Monitoring and biocontrol of paddy fungal pathogens collected from Hassan District

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ABSTRACT

Rice or paddy (*Oryza sativa* L.), one of the staple food crops of 60% of world's population, suffers from different kinds of diseases caused by fungi, bacteria, viruses, nematodes and other non-parasitic disorders. In the present study, four paddy samples were collected from different places of Hassan District, Karnataka, India and screened for fungal pathogens using Agar plate method. Results showed varied Disease Incidence in collected samples ranging from 52 to 85%. The variety BT collected from Sarguru showed a minimum DI of 52% whereas Rajmudi-sanna collected from Doddamagge showed 85% of maximum of total DI, followed by Dappa batha from Basavapatna (74%) and Rajmudi-Dappa from Handrangi which showed 60% DI. The Individual DI of different pathogens, such as *Penicillium, Fusarium* , *Aspergillus niger* and *Aspergillus ochraceus* in all the four samples have been recorded. *Aspergillus niger* was the major fungus found in all the four samples. The pathogen *Aspergillus niger* isolated from all the varieties was subjected to inhibition assay using leaf and stem extract of *Leucas aspera* L and *Datura stramonium* L which showed encouraging results in controlling the pathogen. Among these two plants, *Datura stramonium* controlled the fungal growth to the greater extent when compared to *Leucas aspera*. Among the plant parts selected for study, stem would a ideal for extraction of anti-fungal metabolite study. *Aspergillus niger* isolated from both varieties, Rajmudi-sanna and Dappabatha was controlled effectively by using stem and leaf extract of both *Datura stramonium* and *Leucas aspera*. Our results suggested that for extraction of metabolites, solvents such as alcohol and acetone at 50 or 70% can be effectively employed. Dosage dependent study results indicated that the solvent extract of 80 µl had given better results, so this concentration should be useful for inhibition assay.

Key words: Biocontrol, Fungal Pathogen, Mountory, Paddy,

INTRODUCTION

Rice (*Oryza sativa* L.) is the most widely grown food grain crop. The utilization of rice for various purposes involves milling of paddy to remove husk, bran and germ from the surface. The fruit caryopsis is rich in starch. Rice flour is used in preparing rotes, puddings etc., Paddy husk and straw are used for making hardboards and also as cattle feed. It is used as staple food in South India. The major rice growing states in India are west Bengal, Bihar, Madhya Pradesh, Orissa, Andhra Pradesh and Uttar Pradesh.

Rice is a self-pollinating crop. Rice suffers from many diseases caused by fungi, bacteria, viruses, nematodes and other non-parasitic disorders. Fungal disease is considered as the principal disease of rice because of its wide distribution and its destructiveness under favorable conditions for yield loss. The biotic pathogens can infect the crop at any time from seed germination to harvest. The biochemical changes in seed leading to seed deterioration generally takes place when the seed moisture level is favorable for the growth of storage moulds. Colonization of storage fungi led to decrease in carbohydrate content in most of the cases (Ghosh and Nandi, 1986).Finding healing powers in plants is an ancient idea. There is evidence that Neanderthals living 60,000 yrs. ago in present day Iraq used plants such as Hollyhock (Stockwell, 1988; Thomson, 1978). Disease management strategies include use of resistant hybrids/cultivars, chemical control and cultural practices. It is estimated that there are 297,326 plant species on earth (IUCN, 2007). A relatively small percentage (1-10%) of these is used as foods by both humans and other animal species. It is possible that even more are used for medicinal purposes (Moerman, 1996). Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen substituted derivatives (Geissman, 1963). Most are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total (Schultes, 1978). In many cases, these substances serve as plant defense mechanisms against

predation by microbes, insects and herbivores (Marjorie, 1999). Advice abounds for the amateur herbalist on how to prepare healing compounds from plants and herbs. Water is almost universally the solvent used to extract activity. At home, dried plants can be ingested as teas (plants steeped in water) or, rarely, tinctures (plants in alcoholic solutions) or inhaled via steam from boiling suspensions of the parts. Dried plant parts can be added to oils or petroleum jelly and applied externally. Poultices can also be made from concentrated teas or tinctures (Brantner and Grein, 1994; Thompson, 1978).

In recent years, Integrated Pest Management has identified the use of biocontrol agents and induced systemic resistance as eco-friendly strategies of disease management. There are a number of reports on the possible use of different agents in more than 25 plant species against diseases caused by bacteria, fungi, viruses and nematodes (Hammerschmidt and Kuc, 1995; Shetty and Vasanthi, 2000). *Datura stramonium* concoction has shown antifungal activity against floral malformation in mango caused by *Fusarium mangiferae* (Usha *et al.*, 2009) Methanol extraction of *Leucas aspera* has shown antimicrobial activity against food borne pathogens like *pseudomonas spp*. and *Bacillus subtilis* (Preethi *et al*., 2010). Screening is the method of potentially interesting activity; evaluation is the quantization and characterization of that activity. Now a days, the need for antifungal compounds is increasing due to the increase in fungal infections. Though there are a few of the antifungal compounds, the need for novel antifungal compounds is still going on.

The extensive use of frequent injudicious application of synthetic pesticides and fungicides has led to problems like development of resistance, toxicity hazards to man, plants, domestic animals and wild life (Doult and Smith, 1971). Hence, there is a need to use environmentally safer chemicals or formulations or bio-control agents which can fit into Integrated Pest Management Programme. In the present study, paddy samples were collected in and around Hassan District for screening of fungal pathogens and to record disease incidence. Further, attempts were also made to study the biocontrol of isolated pathogens from collected seed samples of Doddabatta (Basavapatna), BT (Sarguru), Rajmudi - dappa (Handrangi) and Rajmudi –sanna (Dodda magge) using stem and leaves of *Leucas aspera* L. and *Datura stramonium* L. with different solvent extracts.

MATERIALS AND METHODS

Chemicals used

Sodium hypochlorite, Alcohol, Acetone, Agar, Dextrose. All the chemicals were purchased from Hi Media Laboratories Pvt. Ltd., Ranbaxy Laboratories Ltd., Mumbai and E. Merck (India) Ltd., Mumbai.

Collection of seed samples

Paddy samples were collected from in and around Hassan District of Karnataka from local traders as shown in Table 1. Collected seed samples were packed in polyethylene covers and stored at 4Ú C until further use.

Table 1. Collected sample locations.

Sample Name	Place of Collection
Dappa Batha	Basavapatna
BT	Sarguru
Rajmudri(dappa)	Handrangi
Rajmudri(sanna)	Doddamagge

Screening for seed-fungi using Agar plate method

Preparation of media

Potato skin was peeled and cut into small pieces. Two hundred gm of potato pieces was weighed and boiled with known volume of distilled water. The boiled potato was filtrated and the collected filtrate was brought up to 1000 mL with distilled water. Twenty gm of Dextrose and 15 gm of Agar were added to the above extract, and then sterilized at 121ÚC for 15 min. 15 mL of media was poured to sterilize Petri plates, and allowed to cool. Petri plates containing solidified media were used for plating the seeds.

Procedure for plating the seeds

Randomly, 100 seeds were taken from each seed samples and were surface sterilized using 0.5% sodium hypochlorite solution for about 8 min. Seeds were washed further with sterile distilled water repeatedly and blotted.

Further, sterilized seeds were plated using sterile forceps in equidistance on Petri plates containing Potato Dextrose Media and were maintained at room temperature about 25 ± 2 ÚC for seven to ten days for fungal growth. After ten days, Percentage of Disease Incidence (DI) was recorded using the following formula:

Disease Incidence (DI)

$$= \frac{\text{Total No. of diseased seeds X 100}}{\text{Total No. of seeds plated}}$$

Individual Pathogen Disease Incidence:

Individual Pathogen DI

$$= \frac{\text{Total No. diseased seeds of pathogen X 100}}{\text{Total No. of diseased seeds}}$$

The seed samples which showed diseases of different pathogens were selected to isolate the pathogen.

Identification of different pathogens was done based on color, morphology of sporulation structures and mycelial growth using compound microscope (Tsuneo Watanabe, 2002) From the seed mycoflora, *Aspergillus niger* pathogen was selected for further study.

Test organism

Aspergillus niger were cultured on PDA at 28ÚC for 48 hrs. The stock culture was maintained on nutrient agar slants at 4 ÚC.

Extraction of anti-fungal metabolites

The plant parts like stem and leaf of *Leucas aspera* and *Datura stramonium* were collected freshly and used for extraction. Leaf and stems were separated from plants and washed thoroughly with distilled water and sterilized with 0.01% of sodium hypochlorite solution to remove the surface pathogens. Cleaned leaves or stems were cut separately into small pieces and two gm of leaf or stem was used for extraction.

Samples of leaf or stem were homogenized in a cold mortar using pestle by adding 5 mL of different solvents, 0.1 M buffer, 50 and 70% acetone, 50 and 70% alcohol. The extract was than filtered using Whatmann filter paper No.1 and the filtrates were used as anti-fungal metabolites. Buffer or solvents were used for comparison in control experiments. For dose dependent study, aliquots of 20, 40, 60 and 80 µL of antifungal metabolite extracts were used.

Preparation of *Aspergillus niger* inoculum

Fungal spores of *Aspergillus niger* were released in sterile distilled water in a small test tube through gentle shake. Spores were partially purified by filtering through sterilized cheesecloth, and were spread in sterilized Petri plates, then PDA was poured over spores (~$1X10^6$-10^8 cfu/mL) and allowed to solidify.

Antifungal activity of crude extracts

Discs of 0.6cm diameter were prepared using perforator on Whatmann filter paper no.1 and impregnated with known aliquots of each extract (20, 40, 60 and 80 µL). The saturated discs were placed on pre-inoculated-PDA plates in diagonal order. In the centre, proper neutral or negative control was maintained. The plates were maintained under room temperature for 7-10 days, for observation of antifungal activity. The antifungal activity was evaluated by measuring zones of inhibition of fungal growth surrounding the plant extracts (Pawan Kaur *et al.,* 2012). Clear zones of varying size around each disc were observed. The length of this clear zone is the zone of inhibition and measured with ruler scale in mm (Megan Jungwi, 2013). Amphotericin B (10µg/mL) anti-fungal drug was used as positive reference standards to determine the sensitivity of *Aspergillus niger* species tested.

The relative percentage inhibition of the test extract with respect to positive control was calculated by using the following formula (Ajay *et al.*, 2002).

$$\text{Relative percentage inhibition of the test extract} = \frac{100 \times (a-b)}{(c-b)}$$

where a-total area of inhibition of the test extract; b-total area of inhibition of the solvent and c-total area of inhibition of the standard drug.

The total area of the inhibition was calculated by using area = ðr2;

where, r = radius of zone of inhibition.

Results

The paddy samples collected from different places of Hassan District have been screened for fungal pathogens using Agar plate method. The present result showed varied Disease Incidence in collected samples ranging from 52 to 85%. The variety BT collected from Sarguru showed a minimum DI of 52% whereas Rajmudi-sanna collected from Doddamagge showed the highest DI of 85%. Dappa batha has 74% of DI, and Rajmudi-Dappa which has showed about 60% DI (Table 2).

Table 2. Place of sample collection and disease incidence (DI)

Samples*	Place of collection	% of DI
BT	Sarguru	52
Dappa batha	Basava patna	74
Rajmudi (dappa)	Handrangi	60
Rajmudi (sanna)	Doddamagge	85

* Based on 100 seeds

The Individual DI of different pathogens, such as *Penicillium, Fusarium* , *Aspergillus niger* and *Aspergillus ochraceus* in all the four samples have been recorded. The DI varied from 6.66% to 48.33% in BT variety collected from Sarguru for different fungal pathogens. The pathogen *Fusarium* was the pre-dominant when compared to other pathogens and DI recorded up to 48.33%. The DI of *Aspergillus niger* was recorded about 45%. Disease incidence of 6.66% recorded for *Aspergillus ochraceous* as minimum. However, this sample was free from *Penicillium* sp. contamination (Table 3).

In the paddy seed sample (Dappa batha) collected from Basavapatna had Disease Incidence (DI) was between 6.75%

Table 3. Individual pathogen disease incidence in different varieties of paddy.

Samples	Pathogens*			
	Penicillium sp.	*Fusarium* sp.	*Aspergillus niger*	*Aspergillus ochraceous*
Dappa batha	6.75	13.51	56.76	22.97
BT	-	48.33	45	6.66
Rajmudi(dappa)	28.84	23.07	19.07	28.84
Rajmudi(sanna)	-	2.35	97.64	-
Mean % of DI	8.90	21.82	54.62	14.62

* Based on 100 seeds

and 56.76% for different fungal pathogens (Table 3). Disease Incidence of *Penicillium* sp. was recorded as 6.75%, and the highest DI was recorded for *Aspergillus niger* (56.76%), followed by *Aspergillus ochraceous* (22.97%). The lowest DI was recorded for *Fusarium* sp. The DI varied from 19.03% to 28.84% in Rajmudi-dappa variety collected from Handrangi for different fungal pathogens. Disease Incidence of *Penicillium* sp. and *Aspergillus ochraceus* showed a maximum record of 28.84%, followed by *Fusarium* with a record of 23.04%. *Aspergillus niger* showed a minimum DI of 19.03 %. The variety Rajmudi-sanna collected from Doddamagge showed a maximum contamination of *Aspergillus niger* and DI recorded was maximum (97.64%). The DI of *Fusarium* sp. was minimum. There was no contamination of *Penicillium* or *Aspergillus ochraceus* in this sample.

Among the screened pathogens, *Aspergillus niger* species was chosen for biocontrol studies. For inhibition study, two plants of *Datura stramonium* L. and *Leucas aspera* were selected. From these plants, leaves and stem were selected for extraction of antifungal metabolites for testing their efficacy against *Aspergillus niger* fungal pathogen. The antifungal activity of these extracts was qualitatively assessed in the present study. After incubation the antifungal activity of the test material was determined by measuring the diameter of the

Table 4. Inhibition assay of *Datura stramonium* L. stem extract against *Aspergillus niger*

Extract	Concent-ration (%)	Paddy sample	Diameter of Zone of Inhibition in mm			
			Plant extract in impregnated Discs(µl)			
			20 µl	40 µl	60 µl	80 µl
Alcohol	**50**	Rajmudri sanna	0(0)	10(33.3)	8(26.6)	12(40)
		Dappa batha	18(60)	10(33.3)	10(33.3)	18(60)
	70	Rajmudri sanna	16(53.3)	24(80)	18(60)	18(60)
		Dappa batha	22(73.3)	14(46.6)	16(53.3)	20(66.6)
Acetone	**50**	Rajmudri sanna	12(40)	18(60)	20(66.6)	22(73.3)
		Dappa batha	22(73.3)	18(60)	18(60)	26(86.6)
	70	Rajmudri sanna	12(40)	22(73.3)	26(86.6)	28(93.3)
		Dappa batha	24(80)	16(53.3)	18(60)	28(93.3)

Figures in parenthesis indicate relative percentage inhibition of the test extract with respect to positive control.

Table 5. Inhibition assay of *Datura stramonium* L. leaf extract against *Aspergillus niger*

Extract	Concent-ration (%)	Paddy sample	Diameter of Zone of Inhibition in mm			
			Plant extract in impregnated Discs(µl)			
			20 µl	40 µl	60 µl	80 µl
Alcohol	50	Rajmudri sanna	12(40)	10(33.3)	14(46.6)	16(53.3)
		Dappa batha	20(66.6)	14(46.6)	14(46.6)	12(40)
	70	Rajmudri sanna	14(46.6)	16(53.3)	18(60)	12(40)
		Dappa batha	16(53.3)	12(40)	18(60)	16(53.3)
Acetone	50	Rajmudri sanna	10(33.3)	14(46.6)	22(73.3)	18(60)
		Dappa batha	10(33.3)	14(46.6)	16(53.3)	22(73.3)
	70	Rajmudri sanna	0(0)	14(46.6)	16(53.3)	20(66.6)
		Dappa batha	10(33.3)	12(40)	20(66.6)	18(60)

Figures in parenthesis indicate relative percentage inhibition of the test extract with respect to positive control.

zone of inhibition in terms of millimeter with a transparent scale.

As to the standard anti-fungal drug used in the test, the inhibition zones of amphotericin B (10µg/mL) was 30mm, whereas negative control showed no zone of inhibition.

Inhibition assay of *D. stramonium* stem extract against *A. niger* for variety Rajmudri sanna and Dappa batha were evaluated. It was observed that stem extract in 70% acetone showed a maximum of 93.3% relative percentage inhibition and a minimum of 33.3% in 50% alcohol extract (Table 4). Likewise leaf extract of *Datura stramonium* L. showed a maximum of 93.3% relative percentage inhibition in 70% acetone and a minimum of 33.3% in 50% alcohol extract (Table 5). *Leucas aspera* L. stem extract against *Aspergillus niger* for variety Rajmudri sanna and Dappa batha was evaluated and result showed that, 70% acetone showed a maximum relative percentage inhibition of 86.6% and a minimum of 26.6% in 50% alcohol (Table 6), whereas leaf extract showed a maximum of 66.6% in 70% acetone and minimum of 33.3% in 50% alcohol (Table 7). Though there are maximum levels of inhibition in stem and leaf extracts of *Datura* and *Leucas*, *Datura* stem appears to be more efficient in inhibiting *Aspergillus niger* colonization and acetone as a preferred solvent.

DISCUSSION

Paddy is one of the important food crops for world's population and widely grown all over the world. The crop is known to infect by many pathogens including fungi. Fungal disease is considered as the major disease of paddy because of its destructiveness under favorable conditions. Hence disease management strategies include use of resistant hybrids/cultivars, chemical control and cultural practices are regularly employed. But, now a days biological control strategies are gaining more insight for managing of pathogens and disease control.

In fact, there are a lot of different researches available in world literature. Antimicrobial activity of *C. lanceolatus* leaf extract was compared with the antimicrobial activity of standard drugs for evaluating relative percentage inhibition(Veeranna *et al*., 2012).The antimicrobial activity of *Psoralea corylifolia* Linn. was studied by 'Disc diffusion' method using different gram positive and gram negative bacterial and fungal strains, which showed that it has prominent activity compared with the standard used (Bina Gidwani *et al.*, 2011). Aqueous extracts of *Pistacia*, *Tilia argentea* and *Anthemis pungens* had microbial activity against *E.coli, Bacillus subtilis, Klebsiella pneumoniae, Staphylococcus aureus* and environmental *Aeromonas* spp. strains (Nihal Dogruoz *et al*., 2008). Jagessar *et al*. (2008) studies suggest that the ethanol extracts of *Phyllanthus acidus,* can be used as herbal medicines in the control of *E.coli* and *S.aureus*. Sunita Bansod and Mahendra Rai (2008) showed that plant oils can be used to cure mycotic infections and plant oils may have a role as pharmaceuticals and preservatives. Kumar *et al*. (2012) found the aqueous extracts of *Psidium guajava*, *Citrus limonium, Allium sativum* and *Zingiber officinale* were active against both Gram-positive and Gram-negative bacteria. Crude extract of *Terminalia arjuna* showed antagonistic activity against five clinically significant antidermatophytic fungi *i.e., Trichophyton mentagrophytes,*

Table 6. Inhibition assay of *Leucas asperal* L. stem extract against *Aspergillus niger*

Extract	Concent-ration (%)	Paddy sample	Diameter of Zone of Inhibition in mm			
			Plant extract in impregnated Discs(µl)			
			20 µl	40 µl	60 µl	80 µl
Alcohol	50	Rajmudri sanna	12(40)	8(26.6)	8(26.6)	20(66.6)
		Dappa batha	14(46.6)	10(33.3)	12(40)	14(46.6)
	70	Rajmudri sanna	12(40)	10(33.3)	14(46.6)	22(73.3)
		Dappa batha	12(40)	14(46.6)	20(66.6)	16(53.3)
Acetone	50	Rajmudri sanna	10(33.3)	20(66.6)	16(53.3)	18(60)
		Dappa batha	18(60)	18(60)	18(60)	20(66.6)
	70	Rajmudri sanna	14(46.6)	14(46.6)	24(80)	26(86.6)
		Dappa batha	14(46.6)	16(53.3)	12(40)	14(46.6)

Figures in parenthesis indicate relative percentage inhibition of the test extract with respect to positive control.

T. rubrum, T. tonsurans, Microsporum gypseum and *M. fulvum* (Bhattacharyya and Jha, 2011). The ethanol and chloroform extracts of *Declepis hamiltoni* can be used in food treatments to inhibit microorganisms such as *A.flavus, Rhizopus, Yersinia enterocolitica* and *S. typhi*. The extracts can be used as a component of food preservative to enhance the shelf life of the foods (Vimala *et al*., 2007). Aqueous extract of 52 plants namely *Datura stramonium, Leucas aspera, Acacia nilotica, Punica granatum* etc.were tested for their anti-fungal potential against eight important species of *Aspergillus* isolated from *sorghum*, maize and paddy seed samples (Sathish *et al.,* 2007). Hexane, chloroform, ethyl acetate, methanol and water extracts from the flower of *Cassia fistula* (an ethnomedicinal plant) exhibited antifungal activity against *Trichophyton mentagrophytes* and *Epidermophyton floccosum* (Duraipandiyan and Ignacimuthu, 2007) Piper betle extract exhibited antifungal activity towards all oral Candida sp. with various degrees of inhibition (Himratul-Aznita *et al*., 2011). Paola Díaz Dellavalle *et al.* (2011) evaluated *in vitro* the potential antifungal activity of medicinal Uruguayan plant

Table 7. Inhibition assay of *Leucas aspera* L. leaf extract against *Aspergillus niger*

Extract	Concent-ration (%)	Paddy sample	Diameter of Zone of Inhibition in mm			
			Plant extract in impregnated Discs(µl)			
			20 µl	40 µl	60 µl	80 µl
Alcohol	50	Rajmudri sanna	0(0)	10(33.3)	12(40)	8(26.6)
		Dappa batha	10(33.3)	14(46.6)	16(53.3)	16(53.3)
	70	Rajmudri sanna	14(46.6)	12(40)	12(40)	14(46.6)
		Dappa batha	12(40)	14(46.6)	20(66.6)	16(53.3)
Acetone	50	Rajmudri sanna	10(33.3)	16(53.3)	18(60)	18(60)
		Dappa batha	16(53.3)	12(40)	12(40)	18(60)
	70	Rajmudri sanna	12(40)	14(46.6)	12(40)	20(66.6)
		Dappa batha	12(40)	14(46.6)	14(46.6)	16(53.3)

Figures in parenthesis indicate relative percentage inhibition of the test extract with respect to positive control.

extracts against *Alternaria* spp. Shinde *et al*. (2011) showed aqueous, alcoholic and ethyl acetate extracts of leaves of five *Terminalia* species (*T. alata*, *T. arjuna*, *T. bellerica*, *T. catappa* and *T. chebula*) tested were effective against five plant pathogenic fungi like *Aspergillus flavus*, *Aspergillus niger*, *Alternaria brassicicola.* Abdul Rahman Al-Qurashi *et al*. (2007) have observed a dose related anti-aspergillus effect of thymoquinone and amphotericin B.

In the present study, pathogens isolated from paddy were evaluated for its biocontrol using *L. aspera* and *D. stramonium* stem and leaf extracts in different solvents. Results showed that all seed samples were infected with fungal pathogens. Isolated fungal pathogens were identified as *Aspergillus niger, Fusarium* sp., *Aspergillus ochraceous* and *Penicillium* sp. The variety Rajmudi (dappa) was severely infected with all pathogens. The development of fungi is influenced by the moisture content of the stored grain, temperature, condition of the grain going into storage, length of time the grain stored and amount of insect and mite activity in the grain (Rice Knowledge Bank, 2009). Hence, study suggested that paddy samples were needed to be stored in proper condition, and screening is essential before sowing in the field. So, farmers could overcome from the yield loss problem. *Aspergillus niger* is major fungus for production of aflotoxin in food industry, so, before milling of paddy, seed testing would be ideal to avoid aflotoxin problem in food.

The pathogen *Aspergillus niger* isolated from all varieties was subjected to inhibition assay against leaf and stem extract of *L. aspera* and *D. stramonium* which showed encouraging results in controlling of pathogen.

Leaf extract of *Datura stramonium* showed maximum inhibition in 50% of alcohol and 50 and 70% acetone solvent extraction. Hence, both solvents could be effectively used to extract the metabolite from the plant. Stem extract of the same plant (*Datura stramonium*) had maximum inhibition in acetone. Hence *Datura* leaf and stem extract could effectively employed as biocontrol agent against fungal pathogen. Dosage dependent study results indicated that the 80 µL of solvent extract had given better results. So the minimum of 80 µL solvent extract is recommended for inhibition assay.

In stem extract of *Leucas aspera* in 70% alcohol and acetone has given better results when compared to buffer and other concentration of solvent, whereas leaf extract with buffer and 70% acetone recorded maximum inhibition. The present study suggest that *L. aspera* could also be used as biocontrol agent to control the fungus. Cichewicz and Thorpe (1996) have used dried plant parts for extraction of metabolites.

Among these two plants, *Datura stramonium* controlled the fungal growth to a greater extent when compared to *L. aspera*. Among the plant parts selected for study, stem would be ideal for extraction of antifungal metabolite study. Thompson (1978), has given similar results in case of Balsam plant.

Aspergillus niger isolated from both varieties, Rajmudi-sanna and Dappabatha was controlled effectively by using stem and leaf extract of both *D. stramonium* and *L. aspera*. Since these two plants grow in almost all the environmental conditions and are available easily in the fields, they could be used as common biocontrol agent for controlling most of the fungal pathogens not only in paddy, but also for other staple crops. For further investigation antifungal metabolite present in these plants could be isolated and characterization can be done for effective biocontrol remedial measures for most of the food crops.

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