

A METHOD FOR THE EVALUATION OF WATER SOLUBLE AND WATER MISCIBLE FUNGICIDES USED IN THE PREVENTION OF THE SPREAD OF "ATHLETE'S FOOT"

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INTRODUCTION

At the present time no method has been adopted for evaluating the fungicidal properties of water-miscible and water-soluble products used in the prevention of the spread of athlete's foot.

In a study devoted to the investigation of the relationships between the chemical constitution and germicidal action of certain phenol derivatives, E. Klarmann, V. A. Shternov and L. W. Gates used a method of determining the fungicidal effect with respect to certain pathogenic fungi, the details of which have been described in a previous publication (1934). *Trichophyton rosaceum*, *Trichophyton gypseum*, *Achorion schönleinii* and *Monilia albicans* were grown in such a way as to yield a practically constant resistance to phenol which made them suitable for use as test-organisms in studying the fungicidal properties of a number of organic compounds.

This original method, as applied particularly with *Trichophyton rosaceum* as test-organism, has been giving consistently uniform results, not only in the hands of the authors but also in those of others who adopted it as a routine test for evaluating preparations for the control of the spread of pathogenic fungi. At room temperature, phenol was found to kill *Trichophyton rosaceum* in dilutions of 1:70 to 1:80, in 10 minutes, while at 37°C. the dilutions ranged from 1:100 to 1:120 for the same period of

exposure. Of the several pathogenic fungi used as test-organisms, *Trichophyton rosaceum* was found to be at least as resistant to the action of germicidal agents as other pathogenic fungi, and also the most easily cultured at a uniform resistance. Thus, the use of *Trichophyton rosaceum* as standard test-organism for the evaluation of fungicides is proposed, on the basis of the working hypothesis that if any preparation is effective against this fungus in a given concentration, it will be also effective in the same or in a lower concentration against other fungi of pathogenic significance.

As it was desired to change this method to conform more closely with practical conditions, variations of the original procedure were studied. This work has shown that one step, viz. the removing of the fungus growth from the agar slant prior to the preparation of a liquid suspension, could be eliminated. This we succeeded in accomplishing and we were able to grow the fungus directly in a liquid medium. We also found that consistent phenol resistance could be obtained with a lower spore count than that advocated in the original procedure.

DETAILS OF THE PROPOSED METHOD

The culture is a strain of *Trichophyton rosaceum* which, when tested by the method described below, is killed by a 1:70 but not by a 1:90 dilution of phenol in 10 minutes. The nutrient medium is a maltose-peptone broth containing 4 per cent. of maltose and 1 per cent. of peptone (Armour's special peptone for testing antiseptics and disinfectants according to the U. S. Dept. of Agriculture Circular No. 198); pH of the broth is adjusted to 5.6 to 5.8. The nutrient agar (for stock cultures) is made by adding 1.5 per cent. of powdered agar to the broth of the composition stated.

The inoculum is prepared in the following manner: Glass beads 5 to 6 mm. in diameter are placed in 2-ounce flint glass bottles, to a height of about 2 to 3 cm. from the bottom. The broth described above is added carefully to fill all spaces between the beads but not to cover them completely. The bottles are fitted with cotton plugs and sterilized in an autoclave, with the

precautions required for autoclaving sugar media. After inoculation with the stock culture (from the agar slant), the bottles are placed in an incubator and kept at 28°C. for ten days. At the end of this period the cotton plugs are replaced by sterile rubber stoppers and the bottles shaken vigorously for 5 minutes. Then, 5 ml. of sterile saline are added and the bottles shaken again at this point for 5 minutes; finally, an additional 15 ml. of saline are added, the bottles shaken for 3 minutes, and the contents filtered through a 200-mesh monel metal sieve (U. S. Bureau of Standards no. 200). The screen may be soldered into a funnel shape or a disc may be cut which can be folded and fitted into a funnel, and the entire apparatus, viz. funnel, screen and flask wrapped together for sterilization. The suspension obtained by filtration is standardized, using the haemocytometer and counting pipette. The stock suspension should contain approximately 15,000,000 spores per milliliter; by a 1:10 dilution the standard inoculum is obtained containing 1,500,000 spores per ml.

As to the test itself, the temperature of medication is 20°C., and the proportions are 0.5 ml. of the standard inoculum to 5 ml. of fungicide dilution. At intervals of 1, 5 and 10 minutes, a 4 mm. loopful of the organism-disinfectant mixture is inoculated into tubes containing 10 ml. of the maltose-peptone broth of the stated composition. The results are read after 10 days of incubation at 28°C.

Since an experienced worker can make transfers without any difficulty every 20 seconds, a test involving 15 dilutions may be carried out in 20 minutes.

The Disinfectant Scientific Committee of the National Association of Insecticide and Disinfectant Manufacturers proposed that fungicidal solutions used for foot-baths should destroy the inoculating dosage in 1 minute; products used for general disinfecting purposes (on inanimate objects) should destroy this inoculum in 10 minutes.

PRACTICAL ADEQUACY OF THE SPORE COUNT

While the requirements as to the time period in which the fungicidal effect should take place are self-explanatory in view

of the pertinent practical applications, reference should be made here to the adequacy of the specified spore count in the inoculum

TABLE 1
Field studies

INSTITUTIONS*	NUMBER OF STUDENTS	FUNGI COLONIES PER ML.	
		Foot pan, plain water capacity 4 gal.	Swabbing shower and locker room floors, 4 square inches swabbed, placed in 10 ml. sterile N saline
a. Vocational School (girls' department)	57	4	23†
	40	1	12
b. Senior High School (boys' department)	300	31	4
	300	20	3
c. Junior High School (girls' department)	157	2	2
	143	10	2
d. Junior High School, same as c (boys' department)	195	0	2
	180	12	3
e. Junior High School (girls' department)	113	5	3
f. Junior High School, same as e (boys' department)	170	18	29‡
g. Institution (girls)	25	3	24
	30	5	15
h. Institution (boys) same building as g	35	0	5
	70	21	5

* a, b, c, d, e, and f represent Philadelphia public schools; g and h represent the Philadelphia Central Y. M. C. A.

† 3 colonies of *Trichophyton rosaceum*—99.9 per cent of the fungi found in all tests were the common black and blue-green species.

‡ High—58 per ml.

for the purposes of the test. In order to obtain an indication of the number of spores ordinarily encountered under practical conditions, a number of counts were made of water alone con-

tained in foot pans. This was supplemented by spore counts obtained by swabbing the floors of shower and locker rooms in various schools and other institutions. The results given in table 1 indicate that the spore count of the inoculum of 1,500,000 spores is at least 5000 times the number found in field studies herein reported. Thus a safety factor of an adequate magnitude appears to be provided for by this detail of the proposed method.

APPLICABILITY TO OTHER FUNGI

As indicated above, the method lends itself to the evaluation of the fungicidal action with respect to pathogenic fungi other than *Trichophyton rosaceum*. As a matter of record (and for possible reference) the phenol resistance of *Epidermophyton interdigitale* and *Epidermophyton gyseum* is expressed by the minimum fungicidal concentrations in 10 minutes of 1:80 at 20°C. and 1:120 at 37°C. for both organisms.

SUMMARY

The paper describes a method for the evaluation of the fungicidal action of water-soluble or water-miscible preparations, intended particularly for prophylactic purposes (e.g., foot-baths) and for application to inanimate objects. *Trichophyton rosaceum* is used as the test organism. For the sake of uniformity of routine bacteriological testing technic, the operating procedure has been patterned along the lines of that of the Food and Drug Administration method of testing disinfectants.

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REFERENCES

- KLARMANN, E., SHTERNOV, V. A., AND GATES, L. W. 1934 The bactericidal and fungicidal action of homologous halogen phenol derivatives and its "quasi-specific" character. I. Derivatives of parachlorophenol. J. Lab. Clin. Med., 19, 835-851. II. Derivatives of orthochlorophenol. Ibid., 20, 40-67.