Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(4): 399-404
Received: 15-05-2017
Accepted: 16-06-2017

Debjani Choudhury
Department of Plant Protection, Palli Siksha Bhavana, Visva Bharati, West Bengal, India

Yumlembam Rupert Anand
Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Susamoy Kundu
Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Ranjan Nath
Department of Plant Protection, Palli Siksha Bhavana, Visva Bharati, West Bengal, India

Ramen Kumar Kole
Department of Agricultural Chemicals, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Jayanta Saha
Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Correspondence
Debjani Choudhury
Department of Plant Protection, Palli Siksha Bhavana, Visva Bharati, West Bengal, India

Effect of plant extracts against sheath blight of rice caused by *Rhizoctonia Solani*

Debjani Choudhury, Yumlembam Rupert Anand, Susamoy Kundu, Ranjan Nath, Ramen Kumar Kole and Jayanta Saha

Abstract
A total of 11 plant (parts of plants) were extracted with three different solvents (hexane, chloroform and methanol) sequentially and tested against *Colletotrichum musae* (anthracnose of banana) and *Rhizoctonia solani* J.G.Kühn (sheath blight of rice) @ 0.2% concentrations of 20EC formulation. Among the different plant extracts leaves of clerodendrum (*Clerodendrum infortunatum* L.), polyalthia (*Polyalthia longifolia* Sonn.) and rhizomes of ginger (*Zingiber officinale* Roscoe) were found most active against the pathogens. These active plant extracs were tested further in pot culture and field experiment on rice plant against *Rhgizoctonia solani*. In general the Plant extracts showed significant inhibition of radial growth of all the test pathogen but the most effective plant extracts was clerodendrum extract, found to be most effective against rice sheath blight pathogen both in pot culture and field experiment. Carbendazim 50WP (1g/lt) was used as standard fungicides for comparison. Test fungicides at applied concentration also expectedly inhibited the fungal growth and lesion length in pot cultured plants and disease severity under field condition.

Keywords: bioassay, *in vitro*, plant pathogens, plant extracts, Rice, *Rhizoctonia solani*.

Introduction
Rice is an important cereal crop and are affected by various fungal pathogens. Sheath blight caused by *Rhizoctonia solani* is a very destructive disease which develop under favorable weather conditions in rice growing countries causing substantial yield losses (Gautam *et al*, 2003). Management of this disease is difficult due to sclerotia, remains viable in the soil for long. Use of synthetic chemicals causes several adverse effects i.e. development of resistance in pathogen, residual toxicity, pollution in the environment, high cost, resulted more carcinogenic risk than other pesticides which may give rise to undesirable biological effects on animals and human beings etc. (Brant and Hollomon, 1998; Schillberg *et.al*., 2001) [7, 30]. With the increase of interest in antibiotics, plants as a source of potential antimicrobial substances are receiving considerable attention throughout the world (Osman and Abdulrahman 2003) [26]. Plants produce a high diversity of natural products (secondary plant metabolites) that are insignificant for growth and developmental processes (Rosenthal *et al*., 1991) but act against predators and microbial pathogens on the basis of their toxic nature and also important for the communication of the plants with other organisms (Schafer *et al*., 2009). Researches on plant derived chemical to control plant pathogenis fungi is intensified as these have potential to influence modern agrochemical research (Benner *et.al.*, 1993) [3].
Antifungal activity of plant extracts may be more effective than some commercial synthetic fungicides due to presence of naturally occurring substances in plants with anti-microbial properties that have been recognized and tested against a wide range of pathogenic microbes (Tamuli, 2014) [32]. Therefore, it has become necessary to adopt ecofriendly management practices for better crop health management and yield. The systematic search of higher plants have shown that the plant extracts have antifungal activity against many species of fungi (Guerin and Reveille, 1984; Natarajan and Lalithakumari, 1987; Singh and Dwivedi, 1987) [17, 24, 31]. In recent years, plant extracts mainly, neem derivatives gaining importance for the control of plant diseases due to their antifungal and antibacterial properties (Yin and Cheng, 1998; Sahejpal *et al*., 2009) [34, 2].
Therefore, in the present study some locally available plants were tested in vitro, in vivo and field condition against *Rhizoctonia solani*. Thus present effort was given for evaluation of new commercially available botanicals and bio pesticides against sheath rot of rice.

Materials and method

Collection of plant materials

A total of 11 plants were collected from and around the campus, dried, extracted and used for testing the antifungal activities *in vitro* against different test pathogens are presented in table.1.

Table 1: Plants and plant parts used for evaluation of antifungal activities

Sl.no	Common name	Scientific name	Family	Parts used
1	Arjun	*Terminalia arjuna* Roxb.	Combretaceae	Mature leaves
2	Clerodendrum	*Clerodendrum infortunatum*	Verbenaceae	Leaves
3	Dill	*Anethum graveolens* L.	Umbelliferae	Seeds
4	Ginger	*Zingiber officinale* Roscoe	Zingeberaceae	Fresh and dried rhizome
5	Garlic	*Allium sativum* L.	Alliaceae	Fresh and dried bulbs
6	Lantena	*Lantana camara* L.	Verbenaceae	Tender leaves
7	Nicotiana	*Nicotiana tabacum* L	Solanaceae	Leaves
8	Polygonum	*Polygonum hydropiper* L.	Polygonaceae	Leaves
9	Pongamia	*Pongamia pinnata*. Linn.	Leguminosae	Seeds
10	Polyalthia	*Polyalthia longifolia* Sonn.	Annonaceae	Leaves
11	Teak	*Tectona grandis* Linn.	Verbenaceae	Fallen dried leaves

Selection of plant pathogens

The plant pathogens selected and collected from Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya culture collection.

Preparation of Plant Extracts

The extraction of the powdered plants for hot extraction was done following sequential extraction with organic solvents (hexane-chloroform-methanol) based on their polarity using Soxhlet apparatus (capacity 250 ml) for 6-8 hrs. Well dried powdered plant materials (50 g) were packed and used for extraction. Hexane, chloroform and methanol were used for sequential extraction @ 150ml each. The crude extract was collected, concentrated in a Buchi Rotavapor at 45 ^{0}C, transferred in a pre-weighed conical flask and evaporated to dryness.

Preparation of botanical pesticide formulation 20% EC (w/w)

Extracts (2 g) of each plant were taken in a beaker (250 ml). Surfactant mixture of (A) N-Alkaline Sulfonate and (B) K-Alkaline Sulfonate were added @ 4% of the total formulation (20 EC) along with 76 % of light solvent naptha (LSN). The entire formulation procedure was developed and standardized at Bio-formulation Laboratory, Dept. of Ag. Chemicals, BCKV, Mohanpur (Majumder, 2014) [22].

In vitro assay of the plant extracts against radial growth

Preliminary screening of the crude extracts of different plant parts against the selected pathogens was done following poison food technique (Bhutia *et.al.,* 2015, Grover and Moore, 1962) [11, 16] In this method, 40µL of the formulation (20 EC) was pipette aseptically into 20 ml sterile molten Potato Dextrose Agar (PDA) medium (without antibiotic) to get a resulting media with a concentration of 0.2% (400µg/mL) of each formulation. The medium was thoroughly shaken for uniform mixing of the extract and was poured on to the sterile Petri plates under aseptic conditions and allowed to solidify at room temperature for thirty minutes. Carbendazim 50WP @5µg/mL and Mancozeb 75WP @ 25 µg/mL was s*et al*ongside which served as standard fungicide control for comparative studies.

Each of the 9cm Petri Plates (four replications for each treatment) containing sterilized poisoned medium was aseptically inoculated with a 9 mm disc of hyphal mat cut with a sterile cork borer from the edges of actively growing culture. The inoculated plates were then incubated at 28 ±1 °C. The inhibitory effect of the plant extract on fungal growth was determined by measuring the average diameter of the colony at periodic interval till the control plates showed full growth. Efficacy of botanicals was expressed as per cent inhibition of radial growth over the control which was calculated by using the formula (Bhutia *et.al.* 2015) [11]

$$\% \text{ Radial growth inhibition (I)} = \frac{\text{Radial growth in control (C)} - \text{Radial growth in treatment (T)}}{\text{Radial growth in control (C)]}} \text{ X } 100$$

Evaluation of the selected plant extracts on the sheath blight pathogen (*Rhizoctonia solani*) on paddy in vivo and under field condition

Pot and field experiment was conducted in order to test the efficacy of the plant extracts on the sheath blight pathogen on rice. *R.solani* was grown on Petri Plates in the laboratory on PDA media. 20 to 25 pieces of 1 cm long flat wooden toothpicks were pre sterilised twice and placed aseptically on the molten agar medium on the plates. The pathogen was then inoculated on the plates and incubated at 28± 1^{0}C and allowed full growth. The mycelium of the pathogen grew on the toothpicks which served primary inoculums for artificial inoculation. When the pathogen was fully grown in the plates, the small pieces of the tooth picks were picked up with the help of a forcep and inoculated in between the leaf sheath of the rice plant. In order to prevent the inoculums from falling off, the inoculated places were wrapped with cotton. Since the pathogen requires high humidity to start infection, water was sprayed on the plants using a hand sprayer at the point of inoculation in case of pot experiment.

The concentration of the extracts under test was maintained at 0.2% concentration and Carbendazim @1g/lt was also sprayed on the plants for comparative studies. Solvent system used for the preparation of the extract was taken as control. Pots with plants on which only water was sprayed was also maintained alongside. Four replications were maintained per treatment. To study the effect of the extracts on the disease development, the lesion diameter was taken on daily basis. Under field condition reduction of percent disease inhibition was recorded after 10 days for 30 days.

Table 1: Treatment details under pot culture experiments

Sl. no	treatment	concentration of 20 EC formulation
1	Spraying with ginger chloroform +Inoculation	0.2%
2	Inoculation +Spraying with ginger chloroform	0.2%
3	Dipped in ginger chloroform + inoculation	0.2%
4	Spraying with *Polyalthia* Methanol +Inoculation	0.2%
5	Inoculation +Spraying with *Polyalthia* Methanol	0.2%
6	Dipped in *Polyalthia* Methanol + inoculation	0.2%
7	Spraying with *Clerodendrum* chloroform +Inoculation	0.2%
8	Inoculation +Spraying with *Clerodendrum* chloroform	0.2%
9	Dipped in *Clerodendrum* chloroform+ inoculation	0.2%
10	Spraying with Carbendazim (1g/lt)+Inoculation	
11	Inoculation +Spraying with Carbendazim (1g/lt)	
12	Dipped in Carbendazim (1g/lt)+ inoculation	
13	Spraying with water +Inoculation (Control 1)	
14	Inoculation + Spraying with water (Control 2)	
15	Dipped in water + inoculation (Control 3)	

Table 2: Details of treatments under field condition.

Sl. No.	Treatment number	Treatment details	Conc^{n}	Types of treatment
1	T_1	Ginger chloroform (1:0)	@ 0.2%.	**1.** spraying of plant extracts before inoculation with pathogen **2.** spraying of plant extracts after inoculation with pathogen
2	T_2	*Clerodendrum* chloroform	@ 0.2%.	
3	T_3	*Polyalthia* methanol	@ 0.2%.	
4	T_4	solvent	@ 0.1%	
5	T_5	Mancozeb	@0.25%.	
6	T_6	Water (control)	--------	

Results

Initial screening of different plant extracts using three organic solvents sequentially (hexane-chloroform-methanol) against *C. musae* and *R. solani*, obtained on poisoned food plates.

Initially 3 X 11=33 plant extracts were tested against radial growth of *C. musae* (post-harvest pathogen) and *R. solani* (Rice sheath blight pathogen) and results are presented in table 3. In general all the 33 plant extracts have radial growth inhibitory effect at 0.2% concentration of 20% plant extract solution. Out of the 3 solvent extracts, radial growth inhibition activity was maximum in case of chloroform and methanol extracts. Only in case of dill seed (85.93%) and polygonum leaf (51.85%) hexane extracts showed encouraging radial growth inhibition of *R.solani*. Among the choloroform extracts, clerodendrum, ginger, lantena, polyalthia and teak showed 51-87% radial growth inhibition *of C. musae* and chloroform extracts of clerodendrum, ginger, polygonum and polyalthia showed 52-100% radial growth inhibition *of R. solani.* Similarly, methanol extract of Clerodendrum, ginger, polyalthia, and teak showed remarkable inhibitory effect of 53-84% radial growth inhibition against *C. musae* while methanol extracts of Arjun, clerodendrum, dill and polyalthia showed 54-100% radial growth inhibition against *R. soalni*. Besides methanol extracts of arjun and dill had comparable but significantly low inhibitory effect of radial growth of *C. musae.*

From table 3, it revealed that chloroform and methanol extracts of clerodendrum, ginger and polyalthia had uniformly and consistent radial growth inhibitory effect on both the fungi tested primarily. Based on the above results these three plants clerodendrum, ginger and polyalthia were selected for further investigations against wide range of pathogens.

Table 3: Initial screening of different plant extracts extracted sequentially with different solvents (hexane-chloroform-mathanol) against *Colletotrichum musae and Rhizoctonia solani.*

Plant extract	Radial growth inhibition percentage of *colletotrichum musae*			Radial growth inhibition percentage of *Rhizoctonia solani*		
	H	C	M	H	C	Me
Arjun	35.19 (36.38)	46.30 (42.88)	49.63 (44.79)	35.19 (36.38)	19.81 (26.43)	54.07 (47.34)
Clerodendrum	9.63 (18.06)	78.15 (62.14)	53.41 (46.96)	24.07 (29.38)	100.00 (90.01)	87.78 (69.55)
Dill	30.74 (33.67)	38.15 (38.15)	49.67 (44.81)	85.93 (68.04)	76.67 (61.12)	61.11 (51.42)
Ginger	9.26 (17.71)	87.41 (69.22)	55.41 (48.11)	20.37 (26.83)	100.00 (90.01)	58.89 (50.12)
Garlic	28.70 (32.39)	19.63 (26.30)	34.48 (35.96)	26.30 (30.85)	27.41 (31.57)	20.74 (27.09)
Lantena	38.15 (38.15)	51.48 (45.85)	42.33 (40.59)	24.44 (29.63)	18.89 (25.76)	15.19 (22.93)
Nicotiana	15.19 (22.93)	41.48 (40.10)	27.59 (31.69)	26.30 (30.85)	37.78 (37.93)	34.81 (36.16)
Polygonum	19.63 (26.30)	15.19 (22.93)	38.30 (38.23)	51.85 (46.06)	52.96 (46.70)	35.56 (36.61)
Pongamia	30.37 (33.44)	11.48 (19.79)	37.89 (37.99)	18.52 (25.49)	17.41 (24.66)	22.96 (28.63)
Polyalthia	10.37 (18.78)	54.81 (47.77)	84.07 (66.52)	16.85 (24.24)	52.96 (46.70)	100.00 (90.01)
Teak	27.78 (31.81)	71.85 (57.96)	60.59 (51.12)	22.59 (28.38)	34.81 (36.16)	20.74 (27.09)
Carbendazim	41.85 (40.31)	41.85 (40.31)	41.78 (40.27)	30.37 (33.44)	31.11 (33.90)	31.11 (33.90)
Mancozeb	19.63 (26.30)	19.63 (26.30)	19.78 (26.41)	17.41 (24.65)	17.04 (24.38)	17.22 (24.52)
SEm	0.35	0.37	0.47	0.47	0.20	0.27
CD at 5%	1.02	1.08	1.36	1.38	0.58	0.79

The number in parenthesis indicate arc-sin transformed values
Values are the mean of 4 replications.
H-Hexane, C=Chloroform, M=Methanol

Effects of the plant extracts on sheath blight of rice

A pot experiment was carried out to test the efficacy of the selected plant extracts against the sheath blight caused by *Rhizoctonia solani*. Pre-colonised wooden toothpicks (1cm long) were used as pathogen inoculum. Application of plant extract formulations were sprayed before or after inoculation in rice sheaths and impregnated precolonised toothpicks were used as inoculum. Carbendazim 50 WP was used as standard fungicide check are presented in table.4.

Ginger chloroform, *Clerodendron* Chloroform and *Polyalthia* methanol when tested for their efficacy against the sheath blight pathogen showed considerable variability when sprayed before inoculation but Minimum length of the developed lesion was observed when the plant extract impregnated inoculum restricted considerably the disease development as measured by length of lesion among the methods of application.

Among the treatments, Carbendazim 50 WP, showed no lesion development (100% inhibition) after 10 days of inoculation when the inoculums was dipped in it @1g/lt prior to inoculation in the plants followed by clerodendrum-chloroform (76.22% inhibition). After 10 days of treatment it was observed that when the spraying of plant extract was done before inoculation there were 6-62% inhibition of the lesion development but when spraying was done after inoculation efficacy of plant extracts based formulation was reduced.

Table 4: Lesion length in rice plants caused by *Rhizoctonia solani* following spraying with plant extracts under pot culture experiment.

S. No.	Treatment details	Average length of the lesion (cm)			Percent inhibition over control after 5th day of inoculation		
		8 DAI	9DAI	10DAI	Control 1	Control 2	Control 3
1	Spraying with ginger chloroform +Inoculation	4.50±0.32	4.75±0.00	6.25±0.03	35.9		-
2	Inoculation +Spraying with ginger chloroform	5.20±0.00	8.00±0.00	9.58±0.00	-	23.40	-
3	Dipped in ginger chloroform + inoculation	3.90±0.63	4.67±0.14	4.83±0.00		-	51.70
4	Spraying with *Polyalthia* Methanol +Inoculation	5.60±0.29	8.86±0.06	9.13±0.03	6.83	-	
5	Inoculation +Spraying with *Polyalthia* Methanol	6.30±0.00	7.70±0.00	8.90±0.00		28.80	-
6	Dipped in *Polyalthia* Methanol + inoculation	3.50±0.22	4.63±0.05	5.88±0.01		-	41.80
7	Spraying with *Clerodendrum* chloroform +Inoculation	3.00±0.51	3.0±0.070	3.83±0.04	62.07	-	-
8	Inoculation +Spraying with *Clerodendrum* chloroform	4.40±0.14	7.20±0.06	8.10±0.03	-	35.20	-
9	Dipped in *Clerodendrum* chloroform+ inoculation	1.00±0.07	2.17±0.04	2.33±0.07	-	-	76.22
10	Spraying with Carbendazim (1g/lt)+Inoculation	2.50±0.07	2.75±0.07	2.75±0.09	72.80	-	-
11	Inoculation +Spraying with Carbendazim (1g/lt)	4.60±0.50	5.75±0.06	6.13±0.14	-	78.00	-
12	Dipped in Carbendazim (1g/lt)+ inoculation	0.00±0.00	0.00±0.00	0.00±0.00	-	-	100.00
13	Spraying with water +Inoculation (Control 1)	7.50±0.00	9.00±0.03	10.1±1.50	-	-	-
14	Inoculation + Spraying with water (Control 2)	8.00±0.58	10.0±0.00	12.5±1.50	--	-	-
15	Dipped in water + inoculation (Control 3)	8.30±0.58	9.30±0.00	9.80±0.00	-	-	-
	S.Em±	0.87	1.06	0.84			
	CD at 5%	2.45	2.96	2.38			

Percent inhibition of disease severity caused by *R.solani* artificially inoculated in Rice (cv.) following spraying with botanical formulation

Under field condition the experiment was conducted at tillering stage of the crop for two years (2013 and 2014) in Kharif rice season are presented in table.4. In both the years. Percent inhibition of disease severity was observed after 30 days of treatment. In both the years, highest inhibition was observed spraying with clerodendrum chloroform extract while lowest inhibition was observed in solvent treated plants. To record the phytotoxicity, solvents were sprayed in field but no phytotoxicity was recorded. Moreover there were very less disease inhibition has been recorded in solvent sprayed at 0.2% concentration.

Table 5: Percent inhibition of disease caused by *R. solani* artificially inoculated in Rice (cv.) following spraying with botanical formulation (Kharif or winter rice 2013 and 2014)

Sl. No	Treatment	Percent inhibition of disease severity at 30 days of inoculation after spraying	
		2013	2014
1	Ginger chloroform	37.92(38.01)	40.11(39.30)
2	Clerodendrum chloroform	50.34(45.20)	55.10(47.93)
3	Polyalthia methanol	36.12(36.94)	44.23(41.69)
4	Solvent (A+B+LSN)	03.63(10.98)	06.48(14.75)
5	Carbendazim (1g/lt)	28.10(32.32)	36.65(37.26)
	S.Em. (±)	1.8	1.5
	CD 5%	5.7	4.5

A=N-Alkaline Sulfonate, B= K-Alkaline Sulfonate, LSN= light solvent naptha.
Values are mean of four replications ± SD
Values in parenthesis indicate arc-sin transformed values

Discussions

The results obtained from screening of plant extracts with solvent as hexane against different plant pathogens showed comparatively low bioactivity comparing to chloroform and methanol solvents. This suggests that the probable bioactive compounds present are mostly mid to high polar in nature, could be extracted with polar solvents. Ahmad *et al*., 1998 [1]; Eloff *et al*., 1998 [14]; Lin *et al*., 1999 [21] confirms that methanol is a better solvent for more consistent extraction of antimicrobial substances from medicinal plants as compared to other solvents, such as water, ethanol, and hexane. Absence of inhibitory activities of hexane extracts suggested that the antimicrobial compounds of the plants are not soluble in non-polar solvents.

The previous studies showed that successful extraction of active compounds depend on type of solvents used for extraction (Tiwati *et. al.* 2011). The selection of solvents mainly depend on specific nature of bioactive compound present in plant. Methanol extracts of several plants of Zingiberaceae family showed great potential as antifungal and antibacterial properties (Yusuf *et al*. 2001) [35], agreed with the present findings as the extracts of ginger also belonged to the same family. Extracts derived from *Inula viscosa* using several organic solvents (methanol, ethanol, ethyl acetate, acetone, chloroform, and n-hexane) were shown to have antifungal activity against *Phytopthora infestans*, *Colletotrichum cucumerinum*, *Botrytis cinerea*, and *Plasmophora viticola* (Cohen *et al*. 2003) [9]. Plant extracts belonging to different families (Russel *et al*., 1997) [29] were used to control *Fusarium sp*.

In the present study, dil seed extract, obtained in the form of oil showed good inhibitory activity against *R. solani*, agreed with the previous studies which states that plant oils do have great potential for controlling several fungal pathogens such as *Colletotrichum musae Lasiodiplodia theobromae* and *Fusarium proliferatum* (Ranasinghe *et al*. 2002) [28], and also for controlling many fungal and bacterial pathogens (Dorman and Deans 2000) [13].

Though pot experiments like the ones in the present investigation has not been carried out, yet there are many reports of *in vitro* efficacy of plant extracts against this pathogen. Narasimhan *et al*. 1998 studied the effect of neem and pungam oil based EC formulations developed by TNAU and found that these formulations were effective against sheath rot disease of rice under field conditions. Dohroo and Gupta (1995) [12] also reported neem oil to possess inhibitory effect against sclerotia of *Rhizoctonia sp*. and claimed that 0.5% neem oil effectively controlled sheath blight disease caused by *R. solani* (Kandhari and Devakumar, 2003) [19].

Regarding solvent system used for preparation of plant extract, showed no inhibition of disease. From this we can say that, the disease was inhibited by the plant extract and not by the solvent. There were no such huge difference among the three types of inoculation. Inhibition of the pathogen with plant extract may be due to systemic effect of plant extracts on pathogen which does not allow the pathogen to grow further upto a certain point of time. No phytotoxic effects has been observed in plants after application of plant extracts at 0.2% concentration. Bhutia 2013 [4] reported no phytotoxic effects at 0.2% concentration under field condition.

In the present study, it has been observed that *Clerodendrum infortunatum* has more antifungal property while from the reports of Bhutia *et al*. 2015 [11] there were less antifungal effects of another species *Clerodendrum inerme* (Both the species to lamiaceae family) which may be due to the phyto-constituents present in plants because both the species consists of different phyto-constituents (Nayeem, 2015) [25].

In conclusion, the results reported here show that the clerodendrum-chloroform extract was most effective comparing to other extracts in controlling sheath blight disease of rice in pot culture and field experiment caused by *R. solani*. Besides more works have to be done on mechanism of action of plant extracts in molecular level. Further shelf life studies on efficacy of plant extracts are carried in laboratory so that the product should be commercially formulated, explored and confirmed in large-scale experiments in farmer's field as well as storage condition to control sheath blight of rice.

Acknowledgements

Our sincere thanks to DST, Government of India for Rajiv Gandhi National Fellowship as financial support.

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