

***In-vitro* screening of indigenous botanicals of Manipur for anti fungal activities of *Helminthosporium oryzae* an incitant of brown spot disease of rice and efficacy test at different level of concentrations**

David Kamei¹, Archana U Singh^{2*}, Adam Kamei³

¹KVK, ICAR, Senapati District, Manipur, Imphal

²Division of Nematology, ICAR- IARI, New Delhi

³NCIPM&FW, Ministry of Agri, GOI, Manipur Centre, Imphal

Abstract— Eleven indigenous plant species of Manipur viz., *Mariandra benghalensis*, *Millettia pachycarpa*, *Allium hookerii*, *Flogacanthus thyrsoflorus*, *Solanum incanum*, *Tithonia diversifolia*, *Goniolobus sesquipedalis*, *Solanum surattense*, *Artemisia nilagirica*, *Ocimum canum* and *Zanthoxylum acanthopodium* which has been used as local medicine and spices were collected from five district of Manipur viz. Tamenglong, Senapati, Kangpokpi, Imphal east and Imphal West. In –vitro screening of above indigenous botanicals of Manipur was studied for anti-fungal activities of *Helminthosporium oryzae* an incitant of brown spot disease of Rice. The first five botanicals with maximum inhibition was found in *Solanum incanum* with 52.44% followed by *Allium hookerii* (47.77%), *Millettia pachycarpa* (36.66%), *Mariandra benghalensis* (24.44%) and *Flogacanthus thyrsoflorus* (17.77%) over control. Efficacy test at different level of concentrations i.e. 10%, 15% and 20% of standard botanical extracts was evaluated against growth of fungus both in broth and solid culture media. However, among botanicals maximum per cent inhibition on biomass production was found at 20% concentrate of *S. incanum* with growth inhibition of 74.03%, followed by *A. hookerii* (62.66%), *M. Benghalensis* (46.36%) and *F. thyrsoflorus* (42.33%) growth inhibition over the untreated control. In solid media test maximum per cent inhibition on radial growth of test fungi was found at 20% concentration in treatment of *S. incanum* with growth inhibition of 72.70% over control followed by *A. hookerii* (59.81%), *M. Pachycarpa* (45.03), *M. benghalensis* (37.59%) and *F.thyrsoflorus* (28.70%) over the untreated control.

Keywords— Botanicals, Rice, *Helminthosporium oryzae*, Manipur.

I. INTRODUCTION

Brown spot disease of rice incited by *Helminthosporium oryzae* (Breda de Haan) is a major fungal disease of rice. The disease was known to occur in Japan since 1900 and known as nai-yake i.e., seedling blight and sesame leaf spot. The disease has been reported to occur in all rice growing countries including Japan, China, Burma, Sri Lanka, Bangladesh, Iran, Africa, South America, Russia, North America, Philippines, Saudi Arabia, Australia, Malaysia and Thailand [14, 10].

In India the disease was first reported by Sundraman in 1919 from Madras and is known to occur in all rice growing states of India. It was found more severe in dry and direct seeded rice in the state of Bihar, Chhatisgarh, Madhya Pradesh, Orissa, Assam, Jharkhand and West Bengal [5], [14], [17]. Heavy infection significantly reduce the number of tillers and grains and lowered the quality and weight of individual grains resulting in a loss of 30-34% [14]. Kamal and Mia (2009) [9] observed yield reduction of 18.75-22.50% whereas Chakrabarti (2001) [2] recorded 26-52% reduction of yield due to brown spot disease incidence. Brown spot disease of rice can cause enormous losses in grain yield upto 90% particularly when leaf spotting phase assumes epiphytotic proportions as observed in Great Bengal Famine in 1942. [6]

At present indiscriminate use of the chemical compounds has causes great impact on the environmental and hazard. Therefore, plant based pesticides appears as an important alternative to synthetic chemical as they do not pose threat to natural environment, human and animal health. The present work is therefore taken up to screen for anti-fungal activity of some indigenous plants of Manipur state against *Helminthosporium oryzae* an incitant of brown spot disease of rice Many Plant extracts and botanicals have also been found to be effective against brown spot disease. [7] Leaf extracts of *Juglans regia* reduced mycelia growth of *Bipolaris oryzae* by 64% [1].

II. MATERIALS AND METHODS

Eleven indigenous plant species of Manipur viz., *Mariandra benghalensis*, *Millettia pachycarpa*, *Allium hookerii*, *Flogacanthus thyrsoiflorus*, *Solanum incanum*, *Tithonia diversifolia*, *Goniothalamus sesquipedalis*, *Solanum surattense*, *Artemisia nilagarica*, *Ocimum canum* and *Zanthoxylum acanthopodium* which has been used as local medicine and spices were collected from five district of Manipur viz. Tamenglong, Senapati, Kangpokpi, Imphal east and Imphal West. The collected samples were botanically identified with the help of plant taxonomist from the Department of Life Sciences, Manipur University, Canchipur and Botanical Survey of India (BSI), Shillong. The collected samples were brought in the laboratory of Plant pathology, Sam Higginbottom University of Agriculture Technology and Sciences (SHUATS), Prayagraj, Uttar Pradesh, for conducting the experiment.

2.1 Preparation of extracts:

Plant parts such as leaves, fruits, shoots and roots of all botanicals were washed in running tap water and finally by sterile water and air dried for one day to eliminate surface moisture [7]. The samples were packed in envelop and kept in an oven at 60°C temperature for drying and then grinded separately into fine powder in blender. The powdered samples were packed in plastic bags, marked and sealed in air tide and sample bottles kept in refrigerator at 4°C for further experimental purposes.

2.2 Aqueous extracts

For making aqueous extract of all botanicals at 5%, 10%, 15% and 20% concentration methods [15]. This was followed in which 25 g powdered plant material was dissolved in sterilized distilled water to make 100 ml of aqueous extract (25% w/v) or (25:100 w/v) and kept at room temperature for 24 hours in a sterile flask covered with aluminium foil to avoid evaporation then subjected to filtration through sterilized Whatman No.1 filter paper. After filtration, the extracts were evaporated in water bath until 25ml extracts was left in the container. The botanical extracts obtained in this form were taken as standard concentration.

2.3 Isolation of disease pathogen *Helminthosporium oryzae* and maintaining of pure culture:

Helminthosporium oryzae was isolated from infected plant parts of rice by following Motlagh and Kaviani (2008) method [13]. The rice leaves showing characteristic symptoms of brown spot were collected from experimental paddy fields of Sam Higginbottom University of Agricultural, Technology and Sciences (SHUATS), Naini, Prayagraj, (U.P.) and brought into the laboratory. This was then cut into small pieces of 1mm slices and surface sterilized in 0.1% mercuric chloride (HgCl₂) for 1 minute and then rinsed with sterile water for 3 times. Three segments of these disease inoculum leaf pieces were inoculated in a petri plates containing sterilized PDA medium and incubated at 26±1°C. After 48 hours of incubation whitish cottony growth of mycelium appeared in the petriplates. To obtain a pure culture, a tip of this mycelium was cut out with the help of sterilized inoculating needle and transfers it to a fresh sterilized PDA slants and then incubated at 26±1°C in BOD incubator for 144 hour. The isolated pathogen culture was then observed in microscope by preparing slides and pathogen was identified following standard characterization procedures. The pathogen pure culture thus obtained were maintained in PDA slants and stored in refrigerator at 4°C and sub cultured from time to time.

2.4 Morphological characterizations of *Helminthosporium oryzae*:

Under microscopic camera, *Helminthosporium oryzae* mycelium initially was hyaline or white cottony growth turning brown and blue black as it grow older (Photo-1). Mycelium are filamentous, cylindrical with septation and having constriction at the point of branching (Photo-2). The conidia are globule with bulged at the centre and tapering at both ends. The conidia are pale brown or yellowish in colour having 4-9 Pseudo-septation. The conidia length varied from 95.39-96.26 µm and width 18.80-27.43 µm (Photo-3). These characteristics were in accordance with that of Ou (1985) [14] who described that culture of *H. oryzae* was grey to olive or black in colour, conidia are 5-10 septa with slightly curved and widest at the middle. Mature conidia are sub-hyaline. Motlagh and Kaviani (2008) [13] also reported that conidia of *H. oryzae* isolates in India has been observed between 99-135 x 7-11 µm and 24-122x7.23 µm, usually curved, obclavate, sometime almost cylindrical, pale to mid golden brown, 5-12 distoseptate, mycelium grey to dark grey, aerial mycelium was fluffy, cottony, olivaceous with brownish tinge and septate.



PHOTO-1: Brown spot pathogen of (*H. oryzae*) mycelium on PDA



PHOTO-2: Microscopic view of isolated *H. oryzae* mycelium and conidia

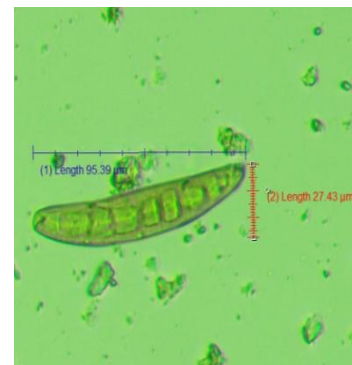


PHOTO-3: Microscopic view conidia of *H. oryzae*

2.5 *In-vitro* anti fungal activities test of botanicals:

2.5.1 Preliminary screening test of botanicals for anti-fungal activities

Eleven botanicals was screened following poisoned food technique of Devi and Chhetry (2013) in PDA media. [3] The 5% (2.5 ml) of standard botanical extracts were added to 50 ml of sterilized molten PDA then after thoroughly mixing 15 ml of the poisoned PDA was poured into sterile petri plates and allowed to solidify. Media without extracts served as control. 5 mm mycelial disc from 5 days old pathogen culture was placed at the centre of each treatment plates. Three replications were maintained for each treatment. The plates were incubated at $27 \pm 1^\circ\text{C}$ in BOD incubator. Observations was taken at 24 hours interval till fungus in the control plates covered the whole surface. Per cent inhibition of fungal growth over control was calculated by using formula, Vincent, (1947).

$$PI = \frac{C-T}{C} \times 100 \quad (1)$$

where, PI-Percent inhibition

C-Growth of fungus in control

T-Growth of fungus in treatment

Based on the preliminary screening results five best performing botanicals with maximum per cent radial growth inhibition of fungus mycelial was selected for further investigation work.

2.6 *In-vitro* studies of botanicals against biomass (dry wt. of mycelium) production of *Helminthosporium oryzae* at different level of concentration (broth medium)

The standard extract of five selected botanicals at 10%, 15% and 20% concentration were calibrated and added to the sterilized flasks containing 50 ml of potato dextrose (PD) broth media separately for each treatment concentration and gently shaken in circular motion for evenly distribution. Flasks containing media without extracts served as control and fungicide propiconazole at 1000 ppm used for comparative studies. Flasks were inoculated with 5 mm mycelium disc of 5 days old pathogen culture. For each treatment four replication were maintained. The inoculated flasks were incubated at $27 \pm 1^\circ\text{C}$ at BOD incubator for 10 days (240 hrs.). Flasks were shaken after every 24 hours for 1 minute. After 240 hours of inoculation mycelium mats was harvested by filtering through Whatman No.1 Filter paper (11 cm diameter), it was dried at 60°C for 72 hours in oven and then cooled in desiccators for 24 hours then weight is taken again and data recorded. Per cent inhibition of fungal growth (biomass or dry wt. mycelium) over control was calculated by using formula given by Vincent (1947). [18]

2.7 *In-vitro* studies of botanicals against radial growth of *Helminthosporium oryzae* at different level of concentration (solid medium)

To study the effect of botanicals against radial growth of fungus standardized botanical extracts was calibrated at three different concentration 10%, 15% and 20% i.e. (2.5 ml, 5 ml and 7.5 ml) to 50 ml sterilized molten potato dextrose agar (PDA) medium was added separately for each concentration and gently shaken in circular motion for evenly distribution and then 15 ml of poisoned media was dispensed in sterilized 9 cm diameter petri plates and allowed to solidify. Plates containing medium without extracts served as control. Then 5 mm mycelial disc of 5 days old pathogen culture was inoculated at the

centre of the plates with mycelium mat. Four replications were taken for each treatment. The plates were incubated at $27\pm1^{\circ}\text{C}$ in BOD incubator and observations was taken at 24 hours interval till the fungus in control plates covered the whole surface of plate. Per cent inhibition of fungal growth over control was calculated by using same formula given by Vincent, 1947 [18].

III. RESULTS AND DISCUSSION

TABLE 1
PRELIMINARY SCREENING OF ELEVEN BOTANICALS AGAINST RADIAL GROWTH OF HELMINTHOSPORIUM ORYZAE (SOLID MEDIA)

S. No.	Treatment				Radial growth at 144hrs (cm)*	(%) growth inhibition
	Local name	Botanical name	Plant part used	(%) conc.		
1.	T ₀ Control	-	-	-	9.00	-
2.	T ₁ Nongmangkha	<i>Flogacanthus thyrsiflorus</i>	Leaves	5	7.40	17.77
3.	T ₂ Mukthrubu	<i>Zanthoxylum acanthopodium</i>	Leaves	5	7.60	15.55
4.	T ₃ Lomba	<i>Mariandra benghalensis</i>	Leaves	5	6.80	24.44
5.	T ₄ Ngamuyai	<i>Millettia pachycarpa</i>	Leaves	5	5.70	36.66
6.	T ₅ Napakpi	<i>Allium hookerii</i>	Roots	5	4.70	47.77
7.	T ₆ Lamnumitlei	<i>Tithonia diversifolia</i>	Leaves	5	7.90	12.21
8.	T ₇ Leikham	<i>Goniothalamus sesquipedalis</i>	Leaves	5	8.30	7.03
9.	T ₈ Khamenkha	<i>Solanum incanum</i>	Fruits	5	4.28	52.44
10.	T ₉ Laibakngou	<i>Artemisia nilagarica</i>	Leaves	5	8.70	3.30
11.	T ₁₀ Leipungkhang	<i>Solanum surattense</i>	Leaves	5	7.60	15.55
12.	T ₁₁ Mayangton	<i>Ocimum canum</i>	Leaves	5	7.50	16.66
13.	T ₁₂ Propiconazole	-	-	1000 ppm	1.02	88.66
	S. Ed (±)	-	-	-	0.40	4.51
	CD (0.05%)	-	-	-	0.92	10.19

*Mean value of three replication

The data presented in Table 1 is the preliminary screening results of botanicals extracts against the radial growth of *Helminthosporium oryzae*. The results found that out of eleven botanicals screened 9 were found significantly reducing the radial growth of *H. oryzae* as compared with the untreated control. Among the botanicals maximum significant reduction on radial growth was recorded in T₈-*Solanum incanum* (4.28 cm) followed by T₅-*Allium hookerii* (4.7 cm), T₄-*Millettia pachycarpa* (5.7) cm, T₃-*Mariandra benghalensis* (6.8cm), T₁-*Flogacanthus thyrsiflorus* (7.4 cm), T₁₁-*Ocimum canum* (7.5 cm), T₂-*Zanthoxylum acanthopodium* (7.6 cm), T₁₀-*Solanum surattense* (7.6 cm), T₆-*Tithonia diversifolia* (7.9 cm). However, treatment T₉-*Artemisia nilagarica* (8.7 m) and T₇-*Goniothalamus sesquipedalis* (8.3 cm) were found not significant as compared with untreated T₀-control (9 cm).

TABLE 2
IN-VITRO BIOMASS PRODUCTION OF *HELMINTHOSPORIUM ORYZAE* AS AFFECTED BY BOTANICALS AT DIFFERENT CONCENTRATION

S. No.	Treatment	Biomass (mycelium dry wt.) of <i>H. oryzae</i> (g)				% growth inhibition over control			
		Concentrations				Concentrations			
		10%*	15%*	20%*	Mean**	10%	15%	20%	Mean**
1.	T ₀ Control	0.33	0.33	0.33	0.33	-	-	-	-
2.	T ₁ <i>Mariandra benghalensis</i>	0.26	0.22	0.17	0.21	21.21	33.33	48.48	34.34
3.	T ₂ <i>Millettia pachycarpa</i>	0.24	0.21	0.15	0.20	27.27	36.36	54.54	39.39
4.	T ₃ <i>Allium hookerii</i>	0.18	0.19	0.12	0.16	45.45	46.39	42.33	44.72
5.	T ₄ <i>Flogacanthus thyrsoiflorus</i>	0.28	0.24	0.19	0.23	15.15	27.27	42.42	28.28
6.	T ₅ <i>Solanum incanum</i>	0.17	0.14	0.10	0.13	48.48	57.57	69.69	58.58
7.	T ₆ <i>Propiconazole</i>	0.05	0.05	0.05	0.05	84.84	84.84	84.84	84.84
	Mean***	0.22	0.19	0.14		50.48	62.42	58.51	-
	S Ed9±1	0.01	0.01	0.006	-	4.50	6.31	1.59	-
	CD	0.04	0.05	0.018	-	13.50	18.00	4.78	-

*Mean value of four replication

** Mean irrespective of concentration

***Mean irrespective of treatment

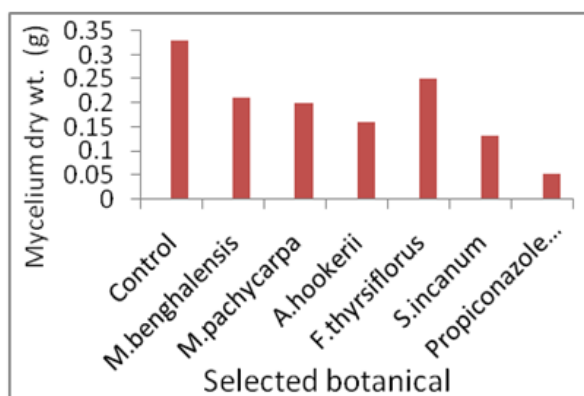


FIGURE 1: Botanicals against biomass production of *H. oryzae*

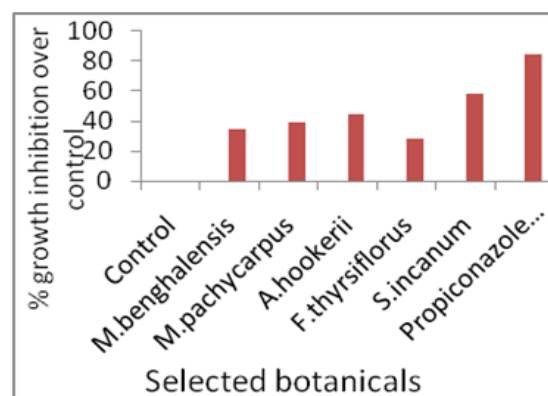


FIGURE 2: Botanicals and per cent inhibition of *H. oryzae* over control

Data presented in Table 2 and Fig.1 & 2 shows the effect of botanicals at different concentration on fungal biomass (dry wt. mycelium) production of *Helminthosporium oryzae*.

At 10% concentration, all selected five botanicals viz. T₅-*Solanum incanum*, T₃-*Allium hookerii*, T₂-*Millettia pachycarpa*, T₁-*Mariandra benghalensis* and T₄-*Flogacanthus thyrsoiflorus* significantly inhibit the fungal biomass (dry wt. mycelium) production of *Helminthosporium oryzae*. Maximum reduction on biomass production was recorded in T₅-*Solanum incanum* (0.17 g) with per cent reduction (58.58%) over control, followed by T₃-*Allium hookerii* (0.18 g) growth reduction(44.72%), T₂-*Millettia pachycarpa* (0.24 g)growth reduction (39.39%), T₁-*Mariandra benghalensis* (0.26 g)growth reduction (34.43%) and least significant effect was found in T₄-*Flogacanthus thyrsoiflorus* (0.28 g) growth reduction (15.15%), over the control T₀(0.33 g).

At 15% concentration, all botanicals significantly inhibit biomass (dry wt. mycelium) production as compared with control. Among the treatments maximum significant reduction on fungal biomass production was recorded in T₅-*S. incanum* (0.14g) with per cent growth reduction of (86.87), followed by T₃-*A. hookerii* (0.19g) and per cent growth reduction (69.11), T₂-*M. pachycarpa* (0.21g) growth reduction (62.82%), T₁-*M. benghalensis* (0.22g) growth reduction(57.30%) and T₄-*Flogacanthus thyrsoflorus* (0.24g) growth reduction (46.39%) over untreated control.

At 20% concentration, all treatments significantly inhibit biomass production. Among botanicals maximum significant reduction was recorded in T₅-*S. incanum* (0.10 g) with per cent growth reduction (74.03) followed by T₃-*A. hookerii* (0.12 g) growth reduction(62.66%), T₂-*M. pachycarpa* (0.15 g) growth reduction(51.16%), T₁-*M. benghalensis* (0.17 g) growth reduction (46.36%) and T₄-*F. thyrsoflorus* (0.19 g) growth reduction (42.33%) over the untreated T₀-control (0.27 g). Propiconazole use as check recorded (0.04 g) with per cent reduction of (85.18) over the untreated control.

TABLE 3

IN-VITRO RADIAL GROWTH OF *HELMINTHOSPORIUM ORYZAE* AS AFFECTED BY BOTANICALS AT DIFFERENT CONCENTRATION

Sl. No.	Treatment	Average radial growth <i>H. oryzae</i> mycelium (cm)				% growth inhibition over control			
		Concentrations				Concentrations			
		10% *	15% *	20% *	Mean**	10%	15%	20%	Mean**
1.	T ₀ (Control)	9.00	9.00	9.00	9.00	-	-	-	-
2.	T ₁ (<i>M. benghalensis</i>)	7.19	7.03	6.94	7.05	20.11	21.88	22.88	21.36
3.	T ₂ (<i>M. pachycarpa</i>)	6.09	6.03	6.01	6.04	32.32	33.00	33.22	32.84
4.	T ₃ (<i>A. hookerii</i>)	5.09	5.01	4.94	5.01	43.44	44.33	45.11	44.29
5.	T ₄ (<i>F. thyrsoflorus</i>)	7.79	7.73	7.64	7.72	13.44	14.11	15.11	14.22
6.	T ₅ (<i>S. incanum</i>)	4.67	4.16	3.78	4.20	48.11	53.77	58.00	53.29
7.	T ₆ (Propiconazole)	1.41	1.45	1.46	1.44	84.33	83.88	83.77	83.99
	Mean***	5.58	5.27	5.17	-	41.28	42.37	42.87	-
	S. Ed (±)	0.18	0.25	0.30	-	0.57	4.10	2.53	-
	CD (0.05%)	0.39	0.53	0.64	-	1.21	8.80	7.59	-

*Mean value of four replication

** Mean radial growth of mycelium irrespective of concentration

***Mean radial growth of Mycelium irrespective of treatment

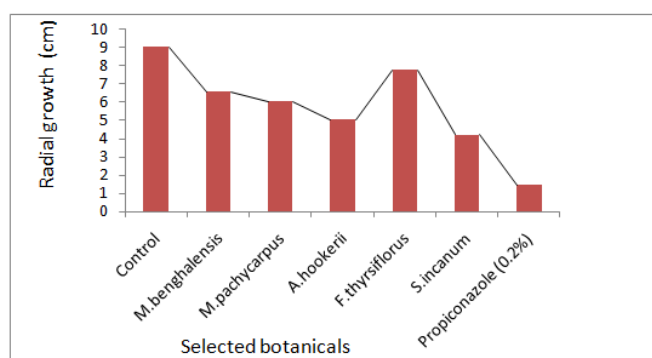


FIGURE 3: Botanicals on radial growth *H. oryzae* at 144 hrs irrespective of conc.

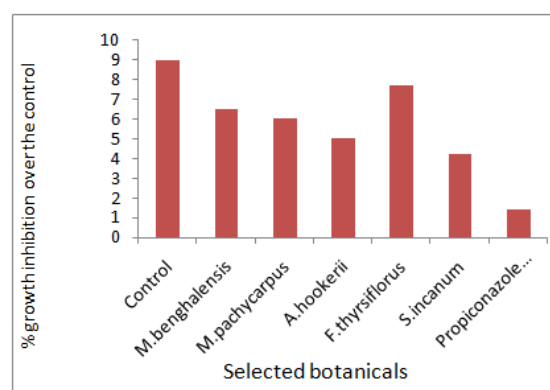


FIGURE 4: Botanicals and percent inhibition of *H. oryzae* over control

The results data presented on Table 3 and Fig. 3 & 4 shows the effect of various botanicals at different concentration on radial growth of *Helminthosporium oryzae*.

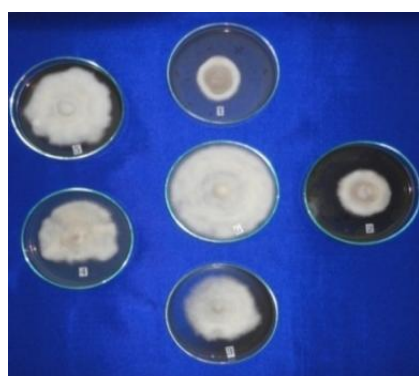
At 10% concentration, all botanicals were found statistically significant in reducing the radial growth of *H. oryzae*. Among the treatments maximum reduction on radial growth of mycelium was found in T₅-*Solanum incanum* (4.67 cm) with per cent inhibition (53.65) over control, followed by T₃-*Allium hookerii* (5.09 cm) growth inhibition (48.98%), T₂-*Millettia pachycarpa* (6.09 cm) growth inhibition (37.87%), T₁-*Mariandra benghalensis* (7.19 cm) growth inhibition (25.65%) and least significant reduction was found in T₄-*Flogacanthus thyrsiflorus* (7.79 cm) with growth inhibition of (18.98%) in compared with T₀-control (9.39 cm).

At 15% concentration, all botanical extracts were found statistically significant in reducing the radial growth of fungus over untreated control. Among the treatments maximum significant reduction of mean radial growth was observed in T₅-*S. incanum* (4.16 cm) with per cent growth inhibition (68.46) over control, followed by T₃-*A. hookerii* (5.13 cm) growth inhibition (57.68%), T₂-*M. pachycarpa* (6.23 cm) growth inhibition (45.66%), T₁-*M. benghalensis* (7.03 cm) growth inhibition (36.57%) and T₄-*F. thyrsiflorus* (7.73 cm) growth inhibition (28.79) in compared to T₀-Control (9.0 cm).

At 20% concentration all treatments found significantly reduced radial growth of fungus over control. Among the botanicals maximum radial growth inhibition was recorded in T₅-*S. incanum* (3.78 cm) with per cent inhibition (72.70) followed by T₃-*A. hookerii* (4.94 cm) growth inhibition (59.81%), T₂-*M. pachycarpa* (6.14 cm) growth inhibition (45.03%), T₁-*M. benghalensis* (6.94 cm) growth inhibition (37.59%) and minimum effect was observed in T₄-*F. thyrsiflorus* (7.74 cm) growth inhibition (28.70%) in compared to with untreated T₀-control (9.64 cm).

The results revealed that effectiveness on radial growth inhibition increases with the increase in dose per cent concentration and found maximum at highest dose of 20% conc. (5.17 cm) followed by 15% conc. (5.21 cm) and 10% conc. (5.58 cm) with per cent reduction of 42.87, 42.31 and 41.28 respectively over control.

It is evident from the above results that all botanicals significantly suppressed biomass and radial growth of *H. oryzae*. However, highest efficacy among selected five botanicals was observed in *Solanum incanum* fruits extracts and root extracts of *Allium hookerii*. The studies also found that all botanicals extracts increased its efficacy with per cent increase in treatment's concentration. (Table.4) Our present finding was in agreement with that of Gaichui (2008) [4] during *in-vitro* study on bio-efficacy of different indigenous plant of Manipur against *fusarium wilt* of chilly reported that Darrek (*Malea azadirach*) can best inhibit the growth of fungus in all the three concentration at 5%, 10%, and 15%, however the best results was recorded in the higher dose concentration of 15% with fungal growth inhibition of 93.71% over the untreated control. Devi and Chhetry (2013) [3] during *in-vitro* test on anti fungal activities of certain indigenous plant extracts of Manipur found that *Acorus calamus* 20% concentration can inhibit mycelium growth of *Drechlera oryzae* a brown spot disease of rice pathogen upto 80%. They also found that other plants extracts such as *Artemisia vulgaris* and *Centelia asiatica* extracts 20% can inhibit growth of fungus upto 40%. Methanolic extracts of medicinal plants viz. *Bergia capensis*, *Marselia quadrifolia*, *Lippiano diflora*, *Eclipta prostrate* and *Commelina clavata* (Manimegalai *et al.*, 2011) an essential plant oils from basil (*Ocimum basilicum*) and sweet fennel (*Ocimum gratissimum*) showed good inhibitory activity against *Bipolaris oryzae* [12]. Neem oils 3% and leaf extracts 3% can inhibit growth of *Bipolaris oryzae* upto (54.25%) and (49.74%) respectively in food poison technique *in-vitro* test [8].



[A] Fungal growth at 10% conc.



[B] Fungal growth at 15 % conc.



[C] Fungal growth at 20 % conc.

[Pt-1.*S. incanum*, Pt-2. *Allium hookarii*, Pt-3.*M. pahycarpus*, Pt-4.*M. Benghalensis*, Pt-5.*F. Thyrsiflorus*, Pt-6. Control]

TABLE 4
LIST OF ELEVEN INDIGENOUS BOTANICALS OF MANIPUR AND ITS MEDICINAL VALUE AND SECONDARY METABOLITES COMPOUND DETECTED USED IN STUDY

S. No.	Local name	Scientific name	Part used in analysis	Collection location and district	Economic utility	Detected secondary metabolites compound
1.	Lomba	<i>Mariandra benghalensis</i>	leaves	Siangai, Senapati	leaves and inflorescence were use as spice and for treatment of sore throat and dry cough	tannin, caumarin, glycoside
2.	Ngamuyai	<i>Millettia pachycarpus</i>	leaves	Siangai, Senapati	leaves were use for fish poisoning and treatment of scabies	phenolic, alkaloid, flavanoid, caumarin, saponin, glycoside
3.	Napakpi	<i>Allium hookerii</i>	roots	Porompat, Imphal East	whole plant is use as spice and for treatment of hypertension	terpinoid, saponin
4.	Nongmangkha	<i>Flogacanthus thyrsoiflorus</i>	leaves	Langol, Imphal west	leave and inflorescence decoction for treatment of high blood pressure, steam inhalation for relief of runny nose, sore throat, cold and cough	phenolic, tannin, terpinoid, glycoside, saponin,
5.	Khamenkha	<i>Solanum incanum</i>	fruits	Longmai, Tamenglong	treatment for stomach ulcer, pile case	phenolic, tannin, caumarin, saponin, terpinoid, glycoside
6.	Lamnumitlei	<i>Tithonia diversifolia</i>	leaves	Changoubung, Kangpokpi	leaves extracts is treated for skin allergy	saponin
7.	Laikham	<i>Ganiothalamus sesqui</i>	leaves	Hengbung, Senapati	dry leaves burning is act as insect repellent, ash is used for treatment of stomach acidity and relief of stomach-aches	flavanoid, caumarin, glycoside
8.	Leipungkhanga	<i>Solanum surattense</i>	leaves	Porompat, Imphal East	use for treatment of toothache, and stomach ulcer	phenolic, saponin, terpinoid, glycoside
9.	Laibak-ngou	<i>Artemisia nilagarica</i>	leaves	Maram, Senapati	used in treatment of wound and arresting of bleeding	tannin, alkaloid, terpinoid
10.	Mayangton	<i>Ocimum canum</i>	leaves	Nungang, Senapati	use as spice, treatment of sore throat, and cough	phenolic, alkaloid, flavanoid, terpinoid
11.	Mukthruhi	<i>Zanthoxylum acanthopodia</i>	leaves	Siangai, Senapati	Use as spice, use in treatment of mouth ulcer and fruits is used for treatment of toothache	phenolic, alkaloids, flavanoid, saponin

REFERENCES

- [1] Bisht, G.S. and Khulbe, R.D. In-vitro efficacy of leaf extracts of certain indigenous medicinal plants against brown leaf spot pathogen of rice. *Indian Phytopath.* **1995**, 48(4):480-188.
- [2] Chakrabarti, N.K. Epidemiological and disease management of brown spot of rice: *Recent advances*, Kluwer Academic publishers **2001**, pp. 293-306
- [3] Devi. O. Jitendiya devi and Chhetry, G.K.N. Evaluation of antifungal properties of certain plants against *Drechlera oryzae* causing brown leaf spot of rice in Manipur valley, *Int.J.of Scientific & Res.Publications* **2013**, 3(5), pp 1-3
- [4] Gaichui, G. Studies on efficacy of plant extracts and used of Panchagavya against wilt disease of chilli caused by *Fusarium oxysporum*, M.Sc.(Agri.) Thesis, submitted to COA, CAU, Imphal **2008**, pp54-65.
- [5] Gangopadhyay, S. Current concepts on Fungal Diseases of Rice. Today and tomorrow's Printers & Publishers, New Delhi **1983**, pp 349.
- [6] Ghose, R.L.M., Ghatge, M.B. and Subramanian, V. Rice in India (revised edn.), New Delhi, ICAR **1960**, pp 474.

- [7] Gurjar Jaya and Talwankar Dhananjay. Antifungal potential of crude plant extract on some pathogenic fungi. *World Journal of Science and Technology* **2012**, 2(6): 58-62.
- [8] Harish, Kr., Shafaat, A., and Suni, Z. Efficacy of fungal, bacterial bioagent and botanicals against brown spot (*Helminthosporium oryzae*) of rice (*Oryza sativa*). *Research Journal of chemicals and environmental sciences* **2015**, 3(2) pp27-31.
- [9] Kamal, M.M. and Mia, M.A.T. Diversity and pathogenicity of rice brown spot pathogen, *Bipolaris oryzae* in Bangladesh assessed by genetic finger printing analysis. *Bangladesh J. Bot.* **2009**, 38:195-200.
- [10] Khalili, E., Sadravi, M., Naeimi, S. and Khosravi, V. Biological control of rice brown spot with native isolates of three trichoderma species. *Braz. J. Microbiol.* **2012**, 43: 297-305
- [11] Manimegalai V., Ambikapathy. V. and Panneerselvam. A. Biological control of paddy brown spot caused by *Bipolaris oryzae* (Breda de Haan), **2011** 2(4):24-28
- [12] Piyo, A., Udomsilp, J., Khang-khun, P. and Thobunluepop, P. Antifungal activity of essential oils from basil (*Ocimum basilicum* Linn.) and sweet fennel (*Ocimum gratissimum* linn.): alternative strategies to control pathogenic fungi in organic rice. *Asian Journal Food Agro. Industry* (Special issue) **2009**, S2-S9.
- [13] Motlagh, M.R.S. and Kaviani, B. Characterization of new *Bipolaris* spp.: The causal agent of rice brown spot disease in the north of Iran. *Intn.J.Agric.Bio.* **2008**, 10(6): pp. 638-642.
- [14] Ou, S.H. Rice diseases 2nd edn. CMI, Kew, England, **1985** pp370.
- [15] Pundir, R. K, and Pranay Jain. Comparative studies on the antimicrobial activity of black paper (*Piper nigrum*) and turmeric (*Curcuma longa*) extracts. *Int. J. of Applied Bio. and Pharmaceutical Techno.* **2010** 1(2): pp 491-501.
- [16] Satish, S.; Mohana, D.C.; Ranghavendra, M.P. and Raveesha, A.K. Anti fungal activity of some plant extracts against important seed borne pathogens of *Aspergillus* sp. *Journal of agriculture technology* **2007**, 3(1):109-119.
- [17] Sunder. S, Ram Singh and Rashmi Agarwal, Brown spot of rice: an over view. *Indian Phytopath.* **2014**, 67(3): 201-215.
- [18] Vincent, J.M. Distortion of fungal hyphae in presence of certain inhibitors. *Nature*, **1947**, 157: 8.