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# Influence of Culture Media on Mycelial Growth and Sporulation of *Pyricularia Oryzae* and Usefulness of Potassium Silicate against the Rice Blast Pathogen

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***ABSTRACT:*** *In 2016, due to epiphytotic formed of rice blast in Bangladesh, the production of Boro rice and transplanted Aman were greatly reduced. Particularly, the severe outbreak was occurred in Boro rice with serious yield losses. Keeping the facts in view, the present investigation was carried out to study the cultural and morphological variability of rice blast pathogen Pyricularia oryzae in selected culture media, and to assays the efficacy of potassium silicate ($K_2SiO_3$) against rice blast pathogen, during Boro season 2018-2019. From the in-vitro study on selected suitable culture media, Carrot Rice straw Dextrose Agar (CRDA) media was found the best for growth and sporulation of rice blast pathogen, Pyricularia oryzae. From the morphological study of the derived pathogen, it was confirmed that the disease was caused by Pyricularia oryzae, as the isolates produced hyaline, pyriform three celled conidia with average size 16.45 × 7.46 µm. They were able to re-establish the disease in in-vitro pathogenicity test. To assess the efficacy of potassium silicate against the rice blast pathogen, mostly cultivated and blast susceptible boro rice variety namely BRRI dhan28 was used as a tested variety and $K_2SiO_3$ applied in different doses (0, 1, 2, 4, 8, and 10 g/L Si) with number of foliar sprayings at pH 5.5. From the in-vivo study it was observed that spraying of $K_2SiO_3$ at 4 g/L significantly reduced the blast disease incidence and severity. From the management study, it's revealed that Potassium (K) and Silicon (Si) containing agro-chemical like $K_2SiO_3$ have direct and indirect benefits in related to induce resistance system in plants (especially grasses crops like rice).*

***Keywords:*** *Rice blast, Pyricularia oryzae, CRDA medium, Potassium Silicate, Induced resistance*

## I. INTRODUCTION

Rice (*Oryza sativa* L.) is the source of subsistence for more than one third of human population in the current world. Rice is grown on about 10.5 million hectares in Bangladesh which has remained almost stable over the past three decades. The country is now producing about 25.0 million tons to feed her 160 million people (Hossain *et al.,* 2017). This increased rice production has been possible largely due to the adoption of modern rice varieties on around 66% of the rice land which contributes to about 73% of the country's total rice production. But rice crop is subjected to attack by 50 diseases including 21 fungal, 6 bacterial, 4 nematodes, 12 viral and 7 miscellaneous diseases and disorders (Jabeen *et al*., 2012). Among the major rice diseases that often cause great economic losses, rice blast (*Pyricularia oryzae*) is a vicious threat to the country's economy (Ganesh *et al.,* 2012). This fungus infects during nearly all growth stages on all aerial parts of rice, leading to leaf blast, nodal blast and neck or panicle blast. The major symptoms of this disease are found on leaves with brownish spot having grey center, sunken lesion on node and brown or black lesion found on neck of the

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panicle. Leaf blast lesions decrease the net photosynthetic rate of individual leaves to an extent far beyond the visible diseased leaf fraction. In case of node blast, sunken lesion appeared at the nodal zone resulting broken the infected plant at node and up part hung up with lower part. Neck or panicle blast is considered as the most destructive phase of the disease and can cause direct yield reduction, since filling of the grains on infected panicles is poor (Gashaw *et al.,* 2014). Outbreaks of rice blast are a serious and recurrent problem in all rice growing regions of the world. It is estimated that each of the three years enough of rice is destroyed by rice blast alone to feed 60 million people (Yang *et al.,* 2011).Out of the total yield loss due to diseases in rice, 35% is by blast, 25% by sheath blight, 20% by BLB, 10% by Tungro and remaining 10% by other diseases. The disease causes yield losses from between 1-100% in Japan, 70% in China, 21-37% in Bali Indonesia (Supriya *et al.,* 2010), and 30-50% in South America and Southeast Asia (Ramesh *et al.,* 2015). In 2016, serious yield losses occurred due to epiphytotic formed of blast diseases caused by *Pyricularia oryzae* in major rice growing regions of Bangladesh such as Dinajpur, Rangpur, Thakurgaon, Panchgar, Kushtia, Jashore, Pabna, Borishal, Mymensingh, Munshigonj ,Chuadanga etc. The production of Boro rice and transplanted Aman in Bangladesh were greatly reduced due to blast infestation with 21.19% and 11.98% disease severity. Different fungicides can be used to control blast disease but they generate additional costs in rice production and chemical contamination to environment and food items (Jamal *et al.,* 2012). Host resistance is the most economically viable and environmentally sustainable practice to manage blast disease, the fungus overcomes blast resistance quickly, and cultivars typically become susceptible to disease within 2-3 years (Singhm *et al.,* 2014).

## II. METHODS AND MATERIALS

**3.1 Planting material and disease samples collection:** Disease free rice seeds were collected from Bangladesh Agricultural Development Corporation (BADC) which supported by Bangladesh government. Mostly cultivated rice cultivar BRRI dhan28 was used as planting material for tested variety because it had been shown high susceptibility to Blast disease caused by *Pyricularia oryzae.* Diseased samples were collected from three blast reported sub-stations; including Dinajpur, Thakurgaon and Panchgar located in northern part of Bangladesh.

**3.2 Isolation and culturing of *Pyricularia oryzae* in selected media:** Lab experiment was carried out during the period of April, 2018 to May, 2019 in Molecular Biology and Plant Virology Laboratory under the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh. Fungi were isolated by tissue planting method. Diseased rice leaves and neck portions of the infected panicle were sterilized by the surface disinfectant, 1% sodium hypochlorite for 3 minutes (Arun, 2011) and placed on blotter paper within sterilized petri-plates labeled with necessary information. Then they were placed in the incubation chamber for 7 days at 25 $\pm 1^{0}$C exposing to UV light for 12 hours per day. The growing mycelia on blotter paper in petri-plates were then placed in selected media viz. PDA (Potato Dextrose Agar), CRDA (Carrot Rice straw Dextrose Agar) and CMA (Corn Meal Agar Media) then incubated at 25 $\pm 1^{0}$C. After 7 days a portion of culture was taken on slide and observed under microscope and identified the pathogenic fungi that is *Pyricularia oryzae* with the help of pertinent literature (Ali, 2009 and Barnwal, 2012). The growing mycelia on potato dextrose agar plates, carrot rice straw dextrose agar plates and corn meal agar plates were incubated at 25 $\pm 1^{0}$C for sporulation. For cultural characterization the plates were incubated for fifteen days. The mycelia which was grown marginally and developed subsequently was picked up aseptically for sub-culturing. The sub culturing was done at an interval 21 days and preserved at low temperature (4±1ºC) in refrigerator.

**3.3 Etiology of *Pyricularia oryzae* and Spores counting:**Mycelial growth, conidiophore and conidia was observed clearly after staining with lactophenol and cotton blue under light microscope at first low power and then with high power objective lens. Microphotographs were taken to show the typical spore morphology of *Pyricularia oryzae* isolates. Pure cultures were stored at 4°C temperature for future research work.The fungus was started to sporulate after 25 days of inoculation on PDA and CRDA media. On CRDA media fungus produces satisfactory amount of spores as it is suitable for its sporulation. For counting spores, petri-plates of CRDA containing pure culture was taken and poured 10 ml distilled water on it and stirred well with a brush.

Then the suspension was filtered with a piece of cotton cloth and harvested the conidial suspension in a beaker. Again added 10 ml more distilled water and repeated the same. The mass of spore sedimentation was collected re-suspended with sterilized distilled water and spore density was adjusted to a concentration of $1\times10^5$ spore/ml using a haemacytometer. A loopful of spore suspension was then placed on a clean slide and a cover slip was placed on it. The rate of sporulation was recorded in three different microscopic fields and rated according to1-4 index, number of spores >30 designate 4 index, 20-30 designate 3 index, 10-20 designate 2 index and <10 sporesdesignate 1 index (Ramesh, *et al.,* 2015).

**3.4 Inoculation of rice plant with *Pyricularia oryzae* and application of $K_2SiO_3$:** The pot experiment was conducted in inoculation chamber at net house. The *Pyricularia oryzae* was inoculated at 40 DAT under proper safety by maintaining inoculation principles.Inoculated plants were kept for incubation in moist chamber at $28^0$ C with >95% RH for 72 hours under darkness for penetration of conidia and disease development. Potassium silicate ($K_2SiO_3$) was sprayed in 6 different doses like 0, 1, 2, 4, 8, or 10 g/L with three replications each number of sprayings at pH 5.5. Three spray were done at 35 days after emergence, before panicle initiation stage and before heading stage in the same dose successively.

**3.5 Assessment of % disease incidence and severity:** Disease incidence was firstly evaluated at ten days after inoculation and also recorded during post flowering stage. Data were recorded visually by observing the symptoms on the basis of dark brown to black lesion at the neck of the panicle. Disease incidence was estimated by using the following formula (Cruz *et al.,* 2009):

% disease incidence = $\frac{Number\ of\ diseased\ tillers}{Total\ number\ of\ tillers\ inspected} \times 100$

The disease severity was examined visually on the whole plants within the quadrants and recorded as the percentage of plant parts (tissue) affected (percentage of blast infection of the plant). Disease severity was recorded in 0-9 scale for predominant lesion type (Singh *et al.,* 2011).

**3.6 Data Analysis**

All data was analyzed using the latest computer based software “Statistix-10”. The mean value was compared according to LSD range test at 5% level of significance($P\leq0.05$).

## III. RESULTS

**4.1 Cultural and Morphological study of *Pyricularia oryzae* in selected media:** The mycelial growth was recorded at 7, 14 and 21 days after inoculation. From the study it was found that the highest growth of 60.60 mm, 70.80 mm and 80.14 mm was recorded in CRDA medium at 7, 14 and 21 days after inoculation, respectively. The second highest growth was recorded in PDA medium 60.16 mm at 21 days after inoculation. There was no mycelial growth found in CMA media. So, CRDA and PDA media were found to be the suitable media for mycelial growth of *Pyricularia oryzae*. Slide culture and microscopic study was done by using the pure culture of *Pyricularia oryzae* from CRDA and PDA media. Mycelia, conidiophores and conidia were found under the light microscope. The length and breadth of the conidia were measured using ocular micrometer. The size of conidia was measured about 17.96-26.64 µm × 7.36-9.22 µm (average 22.42 × 8.59 µm) and 12.06-19.95 µm × 5.38-9.06 µm (average 16.45 × 7.46 µm) in CRDA and PDA media, respectively (Table 1 and Figure 1).

| Selected Media | Growth(mm) | | |
|---|---|---|---|
| | Days of observation | | |
| | 7 DAI | 14 DAI | 21 DAI |
| PDA | I-1= 29.8 c<br>I-2=32.13 b<br>I-3=30.42 c | I-1= 47.6 b<br>I-2= 50.19 a<br>I-3= 50.77 a | I-1= 49.3 b<br>I-2= 50.81 a<br>I-3= 60.16 a |
| CRDA | I-1= 30.8 c<br>I-2=33.83 a<br>I-3=30.83 c | I-1=57.3 a<br>I-2=52.27 b<br>I-3=52.67 b | I-1=70.13 a<br>I-2=66.00 a<br>I-3=80.14 a |
| CMA | 0 | 0 | 0 |

**Table 1. Radial mycelial growths of *Pyricularia oryzae* in selected culture media (I-1= Isolate 1 from Dinajpur, I-2= Isolate 2 from Panchgrah, I-3= Isolate 3 from Thakurgaon)**

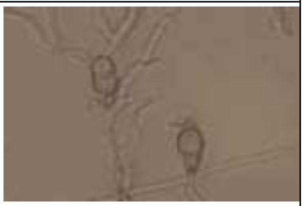
| Media | Day After Initiation(DAI) | | | Microscopic view |
|---|---|---|---|---|
| | 7 DAI | 14 DAI | 21 DAI | |
| PDA |  |  |  |  |
| CRDA |  |  |  |  |

**Figure 1. Growth of *Pyricularia oryzae* in selected media and morphological characters**

**4.2 Usefulness of $K_2SiO_3$on disease incidence and severity of rice Blast in pot culture:**

$K_2SiO_3$ was sprayed as a foliar spray with different doses that had been described in methodology section. The disease incidence was recorded at 45, 60 and 75 DAS of $K_2SiO_3$ spraying and at panicle initiation stage. It was revealed that blast disease incidence significantly decreases with the increase of $K_2SiO_3$ doses. The highest disease reduction was found at 4 g/L $K_2SiO_3$ spraying and disease incidence 1.15-5.5%. It was also observed that $K_2SiO_3$ doses increased up to 10 g/L and after 4 g/L $K_2SiO_3$ dose disease incidence was slightly increased. The highest disease incidence (55.77-90%) was recorded in controlled treatment (Table 2).$K_2SiO_3$ was sprayed as a foliar spray with different doses that had also been described in methodology section. The disease severity was recorded at 45, 60 and 75 DAS of $K_2SiO_3$ spraying and at panicle initiation stage. It was revealed that blast disease severity significantly decreases with the increase of $K_2SiO_3$ doses. The highest disease severity reduction was found at 4 g/L $K_2SiO_3$ spraying and disease severity was 3.33-6.0%. It was also observed that $K_2SiO_3$ doses increased up to 10 g/L and after 4 g/Ldose of$K_2SiO_3$, disease severity was slightly increased. The highest disease severity (33.33-75%) was recorded in controlled treatment (Table 3).

| Application doses of $K_2SiO_3$ | Disease incidence (%)@ 45 DAS | Disease incidence (%) @ 60 DAS | Disease incidence (%) @ 75 DAS | Disease incidence (%) at panicle initiation stage |
|---|---|---|---|---|
| $0\ g\ L^{-1}\ H_2O$ | 55.77 a | 55.77 a | 63.39 a | 90 a |
| $1\ g\ L^{-1}\ H_2O$ | 2.47 b | 3.90 b | 5.75 b | 40 b |
| $2\ g\ L^{-1}\ H_2O$ | 6.52 b | 7.76 b | 7.75 b | 40 b |
| $4\ g\ L^{-1}\ H_2O$ | 1.15 b | 3.45 b | 4.56 b | 5.5 f |
| $8\ g\ L^{-1}\ H_2O$ | 5.71 b | 8.01 b | 12.06 b | 10 d |
| $10\ g\ L^{-1}\ H_2O$ | 3.17 b | 3.17 b | 3.17 b | 20 c |
| LSD(0.05) T | 15.21 | 15.530 | 13.58 | 17.38 |

**Table 2. Efficacy of potassium silicate ($K_2SiO_3$) on disease incidence (%) of rice blast in Pot culture after inoculation with *Pyricularia oryzae***

| Application doses of $K_2SiO_3$ | Disease severity (%) @ 45 DAS | Disease severity (%) @ 60 DAS | Disease severity (%) @ 75 DAS | Disease severity (%) at panicle initiation stage |
|---|---|---|---|---|
| $0\ g\ L^{-1}\ H_2O$ | 33.33 a | 33.33 a | 46.67 a | 75 a |
| $1\ g\ L^{-1}\ H_2O$ | 6.67 b | 8.33 b | 13.33 b | 62 b |
| $2\ g\ L^{-1}\ H_2O$ | 10.0 b | 10.0 ab | 10.0 b | 53 c |
| $4\ g\ L^{-1}\ H_2O$ | 3.33 b | 3.33 b | 5.0 b | 6.0 f |
| $8\ g\ L^{-1}\ H_2O$ | 10.0 b | 15.00 ab | 33.33 ab | 11 e |
| $10\ g\ L^{-1}\ H_2O$ | 10.0 b | 13.33 ab | 16.67 ab | 42 d |
| LSD(0.05)T | 10.0 | 11.47 | 14. 37 | 15.89 |

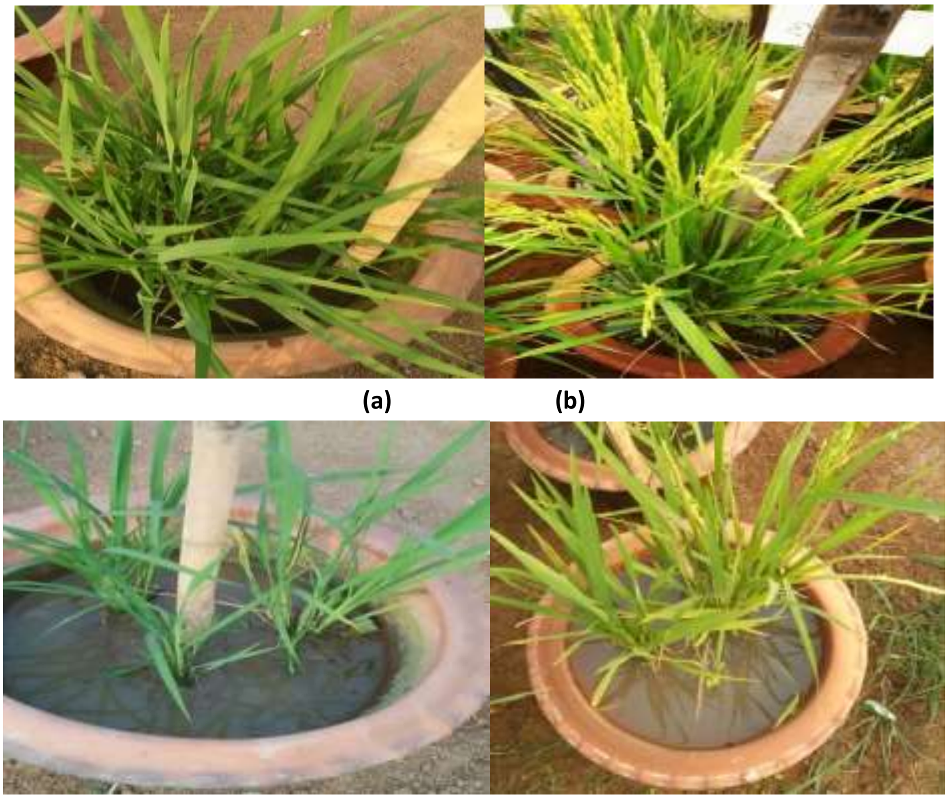
**Table 3. Efficacy of potassium silicate ($K_2SiO_3$) on disease severity (%) of rice blast in pot culture after inoculation with *Pyricularia oryzae***

### 4.3 Effect of $K_2SiO_3$ on rice yield attributes

A comparison of tiller number/pot and panicle mass/ pot at the vegetative stage and panicle initiation stage indicated that there were significant effects of potassium silicate at any of the doses. In case of tiller number/pot, the results showed a significant variation from 9.67 in the control (no $K_2SiO_3$was applied) to 30.0 at 4 g/L$K_2SiO_3$.In case of panicle mass/pot, the results showed a significant varied from 8.67 in the control (no $K_2SiO_3$was applied) to 28.0 at 4 g/L$K_2SiO_3$ (Table 4 and Figure 2).

| Treatment | Number of tiller/pot | Panicle mass/pot |
|---|---|---|
| $T_1$=0 g $L^{-1}$ $H_2O$ (only $H_2O$) | 9.67 c | 8.67 c |
| $T_2$=1 g $L^{-1}$ $H_2O$ | 23.33 ab | 21.33 ab |
| $T_3$ = 2 g $L^{-1}$ $H_2O$ | 25.00 ab | 23.33 ab |
| $T_4$= 4 g $L^{-1}$ $H_2O$ | 30.00 a | 28.0 a |
| $T_5$=8 g $L^{-1}$ $H_2O$ | 25.66 ab | 23.7 ab |
| $T_6$=10 g $L^{-1}$ $H_2O$ | 18.33 b | 17.33 b |
| LSD (0.05) | 3.78 | 3.21 |

**Table 4. Effect of potassium silicate ($K_2SiO_3$) on increase of yield attributes in pot culture**



**(a) (b)**

**(c) (d)**

**Figure 2. Rice plants are showing the highest tiller number and panicles initiation in potassium silicate sprayed pots (a&b) and the lowest tiller number and panicles in control pots (c & d).**

## IV. DISCUSSION

From the present study, it was observed that among the selected media viz. PDA, CRDA and CMA, the best results were found in CRDA medium in considering to cultural and morphological study. In CRDA medium mycelia growth and sporulation rate was higher than PDA media and no mycelial growth and sporulation was found in CMA medium. Sporulation was not observed in PDA even after 25-35 days of culture maintenance. PDA was also used for isolation of *Pyricularia grisea* by Motlagh and Javadzadeh, 2010; Debashis, 2012 and Hajano *et. al*. 2012, they also observed the almost same colored mycelial growth in PDA media but not found any sporulation even after 2-3 months of culture maintenance in proper temperature and moist conditions. These results are also in agreement with Varma (2014) who studied that PDA and Oat meal agar media supported maximum mycelial growth of *P. oryzae* after 168 hours of incubation.

Length and breadth of the conidia of blast pathogen (*Pyricularia oryzae*) was measured from both CRDA media and from lesion developed by inoculation of that culture in the rice leaves. The size of the conidia was much higher from leaf sample than in the media. The size of conidia measured about 17.96 - 26.64 µm × 7.36 - 9.22 µm (average 22.42 × 8.59 µm) and 12.06 - 19.95 µm × 5.38 - 9.06 µm (average 16.45 × 7.46 µm) from leaf sample and media, respectively. At first the mycelia in cultures were hyaline to whitish in color, then changed to olivaceous and gradually turn into pinkish color. Shafaullah (2011) also observed that mycelium in cultures was first hyaline in colour, then changed to olivaceous, 1-5.2 µm in width, septate and branched. MijanHossain (2000) also observed that mycelium in culture was first hyaline then changed to olivaceous, 1– 5.2 µm in width, septate and branched. The spore measurements were 15 – 22 µm× 4 – 7 µm (Average, 17.4 µm × 5.2 µm). Mostly 3 celled conidia were found from both CRDA media and conidia were found from infected leaf samples.

In management study using $K_2SiO_3$, the results showed a significant decrease on blast disease incidence as $K_2SiO_3$ doses increased. Blast incidence varied from 55.77-90% in the control (no $K_2SiO_3$ applied) to 1.15-5.5% at 4 g L−1 $K_2SiO_3$. The effect of blast decrease was observed in treatments with solution pH at 5.5. Similar results were observed by Bowen *et al.* (1992), who stated that the major mechanism for reduction of mildew on grapes was a direct effect of foliar $K_2SiO_3$ hindering the development of the pathogen, thus affecting its propagation. Research done in organic soils in southern Florida (USA), demonstrated that Si containing fertilization on rice reduced blast incidence 17 to 31% and helmintosporiosis 15 to 32% in relation to a non-fertilized control (Datnoff *et al.,* 1997 and You *et al.,* 2012). Therefore, Si containing fertilization can reduce or eliminate fungicide spraying in a culture, depending on disease severity. Studies relating powdery mildew reduction with foliar Si fertilization were done on cucumber, melon, and eggplant by Bowen *et al.* (1992). According to these authors, leaf spraying with potassium silicate reduced the number of fungus colonies on the leaves. Application of $K_2SiO_3$ also increased the number of tiller and panicle per pot.

## V. CONCLUSION

The study was comprised to develop reproducible protocol for the mycelial growth and sporulation of *Pyricularia oryzae* to determine the cultural and morphological variability among the isolates of the pathogen. At the end of the study it was found that the villages of Dinajpur district from where samples were collected were with higher disease incidence and severity, and had lower yield. Among these three media best growth and sporulation were observed in CRDA media. So from this study, it had been clear that the pathogenic races were changed due to the effect of climate change. It had been reported that the fungicides available in Bangladesh market are not working effectively against the new races of *Pyricularia oryzae*. Besides of this development of the new rice variety against the new races of *Pyricularia oryzae* was difficult and time consuming for rice breeders to breed for resistance to current races. In this situation new phyto-chemicals like potassium silicate ($K_2SiO_3$) at 4 g/L showed the best option to control/manage the blast disease of rice.

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