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Studies on the Sporulation of *Pyricularia Oryzae* Cav.

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Introduction : It has been the experience of many Research workers engaged in the study of rice blast disease that considerable difficulties are being encountered in securing sufficient amount of sporulation in culture while multiplying the pathogen for inoculation purposes. It is also known that unless the spore load is of sufficient magnitude successful results are not often obtained in pathogenicity tests. Although certain media like host-leaf extract, rice straw, barley seed, rice polished agar etc., have been suggested by different workers in other countries, it was not always successful to obtain abundant sporulation of the pathogen by using the above mentioned media under conditions existing at this centre. To a certain extent it may perhaps be due to variations in the environmental and other conditions prevailing here. But the suitability of the medium appears to be an important single factor that influences the extent of sporulation. With this object in view, different media were tested to ascertain their suitability for growth and sporulation of *Pyricularia oryzae* under laboratory conditions. Experiments were also conducted to find out the optimum incubation period for maximum sporulation. The size of condia produced in different media was also determined with a view to observe variations if any. The results obtained in these experiments are presented in this paper.

Materials and Methods : Chopped leaves of the following host plants viz., *Oryza sativa*, *Eleusine coracana*, *Brachiaria mutica*, *Panicum repens and Pennisetum purpureum* were tested as media to study the sporulation of *Pyricularia oryzae*. The addition of two synthetic media viz., Richard's solution and Leonian's solution to the leaf bits of the above hosts was also tested for their added effect on sporulation. Addition of mere water to the leaf bits was also included in the experiments for the purposes of control.

The leaf material of the different hosts was cut into small pieces of uniform size as far as possible and then transferred to 250 ml. Erlenmeyer flasks. In each flask 10 gm. of the leaf material was taken and 10 ml. of water or the synthetic media was added. The flasks were then autoclaved at 15 lb. pressure for one hour. The sterilised flasks were inoculated with equal quantities of the inoculum of *P. oryzae* cut out with a sterilised cork borer from a uniformly grown culture in petridishes. The inoculated flasks were

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incubated at 28°C in the laboratory for a period of 14 days. Five days after inoculation the flasks were shaken and again incubated. Three replicates were maintained.

After 14 days incubation period, 50 ml. of water was added to each flask and shaken thoroughly to dislodge the spores from the leaf bits. The suspension was allowed to stand for some time and then filtered through a tea filter to prevent leaf bits and mycelial mats entering the spore suspension. The spore suspension thus obtained under different treatments was then examined and the number of spores per ml. of the suspension was computed by using a Haemocytometer.

Spore measurements were also recorded in each of the above treatments. Twentyfive spores at random were measured in each of the replication and the maximum, minimum as well as the average of the spore measurements were taken.

The number of days required for obtaining maximum sporulation was also ascertained by conducting another experiment using *Brachiaria mutica* leaves plus Richard's solution. Here also the inoculated flasks were incubated at 28°C and number of spores per ml. of suspension was ascertained as before using a Haemocytometer at 2 days interval starting from the eighth day of inoculation. Three replicates were maintained and the spore counts were taken upto 22 days after inoculation.

Results: 1. *Sporulation f P. oryzae in different media:* In this experiment conducted in two separate sets, Richard's solution was added as at base in the other Leonian's solution was added. The data obtained were scrutinised statistically and was found to be highly significant. The details are presented in table 1.

It is seen from Table 1, that in the first set the medium *Brachiaria mutica* + Richard's solution is significantly superior to all the other media producing maximum number of spores, followed by *B. mutica* + water and *Panicum repens* + Richard's solution. The medium paddy leaves + water has recorded the minimum number of spores.

Similarly in the second set also the medium *B.mutica* + Leonian's solution followed by *B. mutica* + water have recorded significantly higher sporulation than all the other media.

The media *Eleusine coracana* + Richard's solution and *E. coracana* + Leonian's solution have also sustained good sperulation of *P. oryzae.*

2. *Size of spores of P. oryzae in different media (in microns).* The data on the average, maximum and minimum length and breadth for 25 spores selected at random from each replicate is finished in Table 2.

TABLE 1. *Mean number of spores of P. oryzae in different media (in ten thousands)*

Treatments (media)		Mean number of spores per ml.	Mean number of spores per ml.
Brachiaria mutica	+Richard's solution	465.8	
,,	+Leonian's solution		365.0
,,	+Water	390.8	313.3
Oryza sativa	+Richard's solution	129.0	
,,	+Leonian's solution		140.0
,,	+Water	92.5	113.3
Eleusine coracana	+Richard's solution	170.8	
,,	+Leonian's solution		145.8
,,	+Water	174.2	121.6
Panicum repens	+Richard's solution	241.7	
,,	+Leonian's solution		79.2
,,	+Water	103.3	73.3
Pennisetum purpureum	+Richard's solution	138.3	
,,	+Leonian's solution		116.7
,,	+Water	108.3	122.5
	S.E.	9.6	8.0
	C.D.	28 5	23.7

TABLE 2. *Size of spores in different media (in microns)*

Treatments (Media)		Average		Maximum		Minimum	
		l	b	l	b	l	b
Brachiaria mutica	+Richard's solution	16.65	6 00	20.0	6 5	14 0	5 0
,,	+Leonian's solution	17.22	6 40	19.5	7 0	14.0	5.5
,,	+Water	16 24	6.15	23.0	7.0	13.0	5.0
Oryza sativa	+Richard's solution	15 84	5.86	21 0	7.0	12.0	5.0
,,	+Leonian's solution	16.48	6.06	19.5	6.5	15.0	5.5
,,	+Water	16 82	6.11	20.0	7.0	13.0	5.0
Eleusine coracana	+Richard's solution	16.20	5.98	20.0	7.0	12.0	5 0
,,	+Leonian's solution	16.44	5.96	18 5	6 5	14 0	5.5
,,	+Water	15.88	5.98	21.0	7.0	12.0	5.0
Panicum repens	+Richard's solution	16.22	6 06	20.0	6.5	12.0	5.0
,,	+Leonian's solution	15.90	5.92	19.0	6.5	14.0	5.0
,,	+Water	15.79	5.90	19.0	7.0	12 5	5 0
Pennisetum purpureum	+Richard's solution	15.84	5 96	18.5	7.0	14.5	5 5
,,	+Leonian's solution	16.16	6.02	19.5	6.5	13.5	5 5
,,	+Water	16.03	6.02	18.0	6.5	14.0	5.0

l = length b = breadth

It is seen from table 2, that there is no significant variation in the size of spores formed in the different media. However, the spores produced in the medium *B.mutica*+Leonian's solution are comparatively larger than those produced in any of the other media,

3. *Sporulation at different intervals of time* :—The data collected on the number of spores produced at different intervals of time from the date of inoculation are presented in table 3.

TABLE 3. *Mean number of spores of P. oryzae produced at different intervals (in ten thousands)*

Treatments (days)	Mean number of spores per ml. (in ten thousands)
8 days after inoculation	21.00
10 days after inoculation	221.67
12 days after inoculation	463.25
14 days after inoculation	489.92
16 days after inoculation	322.83
18 days after inoculation	264.92
20 days after inoculation	234.00
22 days after inoculation	155.42
S. E.	18.47
C. D.	56.03

Table 3 shows that the two treatments 14 and 12 days after inoculation are on par and superior to all the other treatments in recording higher number of spores. A steady increase in sporulation from the eighth day upto the fourteenth day and a gradual decrease thereafter is noticed.

Discussion and conclusion : Various investigators had reported different methods and cultural practices for obtaining good sporulation in the case of *P. oryzae* in culture. According to Ren Jong Chiu *et al.*, (1963), good sporulation was obtained when the blast fungus was inoculated on barley seeds and this procedure had been adopted at the Academia Sinica. Similar methods to obtain abundant sporulation of *P. oryzae* using barley seeds had been reported by Thirumalachar *et al.*, (1963) and Kazuo Goto (1963). Frances M. Latterell *et al.*, (1963) obtained good sporulation when *P. oryzae* was grown on rice polish agar and the spore suspension was also found to be free from any perceptible mycelial fragments and other extraneous matter. Purushothaman *et al.*, (1968) obtained abundant sporulation by using a modified Goto's medium containing barley grains and chopped rice leaves.

n the present investigations, the sporulation of *P. oryzae* on the leaf tissues of some of the plants belonging to the order – Graminae with the addition of either synthetic media or water was studied in detail. Among the different media tried, maximum sporulation was obtained in the media *B. mutica*+Richard's solution, *B. mutica*+Leonian's solution and *B. mutica*+water respectively and the spore suspension was also free from any perceptible mycelial fragments and other leaf tissues.

According to Tanaka *et al.*, (1951,a), addition of a small amount of hot water extract of rice straw stimulated growth and sporulation of *P. oryzae*. This was attributed to the presence of growth promoting factors like biotin in the straw. The effect of biotin and other growth promoting substances on the sporulation of *P. oryzae* had been studied by many investigators. Addition of authentic biotin, even at the concentration of 3 μg. per gram was found to increase the fungal growth (Tanaka, 1963). The large number of spores produced in the case of *B. mutica* leaves+water, in the present studies might also be due to some growth promoting substance present in the leaves of *B. mutica*, but the actual substance responsible had not been ascertained. The maximum sporulation obtained in the case of *B. mutica*+synthetic media might be due to the high nutrient content of the synthetic media besides the growth promoting substance present in the leaf tissue. Similarly the slightly larger size of spores obtained in the medium *B. mutica*×Leonian's solution might be due to the high nutrient content along with the growth promoting substance.

Henry and Anderson (1948), had found out that spores were produced most rapidly and in great quantity at 28°C, but they decreased after 9 days. In the present studies, maximum sporulation was obtained 14 days after inoculation at 28°C. Sporulation was found to increase rapidly after 10 days and gradually decreased after 14 days.

Hence, it can be concluded that abundant sporulation of *Pyricularia oryzae* can be obtained in cultures using the media *B.mutica*+Richard's solution, *B mutica*+Leonian's solution or *B.mutica*+water, 12 to 14 days after inoculation.

Summary: Sporulation studies with the rice blast fungus – *Pyricularia oryzae* Cav. were conducted to find out a suitable medium using leaf tissues of five plants viz., *Brachiaria mutica*, *Oryza sativa*, *Eleusine coracana*, *Panicum repens* and *Pennisetum purpureum* belonging to the Order – Graminae with the addition of either Richard's solution, Leonian's solution or water. Maximum sporulation was obtained in the case of *B. mutica*+Richard's solution, *B. mutica*+Leonian's solution and *B, mutica*+water respectively. The spore suspension was also free from any perceptile mycelial fragments or extraneous

matter. There was no significant variation in the size of spores produced in the different media. Maximum sporulation was obtained 12 to 14 days after inoculation.

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* Original not seen.