Scientific Research Publishing

American Journal of Plant Sciences, 2021, 12, 662-678
https://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

In Vitro Efficacy of Botanicals against Rice Blast Pathogen *Magnaporthe oryzae oryzae*

Z. Nazifa, F. M. Aminuzzaman*, L. Laila, M. K. Rehena

Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh
Email: *aminsaupp@yahoo.com

How to cite this paper: Nazifa, Z., Aminuzzaman, F.M., Laila, L. and Rehena, M.K. (2021) *In Vitro* Efficacy of Botanicals against Rice Blast Pathogen *Magnaporthe oryzae oryzae*. *American Journal of Plant Sciences*, **12**, 662-678.
https://doi.org/10.4236/ajps.2021.124045

Received: February 25, 2021
Accepted: April 26, 2021
Published: April 29, 2021

Abstract

Rice is the most staple cereal crop of Bangladesh and rice blast caused by *Magnaporthe oryzae oryzae* (MoO) has become a major factor limiting rice yield in Bangladesh and throughout the world. Eight botanicals extracted both in water and ethanol namely Kalijira (*Nigella sativa*), Turmeric (*Curcuma longa*), Ginger (*Zingiber officinalis*), Garlic (*Allium sativum*), Onion (*Allium cepa*), Neem (*Azadirachta indica*), Allamanda (*Allamanda cathartica*) and Aloevera (*Aloe vera*) were tested against MoO *in vitro* in the Mycology Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh. All the botanicals significantly reduced radial growth of the tested pathogen. Maximum mycelia growth inhibition of MoO was achieved with water extract of turmeric (1:1 w/v) and ethanol extracts of neem (1:4 w/v) with 86.57% and 92.62% mycelia growth inhibition at 14 DAI, respectively.

Keywords

Rice Blast, *Magnaporthe oryzae oryzae* (MoO), Botanical Extracts, Ethanol

1. Introduction

Rice (*Oryza sativa* L.) is consumed as a staple food for half of the world's population [1]. In Bangladesh, rice is the most staple cereal crop and central to Bangladesh's economy, accounting for nearly 20 percent of gross domestic product (GDP) and providing about one-sixth of the national income of Bangladesh [2]. But it is a great concern that rice blast caused by *Magnaporthe oryzae* has become a major factor limiting rice yield throughout the world [3] [4] [5].

Magnaporthe oryzae (teleomorph) (Herbert) Barr (anamorph: *Magnaporthe oryzae*) [6] is one of the most important plant pathogenic fungi having an excep-

tional capacity of rapidly changing its genetic makeup resulting in new pathogenic variants (races) [7] [8]. It is the causal agent of rice blast, one of the most devastating diseases of rice (*Oryzae sativa* L.) observed in most of the rice growing countries across the world [9]. The pathogen can cause infection on leaves, stems, peduncles, panicles, seeds and even roots. This disease is the potential threat that may cause crop failure and yield loss. Thus it has been ranked among the most important rice diseases. *Pyricularia oryzae* (Po) was isolated from infected leaf and panicle and identified based on cultural characteristics and conidia morphology and mycelia growth of *Pyricularia* isolates varied significantly with fair to excellent sporulation ability [10].

To initiate rice blast, the MoO has evolved a unique mechanism for conidium attachment to rice leaf surfaces. The disease can be severe during periods of cool temperatures and high moisture, while conidia do not germinate under direct sunlight [5]. Cloudy overcast weather and dew encourage blast spread. Conidia remain viable during winter even under snow. Infected host residue is the most important source of the primary inoculum causing epidemics initiation [11]. Survival of the fungus was greatly reduced during winter, but during spring, sporulation of the fungus occurred on plant debris [12]. Dissemination of the fungus also involves a wide range of alternative host plants [13]. In temperate regions, infested rice seed, straw, and residues have been implicated as the most important overwintering sources of primary inoculum, although their impacts on initial disease development and distribution is not fully understood [14]-[19] and [5].

Chemicals are commonly applied for controlling rice blast disease [20] [21]. However, the frequent use of fungicides on crops may cause hazards to human beings, plant health, beneficial micro-organisms, and develop fungicide resistance into the pathogens and residual toxicity in plant parts. On the other hand, some botanical extracts have proved to be most secure and have no adverse impact on environment [22] [23]. Plant extracts like garlic juice successfully reduced the infection caused by *Magnaporthe* sp., on rice [24]. Mycelia growth of rice blast fungus was also significantly reduced by water and ethanol leaf extracts, and oil extract of neem seed [25]. The purpose of this study was to evaluate comparative *in vitro* efficacy of water and ethanol extracts of botanicals against MoO causing rice blast.

2. Materials and Methods

2.1. Experimental Site

The experiment was conducted in the Mycology Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh.

2.2. Experimental Period

The experiment was conducted during the period from June 2018 to December 2019.

2.3. Botanicals Used for *in Vitro* Management of MoO

Aqueous extracts of eight botanicals namely Kalijira (*Nigella sativa*), Turmeric (*Curcuma longa*), Ginger (*Zingiber officinalis*), Garlic (*Allium sativum*), Onion (*Allium cepa*), Neem (*Azadirachta indica*), Allamanda (*Allamanda cathartica*) and Aloevera (*Aloe vera*) (Figure 1 and Table 1) were evaluated against MoO *in vitro* following poisoned food technique [26]. Botanicals have been chosen based on their antimicrobial compounds. These botanicals were collected from Horticulture farm, Sher-e-Bangla Agricultural University, Dhaka.

2.4. Preparation of Botanical Extracts

Botanicals extracted with either water or ethanol in different concentrations were used to understand the efficiency of the botanicals those are:

1:4 (w/v) = 25 g botanical in 100 ml either water or ethanol

Figure 1. Botanicals used for *in vitro* efficacy assessment against MoO.

Table 1. Botanicals used for *in vitro* management of MoO.

Common name	Scientific name	Family	Part used for extracts	Antimicrobial compounds
Kalijira	*Nigella sativa*	Rulunculaceae	Seed	Thymoquinone, Thymohydroquinone
Turmeric	*Curcuma longa*	Zingiberaceae	Rhizome	Curcumin
Ginger	*Zingiber officinalis*	Zingiberaceae	Rhizome	Gingerol, paradol, shogaols and zingerone
Garlic	*Allium sativum*	Amaryllidaceae	Bulb	Allicin
Onion	*Allium cepa*	Amaryllidaceae	Bulb	Flavonoids
Neem	*Azadirachta indica*	Meliaceae	Leaf	Azadirachtin
Allamonda	*Allamanda cathartica*	Apocynaceae	Leaf	Hexanoicacid, Octanoic acid
Aloe Vera	*Aloe vera*	Asphodelaceae	Leaf	Aloesin, Aloin etc.

1:2 (w/v) = 50 g botanical in 100 ml either water or ethanol.

1:1 (w/v) = 100 g botanical with 100 ml either water or ethanol.

2.4.1. Water Extract of Botaticals

In case of water extract of botanicals, 25 g, 50 g and 100 g botanicals were crushed in 100 ml water separately, grinded with mortar and pestle in water temperature (22˚C) and *in vitro* condition for 10 mins. Then the extracted juice were seived and taken in conical flask. Then different concentrations were applied in PDA (Potato Dextrose Agar) culture plates and then 15 days old mycelia discs of MoO were cut using disc cutter and placed in the middle of the petridish. The experiments were done *in vitro* following poisoned food technique [26]. Radial mycelia growth was recorded in 7, 10 and 14 days after inoculation (DAI).

2.4.2. Ethanol Extracts of Botanicals

Incase of botanical ethanol extracts, 25 g, 50 g and 100 g botanicals grinded with mortar and pestle, then mixed with 100 ml ethanol separately. The mixtures were then kept overnight in beakers covered with aluminium foil for increasing the extraction efficiency and next day extracted juice were seived and taken in conical flask. Then different concentrations were applied in PDA (Potato Dextrose Agar) culture plates and then 15 days old mycelia discs of MoO were cut using disc cutter and placed in the middle of the petridish. The experiments were done *in vitro* following poisoned food technique [26]. Radial mycelia growth was recorded in 7 and 14 DAI.

2.5. Experimental Design and Statistical Analysis

The experiment was done following Complete Randomized Design (CRD) with three replications and statistical analysis was done using Statistix10 software. Treatment means were compared by Duncan's Multiple Range Test (DMRT). Least significant differences (LSD) were calculated using significant level at P = 0.05.

3. Results and Discussions

The present study was conducted to evaluate the efficacy of botanical extracts against a virulent isolate of *Magnaporthe oryzae oryzae* MoO19 [27]. The isolate was identified based on three celled pyriform conidia (**Figure 2**). The results with discussion of the experiments conducted on these lines are presented in this chapter.

3.1. Efficacy of Botanicals in Controlling Radial Mycelia Growth of MoO *in Vitro*

3.1.1. Efficacy of Botanical Water Extracts in Controlling Radial Mycelia Growth of MoO *in Vitro*

1) *In Vitro* Mycelia Growth of MoO at 7 DAI

In vitro mycelia growth of MoO on different botanical treatment at 7 DAI was found significantly different (**Table 2**). Lowest mycelia growth was recorded in

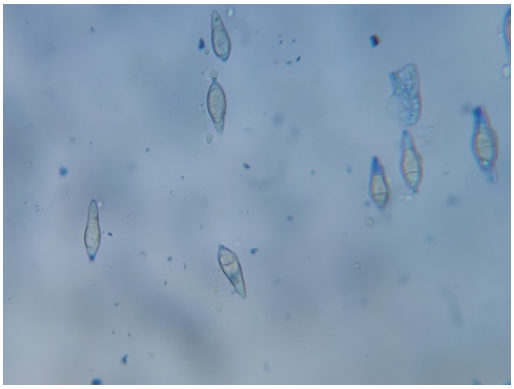

Figure 2. Three celled pyriform conidia of MoO (×40).

Table 2. Efficacy of botanical water extracts against mycelia radial growth of MoO *in vitro.*

Treatments	Treatment conc. (w/v)	Radial mycelia growth (mm) at 7 DAI	Radial mycelia growth (mm) at 10 DAI	Radial mycelia growth (mm) at 14 DAI
	1:4	38.00 c	52.00 a	59.33 b
Kalijira	1:2	23.33 ef	26.00 d	44.00 c
	1:1	19.00 g-j	19.00 g-i	24.33 f-i
	1:4	21.00 f-h	22.00 fg	20.67 hi
Turmeric	1:2	12.00 k	16.00 i	18.00 i
	1:1	8.33 k	9.00 j	9.00 j
	1:4	22.00 e-g	24.00 fg	26.00 e-h
Ginger	1:2	19.00 g-j	19.00 g-i	19.00 hi
	1:1	17.33 ij	17.33 hi	19.33 hi
	1:4	19.00 g-j	26.00 e	38.67 e-g
Garlic	1:2	16.00 j	17.00 hi	29.67 ef
	1:1	29.00 d	38.00 bc	40.00 c
	1:4	19.00 g-j	22.00 fg	24.00 f-i
Onion	1:2	18.00 h-j	20.00 gh	24.00 f-i
	1:1	20.00 f-i	21.67 fg	24.00 f-i
	1:4	40.00 bc	41.00 b	42.00 c
Neem	1:2	29.00 d	30.00 d	32.00 de
	1:1	20.00 f-i	22.67 e-g	30.00 ef
	1:4	37.33 c	40.00 b	42.00 c
Allamonda	1:2	25.00 e	26.00 e	30.67 d-f
	1:1	18.00 h-j	19.00 g-i	22.00 g-i
	1:4	43.00 b	50.00 a	52.00 b
Aloe Vera	1:2	23.00 ef	24.00 ef	26.00 e-h
	1:1	20.67 f-i	36.00 c	38.00 cd
Control		46.67 a	53.33 a	67.00 a
LSD (P = 0.05)		3.49	3.77	7.39

Each value is the mean of three replications. In a column, figure having same letter(s) do not differ significantly at 5% level by DMRT.

Turmeric in 1:1 (w/v) that is 8.33 mm at 7 DAI. Maximum growth inhibition of MoO was achieved with turmeric 1:1 (w/v) with 82.15% mycelia growth inhibition which was significantly different and superior to rest of the treatments (Figure 3). The next botanicals in order of merit were turmeric 1:2 (w/v) with 74.29% growth inhibition compared to control. The next botanicals in order of merit were garlic 1:2 (w/v), ginger 1:1 (w/v), onion 1:2 (w/v) and allamonda 1:1 (w/v) with 65.72%, 62.87%, 61.43% and 61.43% mycelia growth inhibition respectively of the test fungus as compared to control. After that in order of merit were kalijira 1:1 (w/v), ginger 1:2 (w/v), garlic 1:4 (w/v) and onion 1:4 (w/v) with 59.29% growth inhibition of the test fungus as compared to control. After in that order of merit were onion 1:1 (w/v), aloe vera 1:1 (w/v) and turmeric 1:4 (w/v) with 57.14%, 55.71% and 55% growth inhibition of the test fungus as compared to control. Aloe Vera 1:4 (w/v) with 7.86% inhibition was found to be the least effective botanicals. Graphical representation was shown in Figure 4.

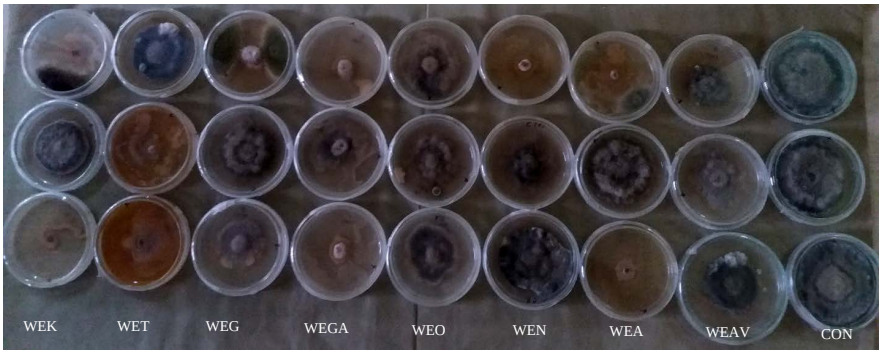

Figure 3. *In vitro* efficacy of botanical water extracts against MoO at 7 DAI; WEK = Water extract of Kalijira, WET = Water extract of Turmeric, WEG = Water extract of Ginger, WEGA = Water extract of Garlic, WEO = Water extract of Onion, WEN = Water extract of Neem, WEA = Water extract of Allamonda, WEAV = Water extract of Aloe Vera and CON = Control.

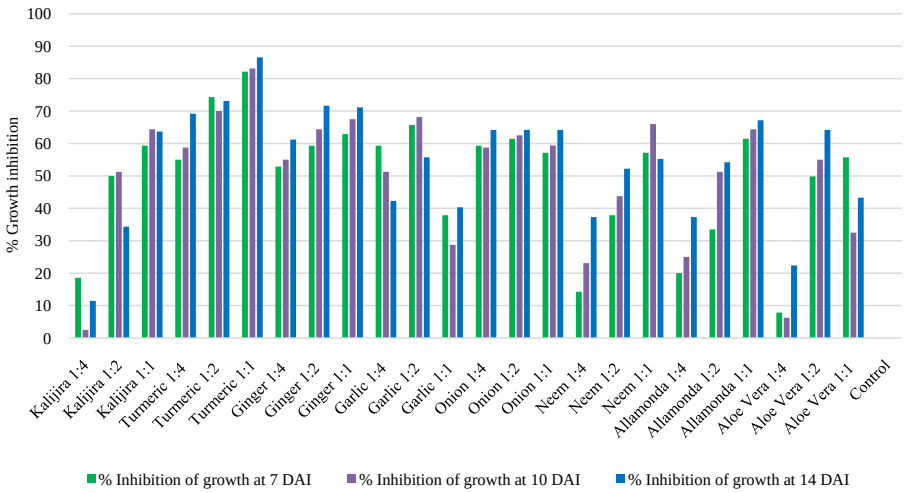

Figure 4. Efficacy of botanical water extracts against mycelia radial growth of MoO *in vitro* showing % growth inhibition at 7, 10 and 14 DAI.

2) *In Vitro* Mycelia Growth of MoO at 10 DAI

In vitro mycelia growth of MoO on different botanical treatment at 10 DAI was found significantly different (Table 2). Lowest mycelia growth was recorded in Turmeric in 1:1 (w/v) that is 9 mm at 10 DAI. Maximum growth inhibition of MoO was achieved with turmeric 1:1 (w/v) with 83.12% mycelia growth inhibition which was significantly different and superior to rest of the treatments (Figure 3). The next botanicals in order of merit were turmeric 1:2 (w/v), garlic 1:2 (w/v) and ginger 1:1 (w/v) with 70%, 68.12% and 67.50% mycelia growth inhibition respectively of the test fungus as compared to control. After that in order of merit were kalijira 1:1 (w/v), ginger 1:2 (w/v) and allamonda 1:1 (w/v) with 64.37% growth inhibition of the test fungus as compared to control. After in that order of merit was onion 1:2 (w/v) with 62.50% growth inhibition of the test fungus as compared to control. After in that in order of merit were onion 1:1 (w/v), onion 1:4 (w/v) and turmeric 1:4 (w/v) with 59.37%, 58.75% and 58.75% inhibition of growth of the test fungus as compared to control. After in that of merit order ginger 1:4 (w/v) and aloe vera 1:2 (w/v) with 55% growth inhibition of the test fungus as compared to control. Kalijira 1:4 (w/v) with 2.5% inhibition was found to be the least effective botanicals in *in vitro*. Graphical representation was shown in Figure 4.

3) *In Vitro* Mycelia Growth of MoO at 14 DAI

In vitro mycelia growth of MoO on different botanical treatment at 14 DAI was found significantly different (Table 2). Lowest mycelia growth was recorded in Turmeric in 1:1 (w/v) that is 9 mm at 14 DAI. Maximum growth inhibition of MoO was achieved with turmeric 1:1 (w/v) with 86.57% mycelia growth inhibition which was significantly different and superior to rest of the treatments (Figure 3). The next botanicals in order of merit was turmeric 1:2 (w/v) with 73.13% mycelia growth inhibition of the test fungus as compared to control. After that in order of merit were ginger 1:2 (w/v) and ginger 1:1 (w/v) with 71.64% and 71.15% growth inhibition respectively of the test fungus as compared to control. After in that order of merit were turmeric 1:4 (w/v) and allamonda 1:1 (w/v) with 69.15% and 67.16% growth inhibition respectively of the test fungus as compared to control. Kalijira 1:4 (w/v) with 11.45% inhibition was found to be the least effective botanicals in *in vitro*. Graphical representation was shown in Figure 4.

Curcuma longa L. (Zingiberaceae family) and its polyphenolic compound curcumin have been subjected to a variety of antimicrobial investigations due to extensive traditional uses and low side effects. Antimicrobial activities for curcumin and rhizome extract of *C. longa* against different bacteria, viruses, fungi, and parasites have been reported [28]. According to [29] fungicidal activity of turmeric rhizome-derived materials was tested using a whole plant method *in vivo* against *Botrytis cinerea*, *Erysiphe graminis*, *Phytophthora infestans*, *Puccinia recondita*, *Pyricularia oryzae*, and *Rhizoctonia solani*. Our result was accordance with [30] who studied on a satisfactory potential of turmeric as a natural pesticide for possible use in crop protection and thus a highly promising future

towards this direction, that is, the possibility of effective control of certain pests of agricultural importance with the use of turmeric products as a cheap and more environment friendly alternative to chemical pesticides already used for the same purpose. Essential oils and oleoresin from *Piper nigrum*, *Coriander sativum* and *Curcuma domestica* were isolated and tested for their effect on *Magnaporthe oryzae* in rice [31].

4) Efficacy of Botanical Water Extracts 1:4 (w/v) in Controlling Radial Mycelia Growth of MoO*In Vitro*

Among those eight botanicals in 1:4 (w/v) at 7 DAI, highest mycelia growth was recorded in Aloe Vera (43 mm) of the test fungus compared to control (46.67 mm) and lowest mycelia growth (19 mm) in garlic and onion extracts was recorded (Table 3).

Among those eight botanicals in 1:4 (w/v) at 10 DAI, highest mycelia growth was recorded in kalijira (52 mm) of the test fungus compared to control (53.33 mm) and lowest mycelia growth (22 mm) in turmeric and onion was recorded (Table 3).

Among those eight botanicals in 1:4 (w/v) at 14 DAI, highest mycelia growth was recorded in kalijira (59.33 mm) of the test fungus compared to control (67 mm) and lowest mycelia growth (20.67 mm) in turmeric was recorded (Table 3).

5) Efficacy of Botanical Water Extracts 1:2 (w/v) in Controlling Radial Mycelia Growth of MoO *in Vitro*

Among those eight botanicals in 1:2 (w/v) at 7 DAI, highest mycelia growth was recorded in neem (29 mm) of the test fungus compared to control (46.67 mm) and lowest mycelia growth (12 mm) in turmeric was recorded (Table 4).

Table 3. *In vitro* mycelia growth of MoO at 7, 10 and 14 days after inoculation in 1:4 (w/v) botanical extracts.

Treatments	Concentration (w/v)	Radial mycelia growth (mm)		
		7 DAI	10 DAI	14 DAI
Kalijira	1:4	38 c	52 a	59.33 ab
Turmeric	1:4	21 d	22 c	20.67 d
Ginger	1:4	22 d	24 c	26 d
Garlic	1:4	19 d	26 c	38.67 d
Onion	1:4	19 d	22 c	24 d
Neem	1:4	40 bc	41 b	42 c
Allamonda	1:4	37.33 c	40 b	42 c
Aloe Vera	1:4	43 d	50 a	52 bc
Control		46.67 a	53.33 a	67 a
LSD (P = 0.05)		3.37	4.93	11.04

Each value is the mean of three replications. In a column, figure having same letter(s) do not differ significantly at 5% level by DMRT.

Table 4. *In vitro* mycelia growth of MoOat 7, 10 and 14 days after inoculation in 1:2 (w/v) of botanical water extracts.

Treatments	Concentrations (w/v)	Radial mycelia growth (mm)		
		7 DAI	10 DAI	14 DAI
Kalijira	1:2	23.33 c	26 bc	44 b
Turmeric	1:2	12 e	16 e	18 e
Ginger	1:2	19 d	19 e	19 e
Garlic	1:2	16 d	17 e	29.67 cd
Onion	1:2	18 d	20 dc	24 de
Neem	1:2	29 b	30 b	32 c
Allamonda	1:2	25 c	26 bc	30.67 cd
Aloe Vera	1:2	23 c	24 cd	26 e
Control		46.67 a	53.33 a	67 a
LSD (P = 0.05)		3.56	4.55	6.64

Each value is the mean of three replications. In a column, figure having same letter(s) do not differ significantly at 5% level by DMRT.

Among those eight botanicals in 1:2 (w/v) at 10 DAI, highest mycelia growth was recorded in neem (30 mm) of the test fungus compared to control (53.33 mm) and lowest mycelia growth (16 mm) in turmeric was recorded (Table 4).

Among those eight botanicals in 1:2 (w/v) at 14 DAI, highest mycelia growth was recorded in neem (32 mm) of the test fungus compared to control (67 mm) and lowest mycelia growth (18 mm) in turmeric was recorded (Table 4).

6) Efficacy of Botanical Water Extracts 1:1 (w/v) in Controlling Radial Mycelia Growth of MoO*In Vitro*

Among those eight botanicals in 1:1 (w/v) at 7 DAI, highest mycelia growth was recorded in garlic (29 mm) of the test fungus compared to control (46.67 mm) and lowest mycelia growth (8.33 mm) in turmeric was recorded (Table 5).

Among those eight botanicals in 1:1 (w/v) at 10 DAI, highest mycelia growth was recorded in garlic (38 mm) of the test fungus compared to control (53.33 mm) and lowest mycelia growth (9 mm) in turmeric was recorded (Table 5).

Among those eight botanicals in 1:1 (w/v) at 14 DAI, highest mycelia growth was recorded in garlic (40 mm) of the test fungus compared to control (67 mm) and lowest mycelia growth (9 mm) in turmeric was recorded (Table 5).

3.1.2. Efficacy of Botanical Ethanol Extracts in Controlling Radial Mycelia Growth of MoO *in Vitro*

1) *In Vitro* Mycelia Growth of MoO at 7 DAI

In vitro Mycelia growth of MoOon different botanical treatment with ethanol extracts at 7 DAI was found significantly different (Table 6). No mycelia growth was recorded in neem and allamonda in all of their concentrations at 7 DAI. Maximum growth inhibition of MoO was achieved with neem and allamonda in all of their concentrations with 100% mycelia growth inhibition which was

Table 5. *In vitro* mycelia growth of MoO at 7, 10 and 14 days after inoculation at 1:1 (w/v) botanical water extracts.

Treatments	Concentration (w/v)	Radial mycelia growth (mm)		
		7 DAI	10 DAI	14 DAI
Kalijira	1:1	19 c	19 cd	24.33 d
Turmeric	1:1	8.33 d	9 e	9 f
Ginger	1:1	17.33 c	17.33 d	19.33 e
Garlic	1:1	29 b	38 b	40 b
Onion	1:1	20 c	21.67 cd	24 d
Neem	1:1	20 c	22.67 c	30 c
Allamonda	1:1	18 c	19 cd	22 de
Aloe Vera	1:1	20.67 c	36 b	38 b
Control		46.67 a	53.33 a	67 a
LSD (P = 0.05)		4.93	4.48	3.69

Each value is the mean of three replications. In a column, figure having same letter(s) do not differ significantly at 5% level by DMRT.

Table 6. Efficacy of botanical ethanol extracts against mycelia radial growth of MoO *in vitro.*

Treatments	Concentration (w/v)	Radial mycelia growth (mm)	
		7 DAI	14 DAI
Kalijira	1:4	20.00 e	53.33 bc
	1:2	36.00 bc	56.00 b
	1:1	37.33 b	48.67 d
Turmeric	1:4	40.67 b	48.00 de
	1:2	16.67 e	38.00 g
	1:1	10.00f	29.33 hi
Ginger	1:4	30.67 cd	44.00 ef
	1:2	08.67 fg	22.67 j
	1:1	10.00 f	21.33 j
Garlic	1:4	8.00 fg	23.33 j
	1:2	6.00 fg	21.33 j
	1:1	4.00 gh	40.00 fg
Onion	1:4	40.00 b	50.00 cd
	1:2	30.00 d	50.67 cd
	1:1	17.33 e	44.00 ef
Neem	1:4	0.00 h	7.33 k
	1:2	0.00 h	6.00 k
	1:1	0.00 h	4.67 k
	1:4	0.00 h	30.00 hi

Continued

Allamonda	1:2	0.00 h	32.67 h
	1:1	0.00 h	21.00 j
	1:4	20.00 e	40.00 fg
Aloe Vera	1:2	18.00 e	28.00 i
	1:1	30.00 e	30.67 hi
Control		54.67 a	63.33 a
LSD (P = 0.05)		5.95	4.02

Each value is the mean of three replications. In a column, figure having same letter(s) do not differ significantly at 5% level by DMRT.

significantly different and superior to rest of the treatments (Figure 5). The next botanicals in order of merit were garlic 1:1 (w/v) with 92.68% growth inhibition compared to control. The next botanicals in order of merit were garlic 1:2 (w/v), ginger 1:2 (w/v) and garlic 1:4 (w/v) with 89.02%, 87.80% and 85.37% mycelia growth inhibition respectively of the test fungus as compared to control. After that in order of merit were turmeric 1:1 (w/v) and ginger 1:1 (w/v) with 81.71% growth inhibition of the test fungus as compared to control. Turmeric 1:4 (w/v) with 25.61% growth inhibition was found to be the least effective botanicals *in vitro*. Graphical representation was shown in Figure 6.

2) *In Vitro* Mycelia Growth of MoO at 14 DAI

In vitro mycelia growth of MoO on different botanical treatment with ethanol extracts at 14 DAI was found significantly different (Table 6). Lowest mycelia growth was recorded in neem that is 7.33 mm, 6 mm and 4.67 mm at 1:4, 1:2 and 1:1 (w/v) respectively at 14 DAI. Maximum growth inhibition of MoO was achieved with neem in all of it's concentrations with 92.62%, 90.52% and 88.42% mycelia growth inhibition respectively which was significantly different and superior to rest of the treatments (Figure 5). The next botanicals in order of merit was allamonda 1:1 (w/v) with 66.84%, garlic 1:2 (w/v) with 66.32% and ginger 1:1 (w/v) with 66.32% growth inhibition compared to control. The next botanicals in order of merit were ginger 1:2 (w/v) and garlic 1:4 (w/v) with 64.20% and 63.16% mycelia growth inhibition respectively of the test fungus as compared to control. After that in order of merit were aloe Vera 1:2 (w/v), turmeric 1:1 (w/v), allamonda 1:4 (w/v) and aloe Vera 1:1 (w/v) with 55.79%, 53.69%, 52.63% and 51.57% growth inhibition respectively of the test fungus as compared to control. Kalijira 1:2 (w/v) with 11.57% growth inhibition was found to be the least effective botanicals *in vitro*. Graphical representation was shown in Figure 6.

[32] Recorded that *A. indica* leaf extract @ 0.5% was found most effective in minimizing the mycelia growth of both the pathogens28.35 mm and 27.12 mm, closely followed by *P. glabra* leaf extract 29.57 and 30.10 mmin the same concentration, 96 hrs after incubation *in vitro*. Our results are in accordance to those [33] who studied that among the botanicals the spraying of Achook, NeemAzal T/S, Neem gold and Tricure shows significant reduction in disease

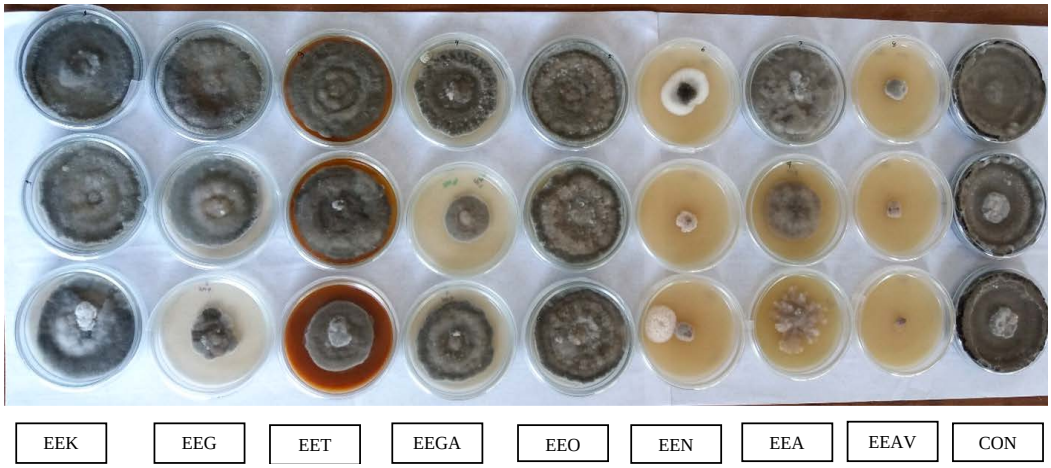

Figure 5. *In vitro* efficacy of botanical ethanol extracts against MoO at 7 DAI; EEK = Ethanol extract of Kalijira, EEG = Ethanol extract of Ginger, EET = Ethanol extract of Turmeric, EEGA = Ethanol extract of Garlic, EEO = Ethanol extract of Onion, EEN = Ethanol extract of Neem, EEA = Ethanol extract of Allamonda, EEAV = Ethanol extract of Aloe Vera and CON = Control.

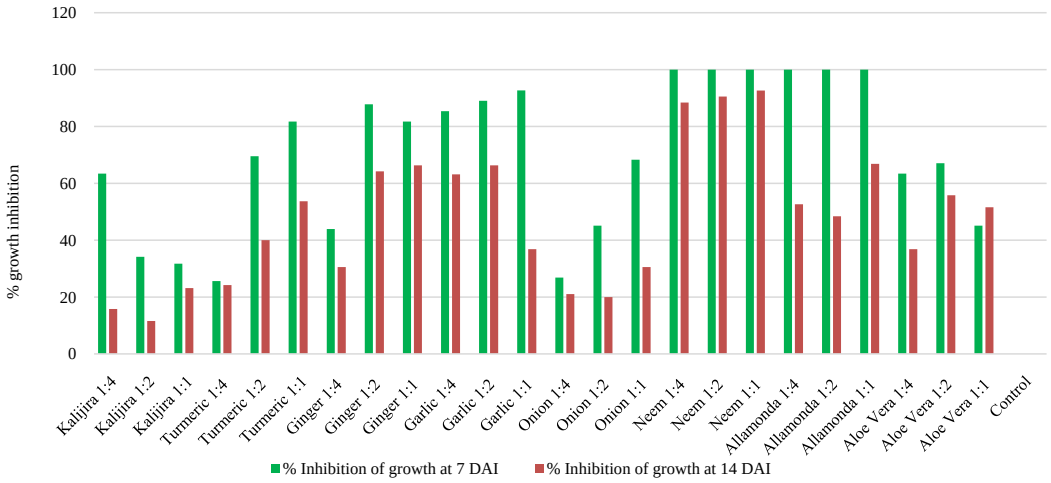

Figure 6. Efficacy of botanical ethanol extracts against mycelia radial growth of MoO *in vitro* showing %growth inhibition at 7 and 14 DAI.

severity against blast of rice, along with improving yield attributes, increasing the 100-grain weight and grain yield. [34] Also reported that neem extracts reduced the mycelia growth of *M. oryzae*. [25] Also had same opinion that water and leaf extracts/oil extracts of seeds of *Azadirachta indica* (neem) reduced radial growth of mycelium of *M. grisea in vitro* and the development and spread of blast disease in green house.

3.1.3. *In Vitro* Mycelia Growth of MoO at 7 and 14 Days after Inoculation at 1:4 (w/v) Botanical Water Extracts

Among those eight botanicals mixed with ethanol in 1:4 (w/v) at 7 DAI, highest mycelia growth was recorded in turmeric (40.67 mm) of the test fungus compared to control (54.67 mm) and lowest mycelia growth (0 mm) in neem and allamonda was recorded (Table 7).

Table 7. *In vitro* mycelia growth of MoO at 7 and 14 days after inoculation at 1:4 (w/v) botanical ethanol extracts.

Treatments	Treatment conc. (w/v)	Radial mycelia growth (mm) at 7 DAI	Radial mycelia growth (mm) at 14 DAI
Kalijira	1:4	20 d	53.33 b
Turmeric	1:4	40.67 b	48 cd
Ginger	1:4	30.67 c	44 de
Garlic	1:4	8 e	23.33 g
Onion	1:4	40 b	50bc
Neem	1:4	0 f	7.33 h
Allamonda	1:4	0 f	30 f
Aloe Vera	1:4	20 d	40 e
Control		54.67 a	63.33 a
LSD value (P = 0.05)		4.12	4.17

Each value is the mean of three replications. In a column, figure having same letter(s) do not differ significantly at 5% level by DMRT.

Among those eight botanicals mixed with ethanol in 1:4 (w/v) at 14 DAI, highest mycelia growth was recorded in kalijira (53.33 mm) of the test fungus compared to control (63.33 mm) and lowest mycelia growth (7.33 mm) in neem was recorded (Table 7).

Highest %growth inhibition was recorded in neem and allamonda at 1:4 (w/v) ethanol extract. The observations supports the findings of [35] who found the efficacy of plant parts extract of neem seed kernel, neem oil, *Pongamia* spp. extracts Panchagavya and *Asafoetida* spp. extract in descending order against rice blast fungus. [36] Observed that extracts from *C. Arabica*, *N. tabacum*, *A. vera*, *A. indica*, were found significant to manage rice blast disease *in vitro* and *in vivo*.

3.1.4. *In Vitro* Mycelia Growth of MoO at 7 and 14 Days after Inoculation at 1:2 (w/v) Botanical Ethanol Extracts

Among those eight botanicals mixed with ethanol in 1:2 (w/v) at 7 DAI, highest mycelia growth was recorded in kalijira (36 mm) of the test fungus compared to control (54.67 mm) and lowest mycelia growth (0 mm) in neem and allamonda was recorded (Table 8).

Among those eight botanicals mixed with ethanol in 1:2 (w/v) at 14 DAI, highest mycelia growth was recorded in kalijira (56 mm) of the test fungus compared to control (63.33 mm) and lowest mycelia growth (6 mm) in neem was recorded (Table 8).

3.1.5. *In Vitro* Mycelia Growth of MoO at 7 and 14 Days after Inoculation at 1:1 (w/v) Botanical Ethanol Extracts

Among those eight botanicals mixed with ethanol in 1:1 (w/v) at 7 DAI, highest mycelia growth was recorded in kalijira (37.33 mm) of the test fungus compared

Table 8. *In vitro* mycelia growth of MoO at 7 and 14 days after inoculation at 1:2 (w/v) botanical ethanol extracts.

Treatments	Treatment conc. (w/v)	Radial mycelia growth (mm) at 7 DAI	Radial mycelia growth (mm) at 14 DAI
Kalijira	1:2	36 b	56 b
Turmeric	1:2	16.67 d	38 d
Ginger	1:2	8.67 e	22.67 g
Garlic	1:2	6 e	21.33 g
Onion	1:2	30 c	50.67 c
Neem	1:2	0 f	6 h
Allamonda	1:2	0 f	32.67 e
Aloe Vera	1:2	18 d	28 f
Control		54.67 a	63.33 a
LSD value (P = 0.05)		3.02	4.07

Each value is the mean of three replications. In a column, figure having same letter(s) do not differ significantly at 5% level by DMRT.

Table 9. *In vitro* mycelia growth of MoOat 7 and 14 days after inoculation at 1:1 (w/v) botanical ethanol extracts.

Treatments	Treatment conc. (w/v)	Radial mycelia growth (mm) at 7 DAI	Radial mycelia growth (mm) at 14 DAI
Kalijira	1:1	37.33 b	48.67 b
Turmeric	1:1	10 de	29.33 d
Ginger	1:1	10 de	21.33 e
Garlic	1:1	4 ef	40 c
Onion	1:1	17.33 cd	44 c
Neem	1:1	0 f	4.67 f
Allamonda	1:1	0 f	21 e
Aloe Vera	1:1	30 c	30.67 d
Control	1:1	54.67 a	63.33 a
LSD value (P = 0.05)		9.36	4.61

Each value is the mean of three replications. In a column, figure having same letter(s) do not differ significantly at 5% level by DMRT.

to control (54.67 mm) and lowest mycelia growth (0 mm) in neem and allamonda was recorded (Table 9). Among those eight botanicals mixed with ethanol in 1:1 (w/v) at 14 DAI, highest mycelia growth was recorded in kalijira (48.67 mm) of the test fungus compared to control (63.33 mm) and lowest mycelia growth (4.67 mm) in neem was recorded (Table 9).

4. Conclusions

Studies were conducted to determine the effect of aqueous extracts of Kalijira

(*Nigella sativa*), Turmeric (*Curcuma longa*), Ginger (*Zingiber officinalis*), Garlic (*Allium sativum*), Onion (*Allium cepa*), Neem (*Azadirachta indica*), Allamanda (*Allamanda cathartica*), Aloevera (*Aloe vera*) for control of rice blast pathogen *Magnaporthe oryzae oryzae in vitro* following poisoned food technique. Maximum growth inhibition of MoO was achieved with water extract of turmeric in 1:1 (w/v) with 86.57% mycelia growth inhibition which was significantly different and superior to rest of the treatments.

In case of botanicals extracted in ethanol, maximum growth inhibition of MoO was achieved with neem in all tested concentrations with 92.62%, 90.52% and 88.42% mycelia growth inhibition respectively which was significantly different and superior to rest of the treatments.

A field trial is suggested for testing the field performance of turmeric water extract and neem ethanol extract along with a chemical fungicide to control rice blast in field condition.

Acknowledgements

We thank anonymous reviewers for their kind review of the manuscript. This research was financially supported by Sher-e-Bangla Agricultural University Research System (SAURES), Project ID: SAU/SAURES/Project/2018-19/4829/1505(13).

Authors' Contributions

This work was carried out in collaboration among all authors. Author ZN conducted the research work. Author FMA designed and supervised the study and edited the manuscript. Author LL and MKR managed the literature searches. All authors read and approved the final manuscript.

Conflicts of Interest

The authors have declared that no competing interests exist.

References

[1] Tobias, A., Molina, I., Valera, H.G., Mottaleb, K.A. and Mohanty, S. (2012) Handbook on Rice Policy for Asia. International Rice Research Institute, Los Baños.

[2] Timothy, S.T., Khandaker, M., Catherine, C., Anwarul, H., Nazria, I., Saad, Q. and Yan, S. (2013) Agriculture and Adaptation in Bangladesh: Current and Projected Impacts of Climate Change. IFPRI (International Food Policy Research Institute) Discussion Paper 01281. 76.

[3] Marchetti, M.A., Rush, M.C. and Hunter, W.E. (1976) Current Status of Rice Blast in Southern United State. *Plant Disease Reporter*, **60**, 721-725.

[4] Mekwatanakarn, P., Kositratana, W., Phromraksa, T. and Zeigler, R.S. (1999) Sexually Fertile *Magnaporthe grisea* Rice Pathogens in Thailand. *Plant Diseases*, **83**, 939-943. https://doi.org/10.1094/PDIS.1999.83.10.939

[5] Ou, S.H. (1985) Rice Disease. 2nd Edition, Commonwealth Mycological Institute, Kew Survey, 380.

[6] Couch, B.C. and Kohn, L.M. (2002) A Multilocus Gene Genealogy Concordant with

Host Preference Indicates Segregation of a New Species, *Magnaporthe oryzae* from *Magnaporthe grisea*. *Mycologia*, **94**, 683-693.
https://doi.org/10.1080/15572536.2003.11833196

[7] Dean, R., Kan, J.A.L.V., Pretorius, Z.A., Kosack, K.E.H., Pietro, A.D., Spanu, P.D., Rudd, J.J., Dickman, M., Kahmann, R., Ellis, J. and Foster, G.D. (2012) The Top 10 Fungal Pathogens in Molecular Plant Pathology. *Molecular Plant Pathology*, **13**, 414-430. https://doi.org/10.1111/j.1364-3703.2011.00783.x

[8] Khan, M.A.I., Ali, M.A., Monsur, M.A., Tanaka, A.K., Hayashi, N., Yanagihara, S., Obara, M., Mia, M.A.T., Latif, M.A. and Fukuta, Y. (2016) Diversity and Distribution of Rice Blast (*Pyricularia oryzae* Cavara) Races in Bangladesh. *Plant Diseases*, **100**, 2025-2033. https://doi.org/10.1094/PDIS-12-15-1486-RE

[9] Kihoro, J., Njoroge, J.B., Murage, H., Ateka, E, and Makihara, D. (2013) Investigating the Impact of Rice Blast Disease on the Livelihood of the Local Farmers in Greater Mwea Region of Kenya. *SpringerPlus*, **2**, 308.
https://doi.org/10.1186/2193-1801-2-308

[10] Rayhanul, M.I., Aminuzzaman, F.M., Chowdhury, M.S.M., Laila, L. and Ahmed, M. (2019) Survey on Rice Blast in Some Selected Area of Bangladesh and *in Vitro* Evaluation of Fungicides against *Pyricularia oryzae*. *Bangladesh Journal of Plant Pathology*, **35**, 59-64.

[11] Jeyanandarajah, P. and Seveviratne, S.N. de S. (1991) Fungi Seed-Borne in Rice (*Oryza sativa*) in Sri Lanka. *Seed Science and Technology*, **19**, 561-569.

[12] Harmon, P.F. and Latin, R. (2001) Perennation of *Magnaporthe grisea* in the Midwest: Disease Management Implications. *Phytopathology*, **91**, S148.

[13] Valent, B. and Chumley, F.G. (1991) Molecular Genetic Analysis of the Rice Blast Fungus *Magnaporthe grisea*. *Annual Review of Phytopathology*, **29**, 443-467.
https://doi.org/10.1146/annurev.py.29.090191.002303

[14] Lamey, H.A. (1970) *Pyricularia oryzae* on Rice Seed in the United States. *Plant Disease Reporter*, **54**, 931-935.

[15] Kingsolver, C.H., Barksdale, T.H. and Marchetti, M.A. (1984) Rice Blast Epidemiology. *Bulletin of the Pennsylvania Agricultural Experiment Station*, **853**, 29.

[16] Agarwal, P.C., Mortensen, C.N. and Mathur, S.B. (1989) Seed-Borne Diseases and Seed Health Testing of Rice. Technical Bulletin No. 3, Phytopathological Papers No. 30. CAB International Mycological Institute, Kew, UK.

[17] Cloud, G.L. and Lee, F.N. (1993) Rice Blast: Factors Influencing Severity and Control Strategies Used to Manage the Disease. Arkansas Cooperative Extension Service, Misc Publ. 341.

[18] Lee, F.N. (1994) Rice Breeding Programs, Blast Epidemics and Blast Management in the United States. In: Zeigler, R.S. and Leong, S.A., Eds., *Rice Blast Disease*, CAB International, Wallingford.

[19] Manandhar, H.K., Jorgensen, H.J.L., Mathur, S.B. and Smedegaard-Petersen, V. (1998) Seed Borne Infection of Rice by *Pyricularia oryzae* and Its Transmission to Seedlings. *Plant Disease*, **82**, 1094-1099.
https://doi.org/10.1094/PDIS.1998.82.10.1093

[20] Anwar, A., Bhat, G.N. and Singhara, G.S. (2002) Management of Sheath Blight and Blast in Rice through Seed Treatment. *Annals of Plant Protection Sciences*, **10**, 285-287.

[21] Gohel, N.M., Chauhan, H.L. and Mehta, A.N. (2008) Bio-Efficacy of Fungicides against *Pyricularia oryzae* the Incitant of Rice Blast. *Journal of Plant Disease Sciences*, **3**, 189-192.

[22] Iftikhar, T., Babar, L.K., Zahoor, S. and Khan, N.G. (2010) Best Irrigation Management Practices in Cotton. *Pakistan Journal of Botany*, **42**, 3023-3028.

[23] Babar, L.K., Iftikhar, T., Khan, H. and Makhdum, A.H. (2011) Agronomic Trials on Sugarcane Crop under Faisalabad Conditions. *Pakistan Journal of Botany*, **43**, 929-935.

[24] Slusarenko, A.J., Patel, A. and Portz, D. (2008) Control of Plant Diseases by Natural Products: Allicin from Garlic as a Case Study. *European Journal of Plant Pathology*, **121**, 313-322. https://doi.org/10.1007/s10658-007-9232-7

[25] Amadioha, A.C. (2000) Controlling Rice Blast *in Vitro* and *in Vivo* with Extracts of *Azadirachta indica*. *Crop Protection*, **19**, 287-290. https://doi.org/10.1016/S0261-2194(99)00080-0

[26] Nene, Y.L. and Thapliyal, P.N. (1979) Fungicides in Plant Disease Control. 2nd Edition, Oxford and IBH Pub. Co., New Delhi.

[27] Nazifa, Z., Aminuzzaman, F.M., Akhter, K., Rehena, M.K. and Laila, L. (2021) Survey on Rice Blast and Morphological Characterization of *Magnaporthe oryzae oryzae*. *Journal of Advances in Microbiology*, **21**, 8-21. https://doi.org/10.9734/jamb/2021/v21i130315

[28] Moghadamtousi, S.Z., Kadir, H.A., Hassandarvish, P., Tajik, H., Abubakar, S. and Zandi, K. (2014) A Review on Antibacterial, Antiviral, and Antifungal Activity of Curcumin. *BioMed Research International*, **2014**, Article ID: 186864. https://doi.org/10.1155/2014/186864

[29] Kim, M.-K., Choi, G.-J. and Lee, H.-S. (2003) Fungicidal Property of *Curcuma longa* L. Rhizome-Derived Curcumin against Phytopathogenic Fungi in a Greenhouse. *Journal of Agricultural and Food Chemistry*, **51**, 1578-1581. https://doi.org/10.1021/jf0210369

[30] Damalas, C.A. (2011) Potential Uses of Turmeric (*Curcuma longa*) Products as Alternative Means of Pest Management in Crop Production. *Plant Omics Journal*, **4**, 136-141.

[31] Sukanya, S.L., Yamini, D. and Fathima, S.K. (2011) Eco-Friendly Management of *Pyricularia oryzae*—The Causal Agent of Blast of Paddy. *Current Botany*, **2**, 46-49.

[32] Pandey, S. (2015) Efficacy of Leaf Extracts in Controlling Leaf Blast and Brown Spot in Rice (*Oryza sativa* L.). *International Journal of Recent Scientific Research*, **6**, 5476-5479.

[33] Sandeep, P. (2018) Effect of Botanicals for Management of Leaf Blast and Enhancing Yield Traits of Rice. *Current Botany*, **9**, 17-21. https://doi.org/10.25081/cb.2018.v9.3548

[34] Rajappan, K., Ushamalini, C., Subramanian, N., Narasimhan, V. and Kareem, A.A. (2001) Management of Grain Discoloration of Rice with Solvent-Free EC Formulations of Neem and Pungam Oils. *Phytoparasitica*, **29**, 171-174. https://doi.org/10.1007/BF02983962

[35] Sireesha, O. and Venkateswarlu, N. (2013) *In Vitro* Evaluation of Botanicals and Panchagavya against Leaf Blast Fungus *Pyricularia grisea*. *Asian Journal of Pharmaceutical and Clinical Research*, **6**, 84-86.

[36] Hubert, J., Mabagala, R.B. and Mamiro, D.P. (2015) Efficacy of Selected Plant Extracts against *Pyricularia grisea*, Causal Agent of Rice Blast Disease. *American Journal of Plant Sciences*, **6**, 602-611. https://doi.org/10.4236/ajps.2015.65065