STUDIES ON THE SPORULATION OF MACROPHOMINA PHASEOLI (MAUBL.) ASHBY. CAUSING STEM-ROT DISEASE OF JUTE WITH REFERENCE TO THE POSSIBLE CAUSES OF OUTBREAK OF THE DISEASE IN NATURE

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The present paper deals with the investigation of factors which might favour the spread of the stem rot, by far the most important disease of jute plant. Studies on temperature and humidity favouring the development of pycnidia on the affected plant and germination of pycnospores have been taken.

It is found that pycnidial pustules of *Macrophomina phaseoli* develop within a wide range of temperature (29-40°C.) and relatively narrow range of humidity (81-100%). Pycnospores on the other hand could germinate in even wider range of temperature (20-41° C.) the range of relative humidity is however, narrower (96-100%).

Introduction

Stem rot is by far the most important disease of jute plant. On the seedlings the disease manifests itself as dark lesions both on hypocotyl and on cotyledons. Throughout the growing period, due to secondary infection by air borne pycnospores the disease may appear on any part of a leaf showing buff coloured lesions. Later, the entire leaf including the mid-rib becomes affected. On the stems, the lesions generally start at the nodes coming in contact with a diseased leaf. These are small at the beginning , but later on may girdle the entire stem, causing the plant to die or a break to appear at the point of girdling (Plate 1).

The disease may spread from several sources as the organism can be seed-borne as well as soil-borne[8,14] through the diseased stubbles. Besides, there are innumerable collateral hosts[6,11,13,15] for this organism, from which the disease may easily spread through air-borne spores. Even with great care in eliminating the primary sources of inoculum, by sowing as far as possible healthy and treated seeds and also adopting crop rotation, there would always remain possibility of the presence of the organism on the seedlings through a certain percentage of infected seeds and from the collateral hosts, which are so many that some one always would be found growing in the neighbouring fields. This spread could be checked beforehand by the application of fungicides at proper moment if all the predisposing factors for such spread are well known.

The purpose of the present investigation is to study the various possible factors which might favour the spread of the disease. For this, studies on the factors such as temperature and humidity favouring the development of pycnidia on the affected plants and germination of pycnospores have been undertaken.

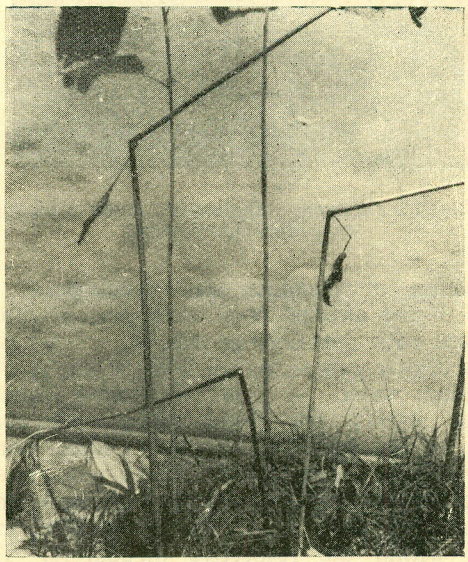

Plate 1.—Jute plants showing break at stem-rot affected region.

Experiments and Results

Effect of Temperature and Humidity on Sporulation—To obtain plants with stem-rot lesion, a number of plants, 21 days old was artificially inoculated with mycelial fragments of *M. phaseoli* grown for 48 hours exposure to 32°C. within a humid chamber and then were removed beside the glass windows inside the laboratory for 4 days.

From each of the affected plants (Plate 2) diseased portion showing black lesion was cut out keeping some healthy tissues at both ends of the stem piece. The size of the individual pieces was about 1.5-2 inches in length.

To find out the effect of temperature on the

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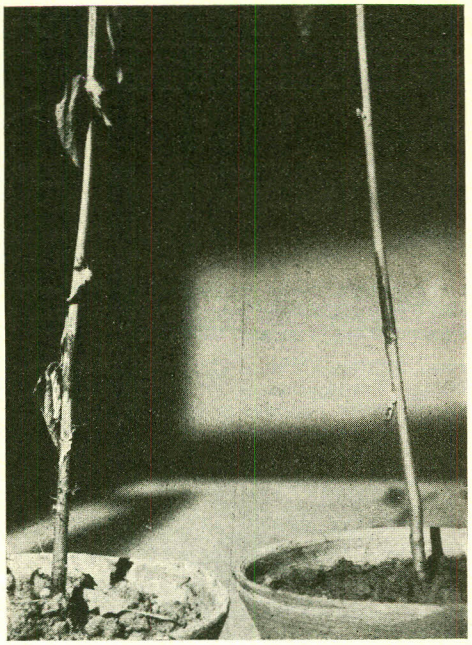

Plate 2.—Jute plants showing stem-rot lesion at the region of artificial inoculation.

production of pycnidia and pycnospores four pieces of the affected stem were placed within a sterilized petri dish having two No. 1 Whatman sterilised filter papers soaked with 10 ml. sterilised redistilled water so that maximum humidity prevails within the dishes. The stem pieces rested on two glass rods to avoid direct contact with soaked filter paper. Three replicates of such dishes were kept under each of the 6 temperatures, such as 2°, 25, 30, 35, 40 and 45°C. maintained within the respective incubators. Three further replicates were kept at room temperature, which fluctuated between 29-37° C. during the period (7 days) under experiment (Table 1).

TABLE 1.—EFFECT OF TEMPERATURE ON THE PYCNIDIAL DEVELOPMENT OF M. PHASEOLI.

No. of cuttings	Temp. in °C	No. of hours for pycnidial development.	Average No of pycnidia per field (1.77 sq. mm.)
12	20	0	0
12	25	0	0
12	29-37	56	16
12	30	96	7
12	35	92	11
12	40	100	3
12	45	0	0

Results show that no pycnidia developed at 25°C. or below. At room temperature which fluctuated between 29-37°C, number of hours required for pycnidial development was much less than those under constant temperature of 30,35 and 40°C. Among the constant temperatures, 35°C. required minimum time for pycnidial formation than either of 30 or 40°C. The number of pycnidia developed per microscopic field was also higher at room temperature.

A second experiment was conducted similarly as above inside sterilised dishes each having two No. 1 Whatman filter papers but this time affected stem cuttings were exposed to a large number of combinations of different relative humidity and temperature. The relative humidity at which stem cuttings were exposed were 65, 81, 88, 92, 96, and 100%. These were prepared by using a volume of 10 ml. redistilled water and respective aqueous saturated solutions of the different chemicals[1] (Table 2) to each of the dishes prepared as above.

TABLE 2.—COMPOSITION AND CONCENTRATION OF SOLUTION USED FOR MAINTAINING SPECIFIC HUMIDITY WITHIN AIR-TIGHT PETRI DISHES.

Materials used	Concentration of solution.	Theoretical R.H. %.
Redistilled water		100
$H_2C_2O_4.2H_2O$	Saturated aq.	96
K_2HPO_4 ..	,,	92
K_2CrO_4 ..	,,	88
$(NH_4)_2 SO_4$ ..	,,	81
$Mg(C_2H_3O_2)_2.4H_2O$	,,	65

In all cases two replicates were used and incubated up to 7 days. Observations were taken periodically and time taken for pycnidial development under each treatment was noted (Table 3).

As the development of pycnidia at 20 and 25°C. was not observed in the previous experiment (Table 1), these two temperatures were not included in this experiment. It was presumed that the exactness of the relative humidity mentioned would suffer as the same saturated solutions were used under different temperatures. But this would be the case with all the relative humidity tested under each temperature.

Pycnidia were found to develop within a wide range of temperature and relative humidity. Fluctuating temperature from 29°C. to 37°C. (room temperature) gave the best result both from the

TABLE 3.—EFFECT OF TEMPERATURE AND RELATIVE HUMIDITY UPON THE DEVELOPMENT OF PYCNIDIA OF M. PHASEOLI.

No. of cuttings per treatment	Temp. in °C.	Percentage of R. H.	Hours taken for pycnidial development	Average No. of pycnidia per microscopic field (1.77 sq. mm.)
4	30	100	96	7
,,	,,	96	100	6
,,	,,	92	104	5
,,	,,	88	116	4
,,	,,	81	132	3
,,	,,	65	0	0
4	35	100	88	8
,,	,,	96	92	12
,,	,,	92	96	10
,,	,,	88	96	10
,,	,,	81	100	8
,,	,,	65	0	0
4	40	100	112	3
,,	,,	96	132	4
,,	,,	92	116	4
,,	,,	88	112	4
,,	,,	81	112	4
,,	,,	65	0	0
4	29-37	100	56	16
,,	,,	96	60	15
,,	,,	92	60	14
,,	,,	88	60	14
,,	,,	81	60	9
,,	,,	65	0	0

stand point of earlier formation as well as greater frequency per unit area. At 65% relative humidity under any of the temperatures tested, no pycnidial appearance was noted.

Effect of Temperature and Relative Humidity on Germination of Pycnospores.—Requirements of favourable temperature and humidity for germination and infection have been studied with spores of fungal pathogens such as uredospores of *P. graminis*,[12] oidia of *Erysiphe polygoni* [10] and with spores of other organisms by different workers. [2, 3, 4, 5, 9].

With the present organism, the first experiment undertaken here was the effect of different temperatures on the germination of pycnospores in hanging drop culture. The temperatures at which the spores were tested for germination were 5, 10, 15, 20, 22 27, 30, 33, 36, 40, 41 and 45°C. regulated by different incubators.

For preparation of hanging drop culture a drop of sterilized water was taken on a clean cover glass and 2-3 pycnidia from affected stem were gently pressed to liberate the spores in water. The cover glass was inverted carefully without disturbing the water drop and placed over a glass ring already stuck on a slide with Vaseline. The cover slip was similarly attached by means of Vaseline, smeared at three places on the upper surface of the ring. The hanging drop culture thus prepared was placed within a pair of petri dishes having two saturated filter papers with redistilled water Four replicates of these hanging drop cultures were put up for each temperature. The hanging drop cultures within the petri dishes were then placed inside respective incubators. Observation under microscope was carried at an interval of 30 minutes up to a period of 6 hrs. After 6 hrs. of incubation the cover slips containing germinating spores were lifted from the ring and mounted on a drop of cotton blue glycerine over a clean microscope slide. Each slide was examined under microscope at different fields. Average of 10 readings of spores counted and number germinated per microscopic field has been tabulated (Table 4) for each temperature.

TABLE 4.—EFFECT OF DIFFERENT TEMPERATURES ON THE GERMINATION OF SPORES AND THE LENGTH OF GERM TUBE IN HANGING DROP CULTURE.

Temperature in °C.	No. of spores counted	No. of spores germinated	Percentage of germination	Time taken for germination of 25% spores (in hrs.)
5	312	0	0	0
10	688	0	0	0
15	712	0	0	0
20	318	133	42.05	—
22	722	329	45.43	4.00
27	705	467	66.25	3.50
30	690	521	75.50	3.00
33	700	525	75.00	3.00
36	688	436	63.37	3.50
40	334	107	32.00	—
41	743	117	15.74	5.50
45	697	0	0	0

The range of temperature from 27° to 36°C. appeared suitable for good germination of pycnospores. No germination was observed at 5, 10 and 45°C.

So far the experiment on germination of spores was done in water suspension at different temperatures. It was intended here to find out the effect of different relative humidity on rates of germination at a constant temperature. The germination tests were carried out with 100, 99.04, 98.65, 98.03, 96 and 93% relative humidity at 20°C.[19].

The humidity chambers were prepared out of fruit jars of 1,000 ml. capacity having their glass lids

grounded, so that airtight conditions could be maintained. Before pouring the respective chemicals in solutions and at saturated condition, the glass jars were thoroughly cleaned, dried and rinsed with redistilled water.

Spores were taken out from pycnidia with the help of a clean and sterilized sharp needle-head and streaked over clean and sterilised cover glass, that served as spore-carrier. Each carrier was then attached with paraffin to one end of a small glass rod. The other end of the rod was heated and embedded in paraffin inside one glass ring that had been sealed with paraffin to the inner part of a glass top of a humidity chamber. This top was then substituted for one on a chamber (Plate 3) kept inside the incubator. A set of two replications were made with each relative humidity and kept at a constant temperature of 20°C. within an incubator for 24 hours. One similar set of two replications from each were kept at room temperature (31-37°C.) and a third set was kept at constant temperature of 30°C. After 24 hours the cover glasses were detached from the glass rod, cleaned thoroughly at the point of attachment and last trace of wax was removed before mounting on a drop of cotton blue, put upon a clean slide. Percentage of germination of spores and length of germ tubes were noted in Table 5.

It appears from this experiment that atmospheric humidity up to 99.24% at 30°C. favoured only a small (11.24%) percentage of spores to germinate even in 24 hours duration, whereas in a fluctuating temperature of 31-37°C. the percentage of spore germination raised sharply (59%). This was found due to the condensation of water in droplets on the spore-carrier.

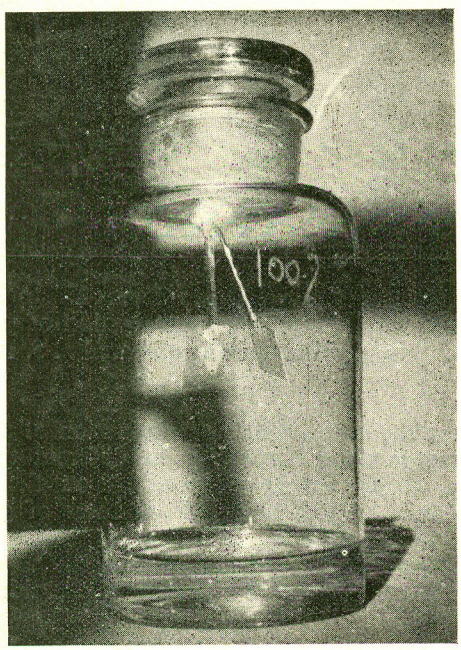

Plate 3.—Humidity chamber with three spore carriers hanging from the lower end of the glass stopper.

Observation on the Occurrence of Stem-rot Disease in Nature.—Regular observations on the occurrence of stem-rot disease in the experimental plots of Pakistan Central Jute Committee was carried out right from the 4th week of April, 1959 when seedlings in most of the fields were 7 days old. Stem-rot affected plants were frequently met with in the plots of 'district trial' as well as in the plots of 'method of sowing' experiment. These two experimental plots were kept under observation. From the first week of May till the 3rd week of June, although there was quite a large percentage of plants both in the 'district trial' and 'method of sowing' trial, were found affected with stem-rot disease, very few plants showed pycnidial pustules on the lesions unless one was completely dead due to heavy attack. Appearance of pycnidial pustules on leaves were seldom met with up to this time. On the June 3, 1959 all the affected plants from 'district trial' fields were rouged out and the fields were kept clean from any undergrowths. This was not done with the plots of the 'method of sowing' experiment. There were profuse undergrowths

TABLE 5.—EFFECT OF RELATIVE HUMIDITY ON THE GERMINATION OF PYCNOSPORES AND DEVELOPMENT OF GERM TUBES.

Temp. °C.	Percentage of R. H.	No. of spores counted	No. of spores germinated	Percentage of germination	Average length of 10 germ tubes after 24 hours in microns
20	100	325	40	12.30	—
,,	99.24	301	21	7.0	104
,,	98.65	316	17	5.38	92
,,	98.03	324	13	4.01	78
,,	96.0	299	7	2.34	40
,,	93.0	307	0	0	0
30	100	320	91	28.44	—
,,	99.24	293	34	11.24	176
,,	98.65	322	29	9.00	149
,,	98.03	319	24	7.52	123
,,	96.00	319	12	3.76	66
,,	93.00	—	0	0	0
31-37	100	303	182	60.00	600
,,	99.24	310	183	59.00	600
,,	98.65	318	130	41.00	140
,,	98.03	309	92	29.76	125
,,	96.0	325	8	2.46	27
,,	93.0	216	0	0.00	0

of grasses together with the accumulation of leaves shed from the standing jute crop. Sporadic stem-rot affected plants were also present in the field. On the June 24, 1959 some leaves of scattered plants were found with the appearance of pycnidial pustules. By 27th of the month the disease appeared on the leaves in epidemic form. In some blocks 50% plants (Table 6) showed most of their lower leaves affected with stem-rot disease with characteristic pycnidial pustules. This was, however, not found with the jute plants of the 'district trial.'

TABLE 6.—PERCENTAGE OF PLANTS SHOWING PYCNIDIAL PUSTULES ON LEAVES.

Block No.	Total plants	No. of plants with leaf infection	Percentage of affected plants
1	813	241	29.64
2	778	198	25.45
3	490	285	58.16
4	1372	432	31.47
5	1421	392	27.58
6	1060	588	55.47
7	1307	405	31.06
8	443	221	50.00

The accumulation of leaf debris and dead affected plants amidst the undergrowths of grasses under the standing crop and the prevailing weather condition obviously influenced the sudden spread of the disease and the appearance of pycnidial pustules on leaves of the plants in the plots of the 'method of sowing' experiment during the 4th week of June. There was plenty of rainfall almost daily during a 12-day period excepting one day in the middle and consequently the field soil was more or less in saturated condition. The above period was followed by six rainless, warm and sultry nights having wind velocity from 1-8 (average 4.6) miles per hour.

Almost every day up to 8 o' clock in the morning during this week leaves of jute plants in the field were found to remain completely soaked with condensed water deposited from the exudation from the leaves. This condensed water on the surface of leaves which might have lasted more than 8 hours (from mid-night till 8 a.m.) would favour the germination of pycnospores[7] which might have been blown to the leaf surface earlier from the undergrowths of the standing crop, where dead plants and fallen leaves with pycnidial pustules were present.

The absence of pycnidial development during the second and third week of June (when almost every day, there was heavy rain) may be explained due to the sweeping down of the pycnospores from the leaves if there was any, deposited on the leaf surface by the air current. Same explanation might be cited for gradual disappearance and from further spread of the disease on leaves of the plants of the same field during the first week of July, 1959, when there was rainfall in almost every day thereby splashing the air-borne spores from leaves on earth or there might be some other explanation for the above observation which could not be put forward in the present work.

From the available data with Jute Research Institute for the preceding years, it was found that the appearance of pycnidial pustules on leaves of jute plant during 1958 was practically nil but in 1957 twice during the growing season, once during the fourth week of May and another time during the 3rd and 4th week of October there were abundant pycnidial pustules on leaves. From weather bulletin it was found that both the times pycnidial pustules appeared during the rainless days which were preceded by periods having heavy shower of rain. The wind velocity in the two periods respectively was from 6-9 (average 8.3) and 1-3 (average 1.5) miles per hour.

Discussion

Among the range of temperature used (Table 1 and Fig. 1) the constant temperature 20 and 25°C. did not favour the pycnidial development. Among the three other constant temperatures used, 35° C. favoured earlier development of pycnidia. This happened within 92-hour exposure of the affected stem pieces to 35°C. The fluctuating room temperature (29-37°C.), however, was found best both from the point of earlier development (56 hours) and frequencey of pycnidia per unit area. As regards effect of humidity on pycnidial development, their appearance was noted within 81 to 100% R. H. (Table 3 and Fig. 2).

Pycnospores were found to germinate from 20°C. to 41°C. in hanging drop culture (Table 4 and Fig. 1) the peak germination was found at 30°C. (75.50). The best range for germination was found between 27 and 36°C. A constant temperature of 30°C. relative humidity as high as 99.24% could not stimulate the germination of pycnospores beyond 11.24% (Table 5) which was very low when compared to that of hanging drop culture under the same temperature. At 100% relative humidity (Table 5) though germination rate accelerated somewhat more, it was still very low in comparison to that of hanging drop culture. Similar observation was also made by other workers,[4,13] with uredospores of *Puccinia glumarum* and *P. graminis tritici* and with many

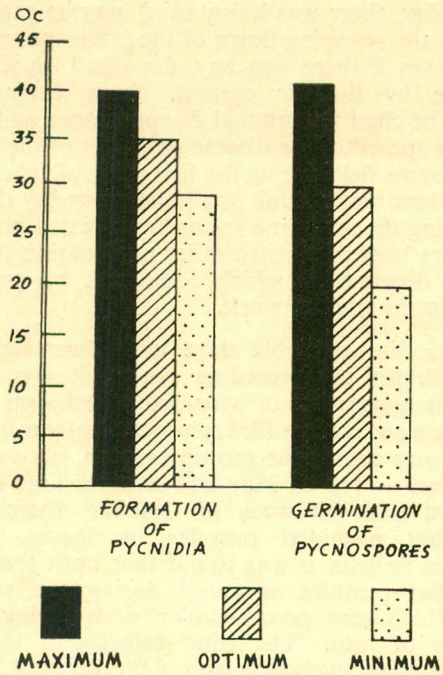

Fig. 1.—Range of temperature for pycnidial formation and germination of pycnospores.

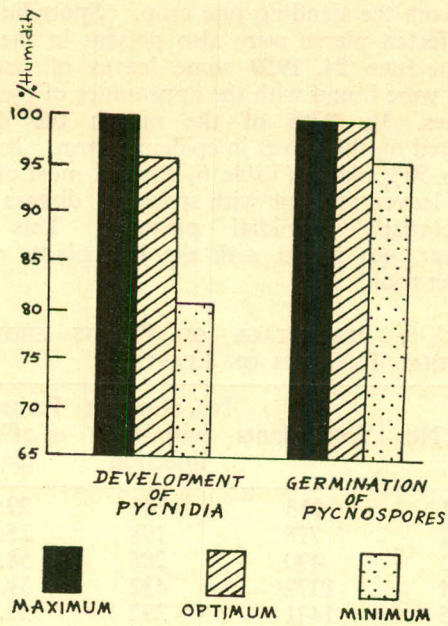

Fig. 2.—Range of relative humidity for pycnidial development and germination of pycnospores.

other pathogenic fungi. At fluctuating room temperature (31-37°C.), however, when there was condensation on spore-carriers kept at 99.24, 98.65 and 98.03% R.H. the germination rates increased decidedly more than those met with under similar relative humidity at a constant temperature of 30°C. (Table 5). At 30°C. constant temperature no condensation of water was noticed.

From the foregoing observations it appeared that pycnidial development could take place within wide range of temperature (29-40°C.) and atmospheric humidity (81-100%), whereas germination of pycnospores though could take place within wider range of temperature (20-41°C.), the range of favourable humidity was very narrow (Table 5 and Fig. 2). The germination was observed much better in presence of free water than at high atmospheric humidity of 99.24% or 100%.

In nature the spread of the disease in an epidemic form was noticed just after a few days when excessive condensation of free water on the surface of leaves occurred almost every day up to 8 o'clock in the morning. Six days before this happened there was heavy rain almost everyday for ten consecutive days. This created optimum condition for the development of pycnidia and pycnospores on the existing scattered diseased plants and on the littered leaf debris amidst the undergrowths of grasses at the foot of the standing crops. The source of inocula thus was ready in the field from which pycnospores were carried by air current to the leaf surface throughout the day and night. During the six rainless and sultry nights in the presence of condensed water on the leaves the germination of pycnospores might have followed by infection and penetration, resulting in epidemic condition in the field.

Summary

Pycnidial pustules of *Macrophomina phaseoli* was found to develop within a wide range of temperature, from 29-40°C. and within 81-100% relative humidity.

Pycnospores on the other hand though could germinate within even wider range of temperature (20-41°C.), the range of favourable atmospheric relative humidity was however found to be very narrow (96-100% R.H.). Most favourable range of temperature for germination was found to be from 27-36°C. At a constant temperature of 30°C. in humid atmosphere (100% R.H.) only 28.44% pycnospores showed their germination within 24 hours whereas in fluctuating temperature of 31-37°C. in similar condition when condensation on spore-carriers occurred about 60% pycnospores germinated. In free water on the other hand at constant temperature of 30°C. over 75% pycnos-

pores put up their germ-tubes.

The above findings corroborated the observation made in a jute field during the month of June, 1959 where a week before there were only a few scattered plants with pycnidial pustules on some of their leaves. In some blocks the disease spread to 50% plants or over following excessive condensation of free water on the surface of leaves which was noted almost every morning during 5 to 6 days time.

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