

STUDIES ON INTERACTIONS BETWEEN *TRICHODERMA VIRIDE* AND *FUSARIUM MONILIFORME*

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ABSTRACT

Interaction between two coinhabiting soil fungi has been studied under various nutritional and environmental manipulations in liquid culture. Significant variation in sporulation and biomass was noted under varying cultural conditions. The sporulation of both the fungi was highly inhibited in mixed cultures. Reasons for their coinhabitation have also been discussed.

INTRODUCTION

Trichoderma viride Pers. ex. Fries, a well known antagonist and mycoparasite (Weindling, 1932 ; Dennis and Webster, 1971 a,b) and *Fusarium moniliforme* Sheldon a root pathogen (Sadasivan and Subramanian, 1954 ; Sharma and Singh 1978 ; Kuhlman *et al*, 1978) are the coinhabitants of agricultural soils of Meghalaya, India. In order to understand their behaviour and ecology it was thought necessary to study their growth and sporulation in pure and mixed cultures under different manipulations of temperature, pH carbon and nitrogen sources.

MATERIALS AND METHODS

The organisms were isolated from soil. Liquid Czapek's sucrose nitrate (NaNO_3 -2g, K_2HPO_4 - 1g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ - 0.5g, KCl. - 0.5g, $\text{Fe SO}_4 \cdot 7\text{H}_2\text{O}$ - 10mg and sucrose 30g in 1000 ml. distilled water, pH 5.4) was used for all the experiments except in the case of carbon and nitrogen source experiments.

Three temperature, 10, 20 and 30°C, were used for the study of the effect of temperature. The pH range (pH 4, 6 and 7) was adjusted by 1N NaOH or HCl. 30.0 g each of sucrose, maltose and glucose separately was used for carbon source experiment. In case of nitrogen source experiment, NaNO_3 of the general Czapek's sucrose nitrate medium was replaced by 3.0g of any of three nitrogen sources viz. NH_4Cl , KNO_3 and NH_4NO_3 .

The inoculum of spore suspension was prepared by transferring the spore mass from a 10 days old culture to sterile distilled water. The spore density of the inoculum was adjusted in between $50 - 100 \times 10^4$ spores per ml. for both the organisms.

50 ml. of the liquid media in 250 ml. conical flask was autoclaved at 15 lb/cm^2 for 20 minutes. To this was added 1 ml. of the aqueous spore suspension of the test fungi for pure culture and 0.5 ml of each fungus in mixed cultures. To prevent bacterial contamination 1 ml.

each of 1% streptomycin and penicillin was added to the media after sterilization. All flasks were incubated at 30°C in diffuse light whereas in the case of temperature experiment, the incubation was done at the three temperatures indicated above. Three replicates were maintained for all the sets. Flasks were handshaken every morning and evening to avoid the adhesion of mycelia to the flask walls or formation of surface mats.

The growth was measured in terms of number of spores and fungal biomass in liquid culture. Flasks were handshaken for ten minutes before the spore count was done by haemocytometer in an aliquot of the suspension under the high power of microscope. Counting was done under ten microscopic fields and a minimum of three haemocytometer preparations were considered for each replicate. After counting the spores, the cultures were filtered through a weighed Whatman No. 1 filter paper and the fungal biomass was estimated by drying the material to constant weight in hot air oven at 60°C. The dried fungal mass was allowed to cool down in a desiccator before weighing on an electric balance. The data were recorded after twenty one days of incubation. The results quoted as significant are statistically significant at 5% level.

RESULTS

Effect of temperature :

The growth of both the fungi increased with increasing temperature. *F. moniliforme* was found to be sporulating much faster as compared to *T. viride* (Table 1). In mixed culture the sporulation was drastically reduced in both the fungi. Maximum sporulation was at 30°C biomass was found to be higher at 20°C and 30°C as compared to value at 10°C. There was not much difference in the values at 20°C and 30°C.

Table 1. Effect of temperature on growth of the fungi expressed in terms of mean \pm SD number of spores ($\times 10^4$ per ml) and mean fungal biomass (mg., in parentheses) after 21 days.

Fungi		10°C	20°C	30°C
<i>F. moniliforme</i>	..	157.8 \pm 21.0 (494.00)	1958 \pm 3.9 (567.50)	2818 \pm 32.8 (569.50)
<i>T. viride</i>	..	21.4 \pm 4.5 (508.80)	110 \pm 7.9 (564.20)	317.8 \pm 11.4 (552.80)
<i>F. moniliforme</i> +	..	35 \pm 3.5	85.6 \pm 8.9	99 \pm 33.9
<i>T. viride</i>	..	+ 10 \pm 0.6 (456.20)	+ 10 \pm 2.9 (567.50)	+ 37.8 \pm 13.1 (564.20)

Effect of pH :

pH 4 was most favourable for the growth of both the fungi in pure as well as mixed cultures. The sporulation at pH 6 and 7 did not differ significantly. Sporulation in both the fungi was reduced in mixed cultures. Biomass decreased significantly with increase in pH (Table 2).

Table 2. Effect of pH on growth of the fungi expressed in terms of mean \pm SD number of spores ($\times 10^4$ per ml) and mean fungal biomass (mg, in parentheses) after 21 days.

Fungi	pH-4	pH-6	pH-7
<i>F. moniliforme</i>	1839.2 \pm 56.9 (505.60)	92.4 \pm 32.7 (381.51)	122.6 \pm 14.2 (300.50)
<i>T. viride</i>	145 \pm 31.5 (355.50)	29.2 \pm 9.1 (321.00)	34.2 \pm 10.5 (295.00)
<i>F. moniliforme</i> +	49 \pm 3.3 +	14.4 \pm 6.2 +	252 \pm 11.1 +
<i>T. viride</i>	16.00 \pm 1.8 (399.10)	7.4 \pm 1.1 (350.10)	21.6 \pm 8.0 (350.20)

Effect of carbon source :

T. viride sporulated best in glucose and least in sucrose, while maltose occupied an intermediate position. *F. moniliforme* showed a preference to sucrose as compared to and glucose maltose. In mixed cultures, glucose and maltose stimulated the sporulation of *F. moniliforme*, while sucrose proved less effective. The sporulation of *T. viride* in maltose was significantly greater in mixed culture than in pure cultures. Mixed cultures in glucose produced maximum biomass throughout the study (Table 3).

Table 3. Effect of carbon source on growth of the fungi expressed in terms of mean \pm SD of spores ($\times 10^4$ per ml) and mean fungal biomass (mg., in parentheses) after 21 days.

Fungi	Sucrose	Glucose	Maltose
<i>F. moniliforme</i>	865.8 \pm 95.0 (555.20)	489 \pm 28.5 (512.00)	329 \pm 77.2 (638.70)
<i>T. viride</i>	23.0 \pm 5.7 (484.20)	302 \pm 48.5 (759.30)	35 \pm 7.9 (535.20)
<i>F. moniliforme</i> +	242 \pm 34.5 +	538 \pm 113 +	389 \pm 67.4 +
<i>T. viride</i>	13 \pm 5.7 (530.20)	289 \pm 22.7 (900.10)	84 \pm 6.5 (518.90)

Effect of nitrogen source :

Ammonium nitrogen was found to be most favourable for the sporulation of *T. viride* while nitrate proved better for *F. moniliforme*. Nitrogen in the form of ammonium nitrate showed intermediate results. Sporulation of both the fungi was drastically reduced in mixed cultures. The reduction, however, was more severe in *T. viride* than *F. moniliforme*. The biomass depicted almost similar trend (Table 4).

Table 4. Effect of nitrogen source on growth of the fungi expressed in terms of mean \pm SD number of spores ($\times 10^4$ per ml) and mean fungal biomass (mg, in parentheses) after 21 days.

Fungi	NH ₄ Cl	KNO ₃	NH ₄ NO ₃
<i>F. moniliforme</i>	195.8 \pm 6.44 (224.70)	728 \pm 43.5 (358.70)	117.8 \pm 496.1 (384.80)
<i>T. viride</i>	9416 \pm 393 (224.70)	65.4 \pm 24.7 (172.20)	3534 \pm 102.3 (226.30)
<i>F. moniliforme</i> +	81.6 \pm 21.2 +	568 \pm 95.2 +	608 \pm 116.8 +
<i>T. viride</i>	21.6 \pm 21.2 (215.60)	25.2 \pm 8.9 (339.00)	22.2 \pm 8.5 (416.70)

In general, *F. moniliforme* produced more spores than *T. viride*.

DISCUSSION

Nutritional and physiological studies on fungi have proved that many fungi are often specific or selective for their growth requirements (Lilly and Barnett, 1951 ; Cochrane, 1958 ; Bilgrami and Dube, 1976). Many plant pathogenic fungi are known to utilize nitrate (Misra and Mahmood, 1960 ; Thind and Randhwa, 1957) which was also found true with *F. moniliforme* in the present study. Better utilization of nitrate by *F. moniliforme* as compared to *T. viride* may probably be due to a better nitrate reductase system in the former. Glucose is a simple and often, a superior carbon source for fungi (Agrawal and Agnihotri, 1970 ; Hasija, 1970). The best growth of *T. viride* in glucose may presumably be due to its better capacity to utilize simple carbon sources or its adaptation to undo the 'glucose effect'. The better growth of *F. moniliforme* in sucrose could be ascribed to its better enzymatic system for sugar metabolism. It appears, therefore, that probably *F. moniliforme* has a wider amplitude and better adaptation for nutrients than *T. viride* (Tables 3, 4).

The manipulations in temperature and pH of the medium affected both fungi in somewhat similar way. Under similar conditions of growth, *F. moniliforme*, generally showed greater sporulation and biomass production both in pure and mixed cultures. This again shows that *F. moniliforme* probably has wider ecological amplitude and is better adapted to the conditions under which the experiment was conducted (Tables 1 & 2).

It may be inferred from the present study that the requirement for similar environmental factors (pH and temperature) and ability to use different nutrients (carbon and nitrogen sources) helps the co-existence of the two fungi in the same habitat. While the former brings them to live together the latter reduces the possible competition. The reduced competition is again evident from the growth of the two fungi in mixed cultures without eliminating or restricting the growth of each other.

As the conclusions are drawn purely from laboratory experiments, their validity as to what actually happens in nature where a complex network of interactions among various physical, chemical and biological factors operate simultaneously, would be tenable only to a limited extent.

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