Effect of Culture Filtrates of Various Fungi on Endogenous Mycelial Respiration and Growth of Some Plant Pathogenic Fungi

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However, there are sufficient examples to suppose that biological control of both soil as well as air borne plant diseases can be made possible with the use of antagonistic microorganisms (Weindling and Fawcett, 1936; Anwar, 1949; Leven, 1965). While on the other hand there are even more instances (Kerr, 1961; Garrett, 1965) to prove that positive results shown by antagonists in the laboratory conditions do not appear to be of much practical values in the field. Recently various aspects of biological control of plant diseases have been critically emphasized by Saksena (1972). The chief principle of microbial control of a plant disease is the phenomenon of antagonism. It would be desirable to give more attention towards the selection of antagonistic organisms before consideration of their applicability in disease control. Majority of the earlier approaches applied to elucidate the antagonistic effect of the organisms were concerned with their adverse effect on mycelial growth of the test organisms and with the studies on cross antagonism (antagonism amongst antagonists). However, a specific understanding of the particular metabolic process of the test organism being affected by the interacting microflora deserves serious consideration. Therefore, the present investigation was made to observe the effect of culture filtrates of various fungi on endogenous mycelial respiration and growth of three plant pathogenic fungi i.e., R. solani, G. papaya and C. capsici.

MATERIALS AND METHODS

 Cultures of various fungi used here were obtained from mycological research labora-

tory. University of Saugar, Sagar, and purified by single spore isolation and or hyphal tip methods. The culture filtrates of these fungal species were obtained by growing each organism in 100 ml flask containing 20 ml of the Czepak’s broth for 10 days at 28 C. The mycelial mats were removed and the culture filtrates were centrifuged at 10,000 rpm in refrigerated centrifuge for 15 minutes. The clear supernatant was taken out and stored in refrigerator until used. The mycelial suspensions of the test organisms for respirometric studies were prepared as previously described (Soni et al., 1972). Determinations of oxygen uptake were made manometrically following the techniques described (by Umbreit et al., 1964). The results obtained have been presented as QO2 (µl) of oxygen consumed per hour per gram dry weight of the mycelium and per cent change over control. The antagonistic activities of various fungi against three test pathogens were determined by noting the zones of inhibition (CIZ & TIZ) in mm following the routine ‘streak methods’ at 28 C.

RESULTS

In case of R. solani the respiration was increased with the addition of culture filtrates of nine fungal species. Among these A. candidus was most effective causing 60%, increase over control. P. nigriceps, A. fumigatus and A. terreus strain III also stimulated the rate of oxygen uptake to a considerable extent. The stimulatory effects shown by others were, however, less significant. The remaining 10 fungi caused very poor inhibition. With regard to their
antagonistic action only T. viride showed good activity. While H. compactum and A. terreus strain III showed moderate action. The poor activity was noticed with rest of the fungal species.

The mycelial respiration of G. papayac was substantially increased by culture filtrates of P. nigricans and A. candidus while A. terreus strain II, P. divericatum, A. terreus strain I and A. nidulans respectively produced considerable inhibitory effects. The stimulatory or inhibitory responses induced by the culture filtrates of other fungi were, however, less significant. With regard to the antagonistic activity, P. nigricans and A. candidus were most effective. T. viride and rest of the fungal species were found to show moderate and poor antagonistic action, respectively.

The respiration of C. capsici was inhibited by the addition of culture filtrates of most of the fungi tested. A. terreus strain II was the most effective with A. terreus strain I, A. niger strain II, A. sydowi, P. divericatum, T. viride, H. rostratum and A. flavus following in the order named. Only P. nigricans was found to cause a substantial increase in fungal respiration while rest of the species were less effective. Out of 19 fungal species six of them showed good antagonistic activity against C. capsici among these A. candidus was most effective which was followed by P. nigricans. A sydowi, H. compactum, H. rostratum and P. divericatum respectively. (Table 1)

DISCUSSIONS

The foregoing results indicate that 3 different strains of A. terreus, strain I and II produced inhibitory action on respiration of G. papayac. C. capsici and negligible activity on R. solani while strain III produced stimulatory action on R. solani and no activity on other two fungi (Table 1). Similar differences among the antagonistic responses of strains I, II and III have also been observed. It appears to be similar to F. oxysporum in which the oxygen uptake was inhibited by strain I and II while stimulated by Strain III (Soni et al., 1972). On the basis of these results it appears that the three strains are biochemically quite different from each other. Which may probably be due to the secretion of different antimetabolites by the different strains of A. terreus. The fungal respiratory stimulation shown by culture filtrates of certain fungi especially those of A. candidus and P. nigricans in the present as well as in previous studies (Soni et al., 1972) seems to be somewhat interesting because these organisms were found to be good antagonists against all the test pathogens. Thus the respiratory stimulation may probably be due to uncoupling action of some chemical substance being present in the culture filtrates of A. candidus and P. nigricans. However, this may not be true for those species which induced high stimulation in oxygen uptake but on the other hand produced poor antagonistic effects. Some fungal species were found to show more or less moderate or good antagonistic effects along with similar inhibitory effects on oxygen uptake of the test pathogens. This may probably be due to the presence of some metabolic inhibitor in the culture filtrates of the fungi concerned. T. viride which was able to show antagonistic action against all the test pathogens with more significantly against R. solani, did not cause similar appreciable inhibition of mycelial respiration, appears due to the fact that either the antagonism of T. viride against the fungi does not involve the antibiotic or if at all antibiotics are involved then their mode of action may be on the site other than that of oxidative metabolism of the test organisms.

ACKNOWLEDGEMENTS

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REFERENCES


Effect of Antifungal Antibiotics on Endogenous Mycelial Respiration of
*Rhizoctonia solani* Kuhn

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Aureofungin and hamycin, two new heptane antibiotic formulations produced by Hindustan Antibiotics Ltd., Pimpri, from *Streptomyces cubosporus* Benedict var. terricola* Thirunalgur et al., 1964* and *Streptomyces pinipoda* Thirunalgur (Vratin et al., 1964) respectively are being widely used for various plant and animal diseases caused by fungal pathogens (Desai et al., 1966; Guinapal and Prasad, 1969; Mathur et al., 1971; Dave and Kaul, 1964; Rana et al., 1968; Dharan Vir and Raychandhuri, 1969; Prati and Mandalakhet, 1969). The survey of available literature reveals that though much work has been done with these antibiotics (Ramechandran, 1961; Sharma and Agarwala, 1966; Rahalkar and Neergard, 1969; Thirunalgur et al., 1969), their effects on fungal respiration are still unknown. Since number of antifungal antibiotics are known to act by affecting the oxidative metabolism of the sensitive organism (Newton, 1965; Gottlieb and Shaw, 1970), the effects of these two antibiotics on endogenous respiration and growth of *Rhizoctonia solani* were investigated in the present study.

**MATERIALS AND METHODS**

Aureofungin and hamycin were obtained through the courtesy of Hindustan Antibiotics Ltd., Pimpri, and 2, 4-Dinitrophenol from Fluka AG, Chemische Fabrik, Buchs S. G. Switzerland. The basal medium was used which contained dextrose 50g; malt extract (Difco) 50g; K$_2$HPO$_4$ 0.012M; KH$_2$PO$_4$ 0.02M; NH$_4$NO$_3$ 0.0375M; MgSO$_4$ 0.009M and 1000 ml distilled water (Durby and Goddard, 1950). *Rhizoctonia Solani* was grown in 100ml Erlenmeyer flasks containing 25ml of the basal medium at 28°C. After 5 days of incubation, the thick mycelial mat was removed, washed in 0.02M phosphate buffer pH 7.0 and then starved in the same buffer for 24 h. About 2 g of starved mycelial mat was fragmented in 50ml of buffer in Waring blender for 30 sec at full speed. The fragments were then washed several times on the refrigerated centrifuge. The supernatant was rejected and the suspension of required dilution was made from the residue for respirometric studies. Determination of oxygen consumption were made following the techniques of Umbreit et al., 1964. Each Warburg flask contained 2.7 ml of mycelial suspension in the main compartment, 0.3 ml of the treatment solution in the side-arm and 0.2 ml of 20% KOH solution in central well. The rate of oxygen uptake was measured as O$_2$ mm (100 ml mg dry wt. at 28°C). A second set of experiment the effects of antibiotics on fungal growth were determined by incubating the mycelium in Petriplates containing 15 ml of the Potato- dextrose-agar medium with a required amount of antibiotic substance for the period of 5 days at 28°C.

**RESULTS AND DISCUSSION**

As evident from the figure both the antibiotics were found to stimulate the oxygen consumption of *R. Solani*. Hamycin in all the concentrations used was very effective with greatest stimulation (195.2%) at 100 PPM, while in case of aureofungin the rate was stimulated between 0.1 and 100 PPM with the maximum stimulation (57.5%) at 10 PPM. The addition of
DNP (0.001 M) with the antibiotic in the system was found to stimulate the aurofungin induced respiration only, whereas no appreciable increase was observed in the hamycin induced fungal respiration. The fungal growth was, however, found to be considerably inhibited by both the antibiotics.

The inhibition of fungal growth and a very poor respiratory response to DNP with an antibiotic in the system indicated that hamycin probably acts as an uncoupler of fungal respiration. Ramachandran (1961), however, reported that antifungal activity of hamycin could be due to leakage of cell contents caused by changes in membrane permeability. It appears, therefore, that besides the alteration in the membrane permeability, the uncoupling of fungal respiration may also be involved in the mechanism of antifungal action of hamycin. A number of other antibiotics have also been reported to act as uncouplers of fungal respiration (Mandels, 1963; Gottlieb and Shaw, 1970).

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REFERENCES


