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# Survival of *Rhizoctonia solani* Kühn Under the Influence of Staling Growth Products of Some Aspergilli and its Growth Response to Some Phenolic Substances

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The fungus *Rhizoctonia solani* Kühn causing damping off disease in various plants was isolated from the forest soil and its efficacy to withstand the staling growth products of some common aspergilli. viz. *Aspergillus niger*, *A. flavus* and *A. candidus* was tested on nutrient agar medium amended with the metabolite in a ratio of 1 : 1. Similarly the pure culture of test pathogen was immersed in the SGP and its capacity of withstanding them was studied. All the culture filtrates were found to check the growth of the test pathogen effectively. In another experiment with phenolic substances (vanillin, ferulic acid and P-hydroxybenzaldehyde) hyphal dry weight yield aud the linear extension of test fungus was recorded by replacing the carbon source of the medium with the phenols. The phenols were noted to be effective fungistatic substances.

## Introduction

*Rhizoctonia solani* Kühn is the most important causal organism of damping off disease in the forest nurseries of India (Bakshi 1976). A pathogen after infecting the host insues a chain of biochemical reactions between the host and pathogen on one hand and the parasite and the adjacent ecological microclimate on the other, thus causing interaction among the local fungi. Fungal interactions has been extensively investigated by Dennis and Webster (1971), Robinson et al. (1968), Shukla (1976), Shukla et al. (1977a) Park (1960) and Singh and Webster (1973). It has been suggested as a means of biological control of the diseases of economically important plants. Similarly, the importance of phenolic compounds in disease resistance has been well recognised (Cruickshank & Perrin 1964, Farkas & Kiraly 1962, Kosuge 1969, Vidhyasekaran 1973). The possibility of obtaining new fungicides or more useful modifications of older materials has motivated considerable research and phenols have been investigated most extensively from this aspect (Cochrane 1958). Deeper insight into fungal interaction and use of phenols as a fungistatic substance

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against *R. solani* forms the theme of the present investigation.

**Material and methods**

*A. Effect of staling growth products*

The soil, infected with *R. solani* was collected from Nangarh forest, Varansi employing standard mycological techniques. The dominant fungal flora of soil was isolated on Czapek-Dox nutrient agar medium (*p*H-5.5 + Streptomycin 10 $\mu g\ L^{-1}$). The test pathogen and three *Aspergillus* species viz. *A. niger*, *A. flavus* and *A. candidus*, which were the most dominant fungal flora of soil, were further purified by transferring them to fresh agar medium plates.

The culture filtrate of different Aspergilli was prepared by the method of Shukla et al. (1977a) and stored at 0°C. The Survival potential of test pathogen against the culture filtrates was studied by two methods (a) Immersion method—The individual culture filtrates (20 ml) were taken in 100 ml flasks and agar blocks cut from the margin of pure colony of test pathogen were immersed in it for 20h. The disks were blotted dry and inoculated into the centre of nutrient medium separately. The growth of the pathogen was recorded after 7 days. The control was prepared by soaking the disk in sterile distilled water only and implanting onto the nutrient medium. (b) Poison food method—The nutrient medium was incorporated with individual culture filtrates in a ratio of 1:1 and the test pathogen was centrally inoculated on to the medium taken in a Petri dish. Fungal disk inoculated on the nutrient medium only were treated as control.

The experiment was done in triplicate and all the plates incubated at 24±1°C under the source of artificial light. The radial growth around the colony after 6 days was recorded.

*B. Growth response to phenolic substances*

Three phenol derivatives viz. vanillin, ferulic acid and p-hydroxybenzaldehyde were employed for this study. The carbon source of original Czapek medium was replaced by 0.005 per cent (w/v) of ferulic acid and 0.001 per cent (w/v) of p-hydroxybenzaldehyde and vanillin. The fungistatic properties of these lignin derivatives were tested by two methods; firstly by harvesting the mycelial growth in liquid nutrient medium. In this method pure disk of the test fungus was inoculated into 25 ml of the treated nutrient medium individually, taken in a 100 ml conical flask. Sugar free medium inoculated with the fungal disk was used as control; secondly, the test pathogen was inoculated into a 45 cm long glass tube (dia 5 mm) containing 35 cm. long medium slent, treated with different phenolic substances. Control was the same, as in the first method.

The flasks and linear tubes were incubated for 10 days at 25±1°C under an artificial light source. The observations were recorded in terms of mycelial dry weight in case of former and in terms of linear mycelial growth in the later experiment.

**Results and Discussion**

The inhibition of the growth of *R. solani* under the influence of various staling growth products of aspergilli has been depicted in figure 1. All culture filtrates proved to be antagonistic to the test pathogen and its growth was drastically reduced in both the methods tested. The poison food method has been found more effective in this study. It may be due to the higher toxicity of the medium which has ultimately retarded the spread of mycelium from the agar block. Shukla et al. (1977a) reported that comparatively immersion plate method was effective than poison food method. Dennis and Webster (1971) working on the antibiotic properties of *Trichoderma viride* claimed that the volatile metabolites of the fungus were responsible for its fungistatic properties against other soil mycoflora. *Aspergillus* sp are known to produce various non-volatile acids (Coch-

rane 1958) and their role in checking the advance of other fungi must be a decisive factor.

The growth response of *R. solani* against the culture filtrate of *A. candidus* showed the maximum antibiotic potential. It completely suppressed the growth of pathogen in the

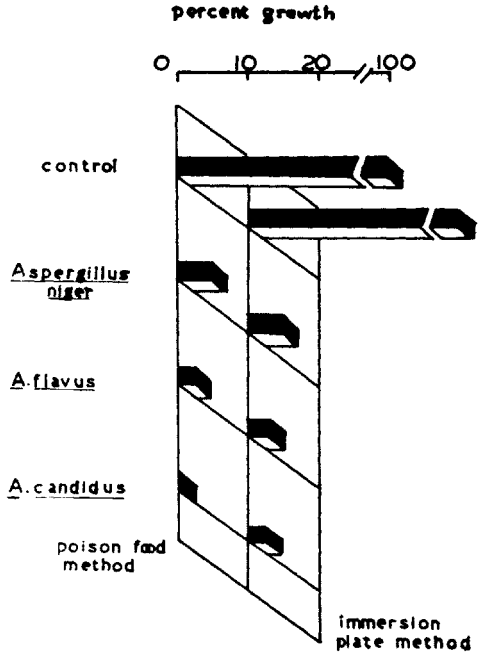


Figure 1

culture-filtrate amended nutrient medium and allowed only two per cent growth after immersing the pathogen in the pure culture-filtrate. In contrast to control, *A. flavus* and *A. niger* supported only 5 and 2% growth in poison food and 6 and 3% growth in immersion plating method respectively. All these results strongly suggest the antibiotic nature of the staling growth products of Aspergilli and a suitable alteration in the microbial population may be helpful in the biological control of a pathogen like *R. solani*.

The influence of phenolic substances on the growth of *R. solani* has been shown in figure 2. All of them effectively checked the dry weight yield of the fungal mycelium. It was observed that vanillin and p-Hydroxy-benzaldehyde allowed the fungus to grow only up to 26% (13mg) and 30% (15mg) res-

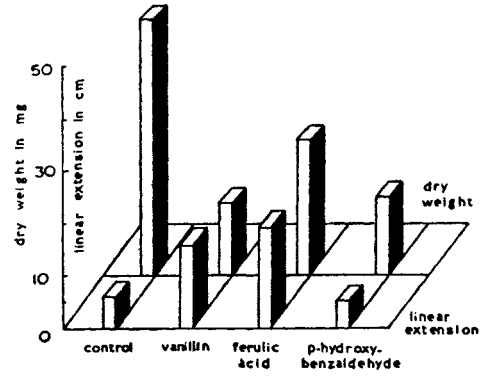


Figure 2

pectively in contrast to control. But ferulic acid was found to be least effective as it allowed growth up to 56% (23mg). The linear extention experiment depicted that the pathogen could advance only up to 7cm on the sugar free control. In contrast to dry weight yield of mycelium the hyphae of *R. solani* traversed up to 18cm in vanillin and 20cm in ferulic acid amended nutrient medium. Only 5cm. growth could be recorded in the medium amended with p-hydroxy-benzaldehyde. Thus the growth response of *R. solani* in agar medium was found to be favourable. This suggests that the pathogen can break up and utilise the phenols in solid medium. Shukla (1976) however, reported that if normal Czapek medium could be employed as control then the growth response of the pathogen to phenols was always negative.

Resistant varities have been found to contain proportionately higher amounts of phenols than the susceptible ones (Vir & Grewal 1974). The same authors have also reported that in the initial stages of disease development phenols participated in checking the advance of the pathogen. Thus, if small amounts of phenolic fungicides may be administered to the young plants, they may

be saved from nursery failures. Vidhyasekaran (1974) is also of the view that resistance of the plants is mainly due to the phenolic contents. Shukla and Dwivedi (1977b) working on the similar pattern reported that the growth of common soil fungi was retarded in the medium supplemented with lignins. It may be due to the spore germination, mycelial growth and enzyme activity of the pathogen (Spurr & Main 1969). It can be presumed, therefore, that the incidence of *R. solani* could be further improved if the phenolic content of its surrounding soil may be enhanced.

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