PART IV

CHAPTER I

GENERAL SUMMARY AND CONCLUSIONS
Oxidative metabolic investigation on pathogenic forms is obviously of great importance both from the viewpoint of fundamental and applied aspects. The determination of whether a substrate is oxidized or not, at a rate higher than of the endogenous rate, is very useful to identify possible metabolic pathways of respiration. This study not only provides a valuable information with regard to the pattern of oxidative metabolism of the test organisms but also the experimental results, especially of the effects of various substances on the endogenous respiration of pathogenic fungi, may be profitably exploited in the selection of more effective chemotherapeutic agents. Moreover, the respiratory responses induced by chemotherapeutic agents very often reveals the valuable clues with regard to the mode of action of some of the fungitoxic substances, particularly those which involve the oxidative metabolism of the sensitive organisms as the main target for their action.

In view of this, a plant pathogen, *Botryodiplodia theobromae* Pat. was selected for the present investigation. This organism may be regarded as one of the most destructive fungal pathogens, particularly in the tropical and subtropical regions.

A detailed study of respiratory characteristics of six
different isolates (i.e., A- Apple (*Pyrus malus* L.); C- Lemon (*Citrus medica* L.); G- Guava (*Psidium guajava* L.); M- Mango (*Mangifera indica* L.); O- Orange (*Citrus aurantium* L.); and Mu- Musambi (*Citrus sinensis* L.) of *Botryodiplodia theobromae* has been made. This study includes the effect of age of the culture, starvation period, pH, carbohydrates, inorganic and organic nitrogen sources, metabolic inhibitors, antibiotics and fungicides on the rate of oxygen consumption by the mycelial suspension of the test isolates. Manometric techniques as described in the manual of Umbreit *et al.* (1964) were adopted during the course of this investigation using Warburg's constant-volume manorespirometers and apparatus. Simultaneously the effects of above mentioned substances on fungal growth of all the test isolates were also observed.

**Effect of Age of the Culture:**

In all the isolates more than one peaks of higher respiratory activity each with an approximate interval of 72 hours were observed. The first peak in almost all the cases was developed in 72 to 96 hours old cultures.

In the same experiment, to determine the relation of respiration to O$_2$ tension, respiration was allowed to proceed in the Warburg flask, without opening the manometer for one hour as described by Allen and Price (1950). In case of A, G and upto some extent in Mu isolate the mycelium showed a poor resistance to a low oxygen concentration at the age with maximum
activity, while a good amount of resistance to low oxygen concentration was evident at the age with minimum respiratory rate. Resistance to survive in low oxygen concentration except at the age of maximum respiration in isolate A, G and Mu indicated their affinity towards anaerobic nature.

To avoid the variations in the rate of respiration due to cellular aging a period of maximum respiratory activity (1st peak of QO_2 values in 3, 3½2 and 4 days old cultures of Mu and A, G, M, O and C isolates, respectively) was chosen for further respirometric determinations.

**Effect of Starvation Period**

With regard to the different starvation periods, the addition of glucose at the time of low endogenous rate resulted in a immediate rise in the rate of oxygen consumption. Starvation was initiated after 1500, 1650, 1290, 1290, 1560 and 1470 minutes aeration of mycelium in case of A, G, M, O, C and Mu isolates, respectively. These isolates required a very long period to starve in comparison to the reports on other fungi (Darby and Goddard, 1950; Pandey, 1977; Kazmi et al., 1980). It was assumed that the biochemical nature of unknown endogenous substrates appears to be responsible for such an abnormally long period needed for starvation. It was also concluded that the capacity of this pathogen, to resist prolonged aeration without exogenous substrate might be a factor for its wide host range and aggressive pathogenic nature.
Effect of pH:

The magnitude of \( QO_2 \) values was very low in acidic pH. Neutral pH (7.0) showed a good amount of respiratory activity. A gradual increase in the rate of respiration was observed above 7.2. In general neutral or alkaline pH range favoured respiratory metabolism of all the isolates of *Botryodiplodia*.

Effect of Carbohydrates:

Majority of the carbohydrates were utilized as good respiratory substrates. The respiratory and growth responses were more or less correlative to each other with all except xylose in case of C; with galactose, glucose and raffinose in case of G; with raffinose in case of Mu; and with sucrose in case of all except Mu isolates. Similar stimulatory responses though comparatively of less magnitude have also been reported in other fungi by Al-Doory (1959), Bechtol and Thomsberry (1966) and by Vyas (1971).

Reduced growth in relation to control was also evident in a few cases. It was concluded that sucrose may well be utilized as respiratory as well as growth substrate by all the test isolates of *Botryodiplodia* except Mu isolate.

Effect of Inorganic and Organic Nitrogen Sources:

These substances produced both stimulatory or inhibitory effects. In case of apple, mango and orange isolates most of the test amino acids and inorganic nitrogen sources were found
to produce very poor stimulatory or inhibitory effects on both the processes.

For the respiration of C isolate methionine was most effective (82% stimulation) which was followed by sodium nitrate, ammonium sulphate, aspartic acid, glycine, tryptophan, cystine, alanine and phenylalanine in the order of decreasing activity. The growth of this isolate was also enhanced upto equal or less extent.

Valine, in C isolate produced considerable inhibition (68%) in respiratory rate while strong stimulation in mycelial growth. Similar responses were evident in case of G isolate with phenylalanine, leucine and cystine, and in case of Mu isolate with tryptophan.

Inorganic sources particularly nitrates of potassium, calcium and ammonium were better substrates for respiration as well as for growth in case of Guava isolate.

The results indicated that all the nitrogen sources were not equally utilized as respiratory and growth substrates by all the test isolates of *B. theobromae*. Some of these substances by producing strong stimulation on both the processes proved to be the best substrates for the isolates concerned. A few amino acids such as threonine in case of Mu isolate by producing strong suppression appeared to be fungicidal in nature for the isolate concerned. Similarly, a number of workers have also reported the fungicidal nature of certain amino acids (Van Andel, 1960).
Effect of Metabolic Inhibitors:

Both the processes, oxygen consumption and growth of all the six isolates were decreased with sodium arsenite and the severity of adverse effect was increased with the increase of concentration. The inhibitory effects showed the presence of pyruvic oxidase system in the respiratory metabolism of these pathogens.

The aside inhibition which was the most significant among test metabolic poisons, may probably be due to the fact that this inhibitor is known to form complexes with metals in cytochrome oxidase, due to which the transfer of electron to oxygen is prevented, consequently the rate of oxygen uptake may be decreased.

The inhibition caused by sodium fluoride indicated the presence of EM pathway in the respiratory metabolism of all except C isolates of B. theobromae. C isolate showed resistant respiratory metabolism to the action of fluoride because none of its concentrations could suppress mycelial respiration. The stimulatory effects caused by fluoride (rather than inhibitory) do not indicate conclusively the presence of EM pathway of prime importance in this isolate.

The rate of oxygen consumption in all the test isolates was strongly suppressed at one or the other concentration of sodium fluoroacetate. This substance is known to exert its
toxicity on aconitase enzyme activity (Lotspeich et al., 1952). Thus the inhibitory effects indicate the presence of functional TCA cycle. Sodium malonate was found to inhibit oxygen consumption by mycelial suspension of A and Mu isolates. The growth was not supported at various concentrations of this inhibitor. In C, M and O isolates, respiration was suppressed at one or the other concentration of substance. As this inhibitor is known to act as competitive inhibitor of succinic dehydrogenase (Quastel and Wooldridge, 1928; Quastel and Wheatley, 1931) the presence of TCA cycle in the oxidative metabolism of these pathogens was evident. However, the malonate stimulation as observed in G isolate at all the used concentrations and in other isolates at a few concentrations may probably be due to the fact that sodium malonate is also known to act as carbon source in certain cases (Clark and Wallace, 1958; Novak, 1959 a,b).

**Effect of Antibiotics**

Aureofungin, nystatin and griseofulvin were most effective than other antibiotics against both the processes, respiration and growth. Among different test isolates A and M were highly susceptible to all the antibiotic substances. Chloramphenicol, erythromycin, gramicidin and penicillin acted more severely against respiration than growth. Some of the test antibiotics showed stimulatory action on mycelial growth while strong inhibitory action on respiration in C, Mu and O isolates.
Uncoupling action of gramicidin, and streptomycin on fungal oxidative metabolism as reported by others, in case of bacteria, could not be proved. Most of the antibiotics showed selectivity in their effects. Griseofulvin (10^0 to 10^2 μg/ml) was strong antirespiratory and growth inhibitory agent for C, A, Mu and M isolates and it appeared that besides the reported alteration in cell wall synthesis and in nucleic acid metabolism, the antifungal action of griseofulvin may also involve the interference in fungal respiratory metabolism.

**Effect of Fungicides**:

Endogenous mycelial respiration and growth were suppressed in all the six isolates. Thiram, blimix and cosan were specifically more active against respiration of G, A and Mu and M respectively than growth. While brassicol, cuman and blitane, captan and thiram were more toxic to growth at and above 10^2, 5x10^2 and 5x10^3 ug/ml concentrations respectively. In general, concentrations which were toxic to growth often affected respiration but the extent of the effect and the relationship between suppression of respiration and that of growth varied with a particular fungicide and the isolate.