INFLUENCE OF HUMIDITY AND AGING ON THE GERMINATION OF THE CONIDIA OF ALTERNARIA TENUISSIMA (Fr.) WILT

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Introduction

Humidity is one of the important factors which influenc e the germination of fungus spores. Some of the spores do not germinate except and unless they are in contact with water, others are capable of germination on dry surfaces in an atmosphere of high humidity, whereas some spores of fungi are able to germinate even at 0% humidity.[5, 6, 16] It could, therefore, be concluded that humidity requirements of fungus spores differ widely from one to another.

It is also a common observation that the age affects both the per cent viability and germination of a sample of fungus spores. However, little quantitative information is available on such effects. Anderson, Henry and Morgan[3] working on the effects of temperature and humidity upon the viability of the conidia of *Piricularia oryzae* state that conidia remained viable longest when stored under cool and dry conditions. Similar results were obtained by Lambert[9] on the teleospores of *Puccinia graminis tritici*, by Angell and Hill[1] on *Peronospora parasitica*, by Noble[11] on *Urocystis tritici*, by Rosen and Weetman[15] on *Puccinia coronata* and by Piemeisel[13] on *Ustilago zeae*.

On the contrary, Hashioka[8] working on *Sphaerotheca fuliginea* and Angeli and Hill[1] on *Perenospora tabacini* have shown that cool and moist conditions were best for the longevity of the spores. Doran[7] stated that the longevity of aeceiospores of *Cronartium ribicola* stored at 70°, 15°, 23°C. was unaffected by the temperature. When aeciospores were stored in moist air, they retained their viability longer than when stored in dry air. These results agree with those of Anderson[4] on the spores of *Cylindrecladium scoparium*, Manaeval[10] and Peltier[14] working on uredospores of *Puccinia graminis tritici* reported that low temperatures and intermediate humidities were best if the spores were to be stored for some time. Naqvi and Good[12] observed that the spores of *Monilinia fructicola* gave best preservation of spore vigour when stored at 75% relative humidity, while humidities higher than 95% and lower than 75% caused injuries. A pronounced delay was observed in all other conditions of storage. The rate at which this delay developed was virtually independent of storage temperature.

The present work deals with the humidity requirements of the conidia of *Alternaria tenuissima* (Fr.) Wilt. for germination which is a pathogen of Brinjal and was collected for the first time by Azmatullah Khan of the Department of Plant Protection, Pakistan, in the year 1950, and later identified by Wiltshire as *Alternatia tenuissima* (Fr.) Wilt. The fungus was selected also for the ease of handling in laboratory, abundant sporulation and its importance as a pathogen.

Materials and Methods

Fungus was isolated from the specimen No. 74 of the Herbarium, Department of Botany, University of Karachi. It was found through a series of experiments that the fungus isolate grew best on potato dextrose agar (Potato 200 gms., dextrose 40 gms., agar 30 gms. and distilled water 1000 mls. at 23-25°C.) and sporulated profusely.

For the study of the effect of humidity on germination fresh spores were needed. They were obtained by adding 30 mls. distilled water to the culture and brushing the colony gently with a fine camel's hair brush. The spore suspension was decanted onto a filter paper placed in a Buchner's funnel. The spores were filtered under pressure by connecting the funnel with a fully released tap by means of a tube. The filter paper became

crisp within 4-6 hours. The filter paper bearing spores was than held in an incubator maintained at 23°C. for about 12 hours to complete the drying. Spores prior to and after the immersion in water were tested for germination. Spores thus obtained are referred to as *fresh.*

The pieces of filter paper bearing the spores were then exposed to different relative humidities. Glass jars containing 250 mls. of saturated solutions of lithium chloride, potassium nitrate, sodium chlorate, zinc sulphate and sodium dihydrogen phosphate were used which maintained 15, 45, 75, 90 and 95% relative humidities respectively. Anhydrous calcium chloride maintained 0% humidity. Watch glasses containing spores were kept on clay pipe triangles fitted in each jar. All the jars were kept at 23°C. Spores were left for two and a half months in these humidities and tested for germination.

Spores stored at the optimum relative humidities were further allowed to remain in the same atmosphere for six and a half months and the effect of the aging on the germination was studied after two, three and a half and six and a half months by counting the number of viable spores.

For germination counts, spores as prepared above were spread over the petri plate containing potato dextrose agar, which was divided into 5 sectors. A sample of 500 spores—100 from each sector—was examined for each determination of the germination to the nearest percentage.

Criterion for the germination was the emergance of a germ tube equal to half the length of the spores. Germination counts were made at regular intervals.

Results.

Spores were stored at 0, 15, 45, 75, 90 and 95% relative humidities maintained at 23°C. for two and half months. The germination counts were taken to the nearest per cent as described under materials and methods. The results are shown in Fig. 1.

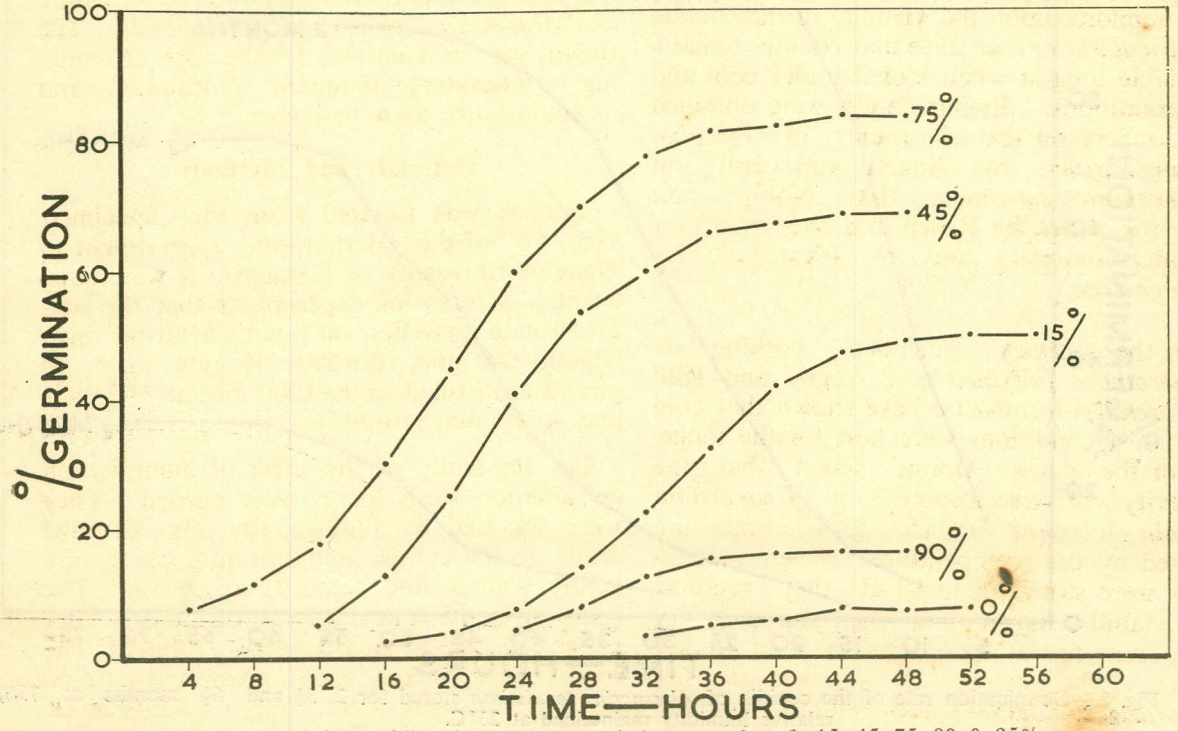

Fig. 1.—Germination rates of the conidia of *Alternaria tenuissima* stored at 0, 15, 45, 75, 90 & 95% relative humidities for 2½ months at 23°C.

The conidia stored at 75% relative humidity started germinating after 4 hours and exhibited a smooth curve and reached the constancy of 83% within 48 hours. Spore samples stored at 45° relative humidity followed the same pattern of the curve and only 69% of the spores germinated in 48 hours and it was initiated around 12 hours.

At 0% relative humidity the spores did germinate but they started after 32 hours and attained only 8% germination after 52 hours. The pattern of the curve was also slightly different from those of 15, 45 75% relative humidities. In the spores sample stored at very high relative humidity such as 90% the curve did not show pronounced rise as in the case of spores stored at 75, 45 and 15% relative humidity. The initiation was after 24 hours and the completion of germination was at 44 hours.

It could, therefore, be briefly stated that after the lapse of 48 hours, 85, 63, 59, 17 and 8% germination was attained by the spore samples exposed to 75, 45, 15, 90 and 0% relative humidities respectively. These observations suggested that very high and very low relative humidities were detrimental to the germination of the conidia.

It is, therefore, concluded that 75% humidity maintained at 23°C. was most suitable for the spores, both for the rate and percentage of germination. Although 75% relative humidity is obviously the best, even then the per cent germination of the conidia was only 83 which is slightly less than the fresh spores This suggested that the viability of conidia decreased during the course of time, though the conditions for storage were most favourable.

This delay is the germination of the spores was probably due to the effect of aging. To ascertain this factor, spores were stored at 75% relative humidity maintained at 23°C. for a period of six and a half months. The germination counts were taken after the intervals of two, three and a half, and six and a half months as described in materials and methods. Results are shown below in Fig. 2.

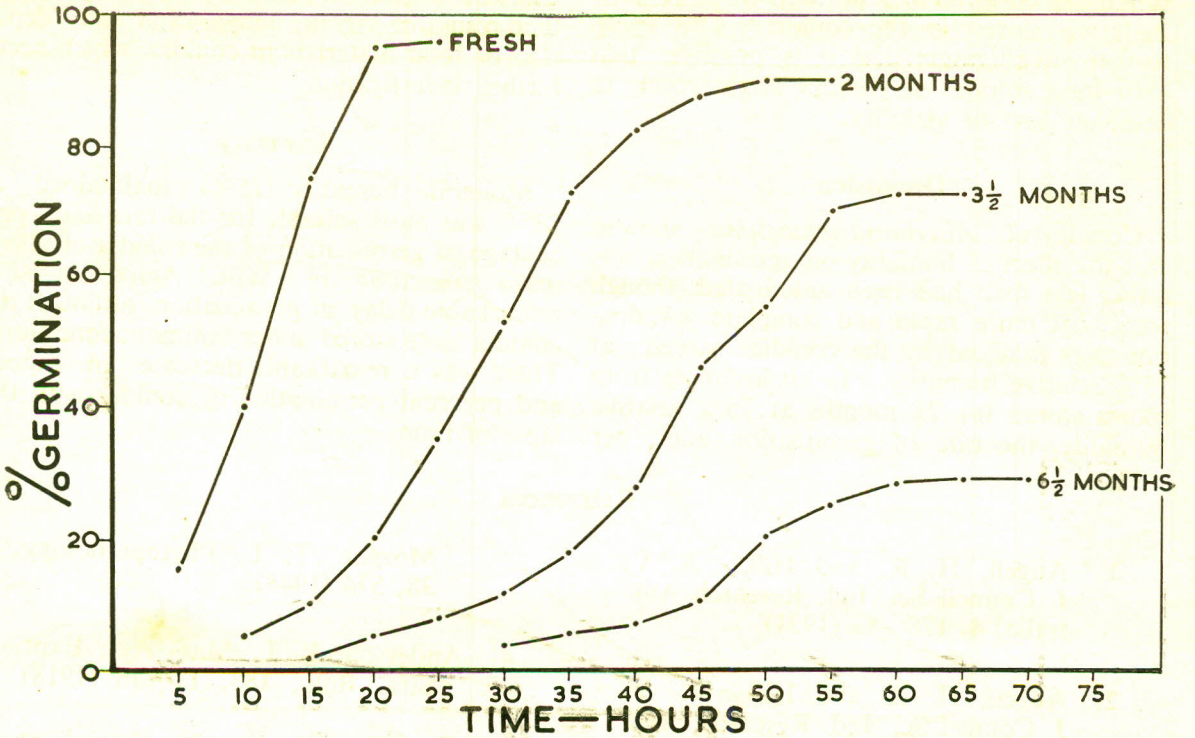

Fig. 2.—Germination rate of the conidia of *Alternaria tenuissima* stored for 2, 3½ and 6½ months at 75% relative humidity maintained at 23°C.

Fresh spores initiated germination at 5 hours and reached nearly 90% within 10-15 hours. After a month of aging, spores showed delay in germination as well as longer time for attaining 85-90% germination. The loss in viability of conidia was not very notable upto two months' aging. Spores stored for $3\frac{1}{2}$ months initiated germination at 15 hours and showed 75% germination at 65 hours. The storage had remarkable effect on the viability of the condia and about 25% conidia became non-viable in a matter of $3\frac{1}{2}$ months. Spores stored for $6\frac{1}{2}$ months showed a great delay in germination. Around 30 hours the spores initially germinated and reached about 30% germination in 70 hours.

Storage for $6\frac{1}{2}$ months had a greater effect on the viability and in this case only about 25% conidia remained viable.

In all the cases of storage a delay in the germination of spores may account for each of vigour desiccation and depletion of food materials stored in the conidia. This needs further investigation and it is possible that extra-long storage of conidia might result in complete loss of viability.

Discussion

Conidia of *Alternaria tenuissima* showed that the effect of humidity on germination was rather less than had been anticipated, though somewhat more rapid and complete germination was attained by the conidia stored at 75% relative humidity. In all instances from spores stored for $2\frac{1}{2}$ months at 75% relative humidity, the rate of germination and per cent viability decreased followed by 45, 15, 90 and 0% relative humidities in descending order. There was clear evidence that both excessively dry and excessively moist conditions were injurious to conidia and that moisture was more injurious than drought. This injurious effect of moisture seemed likely to be tied up with the rapid metabolism of moist spores and the injurious effect of dryness seemed related to dehydration of conidia. These two aspects of physiology of spores need further investigation.

Aging also had a remarkable effect on the onset of germination and viability of conidia. Fresh spores germinated rather quickly (5 hours) and completed 85-90% germination in 10-15 hours, whereas $6\frac{1}{2}$ months old spores at 75% humidity showed onset of germination at 30 hours and 30% germination at 70 hours. The storing not only affected the onset and initial time of germination but also affected the per cent viability of conidia. The loss in viability due to aging may be due to the loss of vigour, dehydration and depletion of food materials in conidia, which needs further investigation.

Summary

Moderate humidity (75%) maintained at 23°C was most suitable for the rate and percentage of germination of the conidia of *Alternaria tenuissima* (Fr.) Wilt. Aging caused a remarkable delay in germination, although the conidia were stored under optimum conditions. There was a remarkable decrease in vigour and per cent germination of conidia with the lapse of time.

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