FUNGAL COLONIZATION OF STALED AGAR DISCS FROM RHIZOSPHERE SOIL INOCULA OF PIGEON PEA

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The mycoflora from rhizosphere of pigeon pea was studied in relation to the competition for colonization of staled agar discs after 24, 48, 72, 96 and 120 hr growth of fungi from mixed inocula of soil. It was observed that species spectrum of fungi on staled agar discs varied in different plates. This study was supplemented with the effect of staled culture filtrates after 120 hr of staling. On the basis of tolerant capacity of staling growth products diffused in agar discs, the fungi are classified in groups I to VI.

INTRODUCTION

The competitive survival of fungi in mixed culture from soil inocula has received little attention. Study of interspecific fungal competition for substrate colonization has been done by Rao (1959) and Wastie (1961), on nutrient agar plates from mixed inocula of soil. Dwivedi and Garrett (1968), working on fungal competition in agar plate colonization from soil inocula, reported that the species spectrum of fungi colonizing nutrient agar changed progressively with the degree of staling caused by earlier established fungal colonies. The present paper deals with the study of effect of staling growth products of the earlier established fungi on agar plates from rhizosphere soil inocula in two different grades of soil : water suspensions.

MATERIALS AND METHODS

In the present investigation the following experiments were performed:

(*a*) *Effect of fungal staling growth products in staled agar on colonization of fungi from rhizosphere soil inocula*

Rhizosphere soil samples of pigeon pea were collected by usual mycological techniques and the excess soil adhering to the root surface was removed by gentle tapping. The soil samples were thoroughly mixed in a sterilized container and sieved. Rhizosphere soil suspensions of 1:1000 and 1: 10000 dilutions were prepared. 15 ml of sterilized and cooled (35°C) nutrient agar medium (Czapek Dox + 0.5 per cent yeast extract) was poured in Petri plates. Twenty-five plates were used for each dilution. After the solidification of agar in the sterilized Petri plates, 1 ml soil suspension of the above dilutions was added in the respective plates. Control was also kept for different rhizosphere soil dilutions. Inoculated plates were incubated at 25 $\pm$ 1°C for 24 hr, 48 hr, 72 hr, 96 hr and 120 hr and thereafter the entire disc

TABLE I

Effect of different staling period on species spectrum of fungi colonizing agar from rhizosphere soil inocula (1:1000 *dilution*) *of* pigeon pea

Name of species	Presence/absence on staled agar after different staling periods (in hr)						Per cent colonization on nutrient virgin agar
	Control	24	48	72	96	120	
Group I							
Rhizopus nigricans Ehrenberg	+	—	—	—	—	—	2.62
Humicola grisea Traaen	+	—	—	—	—	—	1.04
Botrytis cinera Persoon	+	—	—	—	—	—	1.05
Absidia spinosa Lendner	+	—	—	—	—	—	2.10
Group II							
Aspergillus candidus Link.	+	+	—	—	—	—	5.20
A. nidulans (Eidam) Winter	+	+	—	—	—	—	5.78
Penicillium decumbens Biourge	+	+	—	—	—	—	2.63
Mortierella subtillissima Oudemens	+	+	—	—	—	—	1.05
Group III							
Aspergillus luchuensis Inui	+	+	+	—	—	—	10.52
Penicillium funiculosum Thom.	+	+	+	—	—	—	1.05
Curvularia lunata (Walker) Boedijn.	+	+	+	—	—	—	3.15
Group IV							
Fusarium poae (Peck) Wollenweber	+	+	+	+	—	—	4.21
Sterile white mycelia	+	+	+	+	—	—	2.63
Group V							
Alternaria humicola Oudemans	+	+	+	—	+	—	10.52
Aspergillus terreus Thom.	+	+	+	+	+	—	11.56
Group VI							
Fusarium udum (Berkeley) Wollenweber	+	+	+	+	+	+	13.63
Paecilomyces fusisporus Saksena	+	+	+	+	+	+	5.78
Penicillium nigricans Bainier	+	+	—	—	+	+	4.21
Aspergillus niger van Tieghem	+	+	+	+	+	+	10.05

of agar in each Petri plate was placed upside down. The lower surface thus exposed was inoculated by one ml of the respective soil : water suspension.

(*b*) *Growth of various dominant fungi in staled fungal filtrates*

For this study fungi chosen were from those which colonized the highest staled agar after 120 hr. Cultures of different fungi in three replicates were grown in 200 ml liquid Czapek-Dox + 0.5 per cent yeast extract (*p*H 5.0) in Erlenmeyer flasks. One ml spore suspension of individual fungus was inoculated into each flask and incubated for 120 hr at 25 ± 1°C. The metabolites were filtered through seitz-filter and 30 ml

TABLE II

Effect of different staling periods on species spectrum of fungi colonizing agar from rhizosphere soil inocula (1:10000 *dilution*) *of* pigeon pea

Name of species	Presence/absence on staled agar after different staling periods (in hr.)						Per cent colonization on nutrient virgin agar
	Control	24	48	72	96	120	
Group I							
Absidia spinosa Lendner	+	—	—	—	—	—	2.72
Cunninghamella sp.	+	—	—	—	—	—	0.90
Brown sterile mycelium	+	—	—	—	—	—	5.45
Penicillium decumbens Biourge	+	—	—	—	—	—	3.63
Group II							
Aspergillus luchuensis Inui	+	+	—	—	—	—	6.20
Fusarium roseum (Link) Sacc.	+	+	—	—	—	—	3.63
Group III							
Aspergillus candidus Link.	+	+	+	—	—	—	5.78
Rhizopus nigricans Ehrenberg	+	+	+	—	—	—	4.54
Group IV							
Aspergillus nidulans (Eidam) Winter	+	—	+	+	—	—	3.15
Penicillium egyptiacum van Beyma	+	+	+	+	—	—	5.45
Chaetomium globosum Kunze	+	+	—	+	—	—	4.54
Group V							
Fusarium poae (Peck) Wollenweber	+	+	+	+	+	—	5.45
Group VI							
Aspergillus terreus Thom.	+	+	+	—	+	+	7.27
A. niger van Tieghem	+	+	+	+	+	+	8.18
Alternaria humicola Oudemans	+	+	+	+	—	+	4.62
Fusarium udum (Berkeley) Wollenweber	+	+	+	+	+	+	10.00

thereof was transferred to 100 ml flask which was later inoculated with 0.15 ml spore suspension of individual fungus. Seventy-five such flasks including replicates for treated and non-treated ones were prepared for different fungi. All the flasks were incubated at 25 ± 1°C for 15 days. After the said incubation period hyphal mats were separated by filtration and those of respective replicates pooled and dried at 80°C for 24 hr.

RESULTS AND DISCUSSION

A perusal of Table I on opp.pg. reveals that there is retrogression in the number of fungi colonizing the agar discs on the reverse side due to the diffusion of staling products from composite fungal flora. It was observed that the least number of fungi

TABLE III

Growth behaviour of fungi appearing on staled agar discs (after 120 hr staling) in staled liquid culture filtrates (dry weight of hyphal mat in mg)

Name of species	Control	*Aspergillus niger*	*Paecilomyces fusisporus*	*Alternaria humicola*	*Aspergillus terreus*	*Fusarium udum*	Mean growth in all filtrates
Aspergillus niger	213	91.66	51.20	14.80	23.30	47.30	45.45
Paecilomyces fusisporus	172	2.50	30.66	4.10	15.25	4.43	11.35
Alternaria humicola	200	35.33	29.00	82.33	28.42	30.00	41.01
Aspergillus terreus	195	65.30	57.20	63.50	17.25	34.66	47.58
Fusarium udum	205	72.26	10.70	68.00	52.60	59.53	52.61
Mean growth of all fungi	197	53.41	35.75	46.54	27.36	35.18	

could colonize the staled agar after 96 and 120 hr of staling. After the longest period of staling (120 hr) the fungi appearing on the reverse of the agar discs are only *Fusarium udum, Paecilomyces fusisporus, Penicillium nigricans, Aspergillus terreus, A. niger* and *Alternaria humicola.* Fungi appearing after 48 and 72 hr of staling were less in number than those colonizing after 24 hr of staling. Those fungi which appeared in the virgin agar plates but failed to appear on staled agar discs have no tolerant capacity of the staling growth products diffused in the agar discs from the mixed inocula of soil.

In this connection it may be considered that the success in saprophytic colonization of a particular species also depends upon its population level in soil which is related to the inoculum potential. Amongst the fungi listed in Tables I and II several might have colonized the staled agar, perhaps due to the high degree of competitive saprophytic ability for the substrate while it may not be true for remaining ones having lower degree of saprophytic ability.

The growth behaviour of five dominant fungi in their liquid culture metabolites is represented in Table III. It is observed that there is difference in growth supporting values of the culture filtrates of different filtrate producers. Growth supporting values of the various filtrates are given as mean figures for "growth of all fungi". The filtrates of *Fusarium udum, Paecilomyces fusisporus* and *Aspergillus terreus* have less values of mean growth of various fungi. *Fusarium udum* has greater values in all the filtrates as compared to the *Aspergillus* spp., *Paecilomyces fusisporus* and *Alternaria humicola.* It is also noted that culture filtrates of *Aspergillus niger, Fusarium udum* and *Alternaria humicola* reduced the growth of other fungi than their own.

The growth supporting values of culture filtrates depend directly on the concentration of the residual nutrient unused by filtrate producing fungus and inversely on the concentration of fungistatic products due to the staling (Dwivedi and Garrett 1968) The amount of hyphae harvested after growing in culture filtrates for a given time depicts the amount of staling product secreted by the filtrate producing fungus. Higher the values of hyphal mat harvested, lower will be the toxic effects of filtrates.

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