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Effect of substrate moisture on the sporulation of seed-borne Trichoconis Padwickii Ganguly from Nigerian rice (Oryza Sativa L.)

Einfluß des Feuchtigkeitsgehaltes der Substrate auf die Sporenbildung von Trichoconis padwickii Ganguly an nigerianischem Reis (Oryza Sativa L.)

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1. Introduction

Incubation factors in seed health testing have been subjects of various studies. Neergaard and Saad (21), Singh (23), Vargass and Wilcoxson (26), Baker (3), Karlberg (7), Flannigan (4), Kolk and Karlberg (8) have individually studied various aspects of temperature as a vital abiotic factor. Light has also been subject of detailed studies by Leach (9, 10, 11, 12, 13, 14, 15), Aragaki (1), Zimmer and McKeen (27) as well as many others. Substrate moisture has on the other hand not been so systematically studied as other incubation factors.

The view that substrate moisture does influence result of seed health testing (24) emphasizes the urgency of intensive investigation of this ecological aspect particularly in respect of comparative analysis.

Limonard (16) observed that certain seed-borne infections appear in higher or lower percentages depending on the moisture level and that the higher the moisture level, the lower the infection percentages of the seed-borne fungi.

This phenomenon was termed Wet-blotter-Effect, an effect that was however not observed in the infection percentage count of wheat, barley, oats and ryegrass (17).

Rice is one food crop, whose consumption and therefore demand is ever on the increase. One of the constrains in its production is the disease factor. Pyricularia oryzae Cav., Drechslera oryzae (Breda De Haan) Subram. and Jain Trichoconis padwickii. Gang. are among the causal organisms which induce field epiphytotics (22). The use of disease free seeds is no doubt one of the cheapest precautionary measures. To obtain these, however, depends on the seed health test, the use of sensitive culture technique and stimulating incubation factors – like the optimum

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substrate moisture under investigation. The planting value of the seeds can only be established when health and viability are compared. Without the stimulating incubation factors, neither the detection of pathogens that generate true explosive epiphytotics nor the true rate of germination of the seeds is possible. The paucity of information on the effect of substrate moisture on the seed heath testing of the tropical most important cereal crops like rice, calls therefore for urgent attention. This paper aims at reporting the result of an investigation on the influence of substrate moisture an the Sporulation of Trichoconis padwickii, Gang. on rice.

2. **Materials and Methods**

Twenty samples of rice seeds, selected after some screening were used in this investigation. Apart from the moisture factor, the experiments were carried out based on the standard blotter culture technique (6).

Three layers of white filter papers (2.4 gr weight) were placed in clear plastic petri dishes, of 9 cm diametre and moistened with 4, 6, 8, 10 and 12 ml respectively of tap water. These moisture levels represent 167, 250, 333, 417 and 500 percent respectively the weight of the filter papers. Twenty-five seeds were randomly selected from each sample and plated per petri dish to constitute a plot, and this was replicated sixteen times for all moisture levels.

The incubation period was for eight days at a constant room temperature of 20°C under an alternating cycle of 12 hours light and darkness respectively. The irradiation was by means of Philips black light fluorescent lamps (NUV).

Identification exercise based on the diagnostic (growth) characters of the fungus was carried out with the aid of a wild M5 stereoscopic Microscope 6–50 times magnifications. The criteria for the evaluation of moisture effect were:

1. infection percentage and

2. growth intensity (sporulation) of fungus in each moisture level. Infection percentage as distinct from its intensity is calculated as

$$\frac{X}{N} \times \frac{100}{1}$$

whereby N is the total number of incubated seeds and X the number of seeds invaded and effectively colonised by T. padwickii after 8 days incubation. In the blotter testing technique degree of fungal growth and sporulation varies from seed to seed. This according to Neergaard (19) is the function of differences in inoculum harboured in individual seeds which has some direct bearing with inoculum potential (18, 19). Owing to the indirect relationship between spore load (sporulation) and disease severity (infection intensity), the degree of sporulation per seed can in our context be equaled to desease intensity since one gives rise to the other – heavy sporulation generates high disease intensity which is depicted by seed rot on the blotter. The differentiation therefore of both factors-incidence on the one hand and sporulation intensity on the other in seed health test makes

the use of an indexing system as suggested by various researchers (2, 5, 19), imperative.

An indexing system was used, whereby the degree of fungal sporulation was calibrated into: Heavy, moderate and light infections; whereby heavy infection had 3 points, the moderate 2 and light infection 1 point (Tab. 1). The infection of the seed is regarded as heavy when the greater portion of seed surface is effectively colonised. There must be macroscopic evidence of heavy sporulation and profuse mycelial growth. This is mostly accompanied by a characteristic pink purple stains, which sorrounds heavily infected seeds on the blotter. When about 50% of seed surface is covered with moderate sporulation and mycelial development of the fungus this category of infection is regarded as moderate.

It is light, when the infection manifests localised sporulation with little or no mycelial presence and on less than 50% of the seed surface (sparse). The synopsis of this differentiated sum therefore makes up the disease intensity of an infected seed.

3. **Results**

The various moisture levels showed tendentious effect on the infection percentage of T. padwickii. The extreme moisture levels had lowest percentage counts of 32 and 34.6 average for 4 and 12 ml respectively. The highest mean infection percentage was obtained at 8, 10 ml moisture levels respectively. Each had 39.9% (Tab. 2). Furthermore, disease intensity was again lowest at both extreme moisture levels while 8 and 10 ml moisture gave the highest and higher average values (Fig. 1).

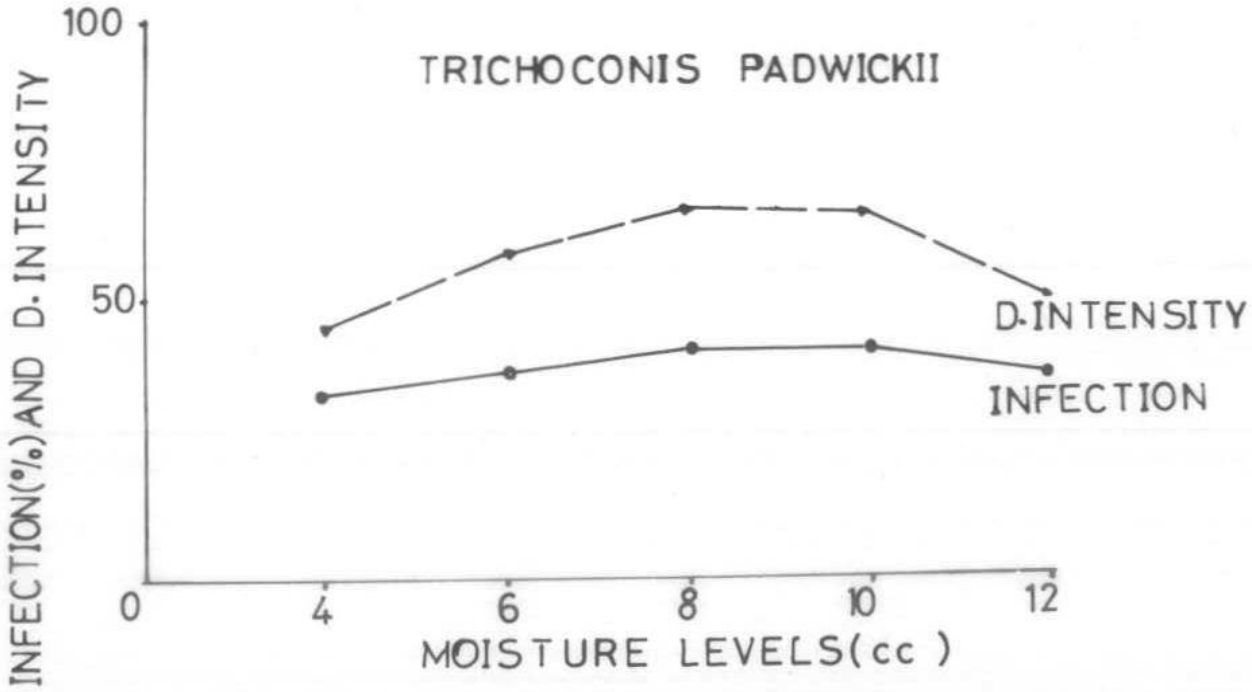

Fig. 1: The moisture effect on Incidence (infection) and Disease or Sporulation Intensity of T. padwickii.

Table 1: Infection percentage and disease index scores of T. padwickii at 3 degrees of infection (sporulation) levels.

Heavy Infection	4 ml		6 ml		8 ml		10 ml		12 ml	
Sample Nos.	Inf.%	SI	Inf.%	SI	Inf.%	SI	Inf.%	SI	Inf.%	SI
1	1.0	3.0	1.5	4.5	3.0	9.0	1.5	4.5	1.5	4.5
2	2.0	6.0	3.0	9.0	0.5	1.5	1.5	4.5	0.0	0.0
3 +	-	-	14.5	43.5	19.5	58.5	20.5	61.5	10.0	30.0
4 +	-	-	13.5	40.5	18.5	55.5	17.0	51.0	8.5	22.5
5 +	-	-	1.0	3.0	4.0	12.0	3.0	9.0	0.0	0.0
6 +	-	-	9.0	27.0	10.5	31.5	9.0	27.0	5.0	15.0
7 +	-	-	15.0	45.0	5.5	16.5	7.0	21.0	3.5	10.5
8	4.0	12.0	6.0	18.0	3.5	10.5	2.0	6.0	1.5	4.5
9	2.0	6.0	2.5	7.5	2.5	7.5	3.0	9.0	1.0	3.0
10 +	-	-	17.5	52.5	23.5	70.5	19.0	57.0	10.5	31.5
11 +	-	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12	4.0	12.0	0.5	1.5	6.0	18.0	2.0	6.0	0.5	1.5
13	3.0	9.0	5.0	15.0	11.0	33.0	10.0	30.0	3.5	10.5
14	0.0	0.0	4.0	12.0	5.0	15.0	2.5	7.5	0.0	0.0
15	1.0	3.0	4.0	12.0	5.0	15.0	5.5	16.5	2.5	7.5
16	3.0	9.0	2.5	7.5	5.0	15.0	4.5	13.5	2.0	6.0
17	2.0	6.0	6.5	19.5	3.0	9.0	4.0	12.0	3.5	10.5
18	3.0	9.0	8.0	24.0	8.0	24.0	9.5	28.5	8.5	25.5
19	3.0	9.0	0.0	0.0	7.5	22.5	4.5	13.5	2.5	7.5
20	1.0	3.0	2.5	7.5	4.5	13.5	2.5	7.5	3.0	9.0

+ = 4 ml Moisture level was not examined.

Moderate Infection	4 ml		6 ml		8 ml		10 ml		12 ml	
Sample Nos.	Inf.%	SI	Inf.%	SI	Inf.%	SI	Inf.%	SI	Inf.%	SI
1	2.0	4.0	2.0	4.0	5.5	11.0	3.5	7.0	3.5	7.0
2	3.0	6.0	3.5	7.0	3.5	7.0	5.5	11.0	2.5	5.0
3 +	-	-	15.0	30.0	14.0	28.0	13.0	26.0	10.0	20.0
4 +	-	-	14.5	29.0	15.0	30.0	19.5	39.0	17.0	34.0
5 +	-	-	5.5	11.0	4.5	9.0	5.0	10.0	4.5	9.0
6 +	-	-	20.5	41.0	13.5	27.0	16.0	32.0	14.5	29.0
7 +	-	-	14.0	28.0	10.5	21.0	9.5	19.0	9.0	18.0
8	6.0	12.0	6.5	13.0	3.0	6.0	8.5	17.0	6.0	12.0
9	4.0	8.0	3.0	6.0	5.0	10.0	2.0	4.0	3.5	7.0
10 +	-	-	18.5	37.0	22.0	44.0	18.0	36.0	21.0	42.0
11 +	-	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12	5.0	10.0	4.5	9.0	11.0	22.0	6.5	13.0	5.0	10.0
13	8.0	16.0	8.5	17.0	10.5	21.0	10.5	21.0	5.5	10.0
14	7.0	14.0	15.0	30.0	13.5	27.0	14.0	28.0	4.5	9.0
15	6.0	12.0	7.0	14.0	15.0	30.0	8.0	16.0	5.0	10.0
16	5.0	10.0	11.0	22.0	12.5	25.0	23.0	46.0	8.5	17.0
17	6.0	12.0	5.0	10.0	8.0	16.0	10.5	21.0	6.0	12.0
18	13.0	26.0	15.0	30.0	17.5	35.0	14.5	29.0	13.0	26.0
19	7.0	14.0	2.5	5.0	9.0	18.0	12.0	24.0	7.0	14.0
20	8.0	16.0	11.0	22.0	16.0	32.0	19.0	38.0	9.5	19.0

+ = 4 ml Moisture level was not examined.

Light Infection	4 ml		6 ml		8 ml		10 ml		12 ml	
Sample Nos.	Inf.%	SI	Inf.%	SI	Inf.%	SI	Inf.%	SI	Inf.%	SI
1	8.0	8.0	10.5	10.5	15.0	15.0	11.0	11.0	12.5	12.5
2	15.0	15.0	10.5	10.5	13.0	13.0	12.0	12.0	9.0	9.0
3 +	-	-	21.5	21.5	20.0	20.0	18.5	18.5	23.0	23.0
4 +	-	-	27.5	27.5	31.0	31.0	23.0	23.0	26.5	26.5
5 +	-	-	12.0	12.0	10.0	10.0	11.5	11.5	12.0	12.0
6 +	-	-	28.0	28.0	31.0	31.0	25.0	25.0	26.5	26.5
7 +	-	-	15.5	15.5	16.0	16.0	22.5	22.5	19.5	19.5
8	7.0	7.0	8.0	8.0	9.0	9.0	8.0	8.0	10.0	10.0
9	4.0	4.0	5.0	5.0	6.5	6.5	9.0	9.0	9.0	9.0
10 +	-	-	30.0	30.0	21.5	21.5	27.5	27.5	29.5	29.5
11 +	-	-	1.5	1.5	0.5	0.5	0.5	0.5	0.0	0.0
12	23.0	23.0	19.5	19.5	22.5	22.5	27.0	27.0	23.5	23.5
13	20.0	20.0	16.5	16.5	18.0	18.0	27.5	27.5	27.5	27.5
14	45.0	45.0	43.0	43.0	40.0	40.0	40.0	40.0	38.0	38.0
15	42.0	42.0	35.0	35.0	31.0	31.0	35.5	35.5	32.0	32.0
16	33.0	33.0	33.0	33.0	35.5	35.5	29.0	29.0	39.0	39.0
17	22.0	22.0	18.5	18.5	21.5	21.5	21.5	21.5	21.5	21.5
18	35.0	35.0	32.0	32.0	29.0	29.0	32.5	32.5	35.5	35.5
19	10.0	10.0	16.0	16.0	28.5	28.5	25.0	25.0	33.5	33.5
20	43.0	43.0	41.0	41.0	43.5	43.5	43.5	43.5	48.5	48.5

+ = 4 ml Moisture level was not examined.

Table 2: Sum of infection percentage and disease index scores of T. padwickii on rice seeds from 20 samples. Moisture levels in ml per Petri Dish

	4		6		8		10		12	
Samples	Inf.%	SI	Inf.%	SI*	Inf.%	SI	Inf.%	SI	Inf.%	SI
1	11.0	15.0	14.0	19.0	23.5	35.0	16.0	22.0	17.5	24.0
2	20.0	27.0	17.0	26.5	17.0	21.0	19.0	27.0	12.0	14.0
3 x	-	-	51.0	95.0	53.5	106.0	52.0	106.0	43.0	73.0
4 x	-	-	55.5	97.0	64.5	116.0	59.5	113.0	52.0	86.0
5 x	-	-	18.5	26.0	18.5	31.0	19.5	30.5	16.5	21.0
6 x	-	-	57.5	96.0	55.0	89.0	50.0	84.0	46.0	70.5
7 x	-	-	44.4	88.5	32.0	53.5	39.0	62.5	32.0	48.0
8	17.0	31.0	20.5	39.0	15.5	25.5	18.5	31.0	17.5	26.5
9	10.0	18.0	10.5	18.0	14.0	24.0	14.0	22.0	9.5	15.0
10 x	-	-	66.0	119.0	67.0	136.0	64.5	120.5	61.0	103.0
11 x	-	-	1.5	1.5	0.5	0.5	0.5	0.5	0.0	0.0
12	32.0	57.0	24.5	30.0	39.5	62.5	35.5	46.0	28.5	35.0
13	31.0	45.0	30.0	48.5	39.5	72.0	48.0	78.0	36.0	48.0
14	52.0	59.0	62.0	85.0	58.5	82.0	56.5	75.5	42.5	47.0
15	49.0	57.0	46.0	61.0	51.0	76.0	49.0	68.0	39.5	49.5
16	41.0	52.0	46.5	62.5	53.0	75.5	56.5	88.0	49.5	62.0
17	30.0	40.0	30.0	48.0	32.5	46.5	36.0	54.5	31.0	44.0
18	51.0	70.0	55.0	86.0	54.5	88.0	56.5	89.5	56.0	87.0
19	20.0	33.0	18.5	21.0	45.0	69.0	41.5	62.5	40.5	55.0
20	52.0	62.0	54.5	70.0	64.0	89.0	65.0	89.0	61.0	76.5
Mean Inf %age	32.0		36.2		39.9		39.9		34.6	
Mean SI Scores	43.5		56.9		64.9		63.5		49.3	

x = 4 ml was not tested. SI* = Sporulation Intensity.

4. **Discussion**

It is evident from the result that the various moisture levels did actually influence the fungus. Though the difference was not statistically significant, varying infection percentages of T. padwickii were recorded at the respective moisture levels. The highest count of the fungus was at 8 and 10 ml respectively. The sporulation intensity which is an accentuation of the fungal incidence also varied with the amount of substrate moisture.

The extreme levels gave lowest sporulation intensity, while the middle ones were higher. 8 and 10 ml gave mean sporulation intensity scores of 65 and 63.5 respectively. It is therefore evident that the optimum moisture for the sporulation and development of T. padwickii orbits around 8 and 10 ml. There is clear evidence of some oerrelation between fungal incidence, depicted by infection percentage and the infection or sporulation intensity. This should not be surprising since inoculum density (Spore load) vary greatly from seed to seed. This difference in amount of inoculum per seed and particularly varying incubation conditions – according to Neergaard and Wallen (20) have been responsible for discrepancies in comparative test results.

Incidence as well as sporulation intensity of pathogenic fungi are all functions of viable inoculum density (18) and also laboratory incubation factor, which in turn could promote growth, fructification and development of symptoms (20). The more favourable the incubation factors are, the faster the growth and more importantly the easier the detection or identification of the fungus on the seeds, and consequently the greater the chance of establishing the true planting value of a seed-lot. This aspect is important as that is the sole objective of seed health tests.

Observational experience has shown that some incubation factors would suppress weak incidences and therefore escape being recorded by the analyst. This situation obviously highlights the importance of incubation factors, such as substrate moisture. Tempe and Limonard (24) have for example shown ample evidence of seed-fungal-bacterial interactions. Earlier, Ledingham et al. (15) and Limonard (16) observed bacterial antagonism against fungi in higher moisture, whereby certain seed-borne infection appeared in lower percentages with increase in substrate moisture. Various temperate seeds have shown this phenomenon which Limonard (17) termed Wet blotter effect. From our results, the moisture effect on T. padwickii is insignificant. Tempe and Limonard (25) found that moisture difference had also no effect on Drechslera oryzae. This does not however negate the importance of optimum moisture level, to which many seed-borne fungi favourably respond. Kolk und Karlberg (8) and Iloba (5) established moisture optima for various other fungi. The higher infection percentage as well as sporulation intensity at 8 and 10 ml respectively indicate the level of optimum substrate moisture for the sporulation and easy detection of T. padwickii by blotter method. Though the inoculum potential of the randomly selected seeds may have been important in accentuating disease intensity, it appears to be proportional to favourable optimum moisture level. This moisture optimum of 8 ml particularly reflected the standard blotter moisture normally used in routine seed health testing (6). It therefore confirms the suitability of the standard moisture level for the detection of T. padwickii. The seeming

insignificant effect of substrate moisture on other fungal microflora of Oryza sativa L. was earlier observed (5). This seeming insignificant effect statistically of substrate moisture on the sporulation on T. padwickii may not be unconnected with the aquatic nature of the host (Oryza sativa). In view of lower count of fungal incidence particularly at the saturated moisture levels, the possibility of bacterial antagonism requires further investigation.

Seed-borne inoculum potential has been expressed not only in terms of percentage of infection but also in terms of the degree of severity (19). Malalasekera and Colhoun (18) established positive correlation between growth rate and sporulation of Fusarium culmorum on individual seeds with inoculated spore load per seed. The severity of disease and seedling losses on the other hand was also positively correlated with the spore load per seed. It is therefore evident that the interpretation of laboratory result, based on only disease incidence expressed in infection percentage may not accurately give the actual degree of seed-borne inoculum potential. Practical experiences have shown that heavy sporulation on infected seeds correlated with high severity of seed infection and as such reflect high disease intensity of such seed.

The importance of Disease Intensity (sporulation) as distinct from infection (fungal presence) in seed health testing has earlier been advocated (5). Aulakah et al. (2) have also demonstrated its importance in forecasting field performance of infected seeds. The correlation of infection and its intensity under optimum moisture level once more emphasizes the importance of integrating both parameters in interpreting the results of seed health tests.

Summary

Twenty samples of rice seeds known to be infected with Trichoconis padwickii were used in this investigation. Substrate moisture levels tested were 4, 6, 8, 10 and 12 millilitres respectively. Though the extreme moisture levels gave lower infection percentages and intensity than others, the difference in general was not statistically significant. Specific moisture effect was therefore not observed on the fungus.

Zusammenfassung

In dieser Untersuchung wurden 20 Reissamenproben verwendet, deren Infektion mit Trichoconis padwickii bekannt war. Die geprüften Substratmengen betrugen 4, 6, 8, 10 und 12 Milliliter. Obwohl die extremen Substratmengen niedrigere Infektionsraten und -stärken ergaben, war der Unterschied statistisch nicht signifikant. Ein spezifischer Einfluß des Feuchtigkeitsgehaltes der Substrate auf die Entwicklung des Pilzes konnte daher nicht nachgewiesen werden.

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