi*. Another explanation for the macronutrient affinity of *A. umbrosus* would be that under glucose-starved state metabolic flux operates through the pentose phosphate (PP) pathway as against the EMP under glucose-maintained cultures (Ng et al. 1972 and Carter and Bull, 1969). With our fungus this gets further corroborated from the fact that it grows better on D-mannitol at very high concentrations of even 12.5%. Varshney (1981) has also observed that *A. Clavatus* preferred D-mannitol for producing its maximum growth. Though several of the Aspergilli are known to accumulate mannitol from glucose (Smiley et al., 1967; Strandberg, 1969; and Lee, 1970), *A. umbrosus* utilizes it as the primary source of carbon. It suggests that operation of mannitol cycle seems to be an important pathway here. Hult et al. (1981) have stated that for some fungal species the mannitol cycle is an important pathway of metabolism.

(2) Growth of *A. umbrosus* on media containing peptone or casein was poor both in vegetative biomass development as well as sporulation. It seemed that it lacked capacity to utilize polypeptides as nutrient and might not be able to hydrolyse them extracellulary. The fungus also did not prefer amino acids as either C or N source. Though, the amino-acids L-arginine and L-aspartic acid are known to be good sole source of nitrogen (Pateman and Kinghorn, 1976). However, inclusion of L-arginine or L-aspartic acid in the medium along with potassium nitrate effected acceleration of biomass production as well as sporulation in *A. umbrosus*. This synergistic relationship between KNO₃ and arginine or aspartic acid might well be functioning as inducer of the involved
pathway(s) in its metabolism. Non-utilization of amino acids as sole nitrogen source here, may well suggest that the fungus *A. umbrosus* lacks the specific system for transport of the amino acids tested. It is quite plausible to accept in the case of *A. umbrosus*, a fungus characterized to possess highly stressed and mutant morphology (see plates 1-11) as has been reported for the mutants of *A. nidulans* (Sinha, 1969 and Kinghorn and Pateman, 1975), where mutation have resulted in the simultaneous loss of different amino acid transport activities. Occurrence of the loss of certain genes that determine and regulate synthesis of carrier proteins (permeases) has been envisaged on mutation.

(3) The optimum temperature for growth and sporulation of *A. umbrosus* was between 26-30°C. On temperatures beyond 35°C the fungus failed to grow, and showed that it was highly intolerant to such temperatures. Temperature is known to have a marked effect on all growth constants. At higher temperatures (> 35°C) a higher maintenance energy requirement gets manifested for higher turnover of proteins and nucleic acids (Trinci, 1969). In respect of the temperature range for physiological activities in fungi, Hagler and Lewis (1974) have stated that "The factor determining minimum growth temperatures appears to be inactivation of solute transport systems, while temperature sensitivity of the protoplasmic membrane in the presence of metabolizable carbon substrates may be an important determinant of the maximum growth temperature". Some growth factors that affect the metabolic efficiency of fungi are the trace elements, vitamins, and hormones. The vitamins and trace elements are prosthetic groups as coenzymes in several of the enzymes operating the pathways of metabolism. Their incorporation into the medium
affects the fungi variously. Such studies on *A. umbrosus* have shown that it was rather adversely affected by the metal salts, vitamins or hormones, though under the influence of ferrous sulphate sporulation was relatively more. The plant growth hormones IAA, IBA, and GA, and the vitamins folic acid, riboflavin, and ascorbic acid were inhibitory for growth. They also affected the fungus in morphological features of its colony and exudation.

(4) The fungus *Aspergillus umbrosus* has antagonistic activity against a number of fungi (Kulshrestha and Ali, 1986). This has been further ascertained towards a few test fungi and bacteria. The positive antifungal and antibacterial activity occurring in the growth medium of the fungus has suggested for the occurrence of an active principle in it. The Aspergilli are known to elaborate a good number of antibiotic substances during growth into the growth medium. Some useful antibiotics of *Aspergillus* species are: echinocandin, a novel polypeptide from *A. nidulans* var. *echinulatus* (Benz et al., 1975); flavipucine and isoflavipucine from *A. flavus* (Findlay et al., 1977); gliotoxin from *Aspergillus* sp. (Okutani, 1977); Versilin from *A. Versicolor* is a new antifungal antibiotic (Basu et al., 1987); and asperlicin from *A. alliaceus* (Monaghan et al., 1989). Quite a large number of secreted metabolites from the species of *Aspergillus* are also known to be toxic to higher organisms, they are commonly called as aflatoxins and mycotoxins depending on their effects and biosynthesis.

Production of dark brown exudate in the medium was found associated with the antagonistic activity of *Aspergillus umbrosus*. It was produced in several of the culture media tested in wholly or partially modified form in the course of
these studies. Fractionated samples were prepared in organic solvents and analysed spectrophotometrically to obtain their UV - scans. There was some similarity of the *A. umbrosus* compounds eluted differently in respect of their peak of maximum absorption and the number of total peaks. The active principle of *A. umbrosus* resembled more with cephalosporin than with the penicillin. Since the cephalosporins are a group of modified dipeptides, the elaborated antibiotic compound of *A. umbrosus* might also belong to the same chemical category.

The biochemistry of antibiotic production here was quite stable as its elaboration was not substantially affected by amendments in cultural condition, excepting that galactose completely inhibited its production when included as the sole carbon source in the growth medium. It was also recorded that galactose changed colony characteristics too as it became loose and floccose (Plate 5). This showed that production of antibiotic was related with the stressed felty growth condition of the fungus.

Kulshrestha and Ali (1986) considered *A. umbrosus* as a potential biofungicide, and have suggested for defining cultural conditions that would give desirable quantities of the viable spore and inoculum of the fungus to be utilized as an agent of biological control against some decomposer and pathogenic fungi. This study has achieved some success towards that objective, though it has to be evaluated for its toxicity, etc.