Effect of fungal staling growth products on colony interaction among *Rhizoctonia solani* Kuhn and some potential rhizosphere fungi

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Summary. Colony interactions between *Rhizoctonia solani* Kuhn: a root pathogen of *Lycopersicon esculentum* Mill and some dominant rhizosphere fungi were assessed in vitro in virgin and staled agar to examine their antagonistic ability and tolerance to antagonism. The range of inhibition of *R. solani* varied widely in competition with some of the antagonists.

In evaluating the antagonistic interactions among fungi, some workers[3,4] noticed different types of colony interaction on virgin nutrient agar. Recently, Skidmore and Dickinson[5] investigated the effect of temperature in light and darkness on colony interaction between some phyllosphere fungi and *Septoria nodorum*. However, no work has been performed to investigate the effect of fungal staling products on microbial interactions. This paper describes experiments which were carried out to investigate the effect of fungal staling growth products on colony interaction between a pathogenic root infecting and some potential saprophytic fungi.

Methods. Dominant rhizosphere fungi such as *Aspergillus candidus, A. fumigatus, Chaetomium globosum, Fusarium chlamydosporum, F. culmorum,* and *Penicillium citrinum* were used for interaction against *Rhizoctonia solani.*

Colony interaction on agar plate staled by mixed rhizosphere and rhizoplane fungi. PDA plates (Oxoid, pH 5.6; 10 ml Petri dish^{-1} of 90 mm diameter) were staled by rhizosphere and rhizoplane fungi for 48 h by cellophane agar technique 6. Staled nutrient agar was inoculated with a 6-mm diameter fresh colony of *R. solani* 3.5 cm apart with an individual antagonist colony and incubated under a bank of light at 25 °C. Assessment of colony interaction was made (after 14 days) when interacting fungi had achieved an equilibrium, i.e. no alteration of growth of interacting fungi was recorded.

Colony interaction on agar plate staled by some potential antagonists. Nutrient agar was staled by individual dominant rhizosphere fungi for 48 h and on the staled agar a 6-mm diameter colony of *R. solani* was opposed 3.5 cm apart with a 6-mm colony of individual antagonist and incubated for 14 days. Controls were set up by single and dual inoculated cultures of the same fungus in nutrient agar but otherwise under identical conditions.

Results. In nutrient virgin as well as in agar staled by mixed rhizosphere and rhizoplane fungi, *R. solani* grew over the colonies of other saprophytic fungi and in most cases attained 100% growth (table 1). Overgrowth of *R. solani* colony was also observed on *A. candidus* and *C. globosum* on all the agar plates diffused with staling growth products of individual antagonist except in staled agar of *P. citrinum* and *F. chlamydosporum* which produced mutual growth inhibition with a very clear narrow demarcating zone (table 2). Overgrowth of *F. chlamydosporum* on *R. solani* was found on agar plates diffused with *P. citrinum* staling growth substances. In the staled agar of some fungi such as *A. candidus, P. citrinum* and *F. culmorum,* a mutual growth reduction value curve was obtained against *R. solani;* this may possibly be due to reduction of growth of both interacting colonies caused by diffused staling products. Thus, the results obtained indicate that *R. solani* possessed strong antagonistic ability, tolerance capacity of staling products and no potential antagonists tested were proved outstandingly successful in reducing the growth of *R. solani*[6,7].

Table 1. Colony interaction between some rhizosphere fungi with *R. solani*

Antagonists	Control (nutrient agar)			Nutrient agar staled by: Rhizosphere fungi			Rhizoplane fungi		
	A	B	C	A	B	C	A	B	C
Aspergillus candidus	3	85	39	3	100	0	3	100	0
A. fumigatus	3	81	25	3	36	0	3	100	0
Chaetomium globosum	3	90	3	3	93	0	3	95	0
Fusarium chlamydosporum	1	66	42	3	71	0	3	68	36
Penicillium citrinum	3	72	29	3	84	8	3	79	26
F. culmorum	3	100	0	3	100	0	3	100	0

A: grade of *R. solani*; B: growth inhibition of antagonists (%); C: growth inhibition of *R. solani* (%). Different grades of colony interactions are according to Porter[8] and Skidmore and Dickinson[5] while percent growth inhibition was calculated by a modified model given by Arora and Upadhyay[9]. Grades: 1: mutual intermingling growth of one colony over another; 2: fungus being observed ceased growth and is being overgrown by another one; 3: overgrowth by *R. solani;* 4: slight growth inhibition of interacting colonies, c 1–2 mm; 5: inhibition of both interacting colonies at a distance > 2 mm.

Table 2. Colony interaction between some rhizosphere fungi with *R. solani* on nutrient agar staled by individual fungi

Antagonists	Nutrient agar staled by: *A. candidus*			*A. fumigatus*			*C. globosum*			*F. chlamydosporum*			*P. citrinum*			*F. culmorum*		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
A. candidus	3	92	10	3	100	0	3	84	33	4	55	54	4	95	54	3	100	0
A. fumigatus	4	85	92	2	0	100	3	84	31	4	54	95	2	0	100	3	100	0
C. globosum	3	100	0	3	84	46	3	100	0	3	100	0	3	100	0	3	100	0
F. chlamydosporum	1	34	48	3	100	0	3	84	32	4	72	78	2	92	22	3	84	52
P. citrinum	3	96	28	3	85	53	4	71	77	3	92	13	4	84	63	3	100	0
F. culmorum	4	96	89	5	86	68	3	80	54	3	100	0	3	100	0	3	100	0

Footnotes as in table 1.

Discussion. The different range of growth variability and antagonistic nature of saprophytic fungi in competition with the test pathogens on agar plates may be commonly ascribed to production of antibiotics or general mycostatic staling growth substances by interacting fungi. However, the possibility of occurrence of competition for nutritions, change in pH, nutrient imbalance, mechanical obstructions and hyperparasitic interactions cannot be ruled out during colony interactions. Several factors such as equal competitive and resistance capacity of interacting fungi to offset the effect of staling products, production of metabolic substances of same biological nature and equal growth rate, may play an operative role in intermingling growth (grade 1) of interacting fungi. Overdominating capacity of one fungal colony on another one (grade 3 or 2) primarily depends upon higher growth rate and tolerance to antagonism. Complete growth inhibition of a fungus may be due to diffusion of active staling growth substances by the resistance fungus which would have proved lethal before the establishment of the sensitive one.

Microscopic examination after 10 days of growth showed lysis and loss of permeability in most of the interacting hyphae. In some cases, swelling and attenuation of hyphal diameter were also noticed. However, when such hyphae were transferred again on fresh agar, normal growth was resumed. Hence abnormality due to mycotoxic substances or change in pH of nutrient broth produced the alteration in the morphological nature of interacting fungi but apparently failed to develop any genetic effect. Park[10] also demonstrated that staling growth substance of *Fusarium oxysporum* was inhibitory to apical growth of several fungal hyphae.

The discrepancy between the ability to inhibit the growth of a fungus in vitro by another one cannot be exactly compared with antagonistic interactions occurring in the actual root system, due to involvement of certain ecological factors in vivo. However, antagonistic effect on microbial colonization in vitro study should be considered as indication of potential antagonistic property of a fungus in vivo.

1 Acknowledgments. I would like to express my appreciation to Dr R.S. Dwivedi for encouragement and CSIR for financial assistance.

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Nuclear degeneration induced by chlortetracycline

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22 June 1978

Summary. After application of chlortetracycline to plants, a degeneration of nuclei, manifested by dilatation and vacuolation of the nuclear membrane, occurs in the mesophyll cells of the sunflower plant.

The tetracycline antibiotics inhibit plant growth and negatively influence the formation of chlorophyll[1-6]. After application of chlortetracycline (CTC) to plants, a marked disturbance of the differentiation of the membranes of chloroplasts occurs and results in their complete degeneration[7]. It follows from the hitherto published cytological studies that tetracycline antibiotics inhibit the course of mitotic division in the cells of the root tip of the broad bean (*Vicia faba* L.)[1] and that especially CTC provokes various disorders of chromosomes[8]. It was therefore interesting to determine how the effect of CTC was manifested in the structure of the nucleus. The results obtained are presented in the paper.

The sunflower plant, *Helianthus annuus* L., cv. Armavirskij 3497 served as experimental material. The achenes of the plants were soused for 12 h in a solution of chlortetracycline hydrochloride of a concentration of 10^{-4}M at 25 °C. The plants were cultivated in a cultivating chamber at a temperature of 25±1 °C, with 14 h of light and 10 h of dark, and using Richter's nutrient solution of half concentration. Material for observation was withdrawn after 7 days, fixed in 5% glutaraldehyde and 2% OsO_4, embedded into Durcupan ACM (Fluka) and examined under the electron microscope Tesla BS 613.

One of the symptoms of beginning degeneration of nuclei in the mesophyll cells of the sunflower plant is the development of protrusions from the nuclear membrane towards cytoplasm (figure 1), of which shapes and sizes vary. Another typical manifestation of degeneration of the nucleus is an occurrence of invaginations in the nuclear membrane (figure 2). These are of oval shape and develop by gradual dilatation of the nuclear membrane. Both outer and inner parts of the nuclear membrane participate in the formation of these structures. Inside these invaginations, which are found around the whole periphery of the nucleus, there are globular particles whose shape, size and density resemble lipidic globules in the cytoplasm. Some invaginations merge with each other, which results in a formation resembling a vacuole in which 2 lipid-like globules are present. Chromatin of degenerating nuclei is considerably condensed and arranged in electrondense clusters located in the peripheral parts of the nucleus (figures 1 and 2). In the period when the nuclear membrane is formed by numerous invaginations, extensive degeneration is observed also in other cellular organelles.

Dilatation of the nuclear membrane in the course of the process of degeneration of nuclei is probably related to the lytic process in the cytoplasm, plastids and mitochondria. This hypothesis seems to be confirmed by the fact that in the nucleus the presence and activity of hydrolytic enzymes[9,10] was found. A similar type of nuclear degeneration combined with vacuolation of the nuclear membrane was observed also in *Myxomycetes*[9], in the microspores of *Tradescantia paludosa*[11], after the application of the herbi-