Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(2): 285-295
Received: 21-01-2019
Accepted: 25-02-2019

Vikas Kumar Ravat
Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur Nadia, West Bengal, India

Amitava Basu
Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur Nadia, West Bengal, India

Correspondence
Amitava Basu
Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur Nadia, West Bengal, India

Supplement of carbon and nitrogen nutrition towards assessment of biomass and virulence of rice fungal pathogen and endophytes

Vikas Kumar Ravat and Amitava Basu

Abstract
Tweleve rice fungal pathogen namely *Rhizoctonia solani*, *Curvularia lunata*, *Alternaria padwickii*, *Nigrospora oryzae*, *Sclerotium hydrophilum*, *Fusarium verticillioides*, *Fusarium* sp, *Rhizopus* sp, *Choanephora cucurbitarum*, *Acremonium* sp, SM 1 and SM 2 were isolated from Swarna cultivar (MTU-7029) of rice at Jaguli instructional farm, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia district of West Bengal. In the present research, experiments were done to evaluate the impact of two different carbon (4 g/l and 8 g/l glucose) and nitrogen (4 g/l and 8 g/l peptone) sources along with control treatment (PD broth). The impact was correlated with the dry mycelial weight of the isolated rice fungal pathogen as well as virulence of the pathogen. Although the fungus could grow in all the carbon and nitrogen sources used here, but the momentum of growth as supported by the dry mycelial weight in different experiments, were not equal. Among all isolated twelve fungal pathogens of rice six (*Rhizoctonia solani*, *Rhizopus* sp., *Choanephora cucurbitarum*, *Acremonium* sp., SM-1 and SM-2) showed maximum biomass against carbon source and remaining six fungus (*Sclerotium hydrophilum*, *Alternaria padwickii*, *Nigrospora oryzae*, *Curvularia lunata*, *Fusarium verticillioides* and *Fusarium* sp.) showed maximum mycelial growth on nitrogen source. Hence, these nutritional behaviours of pathogen may be utilized for their sustainable management.

Keywords: glucose, peptone, rice, MTU-7029, West Bengal

Introduction
Rice plays a key role as it is the staple food for over 2.7 billion people globally. It also delivers employment for over one billion, who either work directly in rice production or in related supported events [1]. Rice production and consumption is rigorous in Asia, where more than 90% of all rice is consumed. To feed a population of 1.4 billion by 2025, India will need to produce 301 million tonnes of food grains in addition to other commodities, at least 45 million tonnes of plant nutrients would be needed [2]. Unfortunately, such a central crop is beneath the threat of abiotic and biotic stresses. The foremost issues are challenging agriculturists to grow supplementary sustainable management systems for rice production like no other time in history. To meet the food and nutritional desires of a growing population, agriculture will essential to move beyond the past emphasis on productivity to encompass improved public health, social well-being and a sound environment [3]. Also, it is important to find alternative measures to control rice diseases which do not damage the environment and at the same time increase yield and improve product value [4, 5, 6]. Nutrients are essential for growth and development of plants and also microorganisms, and they are significant factors in disease control [7]. All the essential nutrients can disturb disease severity [8]. However, there is no general regulation, as a specific nutrient can decrease the incidence of a disease but can also increase the severity of the disease incidence of further diseases [9, 10, 11]. Despite the fact that the reputation of nutrients in disease control has been recognized for some of the most severe diseases, the correct management of nutrients in order to control disease in sustainable agriculture has received little attention [8]. The aim of the present research to understand the nutritional requirement and biology of the rice fugal pathogen.

Materials and Methods
a) Isolation, purification and identification of pathogen
Disease samples consisting on seed, leaf blade and leaf sheath of Swarna cultivar (MTU-7029) of rice were collected from Jaguli instructional farm, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia district of West Bengal, India. These samples were placed in paper bags, which were properly labelled and brought to the laboratory for isolation of disease-causing fungi. Fungus was isolated from rice leaves [12] as well as seed [13].

Different fungal colonies were appeared, which were purified and multiplied on PDA. Pathogenicity of fungus was detected by detach leaf technique. The data on frequency of isolated fungi from seeds and leaves of Swarna cultivar were recorded using the following formula:

Colonization % = {(Number of seeds/pieces colonized by a fungus)/(Total number of seeds/pieces studied)}×100

The isolated fungal species were identified on the basis of their cultural and morphological characteristics with the help of ITCC (Indian Type Culture Collection) and S.H. Ou [14].

b) *In vitro* screening of isolated pathogen based on different carbon and nitrogen doses

To study the growth of different rice fungus they were grown in PD broth, in which two different doses of glucose (4 g/L and 8 g)/L as a carbon source and two different doses of peptone (4 g/L and 8 g/L) as a nitrogen source was added and a control (PDB) was maintained. The pH of the medium was adjusted at the respective pH optimum for the fungus by using 0.1 N HCl and 0.1 N NaOH. A volume of 25 ml each of the aliquots was dispensed in 100 ml Erlenmeyer flasks and autoclaved, inoculated the flasks by adding 7 mm mycelium disc of the fungus. The inoculated flasks were incubated at 28 ±2°C for 15 d as shake culture. After incubation period, the mycelial mat was filtered through Whatman's No. 42 filter paper. Before use, the filter papers were previously oven dried at 70^0C for 3 consecutive days until constant dry weight was achieved and weighed (W1) after keeping them in desiccator. The mycelial mat over the filter paper disc was washed three times with sterilized water in order to remove the traces of salts adhering to mycelial mat and then, filter papers along with mycelial mats were dried up to constant weight in an oven at 70^0C for 48 hours. After drying, the filter papers were cooled in moisture free desiccator and weighed (W2) immediately. The weight of dry mycelium (W3) was calculated as W3=W2–W1. Out of four replications, three were used for recording dry mycelial weight and fourth replicate was used to count the number of spores per microscopic field at low power and compare with spore present in control.

c) *In vivo* screening of isolated pathogen based on different carbon and nitrogen doses

In vivo screening of isolated rice fungal pathogen was performed under controlled condition on Swarna cultivar of rice. Each carbon and nitrogen doses were inoculated on rice plant along with control and Relative lesion height (RLH) was recorded at five-day interval up to fifteen days. Lesion length and number of lesions of different rice fungus were also measured in cm with the help of plastic scale. RLH was measured using following formula [15]:

Relative lesion height (RLH) = (Lesion length / Plant height) x 100

Results

a) Isolation and morphology of pathogen

Twelve sheath infecting fungal pathogen namely *Rhizoctonia solani*, *Curvularia lunata*, *Alternaria padwickii*, *Nigrospora oryzae*, *Sclerotium hydrophilum*, *Fusarium verticillioides*, *Fusarium* sp, *Rhizopus* sp, *Choanephora cucurbitarum*, *Acremonium* sp, SM 1 and SM 2 were isolated from paddy leaves (Figure 1). Among all the 12 fungi the frequency of sheath blight caused by *Rhizoctonia solani* was highest (47.87%) followed by *Sclerotium hydrophilum* (16.4%), *Fusarium* sp (12.99%), *Alternaria padwickii* (12.45%), *Curvularia lunata* (11.23%), *Choanephora cucurbitarum* (6.82%), *Nigrospora oryzae* (5.18%), *Fusarium verticillioides* (3.67%), SM 2 (2.9%), SM 1 (2.7%), *Rhizopus* sp (2.16%) and *Acremonium* sp (2.1%). Ten fungi were isolated from seed (Figure 2) of Swarna cultivar. *Curvularia lunata* was found in maximum frequency (17.5%) followed by *Fusarium verticillioides* (13.16%), *Fusarium* sp (12.6%), *Alternaria padwickii* (12.15%), *Rhizopus* sp (10.12%), SM 1 (8.9%), *Choanephora cucurbitarum* (8.23%), *Acremonium* sp (7.13%), SM 2 (6.45%) and *Nigrospora oryzae* (6.3%). Mycelium of *Alternaria padwickii* is well developed, profusely branched, hyaline at young stage, mature hyphae creamy-yellow, thick, septate at regular intervals of 20-25 µm, branches arising at right-angles to the main axis and constricted at the point of origin (Figure 3, C). Conidia elongately fusoid, with a long appendage at tile tip, non-deciduous, 3 to 5 septate, creamy-yellow, constricted (Figure 3, C-i) at septa, thick-walled, straight with second or third cell. The mycelium of *Rhizoctonia solani* is colourless (Figure 3, A) when young and later becoming yellowish brown with infrequent septations. Sclerotia are superficial, more or less globose but flattened below, white when young and in later stages becoming brown or dark brown. The sclerotia of *Sclerotium hydrophilum* is more or less globose or oblong (Figure 3, B), often aggregated, surface rough, flat at the bottom, white, becoming grey or brownish grey at maturity, 0.3-l.5 mm, sometimes up to 2.5 mm, composed of uniform. Dense mycelium, light yellowish brown, 5.8-9.9µm in diameter. Mycelia of *Choanephora cucurabitarum* are hyaline and non-septate (Figure 3, G). Sporangiophore bearing sporangia is erect, hyaline, unbranched, apically dilated to form a clavate secondary vesicles. From the Figure 3, G (i) it is clearly observed that sporangia were indehiscent, ellipsoid, brown to dark brown with distinct longitudinal striation and measured 15-20 × 10-15 µm. Sporangia are multispored, spherical, initially white to yellow and pale brown to dark brown at maturity. The fungal colonies on PDA are white to pale yellowish brown. Microscopic examination of *Curvularia lunata* revealed the presence of conidia of the fungus. The conidia are four celled and generally curved, second cell larger with two middle cells darker than the paler end cells (Figure 3, F-i). Conidiophores are branched, septate and dark in colour. Isolation of the fungus from the infected parts is carried out on PDA (Potato Dextrose Agar) plates following the standard methodology. After two-three days' whitish mycelial growth appear which gradually become greyish black in colour (Figure 3, F). *N. oryzae* grows rapidly and produces white colonies, initially and then become brown to dark brown due to the abundance of sporulation (Figure 3, D). The species of *N. oryzae* produce a single-cell conidium of 14 -16 µM in diameter; each conidium is born on hyaline vesicle at the tip of the conidiophore of 4.5-6.0 µM. The conidium shape is ranging from spherical to black sub spherical (Figure 3, D-i) with the hyphae diameter at 7 -9 µM. The growth rate of *Acremonium* colonies is moderately rapid, maturing within 5 days. The diameter of the colony is 1-3 cm following incubation at 25°C for 7 days on potato glucose agar. The texture of the colony is compact, flat or folded, and occasionally raised in the centre. Powdery texture may also be observed. By aging, the surface of the colony may become cottony due to the overgrowth of loose hyphae (Figure 3, K). The colour of the colony is white, pale grey or pale pink on the surface. The reverse side is either colourless or a pink to

rose coloured pigment production is observed. *Acremonium* sp. possess hyaline, septate hyphae which are typically very fine and narrow. Unbranched, solitary, erect phialides (Figure 3, K-i) are formed directly on the hyphal tips, the hyphal ropes, or both. The phialides are separated from hyphae by a septum and taper towards their apices. They usually appear in clusters, in balls or rarely as fragile chains (Figure 3, K-ii). The conidia are bound by a gelatinous material. They may be single or multicellular, fusiform with a slight curve or resemble a shallow crescent. The colonies *Fusarium verticillioides* on PDA are creamy to peach to vinaceous on the obverse and pale cream to salmon to violet or red on the reverse (Figure 3, H). This taxon is characterized by mostly zero-septate, clavate microconidia (Figure 3, H-i) with a flat base produced on monophialides in chains in the aerial mycelium. The macroconidia are observing rarely. The mycelium of *Fusarium sp.* is white. It produces pale-red to dark-red pigments on PDA plate (Figure 3, L). Microscopic examinations show the presence of septate, hyaline hyphae. Macroconidia are long slender, abundant and borne on aerial mycelium (Figure 3, L-i). The fungus survives in the soil for long periods by producing resilient, microscopic structures called chlamydospores (Figure 3, L-ii & iii). Colonies of *Rhizopus* sp is very fast growing at 25°C, about 5 - 8 mm high, with some tendency to collapse, white cottony initially becoming brownish grey to yellowish depending on the amount of sporulation (Figure 3, E). Sporangiophores up to 1500 um in length and 18 µm in width, smooth walled, non - septate, simple or branched, arising from stolons opposite rhizoids usually in groups of 3 or more (Figure 3, E-i). Sporangia are globose, often with a flattened base, grayish black, powdery in appearance, up to 175 µm in diameter. SM-1 grew rapidly on PDA and produce dark brown colonies later becoming white and fluffy growth (Figure 3, I), while microscopy the hyphae is non-septate and having 90^0 angle branching patterns (Figure 3, I-i). SM-2 produced spreading type hyphae which initially light green in colour but later becoming dark green (Figure 3, J). Hyphae is hyaline and non-septate (Figure 3, J-i), both of the isolates are non-spore forming.

b) *In vitro* screening of isolated pathogen based on different carbon and nitrogen doses

Among all isolated twelve fungal pathogens of rice six (*Rhizoctonia solani*, *Rhizopus* sp., *Choanephora cucurbitarum*, *Acremonium* sp., SM-1 and SM-2) were showed good mycelium weight against carbon source and remaining six fungus (*Sclerotium hydrophilum*, *Alternaria padwickii*, *Nigrospora oryzae*, *Curvularia lunata*, *Fusarium verticillioides* and *Fusarium* sp.) showed maximum growth on nitrogen source. From the table 2 it is clearly observed that, *Choanephora cucurbitarum* showed maximum dry mycelial weight (524 mg) with 224.33 mg mean mycelial weight after 15 days of inoculation, followed by *Rhizopus* sp. (421 mg), *Rhizoctonia solani* (418 mg), *Acremonium* sp. (409 mg), SM-2 (401 mg) and SM-1 (398 mg) at higher dose of glucose (Treatment 2). *Choanephora cucurbitarum* recorded maximum mean dry mycelial weight (310.67 mg) on media containing 4 g/l of glucose (Treatment 1) followed by *Acremonium* sp. (256.67 mg), SM-1 (247.33 mg), SM-2 (237 mg), *Rhizoctonia solani* (234.67 mg) and *Rhizopus* sp. (231.33 mg). The total mean mycelial growth of treatment 1 was 383.67 mg after 15 days, whereas treatment 2 recorded 428.50 mg total mean mycelial weight after 15 days. Both of the carbon doses (T 1 and T 2) recorded maximum mycelial weight as compare to nitogen doses (T 4 and T 5) and control (T 3). Mean dry mycelial weight of *Nigrospora oryzae* (333.66 mg) was recorded highest, when media supplemented with 8 gm/l peptone (Table 3) afterwards *Fusarium verticillioides* (330.33 mg), *Sclerotium hydrophilum* (316 mg), *Curvularia lunata* (259.67 mg), *Alternaria padwickii* (48.66 mg) and *Fusarium* sp. (13.66 mg) recorded maximum dry mycelial weight respectively. Media containing 4g/l pepton exposed maximum dry mycelial weight of *Curvularia lunata* (328.18 mg) followed by *Fusarium verticillioides* (314.68 mg), *Sclerotium hydrophilum* (308.21 mg), *Fusarium* sp. (307.75 mg), *Alternaria padwickii* (291.08 mg) and *Nigrospora oryzae* (269.61 mg). The overall mycelial weight after 15 days recorded maximum on treatment 3 (397.50 mg) afterwards treatment 4 (355. 56 mg), treatment 5 (342.16 mg), treatment 1 (228.66 mg) and treatment 2 (190.16 mg) recorded maximum dry mycelial weight.

C) *Invivo* screening of isolated pathogen based on different carbon and nitrogen doses

Observation based on RLH data (Table 4) reveals that, *Rhizoctonia solani* recorded maximum RLH (38.76 %) after 15 days of inoculation on treatment 2 (Figure 4f), whereas *Rhizopus* sp. (Figure 4h) recorded 18.01 % RLH at the two-fold increase of carbon source in basel media. The relative lesion height of *Choanephora cucurbitarum* (27.71 %), *Acremonium* sp. (26.31 %), SM 1 (27.14 %) and SM 2 (27.94 %) were increasing with increasing carbon source in media (Figure 4a, 4e, 4k and 4l respectively). These six pathogens (*Rhizoctonia solani*, *Rhizopus* sp., *Choanephora cucurbitarum*, *Acremonium* sp., SM-1 and SM-2) were recorded highest RLH on carbon source as compare to control. The RLH (37.16 %) of *Sclerotium hydrophilum* (Figure 4i) was increasing in increasing in two-fold of the nitrogen source, whereas *Alternaria padwickii* (Figure 4b) recorded RLH when, basel media is supplemented by 4 g/l of nitrogen source (pepton). *Nigrospora oryzae* (Figure 4g) recorded increase in lesion height from 26.85 % (T3) to 29.47 % (T5) at higher dose of nitrogen (8 g/l) source in contrast, *Fusarium* sp. (Figure 4d) recorded increase in RLH from 26.85 % (T3) to 29.47 % (T4) at 4g/l of nitrogen substitution in media. The lesion height of *Curvularia lunata* (Figure 4j) and *Fusarium verticillioides* (Figure 4c) also increasing from 18.76 % (T3) to 23.73 % (T5) and 13.55 % (T3) to 27.83 % (T5) respectively with increasing dose of Pepton supplemented in basel media. The overall maximum RLH was recorded 22.39 % (T3) after 15 day followed by T2 (17.35 %), T1 (17.12 %), T4 (16.46 %) and T5 (11.84 %).

d) Cluster analysis

Hierarchical clustering was calculated for all fungal pathogens of rice based on the *in vitro* dry mycelial weight and *in vivo* virulence level data and presented as a dendrogram (Figure 5). Cluster analysis based on *in vitro* dry mycelial weight and *in vivo* virulence level using distance between clusters, gave three major clusters with distance between clusters ranging from 0.0-25.0. Six fungus (*Rhizoctonia solani*, *Rhizopus* sp., *Choanephora cucurbitarum*, *Acremonium* sp., SM-1 and SM-2) comes under cluster-I and showed maximum dry mycelial weight and high virulence level against substitution of carbon source in broth media. Cluster-II contains four fungus (*Sclerotium hydrophilum*, *Nigrospora oryzae*, *Curvularia lunata* and *Fusarium verticillioides*), which showed high virulence and dry mycelial weight when, nitrogen was substituted in the

broth. *Alternaria padwickii* and *Fusarium* sp. comes under cluster-III because, they are having moderate growth on carbon source as well as first dose of nitrogen source (4 g/l) but, when the nitrogen dose was increasing their dry mycelial weight and virulence level was decreasing drastically.

T**able 1:** Experimental details of different treatments.

Treatment no.	Treatment	Treatment details
T1	Treatment 1	4 g/L glucose added in Potato Dextrose Broth (PDB)
T2	Treatment 2	8 g/L glucose added in PDB
T3	Treatment 3	Control without adding any supplement (Potato Dextrose Broth)
T4	Treatment 4	4 g/L peptone added in Potato Dextrose Broth (PDB)
T5	Treatment 5	8 g/L peptone added in Potato Dextrose Broth (PDB)

Table 2: Effect of carbon source on biomass of different rice fungal pathogens

Treatment 1										
Factor	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**MA**		**SE(m)**	**C.D.**
A1	139.00	124.00	140.00	130.00	123.00	121.00	129.50	(A)	2.323	6.70*
A2	220.00	219.00	294.00	259.00	243.00	239.00	245.67	(B)	3.286	9.48*
A3	345.00	351.00	498.00	381.00	376.00	351.00	383.67	(A X B)	5.691	16.42*
MB	234.67	231.33	310.67	256.67	247.33	237.00	-	-	-	-
Treatment 2										
Factor	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**MA**		**SE(m)**	**C.D.**
A1	145.00	147.00	145.00	139.00	132.00	126.00	139.00	(A)	2.567	7.41*
A2	295.00	298.00	304.00	265.00	261.00	276.00	283.17	(B)	3.631	10.47*
A3	418.00	421.00	524.00	409.00	398.00	401.00	428.50	(A X B)	6.288	18.15*
MB	286.00	288.67	324.33	271.00	263.67	267.67	-	-	-	-
Treatment 3										
Factor	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**MA**		**SE(m)**	**C.D.**
A1	118.00	118.00	123.00	117.00	120.00	116.00	118.67	(A)	1.915	5.52*
A2	206.00	209.00	251.00	213.00	227.00	231.00	222.83	(B)	2.708	7.81*
A3	291.00	287.00	389.00	281.00	279.00	284.00	301.83	(A X B)	4.69	13.53*
MB	205.00	204.67	254.33	203.67	208.67	210.33	-	-	-	-
Treatment 4										
Factor	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**MA**		**SE(m)**	**C.D.**
A1	92.00	89.00	113.00	105.00	114.00	110.00	103.83	(A)	1.6	4.61*
A2	107.00	98.00	229.00	181.00	212.00	201.00	171.33	(B)	2.263	6.53*
A3	152.00	101.00	314.00	269.00	301.00	296.00	238.83	(A X B)	3.92	11.31*
MB	117.00	96.00	218.67	185.00	209.00	202.33	-	-	-	-
Treatment 5										
Factor	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**MA**		**SE(m)**	**C.D.**
A1	89.00	89.00	102.00	117.00	120.00	117.00	105.67	(A)	1.548	4.46*
A2	98.00	94.00	187.00	210.00	217.00	207.00	168.83	(B)	2.19	6.32*
A3	125.00	103.00	273.00	291.00	308.00	291.00	231.83	(A X B)	3.793	10.94*
MB	104.00	95.33	187.33	206.00	215.00	205.00	-	-	-	-

Where,	
Factor	**Treatment**
A1	5 days after inoculation
A2	10 days after inoculation
A3	15 days after inoculation
B1	*Rhizoctonia solani*
B2	*Rhizopus sp.*
B3	*Choanephora cucurbitarum*
B4	*Acremonium sp.*
B5	SM-1
B6	SM-2
MA	Mean of factor A
MB	Mean of factor B
*	P≤ 0.05

Table 3: Effect of nitrogen source on biomass of different rice fungal pathogens

Treatment 1										
Factor	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**MA**		**SE(m)**	**C.D.**
A1	91.00	88.00	112.00	89.00	103.00	102.00	97.50	(A)	1.32	3.81*
A2	107.00	109.00	234.00	91.00	231.00	221.00	165.50	(B)	1.87	5.40*
A3	143.00	153.00	324.00	98.00	342.00	312.00	228.66	(A X B)	3.24	9.35*
MB	113.66	116.66	223.33	92.66	225.33	211.66	-	-	-	-
Treatment 2										

Factor	B1	B2	B3	B4	B5	B6	MA		SE(m)	C.D.
A1	88.00	76.00	106.00	87.00	112.00	109.00	96.33	(A)	1.11	3.21*
A2	101.00	93.00	191.00	92.00	201.00	197.00	145.83	(B)	1.57	4.55*
A3	135.00	110.00	276.00	97.00	271.00	252.00	190.16	(A X B)	2.73	7.88*
MB	108.00	93.00	191.00	92.00	194.66	186.00	-	-	-	-
Treatment 3										
Factor	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**MA**		**SE(m)**	**C.D.**
A1	135.00	105.00	125.00	131.00	125.00	128.00	124.83	(A)	1.95	5.65*
A2	287.00	216.00	253.00	275.00	217.00	231.00	246.50	(B)	2.77	7.99*
A3	437.00	437.00	387.00	416.00	363.00	345.00	397.50	(A X B)	4.79	13.85*
MB	286.33	252.66	255.00	274.00	235.00	234.66	-	-	-	-
Treatment 4										
Factor	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**MA**		**SE(m)**	**C.D.**
A1	189.67	190.38	181.94	207.18	197.13	199.65	194.32	(A)	37.38	NS
A2	356.31	357.21	313.18	392.26	368.03	372.23	359.87	(B)	52.87	NS
A3	378.66	325.65	313.72	385.09	378.87	351.38	355.56	(A X B)	91.58	NS
MB	308.21	291.08	269.61	328.18	314.68	307.75	-	-	-	-
Treatment 5										
Factor	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**MA**		**SE(m)**	**C.D.**
A1	143.00	31.00	152.00	129.00	147.00	10.00	102.00	(A)	1.93	5.59*
A2	299.00	56.00	301.00	263.00	309.00	13.00	206.83	(B)	2.73	7.90*
A3	506.00	59.00	548.00	387.00	535.00	18.00	342.16	(A X B)	4.74	13.69*
MB	316.00	48.66	333.66	259.67	330.33	13.66	-	-	-	-

Where,	
Factor	**Treatment**
A1	5 days after inoculation
A2	10 days after inoculation
A3	15 days after inoculation
B1	*Sclerotium hydrophilum*
B2	*Alternaria padwickii*
B3	*Nigrospora oryzae*
B4	*Curvularia lunata*
B5	*Fusarium verticillioides*
B6	*Fusarium* sp.
MA	Mean of factor A
MB	Mean of factor B
*	P≤ 0.05

Table 4: Effect of different carbon and nitrogen source on virulence level of rice fungal pathogens

Factor	Treatment 1				Treatment 2				Treatment 3				Treatment 4				Treatment 5			
	A1	A2	A3	MB	A1	A2	A3	MB	A1	A2	A3	MB	A1	A2	A3	MB	A1	A2	A3	MB
B1	5.10	20.00	35.10	20.07	9.20	27.12	38.76	25.02	4.12	14.86	20.16	13.04	3.74	7.39	9.24	6.79	2.45	3.16	5.14	3.58
B2	1.30	4.80	7.23	4.44	1.20	3.70	5.10	3.33	3.40	8.90	20.12	10.80	14.2	21.70	34.69	23.53	14.30	28.74	37.16	26.73
B3	8.70	11.34	16.90	12.31	7.10	9.30	12.79	9.73	7.80	19.34	29.67	18.93	8.34	21.87	31.56	20.59	3.10	5.60	5.90	4.87
B4	2.30	5.70	9.60	5.87	1.60	1.90	2.70	2.06	7.12	14.85	21.67	14.54	8.12	14.98	23.56	15.55	8.34	15.73	25.36	16.48
B5	8.12	15.78	22.76	15.55	8.29	18.23	27.51	18.01	7.39	14.85	21.65	14.63	1.30	2.60	3.76	2.553	1.10	2.48	3.12	2.23
B6	1.20	2.89	4.25	2.78	1.10	2.40	3.76	2.42	4.64	10.15	18.76	11.18	5.29	12.87	20.51	12.89	7.43	14.83	22.73	15.00
B7	7.40	15.56	23.98	15.65	8.37	16.29	27.71	17.47	6.93	12.95	20.18	13.35	1.50	2.96	4.17	2.87	1.40	2.60	4.10	2.70
B8	1.80	3.90	5.23	3.64	1.20	2.97	4.54	2.90	7.19	13.36	20.12	13.55	8.35	14.27	23.86	15.49	9.41	16.39	27.83	17.88
B9	6.92	14.87	23.84	15.21	7.51	15.94	26.31	16.57	6.53	13.87	21.85	14.08	2.17	3.76	5.11	3.68	1.94	2.91	3.57	2.81
B10	2.45	4.19	5.19	3.94	1.27	2.74	3.95	2.65	8.12	16.94	26.85	17.30	9.63	18.39	29.47	19.16	0.20	0.70	0.91	0.60
B11	5.71	17.28	26.13	16.37	7.23	18.39	27.14	17.57	5.12	16.32	23.91	15.11	1.90	3.20	5.98	3.69	1.25	2.85	3.16	2.42
B12	6.12	15.93	25.17	15.74	7.18	16.25	27.94	17.13	4.98	14.82	23.83	14.54	1.79	2.97	5.63	3.46	1.31	2.61	3.12	2.35
MA	4.76	11.02	17.12	-	5.10	11.26	17.35	-	6.11	14.26	22.39	-	5.52	10.58	16.46	-	4.35	8.22	11.84	-
	SE(m)		C.D.		SE(m)		C.D.		SE(m)		C.D.		SE(m)		C.D.		SE(m)		C.D.	
(A)	0.078		0.220*		0.084		0.237*		0.087		0.246*		0.070		0.198*		0.056		0.158*	
(B)	0.156		0.440*		0.168		0.475*		0.174		0.491*		0.140		0.396*		0.112		0.316*	
(A X B)	0.270		0.763*		0.291		0.823*		0.301		0.851*		0.243		0.687*		0.194		0.547*	

Where

Treatment	Fungus name	Treatment	Fungus name	Treatment	Fungus name
A1	5 days after inoculation	B4	*Nigrospora oryzae*	B10	*Fusarium sp.*
A2	10 days after inoculation	B5	*Rhizopus sp.*	B11	SM-1
A3	15 days after inoculation	B6	*Curvularia lunata*	B12	SM-2
B1	*Rhizoctonia solani*	B7	*Choanephora cucurbitarum*	MA	Mean of factor A
B2	*Sclerotium hydrophilum*	B8	*Fusarium verticillioides*	MB	Mean of factor B
B3	*Alternaria padwickii*	B9	*Acremonium sp.*	*	P≤ 0.05

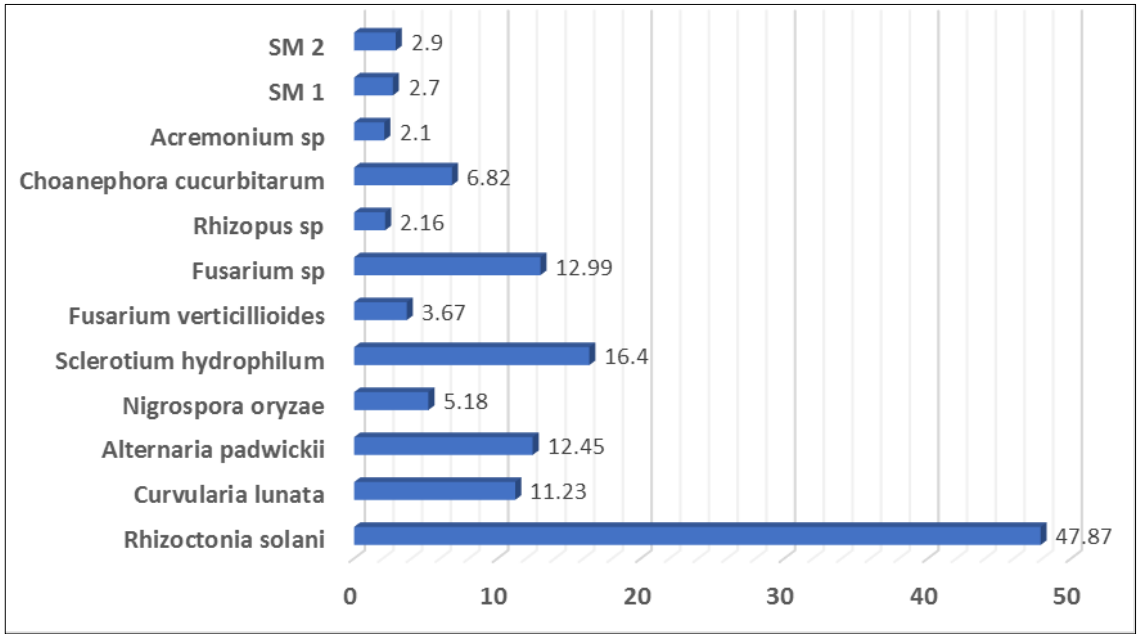


Fig 1: frequency of fungus isolated from rice leaves

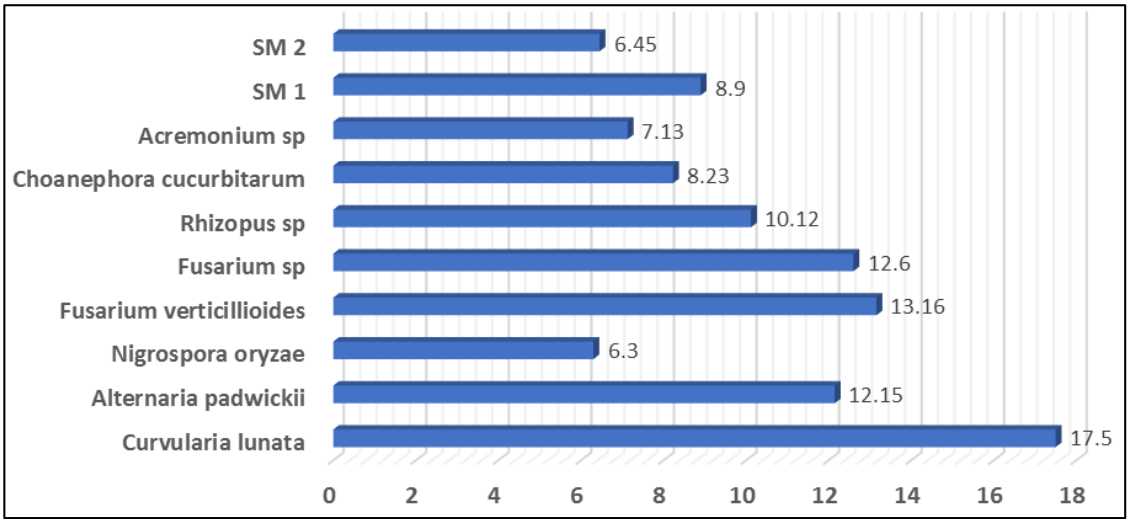
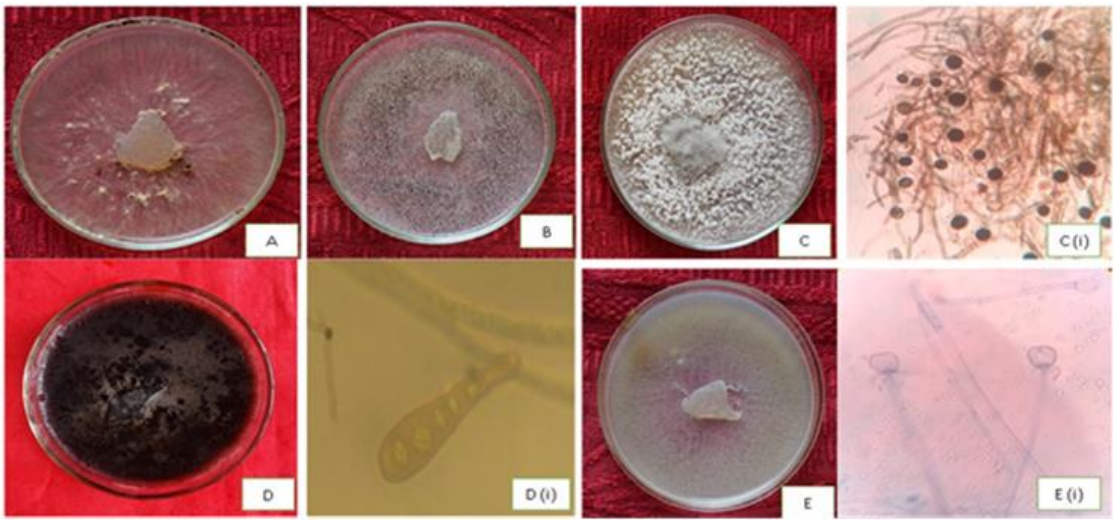


Fig 2: Frequency of fungus isolated form rice seed



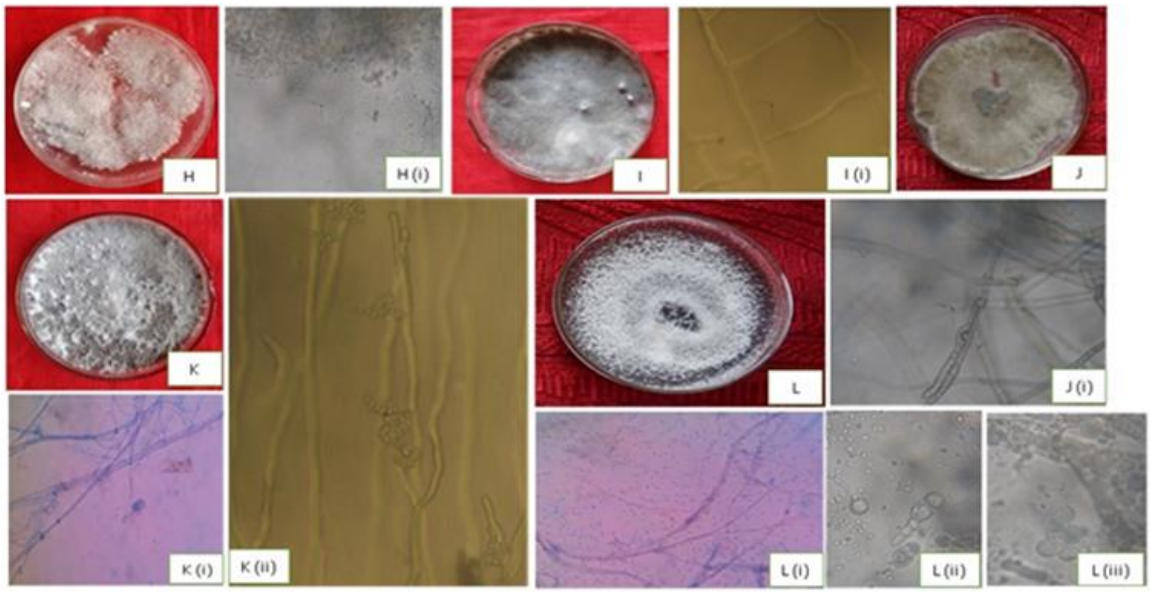


Figure 3. cultural characteristics of (A) *Rhizoctonia solani* , (B) *Sclerotium hydrophilum*, (C) *Alternaria padwickii*, (C- i) Microscopic characteristics of *Alternaria padwickii*, (D) *Nigrospora oryzae*, (D- i) Microscopic characteristics of *Nigrospora oryzae*, (E) *Rhizopus* sp, (E- i) Microscopic characteristics of *Rhizopus* sp(F- i) Microscopic characteristics of *Curvularia lunata*, (G) *Choanephora cucurabitarum*, (G- i) Microscopic characteristics of *Choanephora cucurabitarum*, (H) *Fusarium verticillioides*, (H- i) Microscopic characteristics of *Fusarium verticillioides*, (I) SM-1, (I- i) Microscopic characteristics of SM-1, (J) SM-2, (J- i) Microscopic characteristics of SM-2, (K) *Acremonium* sp., (K- i & ii) Microscopic characteristics of *Acremonium* sp., (L) *Fusarium* sp., (L- i) Microscopic characteristics of Fusarium sp., (L- i & ii) Chlamydospores of *Fusarium* sp.

Fig 4a: Effect of different carbon and nitrogen source on virulence of a) *Choanephora cucurbitarum* b) *Alternaria padwickii* c) *Fusarium verticillioides* and d) *Fusarium* sp.

Fig 4b: Effect of different carbon and nitrogen source on virulence of e) *Acremonium* sp f) *Rhizoctonia solani* g) *Nigrospora oryzae* and h) *Rhizopus* sp.

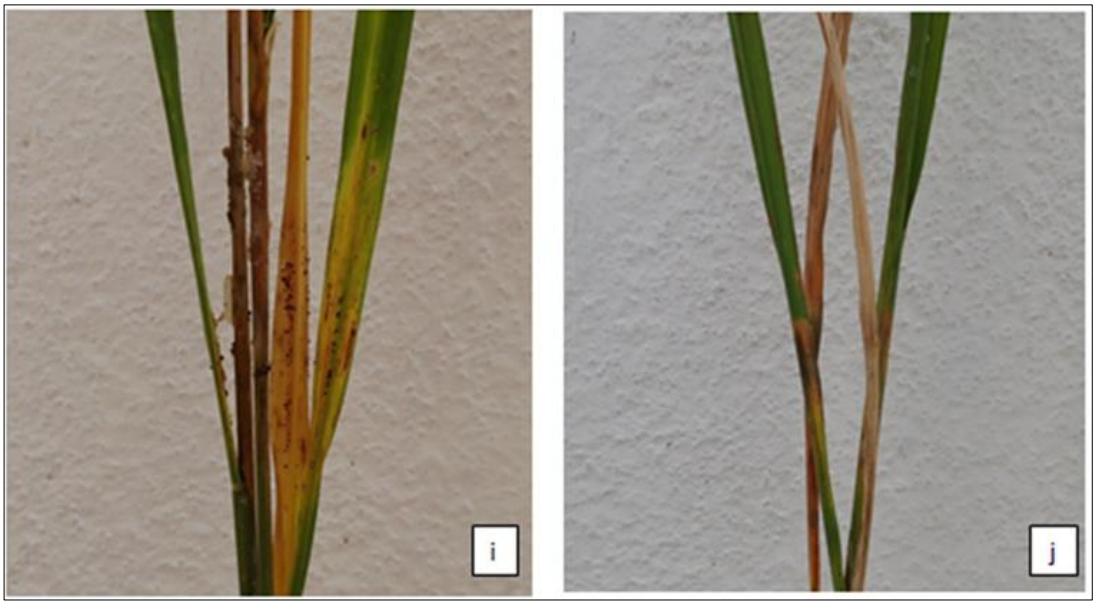


Fig 4c: Effect of different carbon and nitrogen source on virulence of i) *Sclerotium hydrophilum* j) *Curvularia lunata* k) SM 1 and l) SM 2

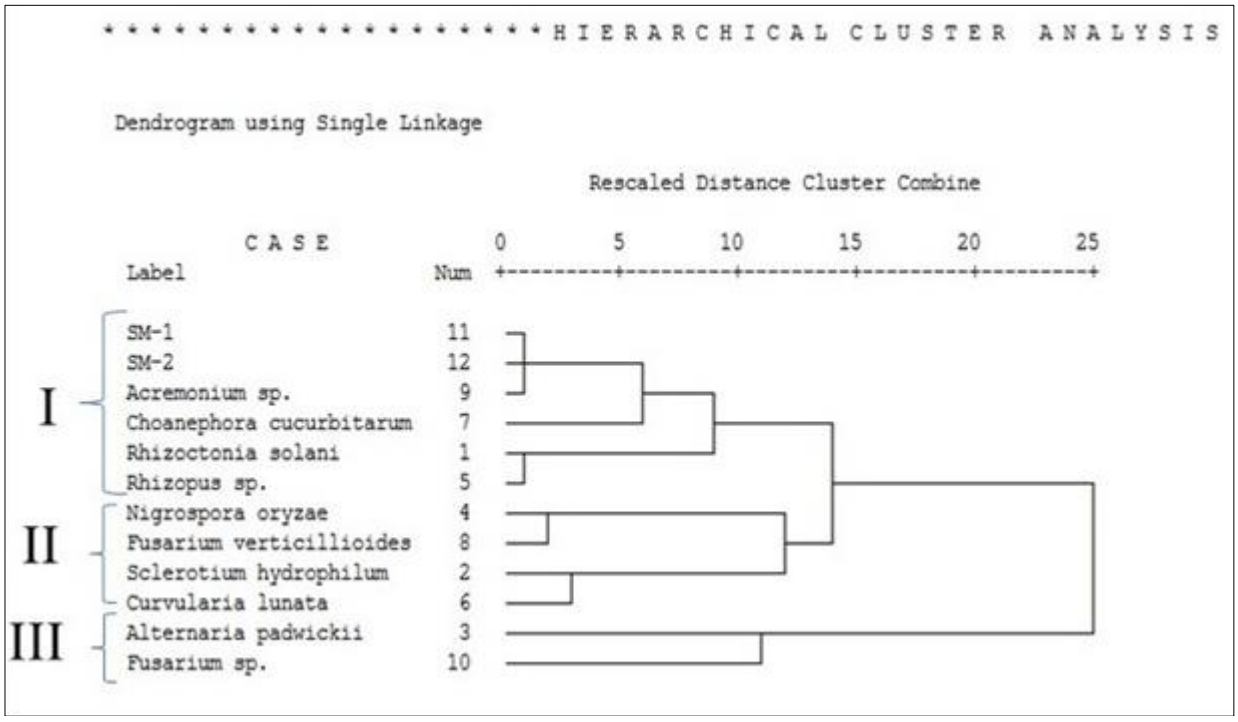


Fig 5: Dendrogram of biomass and virulence level of different rice fungal pathogens

Discussion

The maximum biomass and virulence of *Rhizoctonia solani* was observed in media containing glucose as carbon source which, was in conformity with the earlier reports [16, 17]. From the study of *Fusarium verticillioides* and *Fusarium* sp. it was noticed that that different carbon and nitrogen sources supplemented to the culture broth differentially influenced the growth of the *Fusarium*. Mono- and disaccharides are preferred most by the *Fusarium* isolates in comparison to polysaccharides, whereas, organic nitrogen compounds were of obvious choices for the Fusarium soil spp. regarding nitrogen source utilization [18]. The earlier scientist also reported that, the combination of a particular carbon and nitrogen source markedly increased mycelial growth of the *Fusarium* isolates [19]. Carbon source utilization of the fungal isolates is of diverse type. *Curvularia lunata* was noticed to produce more biomass and high virulence in media containing peptone as nitrogen source, which signifies that, nitrogen is the most important substance for *Curvularia lunata* required by them with regards of vegetative and reproductive growth [20]. Nitrogen source was superior to carbon source for growth and virulence of *Nigrospora oryzae* [21]. The good biomass and virulence of *Rhizopus* sp. was obtained in glucose, indicated that the fungus possessed the necessary growth factors for assimilation of glucose or the pathogen have the ability to form adaptive enzymes. The configuration of the glucose molecule also favoured good growth of *Rhizopus* sp. The ability of the fungus to utilized sucrose and starch was due to the possession of relevant enzymes for the hydrolysis of sucrose and starch into their component sugars. On the other hand, it could also be due to the ability of the fungus to utilize the hydrolytic products of these sugars [22]. Some earlier researchers [23] reported that, maximum growth of *Choanephora cucurbitarum* in potato dextrose agar medium,

which highly supports our finding. *Alternaria padwickii* recorded good biomass on treatment 4 (291.08 mg) followed by treatment 3 (252.66 mg), treatment 1 (116.66 mg), treatment 2 (93 mg) and treatment 5 (48.66 mg) which, signifies that pathogen is more virulence in carbon as well as nitrogen source. This may be also stated that, the combination of different carbon and nitrogen sources are influential for pathogen growth and virulence [24]. The bio mass of *Acremonium* sp. was also favours carbon as well as nitrogen source (25) in contrast the virulence of *Acremonium* sp. was observed maximum when, glucose as a carbon source substituted in broth. *Sclerotium hydrophilum* was noticed maximum growth and virulence on Peptone substitution in medium. Some earlier researchers [26] reported that excessive use of exogenous nitrogen may contribute to the severity of the disease, it is often seen that it became more serious in rice fields where the level of nitrogen was the highest.

The development as well as secondary metabolites production of different species of fungi differs from medium to medium [27, 28, 29]. To enhance the production of these secondary metabolites, a consistent and optimal growth conditions are needed for microbes [30, 31]. Nitrogen and carbon are the most important element for the progress and secondary metabolites construction of fungi. The carbon to nitrogen ratio may contribute in regulating the pH and hence to increase the secondary metabolites construction [32]. Organic acids have an important impact on fungal nutrition and physiology apart from their possible utilization as carbon and energy sources, which comprise contributions to intracellular osmotic potential, charge balance, and pH homeostasis [33, 34]. In addition, the construction of metal-complexing organic acids assists both essential metal and anionic nutrition of fungi and plants via solubilization of phosphate and sulfate, from insoluble metal containing substances, including salts and minerals [35, 36]. Organic acid production is wide spread in fungi with factors disturbing biosynthesis including carbon and nitrogen sources and changes in pH. For example, nitrate acts as a nitrogen source and maintain the pH to enhance oxalate production by *S. rolfsii* [37].

Conclusions

In this experiment, it is clearly indicated that, the different rice fungal isolates had varying growth pattern and virulence according to different carbon and nitrogen doses. Some pathogens prefer lower nitrogen dose for growth and virulence as well as some pathogen prefer higher biomass and virulence on higher nitrogen doses and vice versa. This above finding is helpful to understand the pathogen biology of rice fungal pathogen and it will also helpful to strategies a healthy management practise against isolated fungal pathogen.

References

1. Dat VT. *Rice Agriculture: Prospects and Strategies in Asia*, Paper presented at the workshop on Sustainable Use of Agricultural Resources and Environment Management with focus on the role of Rice Farming Tokyo, Japan, 2004.
2. Patel U, Netam CR, Anurag. Residual effect of organic and inorganic fertilizer nutrients on sulphur content and uptake in wheat crop under Rice-Wheat cropping system. J Soils Crops. 2011; 21:82-5.
3. Hanson JD, Liebig MA, Merrill SD, Tanaka DL, Krupinsky JM, Stott DE. Dynamic cropping systems: increasing adaptability amid an uncertain future, Agron. J. 2007; 99:939-943.
4. Atkinson D, McKinlay RG. Crop protection and its integration within sustainable farming systems. Agr. Ecosyst. Environ. 1997; 64:87-93.
5. Batish DR, Singh HP, Setia N, Kohli RK, Kaur S, Yadav SS. Alternative control of littleseed canary grass using eucalypt oil, Agron. Sustain. Dev. 2007; 27:171-177.
6. Camprubí A, Estaún V, El Bakali M.A, Garcia-Figueres F, Calvet C. Alternative strawberry production using solarization, metham sodium and beneficial soil microbes as plant protection methods, Agron. Sustain. Dev. 2007; 27:179-184.
7. Agrios NG. *Plant Pathology*. 5th ed. (Elsevier-Academic Press), 2005, 635.
8. Huber DM, Graham RD. The role of nutrition in crop resistance and tolerance to disease,in : Rengel Z. (Ed.). Mineral nutrition of crops fundamental mechanisms and implications. (Food Product Press, New York), 1999, 205-226.
9. Marschner H. Mineral Nutrition of Higher Plants (2nd ed., Academic Press, London), 1995, 889.
10. Graham DR, Webb MJ. Micronutrients and disease resistance and tolerance in plants, in: Mortvedt J.J, Cox F.R, Shuman L.M, Welch R.M. (Eds.), *Micronutrients in Agriculture*, 2nd ed., (Soil Science Society of America, Inc. Madison, Wisconsin, USA), 1991, 329-370.
11. Huber DM. The role of mineral nutrition in defense. In Plant Disease, An Advanced Treatise, in: Horsfall J.G., Cowling E.B. (Eds.) Volume 5, *How Plants Defend Themselves*, (Academic Press, New York), 1980, 381-406.
12. Zakaria L, Yaakop AS, Salleh B, Zakaria M. Endophytic Fungi from Paddy. Trop Life Sci Res. 2010; 21(1):101-107.
13. Ora NAN, Faruq MT, Islam N, Akhtar, Rahman MM. Detection and identification of seed borne pathogens from some cultivated hybrid rice varieties in Bangladesh. Middle-East J of Sci. Res. 2011; 10(4):482-488.
14. Ou SH. *Rice diseases*. 2nd Ed. (Commonwealth Mycological Institute, Kew, England), 1985.
15. Singh R, Singh LS, Prasad D, Kureel RS, Sengar R, Singh A. Relationship of susceptibility and growth stages of plant for development of epidemic of sheath blight in rice. J Appl. & Nat. Sci. 2010; 2(2):230-233.
16. Pal P, Kaushik P. Effect of Carbon and Nitrogen Sources on the Growth of *Rhizoctonia solani* Isolated from *Rhynchostylis retusa* Blume. International Journal of Life Sciences. 2012; 2:16-18.
17. Perombelon M, Hadley G. Production of pectic enzymes by pathogenic and symbiotic *Rhizoctonia* strains. New Phytol. 1965; 64(1):144-151.
18. Khilare VC, Ahmed R. Effect of different media, pH and temperature on the growth of *Fusarium oxysporum* f.sp. *ciceri* causing chickpea wilt. International Journal of Advanced Biological Research. 2012; 2(1):99-102.
19. Islam R. effect of various carbon and nitrogen sources on mycelial growth of Fusarium sp. isolated from agricultural fields of Murshidabad. Ind. J Sci. Res. and Tech. 2015; 3(1):71-77.
20. Dandge VS. Effect of nitrogen sources on the growth of different species of *Curvularia*, *Fusarium*, *Phoma* and *Botryodiplodia*. Journal of Experimental Sciences. 2012; 3(3):24-27.
21. Hasan MY, Magid EA. the carbon and nitrogen nutrition of certain fungi. Canadian Journal of Microbiology. 1967; 13(8):1097-1106.

22. Ibrahim M, Shehu K. Effects of Various Carbohydrates on the Growth of *Rhizopus stolonifera*. International Journal of Multidisciplinary Research and Development. 2015; 2(5):306-308.
23. Holcomb GE. First report of *Choanephora* flower spot and blight of periwinkle. Plant Disease. 1998; 82(4):447.
24. Sinha A, Ara A, Kumar A, Alam A. study on effect of different carbon and nitrogen sources on growth and sporulation of *Alternaria solani*. International Journal for Exchange of Knowledge. 2015; 2(2):10-17.
25. Marlida Y, Saari N, Hassan Z, Radu S. Improvement in raw sago starch degrading enzyme production from *Acremonium* sp. endophytic fungus using carbon and nitrogen sources. Enzyme Microb Technol. 2000; 27(7):511-515.
26. Lima A. Current situation of rice production in Portugal Cahiers Options. Méditerranéennes. 199; 15(3):29-40.
27. Rabbani N, Bajwa R, Javaid A. Influence of culturing conditions on growth and sporulation of *Drechslera hawaiiensis*, the foliar blight pathogen of *Marsilea minuta* L. Afr J Biotechnol. 2011; 10:1863-1872.
28. Rodrigues C, Vandenberghe LP, de Oliveira J, Soccol CR. New perspectives of gibberellic acid production. *Cri Rev Biotechnol.* 2011; 32:263-273.
29. Kautharapu KB, Rathmacher J, Jarboe LR. Growth condition optimization for docosahexaenoic acid (DHA) production by *Moritella marina* MP-1. *Appl Microbiol Biotechnol*. 2013; 97:2859-2866.
30. Moges F, Prabhakar T, Sankar G, Latha S, Ramana T. Optimization of media for production of bioactive compounds by *Streptomyces parvullus* SS23/2 isolated from marine algae in the Bay of Bengal, India. Ethiop J Biol Sci. 2012; 11:1-11.
31. Sathiyanarayanan G, Gandhimathi R, Sabarathnam B, Kiran SG, Selvin J. Optimization and production of pyrrolidone antimicrobial agent from marine sponge associated *Streptomyces* sp. MAPS15. Bioprocess Biosyst Eng. 2013; 2:1023.
32. Rossi MJ, Oliveira VL. Growth of the ectomycorrhizal fungus *Pisolithus microcarpus* in different nutritional conditions. Brazil J Microbiol. 2011; 42:624-632.
33. Li HP, Yeager CM, Brinkmeyer R, Zhang S, Ho YF, Xu C *et al*. Bacterial production of organic acids enhances H_2O_2 dependent Iodide oxidation. Environ Sci Technol. 2012; 46:4837-4844.
34. Barros L, Pereira C, Ferreira ICFR. Optimized analysis of organic acids in edible mushrooms from Portugal by ultrafast liquid chromatography and photodiode array detection. Food Anal Methods. 2013; 6:309-316.
35. Cai L, Xiao HR, Huang SM, Li H, Zhou GT. Solubilization of magnesium-bearing silicate minerals and the subsequent formation of glushinskite by *Aspergillus niger*. Geomicrobiol J. 2013; 30:302-312.
36. Wei Z, Liang X, Pendlowski H, Hillier S, Suntornvongsagu K, Sihanonth P *et al*. Fungal biotransformation of zinc silicate and sulfide mineral ores. Environ Microbiol. 2013; 15:2173-2186.
37. Waqas M, Khan AL, Lee IJ. Bioactive chemical constituents produced by endophytes and effects on rice plant growth. J Plant Inter. 2014; 9(1)a:478-487.