FUNGICIDAL PROPERTIES OF SHARIGOL

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Introduction

In an earlier communication, some preliminary results on the fungicidal activity of Sharigol against *Aspergillus niger* and *Alternaria sp.* have already been reported,[1] from which it appears that Sharigol shows promise of being a good fungicide. It was, therefore, considered important to extend this study to human pathogens and other important fungi. The present paper presents a study of the fungicidal effects of Sharigol against the human pathogens, *Aspergillus fumigatus*, *A. flavus*, *Trichopyton metalense*, and two other important fungi, *viz.*, *Helminthosporium oryzae* and *Empusa muscae*.

Effect of Sharigol on the three Human Pathogens

Preliminary tests were done by mixing the chemical with the medium in varying concentrations in the petri dishes, Sabraud's medium being used.

To facilitate a detailed examination of the effect of different concentrations of Sharigol on the growth and sporulation of the three pathogens, a series of experiments was conducted, using Sharigol concentrations from 1 : 1200 to 1 : 40 in 11 steps. In the first series of experiments, Sharigol was mixed in the ratios of 1 : 1200, 1 : 600, 1 : 400 with the medium, and a control was also kept with the series. The medium was then inoculated with a suspension of spores in sterile distilled water prepared by vigorously shaking about 8 ml. of water in a 7-day old culture tube and standardizing the suspension of spores thus obtained so that the number of spores per unit field under the microscope was constant. Three replicates were taken in each series of experiments and observations were made every 24 hours, the minimum and maximum growth for every concentration and every species were noted. The first four rows of Table 1 show the results for the control and the three different concentrations of Sharigol, while the rest of the table shows the data obtained with the higher concentrations.

After 24 hours some growth was observed in the control, but in 1 : 1200 concentration no growth was found. After 48 hours abundant growth was observed and sporulation had started in the control, whereas no growth was observed in the treated petri dishes. After 72 hours the mycelium had spread to a diameter of about 4 to 4.5 cms. in the control and sporulation was abundant, while in the treated petri dishes very small growth had taken place without any sporulation. The inhibitory effect of low concentrations of Sharigol on the mycelial growth and sporulation is thus evident. In the control, sporulation started between the second and third days, while in the petri dishes with Sharigol concentrations of 1 : 1200, 1 : 600 and 1 : 400 sporulation started between the 4th and 6th, 7th and 8th, and 9th and 10th days, respectively.

Inorder to study the effect of higher concentrations of Sharigol, further experiments on the above lines were conducted with concentrations upto 1 : 40, the results of which are also given in Table 1. They indicate that the inhibition of growth as well as the delay in sporulation is proportional to the increase in Sharigol concentration in the medium.

In the concentration of 1 : 80, no growth was observed upto 4 days and on the 5th day, in a few petri dishes (*Aspergillus fumigatus* and *A. flavus*) a minute dirty speck was observed, (Plate No. 1). The increase in size of these specks was extremely

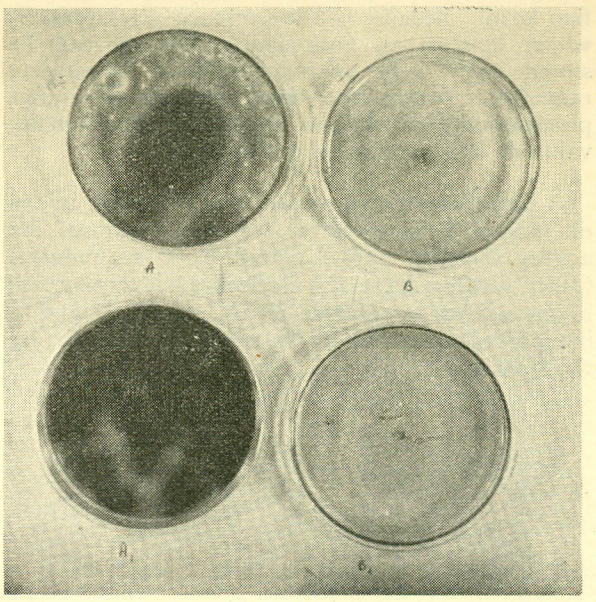


Plate No. 1.—Top - *Aspergillus flavus* A, control; B, 1:80 Sharigol (After 120 hours). Bottom: *Aspergillus fumigatus* A_1 control; B_1 1:80 Sharigol (After 120 hours).

slow. In the case of *Trichophyton metalense*, no growth was observed, and later on it was found that in the concentration of 1 : 60 sometimes the germination fails. In the concentration of 1 : 50 the germination of spores usually fails but sometimes very minute specks of growth may be found. In the concentration of 1 : 40, germination of spores fails and no growth is observed even after keeping the petri dishes for about two months. The time taken for sporulation in the concentrations of 1 : 80 and 1 : 60 is between 23 to 25 and 25 to 28 days respectively. In the concentration of 1 : 50 no sporulation takes place.

Some Abnormalities in Growth due to Sharigol

During the course of study of the effect of Sharigol on fungi some abnormalities in mycelial growth were noted. In concentrations of Sharigol, from 1 : 1200 upwards, the growths were elevated and cushion like, as already reported by Quraishi

TABLE 1.—CONCENTRATION GROWTH.

Sharigol concentration	Test specimen	1 day	2 days	3 days	4 days	5 days	6 days	7 days	Time taken for sporulation
Control	*A. fumigatus* ..	Speck	1.4-2.0	3-4.5	5-6	7-8.5	9-9.5	9.5-10	2-3 days.
	A. flavus ..	,,	2.0-3.0	3.5-4.5	5-5.5	6-6.5	7-8.5	8.5-9.5	
	T. metalense ..	,,	0.5-1.0	1.5-2 5	2.8-3.5	3.5-4.5	5-6	6.5-7.5	
1 : 1200	*A. fumigatus* ..	No	Speck	0.5-1.0	1.5-2	2-2.5	3-3.5	3.5-4.0	4-6 days.
	A. flavus ..	Growth	,,	0.2-0.5	0.8-1.2	1. 5-2	2. 5-3.0	3.5-4.5	
	T. metalense ..	,,	,,	0.2-0.3	0.5-0.8	1-1.8	2. 3-2.8	3.5-4.0	
1 : 600	*A. fumigatus* ..	,,	No	0.4-0.9	0.9-1.1	1. 3-1.5	1. 5-2	2.2-5	7-8 days.
	A. flavus ..		Growth	Speck-0.1	0.5-0.8	1. 0-1.5	1. 8-2	2.5-3.0	
	T. metalense ..		,,	Speck	0.2-0.5	0. 8-1.5	2-2.5	2.8-3.2	
1 : 400	*A. fumigatus* ..	,,	,,	,,	0.2-0.3	0. 4-0.6	0. 8-1	1.3-1.5	9-10 days.
	A. flavus ..	,,	,,	,,	0.1-0.2	0. 5-0.7	0. 8-1	1-1.7	
	T. metalense ..	,,	,,	,,	0.2-0.4	0. 6-1.2	1. 5-2	2.5-3.0	
1 : 300	*A. fumigatus* ..	,,	,,	,,	Speck	0. 2-0.4	0. 5-0.8	1-1.2	10-15 days.
	A. flavus ..	,,	,,	,,	Speck-0.1	0.25-0.35	0. 4-0.6	0.7-1.5	
	T. metalense ..	,,	,,	,,	0.2-0.3	0. 5-1.0	1. 2-1.5	2-2.5	
1 : 200	*A. fumigatus* ..	,,	,,	No Growth	Speck	0. 1-0.2	0.25-0.3	0.5-0.8	15-18 days
	A. flavus ..	,,	,,	Speck	,,	0. 2-0.3	0.35-0.4	0.5-0.9	
	T. metalense ..	,,	,,	No Growth	Speck-0.2	0. 4-0.6	0. 8-1.2	1.5-2.5	
1 : 150	*A. fumigatus* ..	,,	,,	,,	Speck	Speck-0.1	0. 2-0.25	0.3-0.35	18-20 days.
	A. flavus ..	,,	,,	,,	,,	Speck-0.2	0. 3-0.4	0.4-0.8	
	T. metalense ..	,,	,,	,,	Speck-0.2	0. 2-0.4	0. 5-1	1.2-2.0	
1 : 100	*A. fumigatus* ..	,,	,,	,,	No	Speck	0. 1-0.2	0.2-0.3	20-22 days
	A. flavus ..	,,	,,	,,	Growth	,,	0.15-0.3	0.3-0.5	
	T. metalense ..	,,	,,	,,	,,	,,	0. 1-0.2	0.5-0.8	
1 : 80	*A. fumigatus* ..	,,	,,	,,	,,	,,	Speck-0.1	0.15-0.2	23-25 days.
	A. flavus ..					,,	0. 1-0.25	0.15-0.2	
	T. metalense ..					No Growth	Speck-0.1	0.2-0.3	
1 : 60	*A. fumigatus* ..	,,	,,	,,	,,	,,	Speck	Speck-0.2	Slight sporulation only in some cases 25-28 days.
	A. flavus ..	,,	,,	,,	,,	,,	,,	Speck-0.1	
	T. metalense ..	,,	,,	,,	,,	,,	No Growth	0. 1-0.2	
1 : 50	*A. fumigatus* ..	,,	,,	,,	,,	,,	,,	Speck	No Sporulation found after 60 days.
	A. flavus ..	,,	,,	,,	,,	,,	,,	,,	
	T. metalense ..	,,	,,	,,	,,	,,	,,	Speck-0.1	
1 : 40	*A. fumigatus* ..	,,	,,	,,	,,	,,	,,	No Growth	No Growth and no sporulation after 60 days.
	A. flavus ..	,,	,,	,,	,,	,,	,,	,,	
	T. metalense ..	,,	,,	,,	,,	,,	,,	,,	

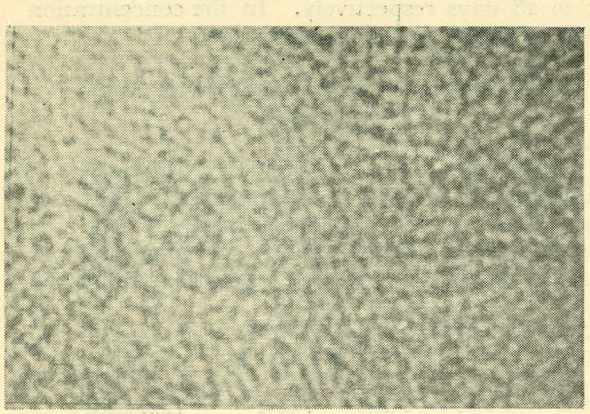


Plate No. 2.—Meshed mycelium of *Aspergillus* Sp. in 1:600 Sharigol.

and Hussain.[1] The contour of the growth was usually irregular and the colour was muddy. The growth of the mycelium was compact and meshed, (Plate No. 2). In the higher concentrations two regions were observed in the cushion-shaped growth, the mycelium being very compact and meshed in the outer region of the cushion forming a pseudoparenchymatous structure, but underneath it the mycelium was loose and individual filaments could be seen under the microscope. Changes in the cell dimensions of the mycelium were also noted and will be reported upon later.

Effect of Concentration on Spore Germination in Helminthosponium oryzae and Empusa muscae

Spores of *Helminthosporium oryzae*, *Aspergillus flavus* and *Empusa muscae* were taken for germination study, which was carried out by the simple method of M. Checogne and Viel[2] on microscope slides ringed with paraffin wax. A suspension of spores was prepared by the method previously mentioned, and was filtered through a pad of sterilized glass wool to remove the pieces of mycelium from the culture. The suspension was standardised as before to obtain a definite number of spores per unit field. Sharigol and a solution of glucose were mixed with portions of the suspension in such a way that the mixture contained 5% of glucose with the following concentrations of Sharigol :

0.01%, 0.02%, 0.03%, 0.06%, 0.09%, 0.18%, 0.25%, 1.0%. Teepol (sodium secondary alkyl (C_{10}-H_{18}) sulphate) was used as an emulsifying agent.

Two drops of each of the above suspensions were dropped inside respective wax rings on glass slides, which were then placed on glass strips and kept in large petri dishes, (Plate No. 3). A slide

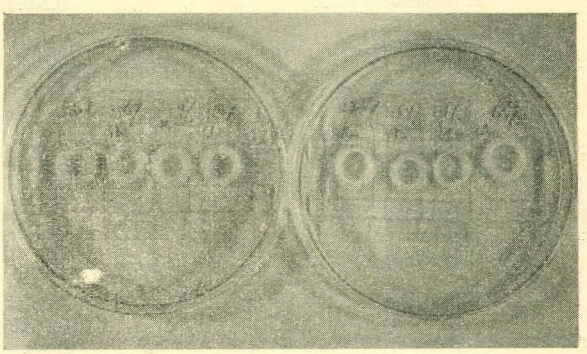
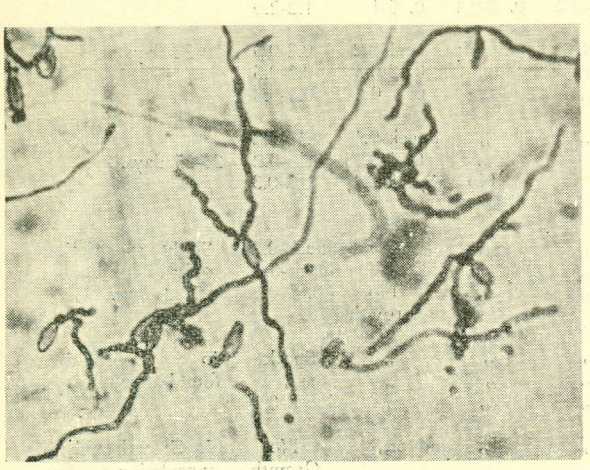


Plate No. 3.—Spore germination on the wax-ringed slides placed on glass strips.



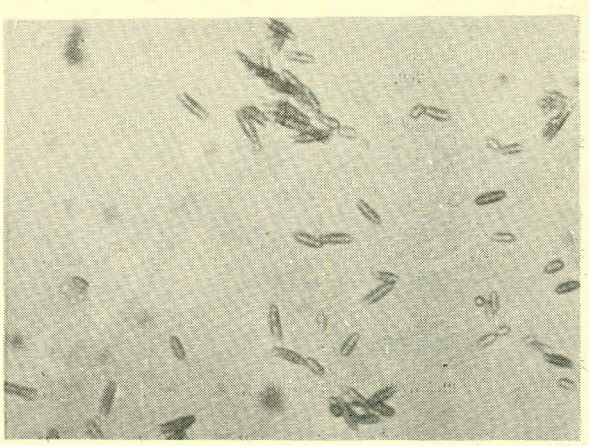


Plate No. 4.—Spores of *Helminthosporium oryzae* after 24 hours incubation at 25-27 °C. (a) without Sharigol, showing germinating spores (b) with 1.5% Sharigol, showing non-germination of spores.

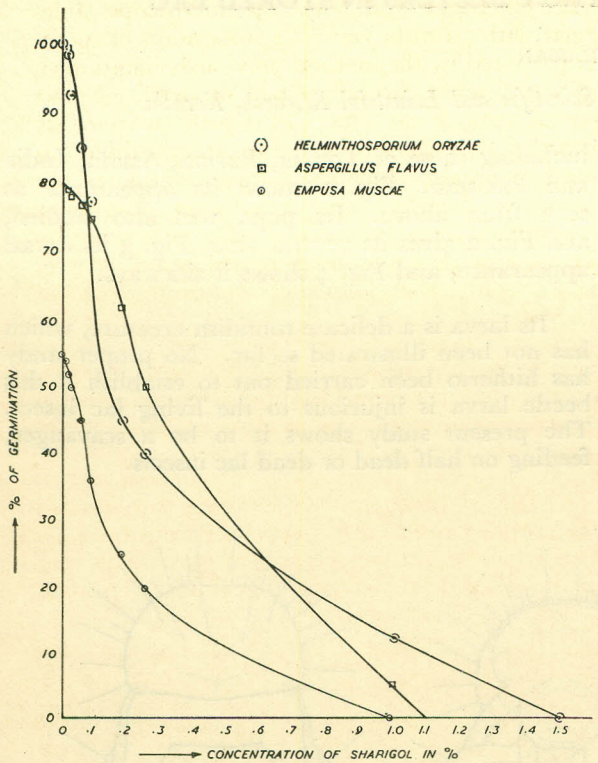


Fig. 1.

without Sharigol was kept as control. To maintain proper humidity, 10 ml. of 20% solution of glycerol were poured into each petri dish and the petri dishes were kept in an air-conditioned room (25°C.-27°C.) for 24 hours. Two replicates were done in each series of experiments and several series of experiments were conducted.

All the slides were studied under the microscope after 24 hours and the percentage of germination was noted (Plate No. 4). These percentages were plotted on the graph against the appropriate concentrations, and three curves, for the three species used, were obtained (cf. Fig. 1).

The graphs show that the percentage of germination decreases regularly with the increase of concentration of Sharigol and at a certain limiting concentration the spores fail to germinate. In the case of *Helminthosporium oryzae* this limiting concentration is 1.5%, in *Aspergillus flavus* 1.1%, and in *Empusa muscae* 1.0%.

Conclusions

Whereas for fungi of agricultural importance, as reported previously by Quraishi and Hussain, small concentrations of Sharigol are enough to check the growth and sporulation, it appears from the above experiments that considerably higher (*about ten times*) concentrations of Sharigol are needed to check the growth of fungi pathogenic to human beings. These results agree with observations previously made on the resistance of fungi pathogenic to man. It may be pointed out here that a certain amount of variation in these experiments is to be expected because the coal supplies from which Sharigol has been obtained tend to differ to some extent, thereby causing some differences in the composition of Sharigol obtained in different batches.

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References

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