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## Antifungal activities of selected indigenous botanicals of Manipur against *Helminthosporium oryzae* of rice

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### Abstract

Indigenous plant of Manipur viz., *Flogacanthus Thyrsiflorus*, *Mariandra benghalensis*, *Milletia pachycarpus*, *Allium hookerii* and *Solanum incanum* were test against *Helminthosporium oryzae* at different concentration i.e., 10%, 15% and 20% in broth and solid media. *In-vitro* test revealed, maximum significant reduction biomass (dry wt. mycelium) was found at 20% conc. *Solanum incanum* (0.10 g) with per cent reduction (64.47) followed by *Allium hookerii* (0.12 g) with biomass reduction (53.57%) over the untreated Control. In solid media maximum significant inhibition on radial growth of mycelium was recorded at 20% conc. *S. incanum* (3.78 cm) with per cent inhibition 59.81 followed by *A. hookerii* (4.94 cm) with per cent inhibition 49.62 in compare with others including Control. Probit analysis revealed among selected botanicals *Solanum incanum* was found most effective with lethal dose ( $L_{50}$ ) inhibiting 50% biomass production of *H.oryzae* with lowest probable concentration of 3.08ppm followed by *A. Hookerii* at 3.1ppm. Likewise in solid media *S. incanum* was most effective with  $L_{50}$  against the growth of *H. oryzae* inhibiting 50% at lowest probable concentration of (8.52ppm) followed by *A.hookerii* (10.65ppm).

**Keywords:** Antifungal activities, indigenous botanicals, Manipur, *Helminthosporium oryzae*

### Introduction

Brown spot of rice is caused by *Helminthosporium oryzae*, is a global diseases of rice causing enormous economic loss in grain yield (upto 90%) particularly when leaf spotting phase assumes epiphytotic proportion as observed in Bengal Famine during 1942 (Ghose *et al*, 1960) [4]. Chakrabarti, (2001) [2] recorded reduction in yield ranged from 26-52%, Kamal and Mia, (2009) [6] reported a reduction in yield ranged from 18.75-50%. Plant metabolites and plant based pesticides appears as an important alternative to synthetic chemical as they do not pose threat to nature 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 the brown spot disease of rice.

### Material and Method

Eleven indigenous plants of Manipur viz. *Flogacanthus Thyrsiflorus*, *Zanthoxylum acanthopodia*, *Mariandra benghalensis*, *Milletia pachycarpus*, *Allium hookerii*, *Tithonia diversifolia*, *Goniothalamus sesqui*, *Solanum incanum*, *Artemisia nilagarica*, *Solanum surattense*, *Ocimum canum*, leaves, fruits and roots were collected and washed in running tap water followed by sterile water and dried in an oven at 40° C for 72 hrs. The oven dried sample materials were ground into fine powder in a blender then packed in polythene bag and sealed in airtight sample bottle for further investigation work. For obtaining aqueous extract the methods of Pundir and Jain, (2010) [8] were adopted, 25 g powdered plant material was dissolved in enough sterilized distilled water to make 100 ml of aqueous extract (25% w/v) or (25:100 w/v). The mixture was kept undisturbed at room temperature for 24 hours in a sterile flask covered with aluminium foil to avoid evaporation and subjected to filtration through sterilized Whatman No.1 filter paper. After filtration, the extracts were evaporated in water bath until 25 ml extracts was left in the container. The botanical extracts obtained in this form are taken as standard full concentration.

### In-vitro test

Preliminary screening test for anti-fungal activities of 11 botanicals was carried out by following poisoned food technique, Devi and Chhetry (2013) [7], in PDA media. 5% (2.5 ml) of standard extracts were added to 50 ml of sterilized molten PDA just before solidify and mixed it by gentle circular motion for uniform distribution of extracts.

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15 ml of this poisoned PDA was poured into sterile petri plates and allowed to solidify. The test media without botanicals extract served as Control. 5 mm mycelial disc of 5 days old pathogen culture were placed at the centre of each treatment plates. Three replications were maintained for each treatment. The plates were incubated in inverted position at  $27\pm 1^\circ\text{C}$  in BOD incubator. Observation was taken at 24 hours interval till the fungus in the Control plates covered the whole surface.

Based on the preliminary screening results, five botanicals that showed maximum growth inhibition, *i.e.* 17.17% and above *viz.*, *Flogacanthus thyrsoiflorus*, *Mariandra benghalensis*, *Millettia pachycarpus*, *Allium hookerii* and *Solanum incanum* were selected for further efficacy test against the growth of fungus at different dose concentration.

The efficacy test for selected 5 botanicals against fungal biomass production and radial growth of *Helminthosporium oryzae* at different concentration was carried out by poisoned food method in broth and solid media.

For biomass production test standard botanical extracts at 10%, 15% and 20% concentration were adjusted and added to the sterilized conical flasks containing 50 ml potato dextrose (PD) broth and gently shaken in circular motion for evenly distribution of extracts. Flasks containing media without extracts were served as control. Fungicide propiconazole at 1000ppm were used as comparative studies. Flasks were aseptically inoculated with 5 mm mycelium disc taken from 5 days old pathogen culture. Each treatment was replicated into four. The whole experiment set up was incubated at  $27\pm 1^\circ\text{C}$  at BOD incubator for 10 days (240 hrs). Flasks were shaken every 24 hours for 1 minute. After incubation period mycelium mats were harvested by filtering through pre-weighted filter paper Whatman No.1 (11 cm diam.), and dried at  $60^\circ\text{C}$  for 72 hours in hot air oven and then cooled it in desiccators for 24 hours and reweighed.

In case of radial growth test, standard botanicals extract were adjusted at 10%, 15% and 20% concentration and were added to 50 ml of sterilized molten potato dextrose agar (PDA) medium and gently shaken in circular motion, 15 ml of these poisoned media were dispensed in sterilized 9 cm diameter petri plates and allowed to solidify. Plates containing the medium without extracts served as control. Then 5 mm mycelial disc of 5 days old pathogen culture were inoculated at the centre of the plates. Each treatment were replicated four times. The whole experiment set up was incubated at  $27\pm 1^\circ\text{C}$  in BOD incubator and observation was taken at 24 hours interval till the fungus in the Control plates covered the whole surface. Per cent inhibition of fungal growth was calculated by using the formula, Vincent (1927) <sup>[10]</sup>.

$$\text{PI} = \frac{C-T}{C} \times 100$$
, where, PI-Percent Inhibition

C-Growth of fungus in control

T-Growth of fungus in treatment

## Results

The data presented on Table 1, is the preliminary screening of 11 botanicals on solid media at 5% conc. against *Helminthosporium oryzae*. Out of 11 botanicals screened 9 were found significantly reduced the radial growth of *H. oryzae* in compared with the Control. Among these nine botanicals maximum inhibition on radial growth of *H. oryzae* was recorded in T8-*Solanum incanum* (4.28 cm) followed by T5-*Allium hookerii* (4.7 cm) with growth reduction of 52.44% & 47.77 % respectively over the untreated control. Treatment (T6, T10, T2, T11, T1, T3), (T5, T8) and (T4, T12) were none

significant among themselves but they are significant to each other. The test also found T9-(*Artemisia nilagarica*) and T7-(*Goniothelamus sesqui*) none significant in compared with the untreated Control (T0). However, chemical Propiconazole can inhibit the radial growth upto 88.66%.

The data presented on Table 2, showed that at 10% concentration, all selected five botanicals treatments *viz.* T5-*Solanum incanum*, T3-*Allium hookerii*, T2-*Millettia pachycarpus*, T1-*Mariandra benghalensis* and T4-*Flogacanthus thyrsoiflorus* significantly inhibit biomass production of *H. oryzae*. Among the treatments maximum reduction on biomass was recorded in T5-*Solanum incanum* (0.17 g) followed by T3-*Allium hookerii* (0.18 g) with growth reduction of (68.79%) and (65.22%) respectively over control. Among all botanicals treatment, (T1, T2) and (T3, T5) was found none significant among themselves but significant with T6, T4. However, all treatments were found significant over the untreated Control (T0). Similarly at 15% conc. maximum reduction on radial growth was on T5-*Solanum incanum* (0.14 g) followed by T3-*Allium hookerii* (0.19 g) with growth reduction 86.87% and 69.11% over the Control Among the treatments (T1, T2, T4) and (T3, T5) were found non-significant among themselves but significant with T6, T4.

Likewise at 20% conc. has shown maximum growth reduction T5-*Solanum incanum* (0.10 g) followed by T3-*Allium hookerii* (0.12 g) with growth reduction (74.03%) and (62.66%) respectively over control. It was found that all treatment were found significant among themselves and over the untreated control.

The test results data also revealed that irrespective of concentration maximum mean inhibition on fungal biomass production among botanicals was recorded in *Solanum incanum* (64.47%) followed by *Allium hookerii* (53.57%), *Millettia pachycarpus* (41.66%) over Control. The results data further revealed that irrespective of the treatments, mean fungal biomass production was found lowest at higher respective dose of concentration *i.e.*, at 20% (0.14 g), at 15% (0.19 g) and 10% (0.22 g). The test results also found Propiconazole recorded a minimum mean mycelium dry weight (0.02 g) with mean biomass growth inhibition of (96.47%) over untreated control.

Perusal in the above probit analysis Table 3, indicated that *Solanum incanum* was found most effective with lethal dose ( $L_{50}$ ), inhibiting 50% biomass production of fungal pathogen *Helminthosporium oryzae* with lowest probable concentration (8.52ppm) and *Allium hookerii* (10.65ppm) among five selected botanicals extract. Whereas *Flogacanthus thyrsoiflorus* (16.87ppm) indicated least significant effect against the growth of *H. oryzae* in the dose response relationship in broth media test.

The results data presented on Table 4, of selected botanicals revealed at 10% concentration, all selected botanicals significantly inhibit radial growth of *Helminthosporium oryzae*, and among the botanicals minimum radial growth of *H. oryzae* was recorded in T5-*Solanum incanum* (4.67 cm) followed by T3-*Allium hookerii* (5.09 cm) with per cent growth reduction of (53.65) and (48.98) respectively over untreated Control. Similarly, at 15% conc. minimum radial growth was recorded in T5-*Solanum incanum* (4.16 cm) followed T3-*Allium hookerii* (5.13 cm) with per cent reduction of (86.46) and (57.68) respectively over control. Similarly, at 20% conc. minimum radial growth was recorded in T5-*Solanum incanum* (3.78 cm) followed by T3-*Allium hookerii* (4.94 cm), with per cent growth reduction of (72.7)

and (59.81) over untreated Control.

Results data further revealed that irrespective of concentration, among botanicals, mean radial growth inhibition was found highest in *Solanum incanum* (59.07%), followed by *Allium hookerii* (49.62%), *Millettia pachycarpus* (36.92%), *Mariandra benghalensis* (27.40%) and least inhibition was found in *Flogacanthus thyrsoiflorus* (19.62%). It is also observed that mean radial growth of fungus was lowest at higher respective dose of concentration i.e., at 20% (5.17 cm), at 15% (5.27 cm) and 10% (5.58 cm). The test also found Propiconazole recorded a mean radial growth of (1.02 cm) with per cent growth inhibition (88.66) over untreated Control.

Perusal of the above probit analysis Table 5, indicated that in solid media test among five selected botanical extracts *Solanum incanum* was found most effective with lethal dose ( $L_{50}$ ) against the growth of fungus, *Helminthosporium oryzae*, inhibiting 50% with lowest probable concentration (3.08 ppm). Least effective against the pathogen was recorded on *Flogacanthus thyrsoiflorus* (3.4ppm), where as *Mariandra benghalensis*, *Millettia pachycarpus*, *Allium hookerii*, were intermediate in their effectiveness against radial growth of fungus in dose response relationship.

### Discussion

It is evident from preliminary *in-vitro* screening test that out of eleven botanicals screened nine were found significantly inhibit the radial growth of *Helminthosporium oryzae*, among them highest efficiency was recorded in *Solanum incanum*, *Allium hookerii* and *Millettia pachycarpus* in compared with others, over the Control. The results data also revealed higher efficacy of biomass and radial growth inhibition increased with increases in the extracts dose concentration, at 20% and 15% concentration. The probit analysis of broth media test revealed among botanicals *Solanum incanum* was most

effective with lethal dose ( $L_{50}$ ) inhibiting 50% biomass production of *H.oryzae* with lowest probable conc.(8.52ppm) followed by *Allium hookerii* (10.65ppm), whereas in solid media it can inhibit 50% radial growth at lowest probable conc. (3.08ppm) and (3.1ppm) respectively. The finding is in agreement with that of Gaichui, (2008) [3], during *in-vitro* study on bio-efficacy of different indigenous plant of Manipur against *fusarium wilt* of chilly found 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 highest concentration of 15% with 93.71%, followed by Wild sage or *Lantana camera* (57.60%), *Flogacanthus thyrsoiflorus* (57.77%), growth inhibition over the untreated control. Hajano *et al.*, (2012) [5] studied efficacy of extracts of garlic, neem and Calatopsis by food poisoning methods and observed that only higher dose of garlic completely inhibited the mycelium growth *Magnaporthe oryzae*. Al-Hazmi, (2013) [1] reported that neem leaf extracts were mostly effective in growth retardation of the *Helminthosporium spp.* fungi when applied at higher concentration (1:1, V:V). Similarly, Jitendiya and Chhetry, (2013) [7] during *in-vitro* test on anti fungal activities of certain indigenous plant extracts of Manipur found *Acorus calamus* 20% concentration can inhibit mycelium growth of *Drechlera oryzae* a brown spot disease of rice pathogen upto 80%. *Artemisia vulgaris* and *Centelia asiatica* extracts 20% can inhibit the growth of the fungus upto 40%, *Flogacanthus thyrsoiflorus* and *Allium hookeri* 20% can inhibit upto 38.60% and 24.40% respectively over the untreated control. Sandeep, (2015) also studied an *in-vitro* efficacy of leaf extracts of four plants against leaf blast and brown spot disease of rice at 0.2% and 0.5% concentration using food poisoned technique and leaf extracts of *Azadirachta indica* (neem) at higher concentration of 0.5% was most effective in minimising the mycelial growth of both the pathogen.

**Table 1:** *In-vitro* evaluation of botanicals on the radial growth of *Helminthosporium oryzae*

S. No	Treatment				Radial growth of mycelium at 144hrs (cm)*	(% Growth inhibition)
	Local/ common name	Botanical name	Plant part used	(%) conc.		
1.	T0 Control	-	-	-	9.00	-
2.	T1 Nongmangkha	<i>Flogacanthus thyrsoiflorus</i>	Leaves	5	7.40	17.77
3.	T2 Mukthubi	<i>Zanthoxylum acanthopodia</i>	Leaves	5	7.60	15.55
4.	T3 Garden sage (Lomba)	<i>Mariandra benghalensis</i>	Leaves	5	6.80	24.44
5.	T4 Ngamuyai	<i>Millettia pachycarpus</i>	Leaves	5	5.70	36.66
6.	T5 Napakpi	<i>Allium hookeri</i>	Root	5	4.70	47.77
7.	T6 Mexican sun flower (Lamnumitlei)	<i>Tithonia diversifolia</i>	Leaves	5	7.90	12.21
8.	T7 Leikham	<i>Goniothalamus sesqui</i>	Leaves	5	8.30	7.03
9.	T8 Khamenkha	<i>Solanum incanum</i>	Fruits	5	4.28	52.44
10.	T9 Laibakngou	<i>Artemisia nilagarica</i>	Leaves	5	8.70	3.30
11.	T10 Leipungkhanga	<i>Solanum surattense</i>	Leaves	5	7.60	15.55
12.	T11 Hoary basil (Mayangton)	<i>Ocimum canum</i>	Leaves	5	7.50	16.66
13.	T12 Propiconazole	-	-	1000 ppm	1.02	88.66
	S. Ed ( $\pm$ )				0.40	4.51
	CD (0.05%)				0.92	10.19

\*Mean of four replication

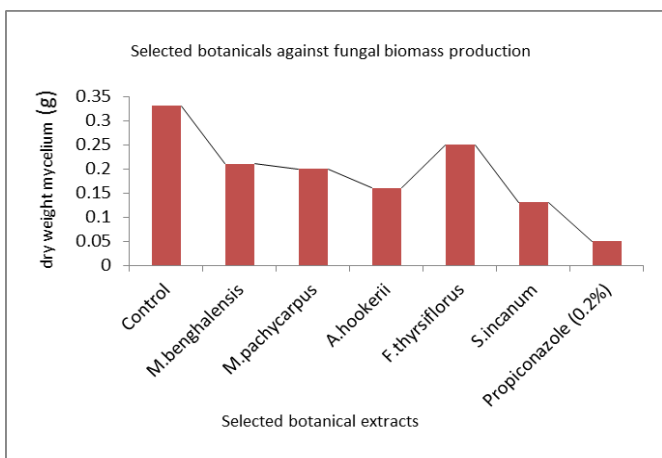
**Table 2:** Effect of different concentration of botanical extracts on biomass production of *Helminthosporium oryzae* (Broth media test)

S. No	Treatment	Average dry weight mycelium (g)*				Per cent growth inhibition over control			
		Concentration				Concentration			
		10%	15%	15%	Mean **	10%	15%	20%	Mean ***
1.	T0 Control	0.33	0.32	0.278	0.27	-	-	-	-
2.	T1 <i>Mariandra benghalensis</i>	0.26	0.22	0.178	0.18	37.28	57.3	46.36	38.88
3.	T2 <i>Milletia pachycarpus</i>	0.24	0.21	0.158	0.16	47.98	62.82	51.16	41.66
4.	T3 <i>Allium hookeri</i>	0.18	0.19	0.128	0.13	65.22	69.11	62.66	53.57
5.	T4 <i>Flogacanthus thyrsoiflorus</i>	0.33	0.24	0.198	0.22	29.37	46.39	42.33	27.26
6.	T5 <i>Solanum incanum</i>	0.17	0.14	0.108	0.09	68.79	86.87	74.03	64.47
7.	T6 Propiconazole (1000ppm)	0.05	0.06	0.02	0.01	109.84	114.42	101.36	96.47
	Mean***	0.22	0.19	0.14		50.49	62.41	58.51	
	S.Ed (±)	0.01	0.01	0.006	-	4.5	6.31	1.59	-
	CD (0.05%)	0.04	0.05	0.018	-	13.5	18	4.78	-

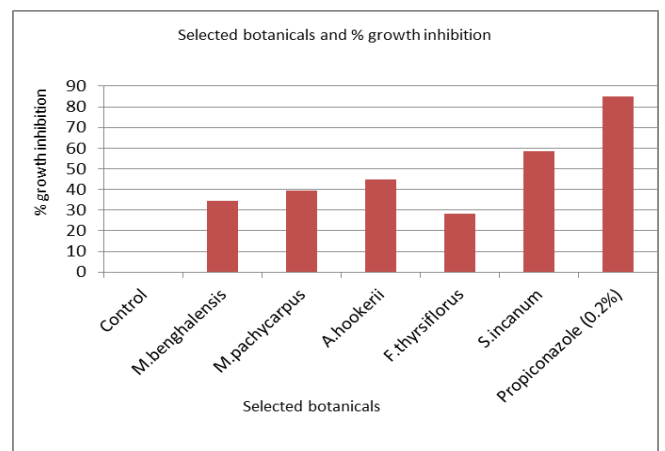
\*Mean of four replication

\*\*\* Irrespective of concentration

\*\*\* Irrespective of treatments



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



**Fig 2:** Botanicals and per cent growth inhibition of *H.oryzae*

**Table 3:** Efficacy of various botanical extracts against *Helminthosporium oryzae* on the basis of LD<sub>50</sub> and LD<sub>99</sub> probit value (broth media).

S. No	Treatments on radial growth	L <sub>99</sub>	L <sub>50</sub>
1.	T1 ( <i>Flogacanthus thyrsoiflorus</i> )	6.75	3.40
2.	T2 ( <i>Mariandra benghalensis</i> )	6.12	3.21
3.	T3 ( <i>Milletia pachycarpus</i> )	5.70	3.11
4.	T4 ( <i>Allium hookeri</i> )	5.67	3.10
5.	T5 ( <i>Solanum incanum</i> )	5.62	3.08

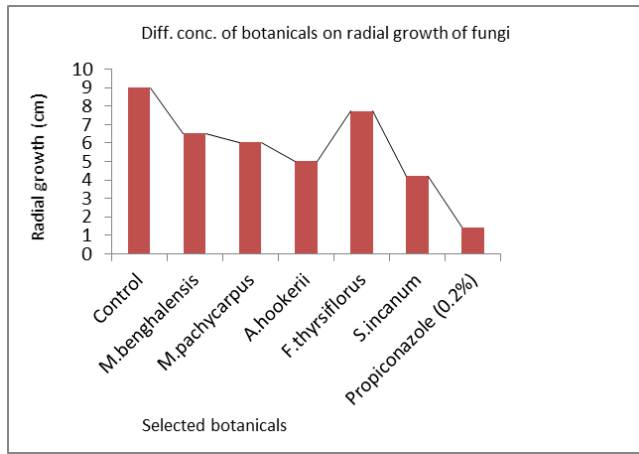
**Table 4:** Effect of different concentration of botanical extracts on radial growth of *Helminthosporium oryzae*

S. No	Treatment	Average radial growth of mycelium (cm)*				% growth inhibition			
		Concentrations				Concentrations			
		10%	15%	20%	Mean **	10%	15%	20%	Mean **
1.	T0 Control	9.00	9.00	9.00	9.00	-	-	-	-
2.	T1 <i>Mariandra benghalensis</i>	7.19	7.03	6.94	6.53	25.65	36.57	37.59	27.40
3.	T2 <i>Milletia pachycarpus</i>	6.29	6.12	6.18	5.63	37.87	45.01	45.06	36.92
4.	T3 <i>Allium hookerii</i>	5.19	5.13	4.94	4.53	48.98	57.68	59.81	49.62
5.	T4 <i>Flogacanthus thyrsoiflorus</i>	7.79	7.76	7.74	7.23	18.98	28.19	28.70	19.62
6.	T5 <i>Solanum incanum</i>	4.67	4.16	3.78	3.68	53.65	68.46	72.70	59.07
7.	T6 Propiconazole (0.2%)	1.41	1.55	1.66	1.02	89.87	97.46	96.25	88.66
	Mean***	5.94	5.83	5.83	-	39.28	47.80	48.58	-
	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	-

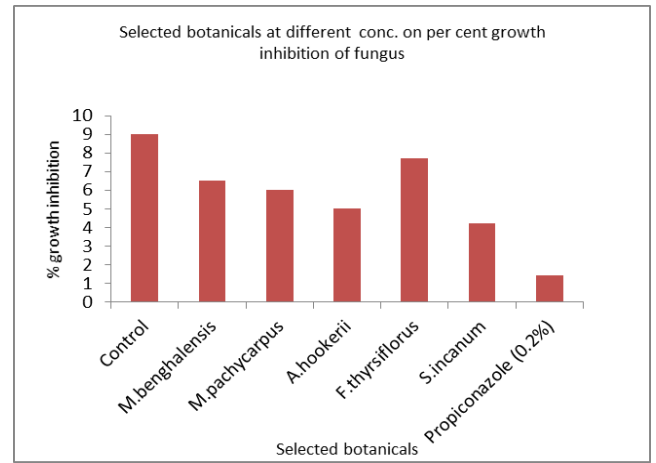
\*Mean of four replication

\*\*\* Irrespective of concentration

\*\*\* Irrespective of treatments



**Fig 3:** Different conc. of botanicals on radial growth of *H.oryzae* at 144 hrs



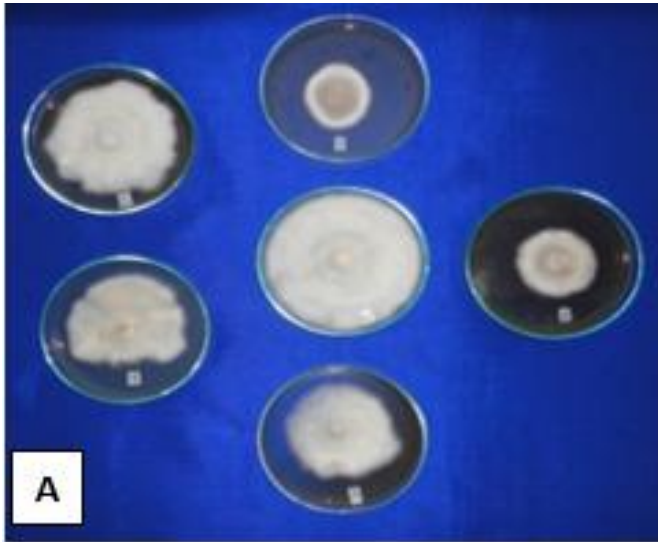
**Fig 4:** Diff. conc. of botanicals and percent radial growth inhibition of *H.oryzae* at 144 hrs

**Table 5:** Efficacy of various botanical extracts against *H. oryzae* on the basis of LD<sub>50</sub> and LD<sub>99</sub> probit value (solid media)

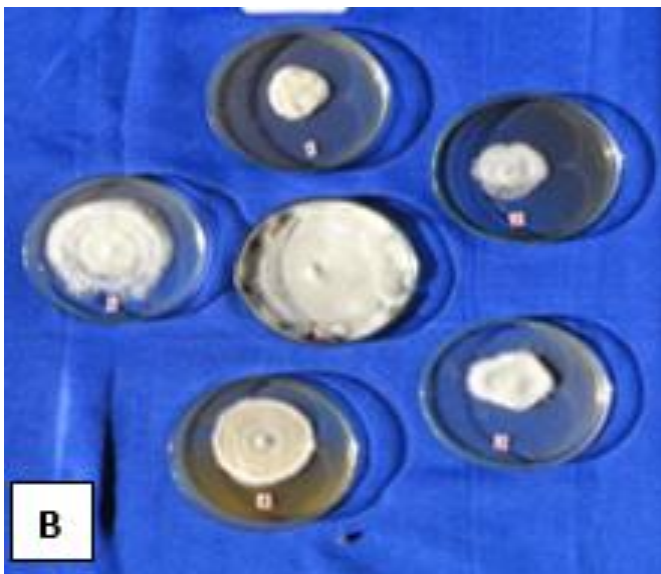
S. No	Treatments on broth growth	L <sub>99</sub>	L <sub>50</sub>
1.	T1 ( <i>Flogacanthus thyrsoiflorus</i> )	41.05	16.87
2.	T2 ( <i>Mariandra benghalensis</i> )	36.75	15.12
3.	T3 ( <i>Millettia pachycarpus</i> )	35.77	14.28
4.	T4 ( <i>Allium hookeri</i> )	32.67	10.65
5.	T5 ( <i>Solanum incanum</i> )	27.11	8.52



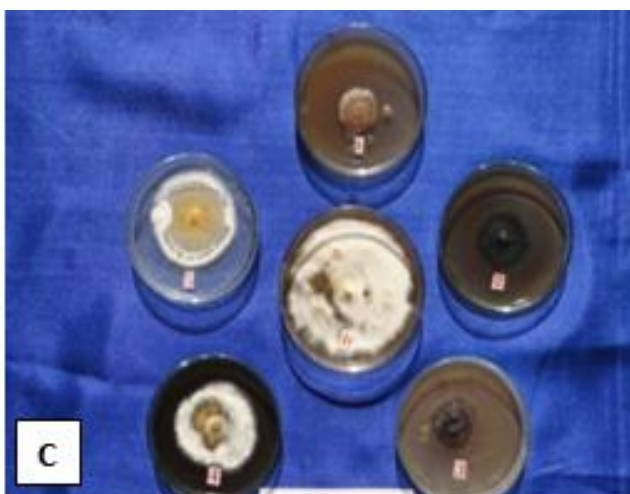
Botanicals of Manipur state



(A). Fungal growth at 10% conc. at 114 hrs 1. *S. incanum*, 2. *Allium hookarii*, 3. *M. pahycarpus*, 4. *M. Benghalensis* 5. *F. Thyrsoflorus* 6. Control.



(B). Fungal growth at 15 % conc. at 114 hrs 1. *S. incanum*, 2. *A. hookarii*, 3. *M. pahycarpus*, 4. *M. Benghalensis* 5. *F. thyrsoflorus* 6. Control



(C). Fungal growth at 20 % conc. at 114 hrs 1. *S. incanum*, 2. *A. hookarii*, 3. *M. pahycarpus*, 4. *M. benghalensis*, 5. *F. thyrsoflorus*, 6. Control.

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