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Cultural and morphological characteristics of *Microdochium oryzae* inciting leaf scald disease in rice and effect of culture media on its growth and sporulation

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Abstract
Rice leaf scald disease, caused by the fungal pathogen *Microdochium oryzae*, is worldwide important disease of rice (*Oryza sativa* L.). The disease has been reported to causes yield losses of up to 23.4% in India. Hence, in this study, morphological and cultural studies were done to understand the pathogen behaviour. The results revealed that *M. oryzae* produced white to off white, fluffy, cottony, septate mycelial growth which later turned to dull white with deep orange or salmon or pink colored droplets of conidial masses. Mycelium was hyaline, septate, irregularly branched and breadth measured 1.89 to 6.09 µm (Ave. 4.15 µm). Conidia were hyaline, bicelled, without constriction at septum, oval to oblong, slightly curved, tapering at both ends and deep orange or salmon coloured when in mass and measured 9-13 µm × 3-4 µm in size. Among the seven different culture media evaluated, potato dextrose agar medium and carrot agar medium were found most suitable and were significantly superior over rest of media and encouraged maximum radial mycelial growth (90mm) of *M. oryzae*. Mycelial growth of test fungus on carrot agar medium was maximum (90mm) but it was sparse, whereas on host leaf extract agar medium fungal sporulation was fair. Asthana and Hawker's agar and carrot agar medium were poor in the conidia formation.

Keywords: Rice, *Oryza sativa*, *Microdochium oryzae*, Conidia, sparse, sporulation

Introduction
Rice (*Oryza sativa* L.) is one of the most important and oldest cereal crop in the world. Rice is a staple food for more than half of the world's population and 90% world's rice comes from Asia. Rice is the good source of proteins, phosphorus and iron. It also contains some amount of calcium. No other food matches the calories and energy provided by rice (345 Kcal/100g). Rice crop is has various disease caused by diverse types of pathogen. Diseases of rice estimated to cause annual loss about 15.6 per cent (Mondal *et al.* 2017) [7]. Among the various fungal diseases of rice leaf scald caused by *Microdochium oryzae* is the most important disease. Incidence of leaf scald infection in an area can reach high levels under favorable conditions resulting in significant loss of yield, as 23.4 per cent yield reduction has been reported in India due to of leaf scald disease (Srinivasan, 1981) [13]. The symptomatology and detail study of the disease revealed that disease is caused by *M. oryzae*. The fungal pathogen *M. oryzae* affects the foliar parts causing zonate lesions on growing tips and alternating light tan and dark brown lesions originating from leaf tips or edges with light brown halos particularly in mature leaves. The affected area dry out giving the leaf scalded appearance. The infected tips also splits near the midrib especially when there were strong wind. Fungus spreads through winds and splashing water. The wet weather with temperature range between 20-30 ˚C and high relative humidity coupled with high doses of nitrogenous fertilizers supports disease development. In recent past leaf scald disease *Microdochium oryzae* is becoming major problem in healthy cultivation of rice. In order to find out effective control measures against the fungal pathogen it was felt to conduct study on morphological cultural characters of the fungal pathogen.

Materials and Methods
Cultural study
Potato dextrose agar medium was used as basal medium for present investigation. The medium was prepared and dispended in conical flask.

The flasks were plugged with non-absorbent cotton plugs and sterilized in an autoclave at 1.054 kg / cm^2 for 20 minutes. Petri plates were sterilized in hot air oven at 160 ^{0}C for 1 hour. Each Petri plate was poured with 20 ml of molten potato dextrose agar medium and allowed to solidify. Five millimeter diameter culture discs of 7 day old test fungus were cut with the help of incinerated cork borer and inoculated at the centre of Petri plates. Inoculated plates were then incubated at room temperature (27 ± 2 ˚C) for 7 days. Cultural characteristics such as type of mycelial growth, color, appearance, *etc.* were recorded.

Morphological study

Slide culture technique (Booth, 1971) [2] was employed to study morphological character of the fungus. Sterilized Petri plates were poured with 20 ml of molten potato dextrose agar medium and allowed to solidify. Small square blocks of solidified medium of size 5mm were cut with the help of sterilized scalpel and transferred onto the centre of the micro slide. The square of medium was inoculated with fungal inoculums and covered with a sterilized cover slip. Micro slide was then place on a pair of glass rod in the sterilized Petri plate internally lined with moist blotting paper. The plates were incubated at room temperature (27±2 ˚C). After obtaining required growth of the fungus, square block was removed from micro slide with minimum disturbance. A drop of lactophenol cotton blue stain was placed on the fungal growth and covered with a cover slip and observed microscope for different morphological characters such as type of hyphae, septation, type of conidia, shape of conidia and size of the conidia.

Effect of culture media on growth and sporulation of *M. oryzae*

Seven different culture media *viz*., Potato dextrose agar, Asthana and Hawker's agar, Richard's agar, Elliot's agar, Czapek's dox agar, Carrot agar and Host leaf extract agar were used in the present study. Twenty milliliter of each medium as was poured into sterilized Petri plate, separately. After solidification, 5 mm cultural discs of the test fungus from actively growing 7 days old culture were cut using sterilized cork borer and single disc was placed at the centre of each Petri plate and incubated at 27±2 ˚C. Each treatment was replicated thrice. The measurement of the colony diameter was taken when the maximum growth was achieved in any one of the media tested. The cultural characters such as colony diameter, colony color and degree of sporulation were recorded.

Results and Discussion

Cultural and morphological characteristics of *M. oryzae*

From the results it was observed that on PDA *M. oryzae* produced white to off white, fluffy, lanose to loose, cottony, septate mycelium and was radiating towards periphery. Later the colony turned dull white with salmon or pink colored droplets of conidial masses on the surface. This confirms the earlier findings of Ou *et al.* (1978) [8] reported that on PDA medium, fungus produced luxuriant white mycelium, it was tinted cream or buff when younger but as the culture grew old, pink to salmon colored conidial masses were produced. The findings are also in accordance with Liang (2019) [6] who found that colonies of *Microdochium* were whitish coloured with hyaline, branched superficial mycelium on potato dextrose agar medium.

Morphological characters from the the primary basis for the identification of fungus Mycelium of the test fungus was white in color which turn dull white with age. Microscopic observations showed that the mycelium was hyaline and septate. Branching of mycelium was irregular. The mycelial breadth measured 1.89 to 6.09 µm (Ave. 4.15 µm). Conidia borne singly or in group of many superficial stromata. Conidiophore was absent. Conidia were oval to oblong, slightly curved, tapering at both ends. They were deep orange in color when observed in mass. Under microscope conidia were hyaline, two celled, without constriction at septum. Conidia measured 9-13 µm × 3-4 µm in size. Characteristics of test fungus observed under laboratory are in close conformity to characters reported for *M. oryzae* by Chin (1974) [3] reported that mycelium of *M. oryzae* was colorless to cottony, hyaline and branched measuring about 1.8 µm × 2.6 µm in width. Conidia were hyaline, curved without beak measuring about 11µm – 12.1 µm × 2.4 µm – 3.4 µm. this findings are also in accordance with Quintana *et al.* (2018) [10] who reported that conidia of the fungus were sickle and half-moon shaped, simple cells occasionally two celled, thin wall and hyaline at microscopy size between 8-13 µm × 3-5.5 µm.

Effect of media on growth and sporulation of *M. oryzae*

The data from table 1, revealed that, among the seven different culture media screened, potato dextrose agar medium and carrot agar medium were found most suitable and were significantly superior over rest of media and encouraged maximum radial mycelial growth (90mm) of *M. oryzae*. Mycelial growth of test fungus on carrot agar medium was maximum (90mm) but it was sparse whereas, on host leaf extract agar medium fungal sporulation was fair. Asthana and Hawker's agar and carrot agar medium were poor in the conidia formation.

All the culture media tested, exhibited a wide range of growth characters the mycelial growth produced on all the culture media tested mostly fluffy, lanose to loose and cottony. Whitish colonies were produced on Elliot's agar (72.50 mm) and Potato dextrose agar medium (90.00 mm) while white to orange colored colonies were produced on Asthana and Hawker's agar (69.10 mm), Czapek's dox agar (58.56 mm) and Richard's agar medium (61.90 mm). On the host leaf extract agar medium, fungus produced off white to grayish colored, flat and scanty growth (58.30 mm). On the carrot agar medium, fungus produced sparse and flat growth with fair sporulation (90.00 mm). The results of present findings are comparative with that of Reed (1959) [11], Parkinson (1980) [9], Singh and Gupta (1983) [12] and Baghela and Singh (2017) [1] who reported that potato dextrose agar was good medium for luxuriant vegetative growth and sporulation of *M. oryzae*. However, the results of present study was differ to earlier findings by various scientists worked on *M. oryzae*. Thomas 1981 [14] found that V_8 vegetable juice agar was most suitable for maintaining a good growth and abundant sporulation of fungus. Singh and Gupta (1983) [12] found that potato dextrose agar with 2 per cent rice leaf extract promotes growth and sporulation of the fungus.

Table 1: Effect of culture media on radial mycelial growth and sporulation of *M. oryzae*

Tr. No.	Cultural media	Colony dia.(mm) *	Colony character	Sporulation
T_1	Elliot's agar medium	72.50	Fluffy, lanose, white colored cottony growth	+++
T_2	Asthana and Hawker's agar medium	69.10	White colored, flat growth with regular margins	+
T_3	Host leaf extract agar medium	58.30	Off white colored and flat growth	++
T_4	Carrot agar medium	90.00	Off white to grayish colored, flat and sparse growth	+
T_5	Richard's agar medium	61.90	Fluffy, lanose to loose whitish to orange cottony growth, pure white at periphery	++++
T_6	Czapek's dox agar medium	58.56	Fluffy, flat and white colored colony	++++
T_7	Potato dextrose agar medium	90.00	Fluffy, lanose to loose whitish orange, cottony growth with salmon or pink colored spore masses	++++
	S.E (m) ±	0.71		
	C.D at 1%	2.85		

*mean of three replications

Sporulation: No sporulation +: Poor ++: Fair +++: Good ++++: Excellent

Conclusion

Among the different culture media tested, maximum growth and sporulation of *M. oryzae* was observed on potato dextrose agar medium. Excellent conidia formation was recorded on potato dextrose agar.

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