

Occurrence, Transmission and Remedial Aspects of *Drechslera oryzae* in Paddy (*Oryza sativa* L.)

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ABSTRACT Among the paddy seed samples, Jaya variety showed high incidence of *Drechslera oryzae*. The severity of the pathogen expressed an array of seedling symptoms like yellowing, brown necrosis, spots, streaks and decaying. The rate of symptom expression was more in case of artificially coated samples than its corresponding control. Hot water treatment at 60°C remained most effective in suppressing the colonization of *D. oryzae*. However, 55°C was found to enhance the seed germination and seedling growth. In case of three fungicides, Hadron at 0.1 % level reduced the incidence of target pathogen and enhanced the seed germination percentage and seedling growth over control. *Trichoderma pseudokoningii* was most useful not only in controlling the incidence of *D. oryzae* but also increased the percentage of seed germination and seedling growth over control and other antagonists. Aqueous leaf extracts of *Thuja orientalis* proved a better treatment for the minimization of *D. oryzae* which in turn enhanced the seed germination and seedling growth compared to its control.

Rice (*Oryza sativa* L.) is the most widely grown cereal crop serving as the staple food for more than half of the world population. In India, rice occupies the first place both in area of about 42.24 million hectares and produces about 82 million tones [1]. Rice is prone to several diseases caused by fungi, bacteria, viral and mycoplasmal pathogens. Among various disease causing organisms, *Drechslera oryzae* is one of the most important fungus known to cause brown spot disease of rice [2]. Padmanabhan *et al.* [3] have reported 40 to 90 per cent loss in yield due to grain infection. So the present study deals an array of paddy seed samples screened for the occurrence of *D. oryzae*, its transmission and also the effect of different seed treatments.

MATERIALS AND METHODS

I Field Survey and collection of seed samples

Having survey made in Mandya, H.D. Kote, Mysore, Jaipura (Mysore District), Darwad, Davanagere and Shivamoga districts of Karnataka, the paddy samples of Jyothi, Jaya, IR- 64 and IR-20 were collected and stored in polythene bags at room temperature (28±2°C) for further use.

II Determination of per cent incidence of *Drechslera oryzae* in rice

Standard blotter method (SBM), Deep-freeze and Potato dextrose agar method were employed to

determine the per cent incidence of *D. oryzae* in rice [4].

III Isolation and maintenance of *D. oryzae* in Rice

Dominant seedborne fungus *Drechslera oryzae* was isolated from the samples (Jaya variety) with relatively high incidence of the pathogen and maintained on PDA slants and was preserved at 5±2°C.

IV Seed Transmission of *Drechslera oryzae* in paddy

Water agar seedling symptom test was adopted. 10% of water agar was prepared and poured in to a series of sterilized Petri plates. Then the seeds of Jaya variety after having inoculated with the test fungus (*D. oryzae*) and plated on the water agar (10 seeds/plate) in four replicates of 10 seed each were maintained under standard conditions of light and darkness along with proper control. At every intervals of 2 days the seedlings were examined for the occurrence of symptoms such as browning, necrosis, yellowing, spots, streaks, seedling decay etc., the results were recorded for 2 weeks of incubation and were tabulated.

V Effect of seed treatment on *Drechslera oryzae* in paddy

Physical treatment

Seed sample (Jaya) with relatively high incidence of *D. oryzae* were selected to evaluate the efficacy of hot water treatment on the incidence of the fungus.

Hot water treatment

200 seeds from selected sample (Jaya variety) were suspended separately in hot water at different temperatures i.e., 50, 55 and 60°C for 10 minutes. These seeds were then plated on the perspex plates containing 3 layers of moistened blotters and were incubated for 7 days at 22±2°C under 12/12h alternate cycles of NUV and darkness along with proper control. On 8th day of incubation, seeds were carefully examined with the aid of stereobinocular microscope for the occurrence of *D. oryzae*. Percentage of seed germination and seedling growth were also recorded.

Chemical treatment

Seeds of same variety with relatively high incidence of *D. oryzae* were treated with Bavistin, Hadron and Benlate each @ 0.1, 0.2 and 0.3g/100g of seeds.

200 seeds of each treatment were plated on wet blotters in the perspex plates and incubated according to standard procedures. On the 8th day, the seeds were evaluated for the occurrence of *D. oryzae*, germination percentage. Root-shoot length of the seedlings were also measured and the vigour index was calculated. The seeds not treated with fungicide served as corresponding control.

VI Biological Control of *D. oryzae*

Antagonist

Seed samples of Jaya variety having high incidence of *D. oryzae* were treated with different antagonists, *Trichoderma harzianum*, *T. pseudokoningii* and *Pseudomonas fluorescens*.

Seed treatment with *Trichoderma harzianum* and *T. pseudokoningii*

The seeds were rolled on 8-day old actively growing sporulated colonies of *T. harzianum* and *T. pseudokoningii* on PDA plates. The spore load was assessed haemocytometrically and was fixed to the range of 5×10^8 and 3×10^8 spores/g of seeds, respectively. The treated seeds were plated on moistened blotters in plastic plates and incubated for the target fungus, seed germination and seedling growth. The seeds without bioagent treatment served as corresponding control.

Seed treatment with *Pseudomonas fluorescens*

Seeds (200) were suspended for 18h at room temperature in the culture suspension of

P. fluorescens. Such inoculated seeds were then air dried and were placed on wet blotters in plastic plates for incubation. After incubation the incidence of *D. oryzae*, per cent seed germination and vigour index were calculated. Seeds untreated with bacterium incubated under similar conditions served as control.

Plant extracts

Seed samples with high incidence of *D. oryzae* were treated with aqueous leaf extracts of some common plants like *Azadirachta indica*, *Clerodendron inermiae*, *Thuja orientalis*, *Catharanthus roseus*, *Tridax procumbens*, *Coleous aromaticus*, *Ruta graveolens* and *Lucas aspera*. For the preparation of leaf extract of the above mentioned plants, fresh leaves were harvested, washed in water thoroughly. 100g of leaves of each plant was macerated with the help of a mortar and pestle to the thick paste, 100ml of distilled water was added and it was filtered through the muslin cloth. The extract was then centrifuged at 5000 rpm for 15 minutes. The supernatant obtained was collected and stored at 5°C for further use. 200 seeds of selected samples were soaked in separate conical flask containing different leaf extract at 28±2°C for a period of 18 hours. Seeds soaked similarly in distilled water served as control. The treated seed samples were air dried and plated on wet blotters in perspex plates for incubation. On 8th day of incubation, the seeds were examined critically for the occurrence of *D. oryzae* and per cent seed germination. The vigour index was also determined based on the seedling growth.

RESULTS AND DISCUSSION

Out of four seed samples, Jaya variety showed higher incidence of *D. oryzae* and Deep-freeze method proved to be the best (Table 1). As a result of *D. oryzae* infection seedlings showed the symptoms such as browning, necrosis, yellowing, spots, streaks and decaying with varied intensity. On 2nd day of incubation, brown necrosis, wilt and spots on leaves were recorded. On 6th day and 8th day of incubation yellowing, wilt, spots and brown streaks were observed on coleoptile as also the leaves of the seedlings. 10th day onwards, increased severity of disease symptoms were noticed (Table 2, Fig. 1). Comparatively, the seedlings with added inoculum showed high intensity of disease expression with respect to uninoculated sample. Seedlings colonized by the fungus resulted in complete decay with in a short duration of 1 or 2 days.

Table 1. Screening of paddy seed samples for *Drechslera oryzae*

Paddy varieties	Standard blotter method		Deep-freeze method		Potato dextrose agar method	
	% Seed germination	Occurrence of <i>D. oryzae</i> (%)	% Seed germination	Occurrence of <i>D. oryzae</i> (%)	% Seed germination	Occurrence of <i>D. oryzae</i> (%)
Jyothi	30.5	44.5	11.0	46.0	42.0	45.0
Jaya	59.0	61.0	7.5	66.0	30.0	62.0
IR-64	57.5	11.0	12.0	14.5	38.0	12.0

Data based on 200 seeds.

Table 2. Per cent occurrence of disease symptoms in seedlings of 4, 8, 12 and 16 days old seedlings due to *Drechslera oryzae* in paddy cv. Jaya (Water agar seedling symptom test)

Seedling symptoms	% occurrence of disease symptoms							
	Control				Inoculated			
	4 day	8 day	12 day	16 day	4 day	8 day	12 day	16 day
Yellowing	0	2.5	2.5	7.5	0	2.5	7.5	20.0
Brown necrosis	20.0	20.0	20.0	25.5	15.0	15.0	15.0	17.5
Wilt	7.5	12.5	12.5	12.5	10.0	20.0	20.0	25.0
Spots	7.5	15.0	15.0	15.0	7.5	10.0	17.5	17.5
Brown streak	0	17.5	17.5	17.5	0	20.0	20.0	20.0

Data based on the average of 4 replicates.

Table 3. Impact of Hot water treatment on the incidence of *Drechslera oryzae* and seed quality of paddy cv. Jaya

Hot water treatment	Incidence of <i>D. oryzae</i> (%)	Seed germination (%)	Shoot length (cm)	Root length (cm)	V.I.
Control	59.0	87.0	1.7±0.17	2.96±0.11	405.42
50°C	32.0	87.5	2.64±0.13	4.10±0.11	589.75
55°C	24.0	92.0	2.44±0.06	4.40±0.19	629.28
60°C	22.0	90.0	2.81±0.16	4.34±0.19	43.5
C.D.	36.24	N.S.			

Data based on 200 seeds.

N.S. = Non significant.

In the variety Jaya, the incidence of *D. oryzae* was decreased by hot water treatment at 50, 55 and 60°C. However, at 60°C its incidence was minimised. The incidence of *D. oryzae* was depleted by more than two fold over control at 60°C. However, 55°C treatment was promising in reducing the fungal incidence which in turn enhanced the seed germination. On the other hand, hot water treatment also enhanced the root-shoot growth of the seedlings. Correspondingly, the vigour index was also increased over control (Table 3).

Bavistin and Benlate suppressed the establishment of *D. oryzae* at 0.3% concentration. However, Hadron a newly formulated compound @ 0.1% concentration was superior over Bavistin and Benlate which not only controlled the target fungus but also improved seed germination and seedling growth (Table 4).

Compared to *T. harzianum*, *T. pseudokoningii* treatment exhibited minimum occurrence of *D. oryzae*. The seeds treated with bioagents indicated enhanced germination. *Trichoderma pseudokoningii*

Table 4. Efficacy of different fungicides on the incidence of *Drechslera oryzae* and seed quality of paddy

Variation in the occurrence of <i>Drechslera oryzae</i> and seed quality of paddy						
Fungicides	Concentration (%)	% Incidence of <i>D. oryzae</i>	% Seed germination	Shoot length (cm)	Root length (cm)	V.I.
Bavistin	0.1	54.5	79.0	1.51±10.08	2.45±0.08	312.84
	0.2	51.0	75.0	1.74±0.14	2.39±0.19	309.75
	0.3	49.0	83.0	1.79±0.06	2.45±0.09	351.92
C.D.		N.S.	3.25			
Hadron	0.1	50.0	89.0	2.30±0.11	4.21±0.12	579.39
	0.2	57.5	78.0	2.63±0.10	2.79±0.10	422.76
	0.3	62.0	76.0	1.9±0.03	2.04±0.02	299.44
C.D.		N.S.	5.88			
Benlate	0.1	53.0	82.0	1.57±0.06	3.99±0.17	455.92
	0.2	50.0	86.0	1.56±0.00	3.95±0.10	473.86
	0.3	38.0	88.0	1.76±0.04	3.72±0.12	482.24
C.D.		7.65	N.S.			
Control	-	59.0	87.0	1.7±0.17	2.96±0.11	405.42

Data based on 200 seeds

N.S.= Non significant.

Table 5. Role of bioagents on the incidence of *Drechslera oryzae*, seed germination and seed quality of paddy cv. Jaya

Bio agents	% Occurrence of <i>D. oryzae</i>	% Seed germination	Shoot length (cm)	Root length (cm)	V.I.
<i>Trichoderma harzianum</i>	48.0	97.0	2.0±0.03	1.73±0.09	361.81
<i>Trichoderma pseudokoningii</i>	38.0	90.0	2.80±0.12	1.75±0.08	409.5
<i>Pseudomonas fluorescens</i>	51.0	88.0	1.92±0.15	3.03±0.07	435.6
Control (1)	45.0	84.0	1.79±0.03	3.65±0.20	456.96
Control (2)	59.0	87.0	1.7±0.17	2.96±0.11	405.42
C.D.	4.86	4.71			

Data based on 200 seeds

Control (1) = Seeds treated with distilled water.

Control (2) = Seeds without distilled water treatment

Table 6. Effect of leaf extracts of some plants on *Drechslera oryzae*, seed germination and seedling growth of paddy cv. Java

Plant Species (leaf extracts)	% Occurrence of <i>D. oryzae</i>	% Seed germination	Shoot length (cm)	Root length (cm)	V.I.
<i>Azadirachta indica</i>	64.0	88.0	1.77±0.06	3.09±0.22	427.68
<i>Clerodendron inermae</i>	54.0	88.0	1.90±0.07	3.02±0.09	432.96
<i>Thuja orientalis</i>	55.0	93.0	1.76±0.07	2.94±0.11	437.1
<i>Catharanthus roseus</i>	60.0	87.0	1.73±0.06	3.09±0.12	419.34
<i>Leucos aspera</i>	57.0	89.0	1.59±0.11	2.29±0.11	345.32
<i>Tridax procumbens</i>	54.0	82.0	1.98±0.08	2.31±0.25	351.78
<i>Coleus aromaticus</i>	54.0	86.0	1.73±0.14	2.26±0.14	343.14
<i>Ruta graveolens</i>	49.5	96.0	1.38±0.07	1.54±0.06	280.32
Control	63.0	75.0	1.75±0.10	2.53±0.13	321.00
CD. (P=0.05)	N.S.	2.05			

Data based on 200 seeds

N.S.= Non significant.

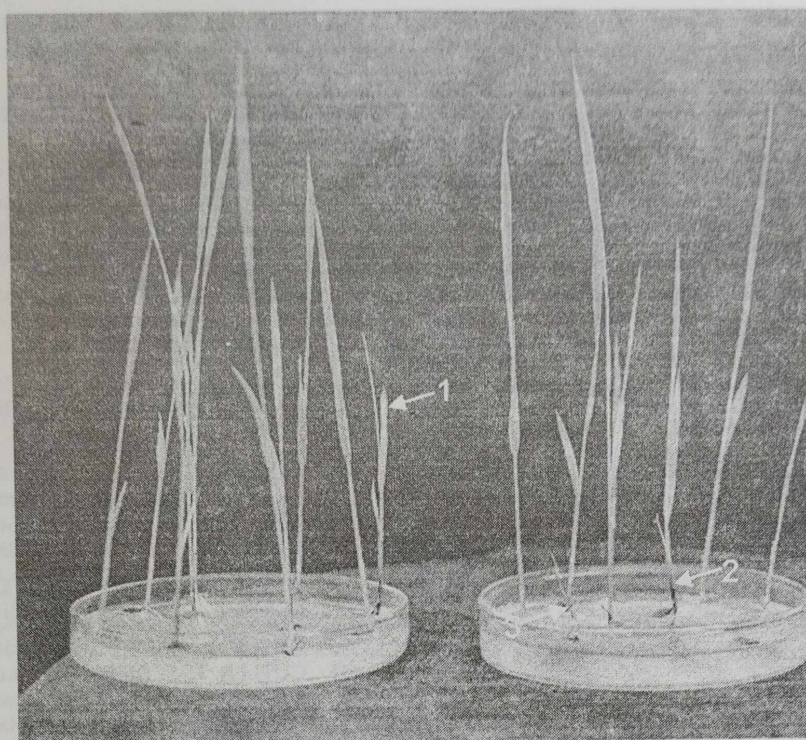


Fig. 1. Paddy seedlings showing different symptoms due to *Drechslera oryzae* infection

1. Seedlings showing brown spots.
2. Seedlings showing water soaked lesions.
3. Seedlings showing wilt symptom.

enhanced the shoot growth as compared to any other treatment including control. In case of *P. fluorescens*, the seed germination and seedling growth were enhanced over control. However, it was inferior to that of *T. pseudokoningii* (Table 5).

All the leaf extracts except *Azadirachta indica* reduced the infection of *D. oryzae* over control. *Thuja orientalis* and *Tridax procumbens* were next to *Ruta graveolens*. However, it failed to stimulate the seedling growth. *Thuja orientalis* extract was found to be an efficient treating agent which suppressed the *D. oryzae* to an higher extent which in turn facilitated the stimulated growth over any other treatment including control (Table 6).

The variation in the occurrence of fungi in the different variety usually depends upon the varied agro-climatic conditions including variety and its ingredients, which have strong affinity with that of fungal species. Dasgupta and Chattopadhyaya [5] reported the nature of variation in susceptibility due to aging of plants. Pathogenicity of fungi depends upon its virulence through the production of some metabolites into the host system. As a result of aggressive nature of virulent pathogen, different

types of symptoms are expressed on the host species. In the present investigation many symptoms like yellowing, brown-necrosis, spots and streaks were observed and recorded in paddy variety due to *D. oryzae* which are in confirmation with the findings of Bedi and Gill [6].

Physical factors like light, temperature, humidity, rain splash, blowing wind play an important role in building up of the inoculum. Higher temperature results in the loss of viability among field fungi. In the present study, 55°C hot water treatment reduced the incidence of *D. oryzae* in paddy cultivar of variety Jaya. The suppression of the fungus due to hot water treatment varies depending upon the site of location of organism in the seed. If the fungus is deep seated it may not be fully suppressed. The seeds treated at 60°C showed slightly inhibited germination. Thus, it indicates the possibility of getting better results at 55°C with respect to suppression of fungus and with enhanced seed germination.

In the present study, seeds treated with *Trichoderma pseudokoningii* might have produced some antifungal compounds resulting in the suppression of *D. oryzae* as there are several reports

showing the occurrence of antifungal compounds in the culture filtrates of some bioagents. [7, 8, 9, 10] Moreover, the enhanced growth in the seedlings of *T. pseudokoningii* treatment is probably due to the release of phytoestimulatory compounds into the seed tissues. This supports the findings of several workers [11, 12].

In the present study leaf extracts of *Thuja orientalis* showed better results compared to other plant extracts. Ward *et al.* [13] reported the antifungal property of some of the purified compounds obtained from common plants. Leaf extract of *Tridax procumbens* and *Ruta graveolens* also suppressed the expression of *D. oryzae* in paddy. Occurrence of alkaloids in some plant extracts are known to inhibitory to a few plant pathogens. Simultaneously, plant extracts may have some growth promoting compounds which help not only in improving the seed germination but also seedling growth. Mohanraj *et al* [14] also reported the occurrence of stimulatory chemicals in plants. The findings of present work indicates the beneficiary effects of some bioagents and plant extracts over routine type of remedial measures.

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