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Effect of Different Culture Media on the Growth and Development of *Pyricularia oryzae* from Different Rice Growing Regions of Karnataka

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A B S T R A C T

The study was conducted to describe the growth characteristics of the leaf blast pathogen *Pyricularia oryzae* isolates on different solid media *viz*., Host Extract Agar (HEA), Oat Meal Agar (OMA), Potato dextrose agar, Richard's Agar medium. Among all the solid media the highest mean mycelial growth of the fungus *Pyricularia oryzae* was recorded on Host Extract Agar (4.08 cm) followed by Oat meal agar (3.83 cm) and least mean mycelial growth of the *P. oryzae* on Richard's agar media (3.21). In general, among all solid media the Potato Dextrose Agar media is more appropriate for cultural and morphological study of rice blast fungus *P. oryzae*.

Introduction

Rice is the most important food crop of India covering about one-fourth of the total cropped area and providing food to about half of the Indian population. This is the staple food of the people living in the eastern and the southern parts of the country, particularly in the areas having over 150 cm annual rainfall. There are about 10,000 varieties of rice in the world out of which about 4,000 are grown in India. They are important source of forage for herbivorous animals. It is spectacularly diverse, both in the way it is grown and how it is used by humans. Rice is unique because it can grow in wet environments that other crops cannot survive in. Such wet environments are abundant across Asia. Rice is life for thousands of millions of people. In Asia alone, more than 2,000 million people obtain 60 to 70 per cent of their calories from rice and its products. Recognizing the importance of this crop, the United Nations General Assembly declared 2004 as the "International Year of Rice" (IYR). The theme of IYR—"Rice is life" reflects the importance of rice as a primary food source, and is drawn from an understanding that rice based systems are essential for food security, poverty alleviation and improved livelihood. The world's estimated rice production is 496.0 million metric tons during 2016 (Anon, 2016). India is the largest rice growing country accounting for about one third of the world acreage under the crop. In India's annual rice production is 103.6 million tons during 2016 (Anon, 2016).

Rice is grown throughout India in all the states. The major rice growing states of India are West Bengal, Uttar Pradesh, Bihar, Madhya Pradesh, Orissa, Andhra Pradesh, Karnataka and Chhattisgarh. Rice suffers from many diseases caused by fungi, bacteria, viruses, phytoplasma, nematodes and other non-parasitic disorders. Among the fungal diseases, blast is considered as a major threat to rice production because of its wide spread distribution and its destructiveness under favourable conditions. The Commonwealth Mycological Institute has recorded its presence from 85 countries throughout the world. Paddy blast is generally considered as the principal disease of rice and is caused by a fungus belonging to the Ascomycete *Pyricularia oryzae* Cavara (teleomorph= *Magnaporthe grisea* (Hebert) Barr Comb nov.). Losses due to the blast disease may range up to 90 per cent depending upon the component of the plant infected. *M. grisea* infects above ground parts of the plant, but neck blast and the panicle blast are the most damaging phases of the disease and have been shown to significantly reduce yield, grain weight and milling quality.

The pathogen may infect all the above ground parts of a rice plant at different growth stages *viz.*, leaf, collar, node, internodes, base or neck and other parts of the panicle and sometimes the leaf sheath. A typical blast lesion on a rice leaf is gray at the centre, has a dark border and it is spindle-shaped.

Materials and Methods

Effect of different media on the growth of *P. oryzae* Culture discs of pathogen (5mm) was inoculated separately on different media and incubated at 28±1ºC for 15 days. The cultural characters and the colony diameter (mm) on each medium were recorded. Fifteen ml of each medium (Table 1) was poured into each of sterilized petriplates. Inoculation was made by transferring the five mm disk of mycelia mat, taken from the periphery of ten days old culture of each seven isolates. Each treatment was replicated thrice. The plates were incubated at 28±1ºC. Observation of colony growth was taken when the maximum growth was attained in any one of the media tested. Other cultural characters viz., rate of growth, type of margin, colony colour and sporulation were also recorded. The pathogen was multiplied by transferring a loop full of the stock culture to 250 ml of potato dextrose broth taken in a 1000 ml flask. The inoculated flask was incubated at 28 ± 1^{0}C for fourteen days. The fungal culture growing on broth was passed through double layered muslin cloth. The concentration of spore suspension was adjusted to 50 spores/microscopic field by adding sterilized distilled water. The spore suspension was collected separately in an atomizer and incubated on to the foliage of 20 days old rice (3-4 leaf stage) seedlings of rice. The seedlings after spray inoculation were kept in green house condition with water sprayed regularly both during morning and evening hours to maintain relative humidity and pots were covered with polythene bags. After 48 hours, the polythene bags were removed. Periodical observations were made for the development of typical blast symptom on the inoculated plants. The pathogen from typical blast symptom was re-isolated and compared with the original culture as well as published literature to confirm the identity of the pathogen.

Results and Discussion

Growth of *P. oryzae* on four different solid media

The experiment was conducted as detailed in material and methods‟ to acertain the period when the maximum growth of the fungus could occur among all the solid media the highest mean mycelial growth of the fungus

Pyricularia oryzae was recorded on Host Extract Agar (4.08cm) (Plate 1), Oat meal agar (3.83 cm) (Plate 2) followed by Potato Dextrose Agar media (3.41 cm) (Plate 3) and least mean mycelial growth of the *P. oryzae* on Rechard's Agar medium (2.84 cm) (Table 2, Fig. 1, Plate 4) (Vanaraj *et. al.*, 2013). The growth assessment of *Pyricularia oryzae* is done to all fifteen isolates generally out of four different media only on Potato Dextrose Agar media sporangias are grown and divided them as good, fair and poor growth of colony other medias are not supported to *P.oryzae* sporangia because the nutrients present in media are not suitable for the *P.oryzae* (Netam *et al.*, 2013) (Table 3).

Cultural characteristics studied on different media showed the variation among fifteen isolates of *P. oryzae* with respect to colony growth (cm) and assessment of colony growth and colony margin (Ou, 1985). Colour varied from grayish black to dark jet black colour, smooth to irregular margin, medium to good growth of the pathogen was observed (Srivastava *et al.*, 2009).

Table.1 List of media used to study the growth characters of *Pyricularia oryzae*

Sl.No.	Media	Type of media
1	Host Extract Agar (HEA)	Semi-synthetic
2	Oat Meal Agar (OMA)	Synthetic
3	Potato Dextrose Agar (PDA)	Semi-synthetic
4	Richard‟s Agar (RA)	Synthetic

Table.2 Growth characteristics of *Pyricularia oryzae* Isolates on different media

***Pyricularia oryzae* Isolates**	**Colony Growth(cm) on HEA**	**Colony Growth(cm) on OMA**	**Colony Growth(cm) on PDA**	**Colony Growth(cm) on RA**
Po1	3.8	4.6	4.0	3.5
Po2	4.4	4.5	4.2	4.2
Po3	4.4	3.9	3.9	2.0
Po4	3.0	4.4	3.3	1.5
Po5	3.5	4.3	3.5	3.5
Po6	3.0	4.6	2.4	3.4
Po7	3.8	1.2	3.7	2.0
Po8	4.5	4.7	3.3	2.0
Po9	3.9	3.7	3.5	3.5
Po10	4.4	3.6	3.8	2.5
Po11	4.4	4.5	3.0	3.5
Po12	4.5	3.2	3.6	2.5
Po13	4.6	3.3	3.8	3.5
Po14	4.6	3.6	3.3	3.0
Po15	4.4	3.4	1.9	2.0
Mean	4.08	3.83	3.41	2.84

Table.3 Growth assessment of *Pyricularia oryzae* isolates on different media

Pyricularia oryzae Isolates	Host Extract Agar (HEA)		Oat Meal Agar (OMA)		Potato Dextrose Agar (PDA)		Rechard's Agar (RA)	
	Number of sporangia/field (n=10)	Growth assessment	Number of sporangia/field (n=10)	Growth assessment	Number of sporangia/field (n=10)	Growth assessment	Number of sporangia/field (n=10)	Growth assessment
Po1	0	P	0	P	14	F	0	P
Po2	0	P	0	P	0	P	0	P
Po3	0	P	0	P	3	P	0	P
Po4	0	P	0	P	32	G	0	P
Po5	0	P	0	P	0	P	0	P
Po6	0	P	0	P	0	P	0	P
Po7	0	P	0	P	0	P	0	P
Po8	0	P	0	P	50	E	0	P
Po9	0	P	0	P	8	P	0	p
Po10	0	P	0	P	0	P	0	p
Po11	0	P	0	P	0	P	0	p
Po12	0	P	0	P	38	G	0	p
Po13	0	P	0	P	0	P	0	p
Po14	0	P	0	P	25	G	0	p
Po15	0	P	0	P	0	P	0	p

G: Good, F: Fair, P: P

Fig.1 Graph showing that growth of fifteen isolates colony on different media

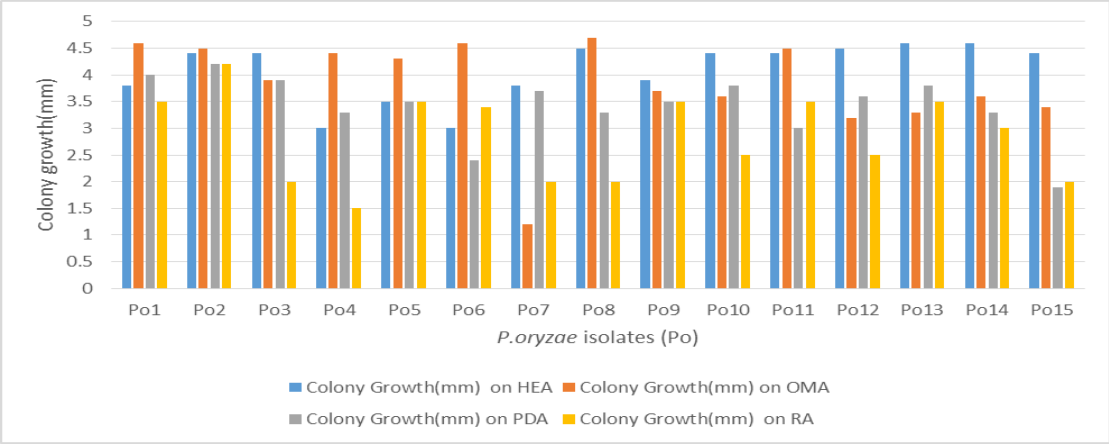


Plate.1 Growth characteristics of *Pyricularia oryzae* isolates in (Host Extract Agar) HEA medium

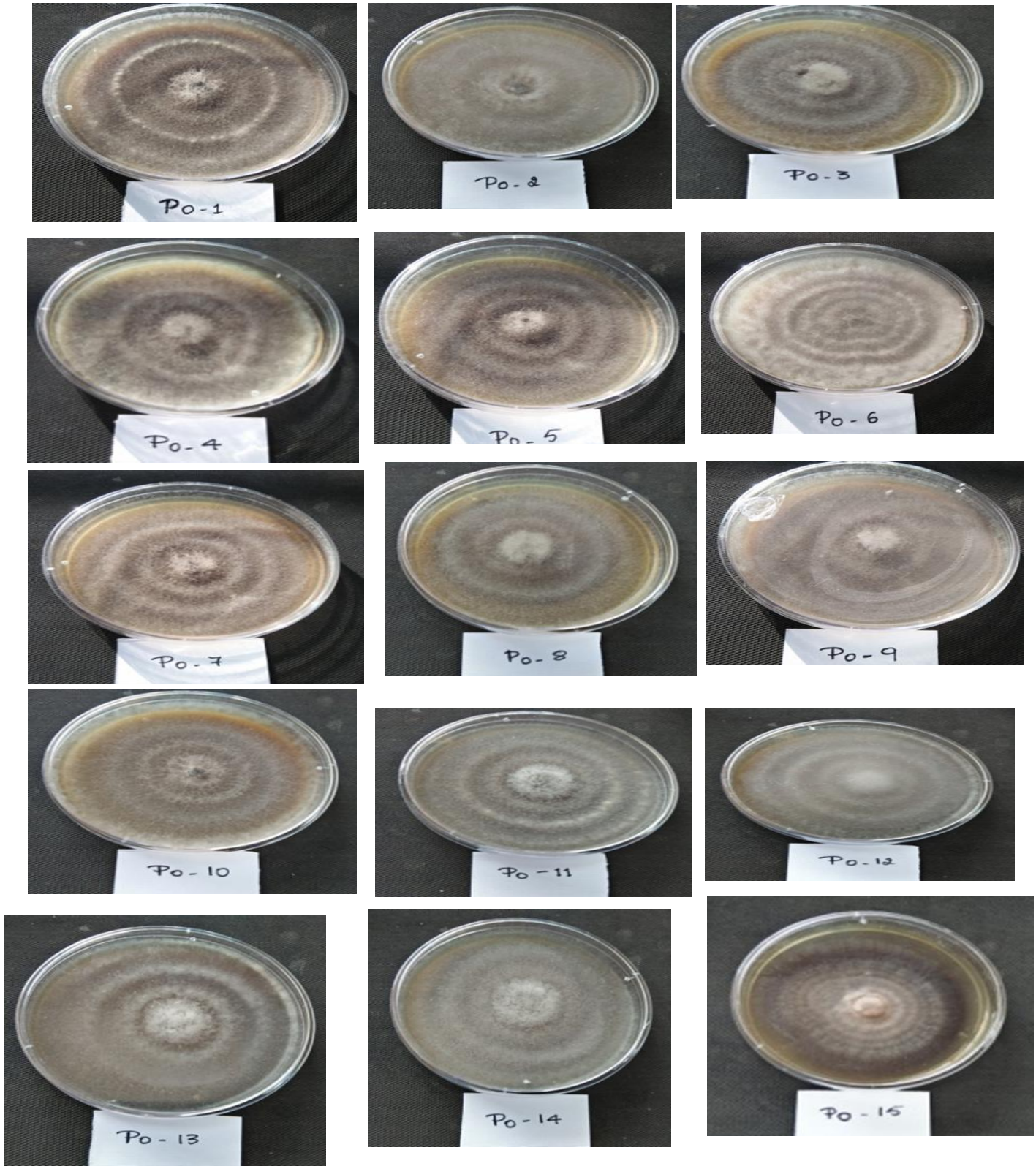


Plate.2 Growth characteristics of *Pyricularia oryzae* isolates in (oats meal Agar) OMA medium

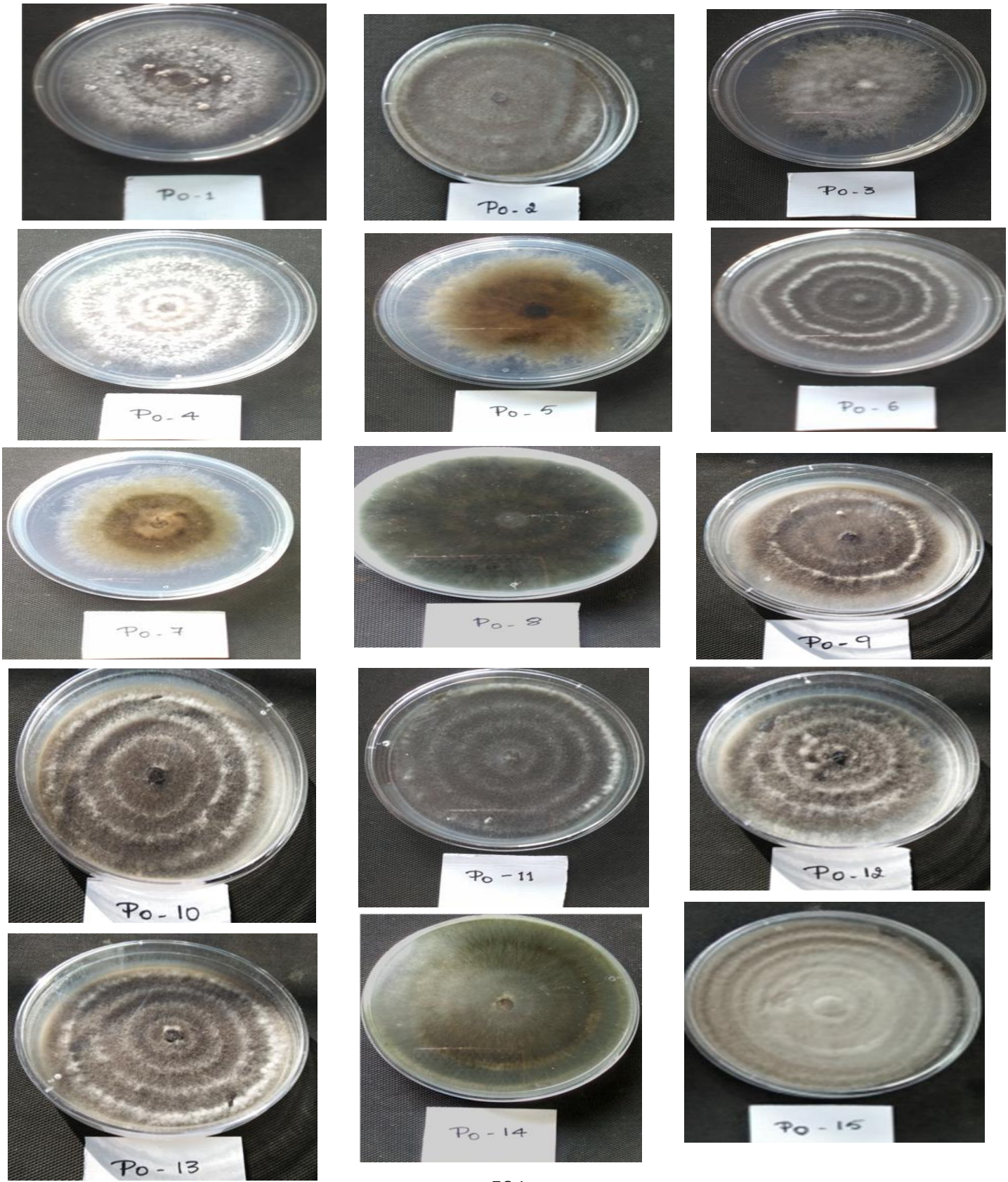


Plate.3 Growth characteristics of *Pyricularia oryzae* isolates in (potato dextrose Agar) PDA medium

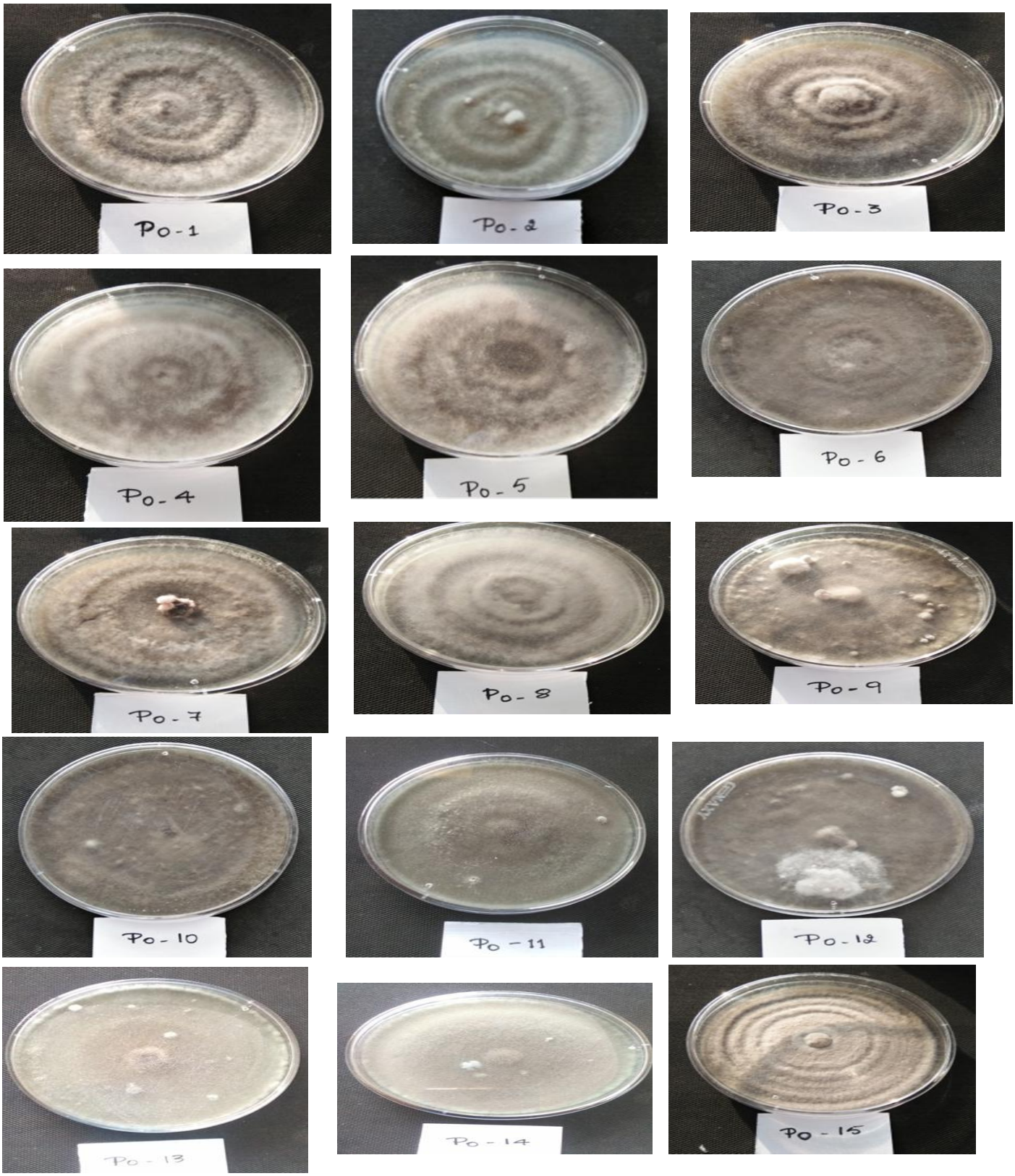
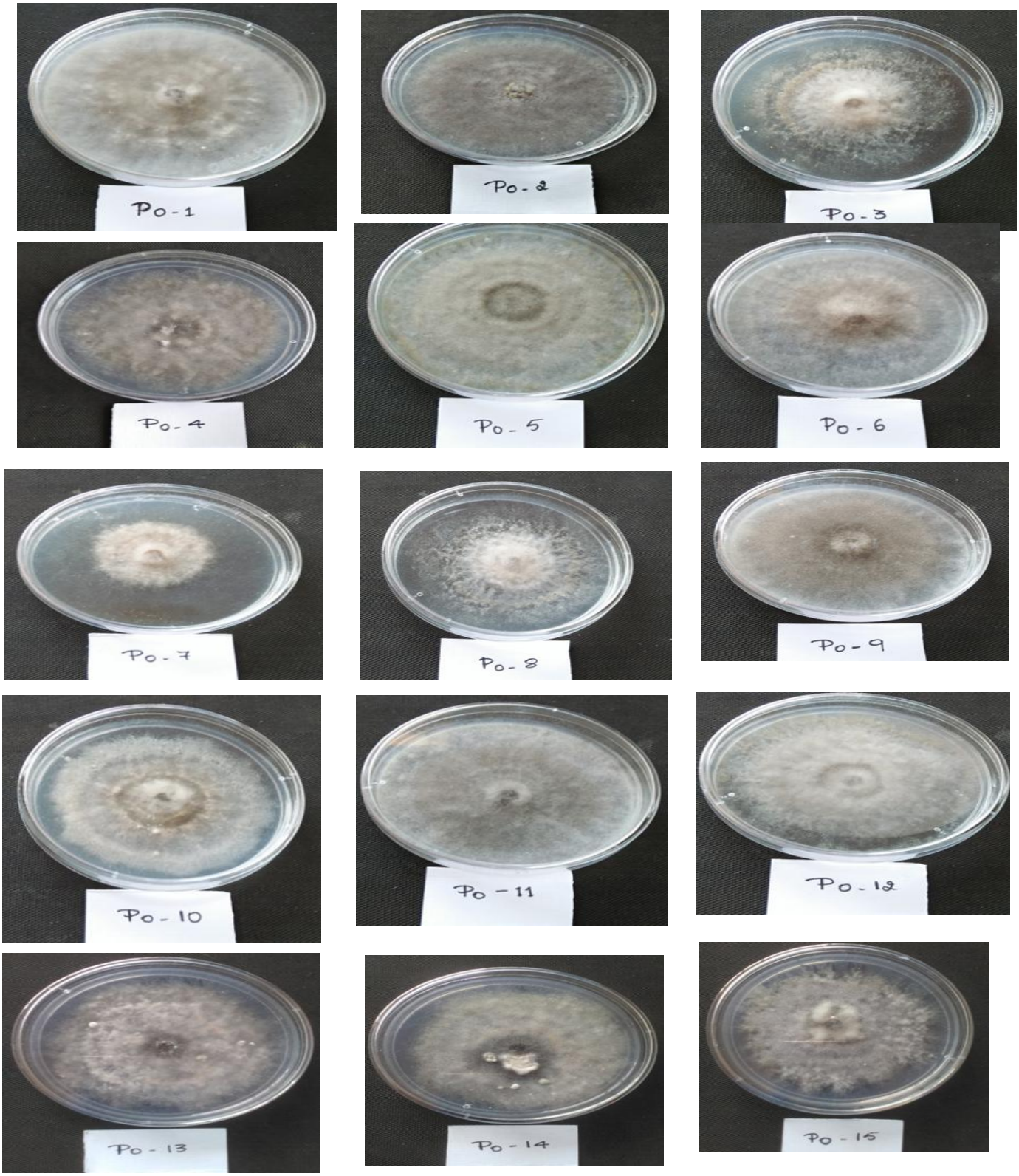


Plate.4 Growth characteristics of *Pyricularia oryzae* isolates in (Richards Agar) RA medium



These fifteen isolates exhibited considerable variation in colony type and colour when grown on different nutrient media. Isolate 2, 6 and 5 developed grayish white colonies on PDA medium while isolate 3, 4, 8 and 7 developed white colonies on Host Extract Agar media. On Oat Meal Agar media fifteen isolates produced white colonies with respect to the Recjard's Agar media produced greyish white colour colonies. The isolate 1, 6 and 7 developed dark grayish colonies while 1, 2, 3 and 5 developed grayish white colonies. Morphological characters viz., size and shape of conidia were studied for identification of the fungus. Conidia were pyriform, almost hyaline to pale olive, 2-septate and 3 celled. The shape, size, septation and colour characters are in agreement with those described by Nishikado (1926) and Mijan Hossain (2000).

Potato dextrose agar media shown positive growth of *P.oryzae colony* (sporangia) growth of colony of all the fifteen isolates were divided into three groups Good growth, Fair growth and Poor growth. PDA is good for cultural and morphological study of *P.oryzae* (Kulkarni, 1973).

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