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Studies on saprophytic survival of *Fusarium oxysporum* using precolonized paddy straw bits

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ABSTRACT

The saprophytic colonization and saprophytic suppression of the test organism of Fusarium oxysporum was studied in relation to soil moisture, pH and temperature. Survival of the pathogen increased when temperature was increased. The percentage survival of F. oxysporum in the pre-colonized paddy straw bits buried in soil was recorded. The fungal population was increased upto 75 per cent MHC. The competitive saprophytic colonization of the test organisms over paddy straw bits was favoured by 30% moisture. The acidic range of pH was favourable for the colonization of the substrate for the test organism.

Key words : *Fusarium oxysporum,* Saprophytic survival, Paddy straw bits.

INTRODUCTION

Garrett's (1956, 1970) concept of saprophytic behaviour include (a) saprophytic survival in the dead host tissues and (b) competitive saprophytic colonization of the substrate. The saprophytic activity and duration of survival of the pathogens in the soil are determined by the nature of the soil, available substrate, environmental factors, substrate utilizing ability of the pathogens and the background antagonism. The fluctuations in environmental parameters like soil moisture, soil reaction temperature and soil aeration influence the saprophytic behaviour either directly acting upon the survival structures of pathogens or indirectly through antagonistic soil microorganisms. The saprophytic growth and activity of the pathogens vary depending upon the environmental conditions and soil.

The differences in the saprophytic behaviour of the pathogens in the soil should be due to variations in the cellulolysis rate of the organisms as suggested by Garrett (1975, 1984, 1985). Though Garrett is pioneer in the studies on the various aspects of saprophytic behaviour of pathogens in soil, Lockwood (1986, 1988) emphasized that there are much works needed to be done on these aspects.

MATERIALS AND METHODS

Influence of soil fungi on the saprophytic survival of *Fusarium oxysporum*

Saprophytic survival of the test organism of F*usarium oxysporum* was studied using precolonized paddy straw bits with the pathogen. Pure sand inocula of the antagonistic fungi *P. janthinellum, P. chrysogenum, T. viride* and *T. harzianum* were prepared individually as described by Garrett (1963). Acid washed sand (200g) and 3 per cent maize meal were taken in seven 500 ml conical flasks and sterilized. Each flask was inoculated with three blocks (10mm) of pure culture of the individual species of antagonistic organisms. pH was adjusted to 6.5 and the moisture was maintained at 45% MHC. The flasks were shaked regularly to maintain a homogenous continuum of propagules.

The sand inoculum of the antagonistic fungus was mixed with hand picked and air dried natural soil (paddy field soil) at the proportion of 1:3 in a 500ml beaker. The moisture level was adjusted to 45% MHC. Saprophytic survival of the pathogen was studied using pre-colonized paddy straw bits with *F. oxysporum* (Garrett, 1956). These pre-colonized bits were buried in the soil amended with individual species of antagonistic fungi.

Soil moisture

The moisture content of the soil was adjusted to 25, 50, 75 and 100% MHC on oven dry weight basis. Hundred paddy straw substrate units pre-colonized with the test organism were buried in each plastic container. The containers were incubated at laboratory temperature for 3 months. The bits were recovered after 4, 8 and 12 weeks, washed in the sterile distilled water, surface sterilized with 0.1 per cent mercuric chloride solution, and the percentage survival was assessed on PDA medium, S_{50} value and critical difference were calculated.

Soil pH

The pH of the soil, distributed in plastic containers was adjusted to 4, 5, 6, 7, 8 and 9 with lime and gypsum. The moisture content of the soil was maintained at 45% MHC. The percentage survival of the pathogen in pre-colonized substrates buried in soil was assessed as described earlier. S_{50} values and critical differences were determined.

Soil temperature

The temperature of the test organism in the pre-colonized paddy straw bits buried in soil and incubated at 20, 30 and 40 ± 2 ^{o}C was studied as described previously. The moisture content of the soil was maintained at 45% MHC. S_{50} value and critical difference were determined.

RESULT

Effect of soil physico-chemical factors on the saprophytic survival of *F. oxysporum*

Effect of soil moisture on the saprophytic survival

The percentage survival of the pathogen in the dead host tissue substrate was maximum 12 CFU in the soil with moisture content 100% MHC, increased with fungal colonies. It was 88 Cfu in the soil with 25% MHC in the precolonized substrate recovered after 4 weeks. The percentage of survival increased with the increase in moisture content and the period of incubation. S_{50} value was greater than 12 weeks in the soil with moisture content 25% MHC and 10 to 12 weeks in the soil with moisture content 100% MHC (Table 1).

Effect of soil pH on the saprophytic survival

The saprophytic survival of test pathogen was more favoured by alkaline range than the acidic range of pH. The percentage survival of the pathogen was 48, 62, 70, 64, 53 and 32 in the pre-colonized substrate recovered from the soil adjusted to pH 4, 5, 6, 7, 8 and 9 respectively after 4 weeks of incubation. The saprophytic survival of the pathogen decreased with increase in the period of incubation (Table 1).

Effect of soil temperature on the saprophytic survival

The saprophytic survival of the test pathogen was maximum in the soil incubated at 30 ± 2°C after 4 weeks of incubation. The percentage survival of the pathogen was 68, 76 and 54 in the pre-colonized substrates recovered from the soil incubated at 20 ± 2, 30 ± 2 and 40 ± 2°C respectively. S_{50} value was decreased with the increase in temperature (Table 1).

Table 1. Effect of soil moisture, pH and temperature on the saprophytic survival of *Fusarium oxysporum*

Treatment	Sampling (After)			S 50 value (weeks)
	I (4 weeks)	II (8 weeks)	III (12 weeks)	
Moisture (% MHC)				
25	88	79	71	>12
50	72	68	43	<4
75	32	25	14	<4
100	12	---	---	<4
pH				
4	48	42	35	>12
5	62	56	42	>12
6	70	66	52	>12
7	64	58	48	>12
8	53	42	35	>12
9	32	30	25	>12
Temperature (°C)				
20 ± 2	68	61	23	>12
30 ± 2	76	64	42	>12
40 ± 2	54	48	35	>12

--- *No growth*

DISCUSSION

Saprophytic behavior of the organism is of two kinds. They are (1) they may compete with obligate saprophyte, and with some other root infecting fungi, for colonization of a carpus of dead plant tissue lying in or on the soil, and (2) the survival of the pathogen sometime prolonged in the dead host tissues invaded during the parasitic phase (Garrett, 1975; Duczek *et al*., 1999).

Saprophytic behavior of the test pathogen was carried out in the present study, and it was observed that pH 8 was favourable for the growth of *F. oxysporum* and the percentage survival of the pathogen was maximum at pH 8 of the soil. Increase in temperature upto 30°C favoured the survival of the pathogen beyond 30°C, the percentage survival was decreased. The soil is more stable than the aerial environment, particularly in all respects, but also changed by many factors. Some changes in one or more factors may exert a profound effect on soil microorganisms (Baker and Cook, 1974).

The nature of survival of pathogens depends upon the interaction of numerous environmental conditions and organismal elements. In the present study it was found that the percentage colonization of *F. oxysporum* increased with increase of the soil moisture up to 75% MHC. S_{50} value of the pathogen was maximum at 30% moisture content of the soil.

Soil does not permit wide range of fluctuation as it better buffered than the sub aerial habitat (Garrett, 1956; 1970). However, the limits of tolerance to the various factors vary depending upon the species. In the present study, it was found that the maximum percentage survival of *F. oxysporum* in the pre-colonized substrate buried in the soil was at pH 5. Papavizas and Davey (1960) emphasized that the competitive saprophytic activity of the pathogen is influenced by soil reactions. In the present investigation, it was found that the survival of the pathogen was influenced by soil reaction. C_{50} values of *F. oxysporum* was maximum at pH 7-8. Sadasivan (1939) and Walker (1941) isolated *Fusarium culmorum* from fresh wheat straw substrate units buried in soils with different pH. The volatile substance emanating from the soil may possibly be the reason for greater suppression of *F. oxysporum* in the acidic pH than the alkaline range.

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