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Antifungal activity of plant oils against major seed-borne fungi of rice

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ABSTRACT

A total of four fungal genera were isolated and identified from rice as Curvularia lunata,Fusarium moniliforme, Helminthosporium oryzae and Sarocladium oryzae. Among them, the most predominant one was H. oryzae. This was followed by S. oryzae and F. moniliforme. Least incidence was observed with C. lunata with the variety ADT 45 and White ponni. Six essential oils viz., Lemongrass (Cymbopogon citratus), Palmarosa (Cymbopogon martinii), Citronella (Cymbopogon nardus), Eucalyptus (Eucalyptus globulus), Geranium (Pelargonium graveolens) and Tulasi (Ocimum sanctum) extracted by hydro distillation process were screened against four major seed borne pathogens of rice. In vitro evaluation of six essential oils at 0.1 per cent by poisoned food technique showed varied fungicidal properties against C. lunata, F. moniliforme, H. oryzae and S. oryzae. Among them, C. citratus, C. martinii and P. graveolens oils were found to be more effective and caused complete mycelial growth inhibition of pathogen even at 0.1 per cent concentration. However, oils of C. nardus, E. globulus and O. sanctum were not resulted in complete inhibition of mycelial growth of C. lunata, F. moniliforme, H. oryzae and S. oryzae at 0.1 per cent concentration.

Key words: *Rice,seed-borne fungi, in vitro, plant oils,antifungal activity*

Rice (*Oryzae sativa* L.) serves as a major carbohydrate source for nearly half of the world's population. In India, rice production area is around 43 million hectares. Rice accounts for 42% of food grain production and 55% of cereal production in India. With continuous increase in population, the food grain requirement is also increasing. India has to produce 135-140 million tons of rice by 2030 (Nayak *et al*. 2015). In India, more than half of the population depends on rice for their food and provides livelihood for about 70% of the population (Prasad *et al*. 2012). The majority of the rice (90%) is being produced in Asian countries with China and India being the major producers. The other major producing countries are Indonesia, Bangladesh, Vietnam, Thailand, Myanmar, Philippines, Brazil and Japan (IRRI, 2008).

The crop is affected by as many as 36 seed-borne diseases, of which 31 were caused by fungi (Ou 1985). Before its harvest, the reduction in the quality of the crop is caused by major fungi due to high moisture and temperature conditions. They cause seed abortion, seed rot, seed necrosis, reduction or elimination of germination capacity as well as seedling damage resulting in development of disease at later stages of plant growth by systemic or localized infection (Khanzada *et al*. 2002). Apart from being seed borne pathogens, fungi may grow on storage products. These fungi may decrease the ability of seed germination, can cause seed discoloration, produce toxins that may be injurious to man and domestic animals and may reduce seed weight too (Uma and Wesely 2013). Seed borne diseases caused by fungi are relatively difficult to control as the fungal hyphae get established and become dormant.

A total of more than 100 fungi were detected on rice seeds. However, the detection frequency varied considerably and about 20 species of fungal pathogens were detected from rice seed at any one time (Mew

and Gonzales 2002). The yield loss due to seed borne disease was approximately estimated from 20 to 50 per cent because of chaffy and unfilled grains (Tuat 1997). Zolkifli *et al*. (1991) reported that due to *H. oryzae* infection the seed germination also reduced to 40 per cent and average grain yield loss of 8.2 to 28 per cent was recorded. Rice seed samples of four widely cultivated varieties *viz*., IR 20, Ponni, Co 43 and MDU 5 collected from Tamil Nadu has recorded 60.6, 46.2, 47.6 and 51.4 per cent of *S. oryzae* seed infection, respectively (Shanmugam 2004). Utobo *et al*. (2011) reported that *F. moniliforme* was the major seed-borne pathogen that caused devastating seedling diseases of rice in the field and has recorded 40 per cent seed infection.

So far, chemical and biological methods have been used to alleviate and control seed borne diseases in rice. Synthetic chemical fungicides do not provide adequate control of the pathogen, besides being toxic to soil microflora, and hazardous to human and animal health (Gupta *et al*. 2001). The recent efforts have focused on developing environmentally safe, long lasting and effective essential oils for the control of plant diseases. Use of essential oils for the control of plant disease is desirable as many plant essential oils have been reported to be effective antimicrobial agents against several seed-borne, soil-borne and foliar pathogens (Ibiam *et al*. 2008; Hashem *et al*. 2010; Muthukumar and Sanjeevkumar 2012; Nguefack *et al*. 2013). Essential oils are long been studied for their characteristic bactericidal and fungicidal properties (Oka *et al.* 2000; Nguefack *et al*. 2005) due to the presence of active compounds as alkaloids, phenols, flavanoids, monoterpenes, sesquiterpenes and isoprenoids (Alilou *et al*. 2008).

The objectives of the present studies are i). Isolation and identification of major seed borne fungi of paddy varieties such as ADT 45 and White Ponni. ii). Screening the antifungal activity of the six plant oils against the major seed borne fungi of rice.

MATERIALS AND METHODS

Collection of seed sample

Two rice varieties (ADT 45 and White ponni) were collected from Experimental farm, Department of Agronomy, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India. The seeds were collected in sterilized polythene bags and stored at 4-5°C until further use. 400 seeds were taken from each variety and randomly picked out 100 seeds. These seeds were further divided into two categories such as normal seeds and discoloured seeds. Based on these visual observation the per cent discolouration was calculated.

The experiment was conducted in the Department of Plant Pathology, Faculty of Agriculture, Annamalai University during the year 2014-2015.

Isolation and identification of pathogen associated with rice seeds (Blotter paper method)

The standard blotter method was used to determine the occurrence of seed borne mycoflora on rice seeds (Mathur and Kongsdal 2003). From each variety 400 seeds were collected and 100 seeds were picked out randomly and they were subjected to isolation. A set of three plates (100 seeds) were considered as replicates. Petri plates containing 25 seeds were considered as sub-replicates. Three pieces of filter paper were soaked in sterilized water and were placed at the bottom of a 9 cm well labeled Petri dishes. The seeds were placed and spaced in each Petri plate using a pair of forceps, making sure that seeds are placed equidistantly with 15 seeds on the outer ring, 9 seeds at inner ring and 1 seed in the middle. The lids of each Petri plates were held in place with gummy cello tape. The Petri plates containing seeds were incubated at 28 ±2° C for 7 days under alternating cycles of 12 hour near UV light and 12 hour darkness.

After the 7-day incubation period, individual rice seeds were examined under Stereo Binocular microscope (CX 21i) in order to record the incidence of different seed borne fungi. The fungi associated with rice seed samples were recorded and expressed in percentage individually. With flamed-sterilized transfer needle, fungal growth on the grains were aseptically mounted in lacto phenol blue on slides and examined under the stereo-binocular microscope for fungal diagnostic characteristics. For proper identification of fungi, semi-permanent slides were prepared from the fungal colony and observed under compound microscope. Pure cultures of isolated fungi were obtained through transfer on Potato Dextrose Agar (PDA) medium (Ainsworth 1961). Fungi were identified

on the basis of their typical structure and basic characters as suggested by (Booth 1971; Watanabe 2002).

Collection of essential oil

The locally available six essential oils *viz.*, Citronella, *Eucalyptus,*Geranium, Lemongrass, Palmarosa, and Tulasi were purchased from Citro Essential Oils Distillery Industry, Erankuttai, Vellithirupur, Bhavani, Erode, Tamil Nadu, India. These essential oils were selected on the basis of the local availability and previous knowledge on their antifungal activities (Ibiam *et al*. 2008; Nguefack *et al*. 2008)

Antifungal activity of essential oils against major seed borne fungi of rice

The antifungal activities of six essential oils were assessed against the four major seed borne fungi by radial growth assay following poisoned food technique (Nehal and EI-Mougy 2009). The plant oils were tested in the concentration range of 0.06 to 0.2 per cent (v/v). To 50 ml sterilized PDA, different concentrations of plant oils were mixed separately and dispensed to sterile Petri plates. All the plates were gently rotated for even dispersal of oil. Plates without oil served as control. Eight mm discs of the test fungi taken from the advancing edge of test pathogens were placed in oil-containing PDA plates and incubated at 28±2°C. The incubation period differed depending upon the pathogen. Three replicates for each treatment were maintained. At the end of the incubation period, the colony diameter (mm) of the test pathogens was measured to determine the Minimum Inhibitory Concentration of respective oil (MIC).

Statistical analysis

All the experiments were of completely randomized design (CRD) and repeated twice. Data were subjected to analyses of variance and treatment means were compared by an appropriate Duncan's multiple range test ($P<0.05$). The IRRISTAT package version 92-1, developed by the International Rice Research Institute Biometrics Unit, Philippines, was used for analysis (Gomez and Gomez 1984).

RESULTS AND DISCUSSION

Pathogen associated with rice grains

A total of four fungal genera were found to be associated with the seeds of two different varieties of rice (Fig. 1). The associated fungi were *C. lunata, F. moniliforme, H. oryzae* and *S. oryzae*. Among them, the most predominant one was *H. oryzae* which was associated with 15.3 per cent (white ponni) and 13.0 per cent (ADT 45) of seed samples, followed by *S. oryzae* (10.6 % and 10.3 %, respectively) and *F. moniliforme* (8.3 % and 7.6 %). Least incidence of 6.3 and 5.3 per cent was observed with *C. lunata* with the variety ADT 45 and white ponni, respectively. Among these, *H. oryzae* was the most frequently isolated seed-borne fungus irrespective of the source of the two different rice varieties tested in the present study.

A similar observation was made by Gopalakrishnan *et al.* (2010) stated that totally eight genera of fungi were found to be associated with rice

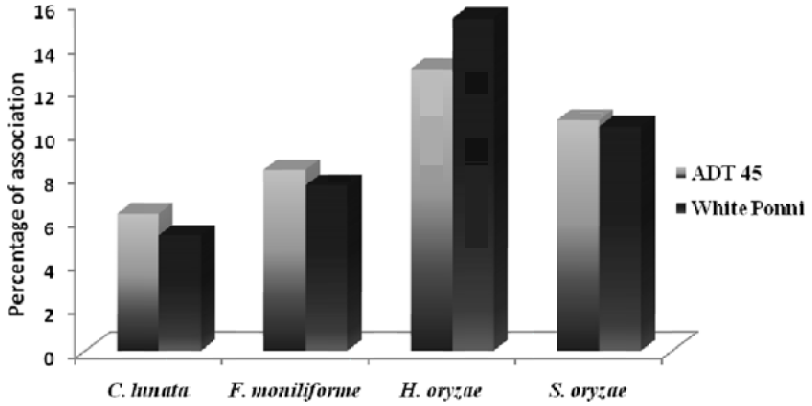

Fig. 1. Occurrence of fungi associated with grains of rice variety (ADT 45 and White ponni)

seeds. Among them, the most predominant one was *H. oryzae* which was associated with 58.59 per cent seed samples, followed by *A. padwickii* (52.96%). Archana and Prakash (2013) reported that total of 69 rice seed samples comprising of six genera of fungi were found to be associated with the rice seed samples. Among them, the most predominant one was *H. oryzae* which was associated with 82. 08 per cnet seed samples, followed by *A. padwickii* (63.36%).

In vitro antifungal activity of essential oils against major seed borne fungi

Curvularia lunata

The inhibitory effects of six essential oils were tested against *C. lunata* following poisoned food technique. All the plant oils were tested at 0.06 to 0.2 per cent concentrations. Among them, *C. citratus, C. martinii,* and *P. graveolens* oils were found to be more effective and caused complete mycelial growth inhibition of pathogen even at 0.1 per cent concentration (Table 1). However the oils of *C. nardus, E. globulus* and *O. sanctum* were not resulted in complete inhibition of mycelial growth of *C. lunata* at 0.1 per cent.

Results of the present study agreement with the findings of Sarala *et al*. (2002) reported that among the four concentrations of palmarosaa oil tried, 0.1 per cent was found to be effective, which recorded 100 per cent growth inhibition of *S. oryzae, D. oryzae* and *C. lunata* over control. Palmarosa oil (0.01%) was the most inhibitory to the growth and spore germination of *S. oryzae* and *D. oryzae* follwed by 3 per cent neem, pungam and mahua oil (Vijayakumar 1998).

Fusarium moniliforme

All the essential oils showed antifungal activity *in vitro.* However, among the essential oils tested, *C. citratus, C. martinii* and *P. graveolens* exhibited complete mycelial growth inhibition of pathogen at 0.1 per cent, compare to control (Table 2). The other oils *viz*., *C. nardus*, *E. globulus* and *O. sanctum* were not effective against the test pathogens at the same concentrations.

Similar observation was made by Somda *et al*. (2007) reported that essential oil from *C. citratus* exhibited a strong antifungal activity against *F. moniliforme* cause of sorghum seed borne fungi. Kishore *et al.* (2007) recorded that *C. citratus* oil was completely inhibited the mycelial growth of *F. moniliforme, A. alternata* in paper disc assay.

Helminthosporium oryzae

The mycelial growth of *H. oryzae* was reducedwith the increase in concentration of essential oils. The oils *viz., C. citratus, C. martinii* and *P. graveolens* were found to be most effective and caused 100 per cent mycelial growth inhibition of pathogen at a concentration of 0.1 per cent (Table 3). However, the oils of *C. nardus, E. globulus* and *O. sanctum* were not effective at the same concentrations when compared to other plant oils tested.

Similarly, Nguefack *et al*. (2008) reported that essential oils from *C. citratus, O. gratissimum* and *T. vulgaris* were highly effective in inhibiting the mycelial growth of *H. oryzae* the cause of rice seed borne pathogen. Nguefack *et al*. (2013) reported that essential oil from *C. citratus* at 425 µg/ml totally inhibited the mycelia growth of *H. oryzae* and *A. alternata* the cause of rice seed borne pathogen. The above results lend support to the present findings.

Table 1. Efficacy of essential oils on the radial growth of *Curvularia lunata* (Poisoned food technique)

	Radial growth of pathogen (mm)					
Essential oils	0.06%	0.07%	0.08%	0.09%	0.1%	0.2%
Cymbopogan citratus	14.3^{a}	10.6^{b}	5.3^{b}	1.0^{a}	0.0^{a}	0.0^{a}
Cymbopogan martinii	16.0^{b}	9.3^{a}	4.3^{a}	1.3^{a}	0.0^{a}	0.0^{a}
Cymbopogan nardus	46.3^{e}	38.6^{e}	29.0^{e}	21.6^{d}	17.6^{d}	11.0^{d}
Eucalyptus globulus	39.6^{d}	28.6^{d}	22.6^{d}	17.0^{c}	12.3^{c}	9.6^{c}
Ocimum sanctum	32.6^{c}	26.0^{c}	20.0^{c}	14.3^{b}	8.6^{b}	2.3^{b}
Pelargonium graveolens	14.0^{a}	9.6^{a}	5.0^{b}	1.2^{a}	0.0^{a}	0.0^{a}
Control	88.0^{f}	89.0^{f}	88.5^{f}	88.3^{e}	88.6^{e}	89.0^{e}

*Values are mean of three replications

*In a column means followed by a common letter are not significantly different at 5 per cent (P=0.05) level by DMRT.

Table 2. Efficacy of essential oils on the radial growth of *Fusarium moniliforme* (Poisoned food technique)

	Radial growth of pathogen (mm)					
Essential oils	0.06%	0.07%	0.08%	0.09%	0.1%	0.2%
Cymbopogan citratus	14.6[b]	8.0[a]	4.3[a]	1.6[a]	0.0[a]	0.0[a]
Cymbopogan martinii	14.0[b]	8.3[a]	5.0[b]	2.0[b]	0.0[a]	0.0[a]
Cymbopogan nardus	36.0[c]	27.0[c]	21.6[c]	17.3[c]	12.0[b]	8.6[b]
Eucalyptus globulus	42.0[e]	36.0[e]	28.0[d]	21.6[d]	17.3[d]	12.0[c]
Ocimum sanctum	40.0[d]	33.3[d]	27.6[d]	22.0[d]	16.0[c]	12.3[c]
Pelargonium graveolens	13.3[a]	8.6[ab]	5.3[b]	2.0[b]	0.0[a]	0.0[a]
Control	88.5[f]	88.6[f]	89.0[e]	88.6[e]	88.0[e]	90.0[d]

*Values are mean of three replications
*In a column means followed by a common letter are not significantly different at 5 per cent (P=0.05) level by DMRT.

Table 3. Efficacy of essential oils on the radial growth of *Helminthosporium oryzae* (Poisoned food technique)

	Radial growth of pathogen (mm)					
Essential oils	0.06%	0.07%	0.08%	0.09%	0.1%	0.2%
Cymbopogan citratus	15.3[a]	11.3[a]	7.0[b]	1.6[a]	0.0[a]	0.0[a]
Cymbopogan martinii	17.6[b]	12.0[b]	6.3[a]	2.0[b]	0.0[a]	0.0[a]
Cymbopogan nardus	65.0[d]	57.0[d]	50.0[d]	39.0[d]	27.0[c]	21.0[c]
Eucalyptus globulus	90.0[e]	72.0[e]	63.0[e]	52.0[e]	48.0[d]	32.0[d]
Ocimum sanctum	47.0[c]	36.6[c]	27.3[c]	19.0[c]	11.3[b]	7.6[b]
Pelargonium graveolens	15.6[a]	12.0[b]	6.0[a]	2.3[b]	0.0[a]	0.0[a]
Control	88.6[f]	88.3[f]	88.9[f]	89.0[f]	88.6[e]	89.0[e]

*Values are mean of three replications
*In a column means followed by a common letter are not significantly different at 5 per cent (P=0.05) level by DMRT

Sarocladium oryzae

Of the six essential oils were tested for their effectiveness against the mycelial growth of *S. oryzae*, *C. Citratus, C. martinii* and *P. graveolens* oils caused complete inhibition on the mycelial growth of pathogen at a concentration of 0.1 per cent (Table 4) . The other oils *viz*., *C. nardus, E. globules* and *O. sanctum* were not effective against the test pathogens at the same concentration.

Similarly, Sarala *et al.* (2002) reported that palmarosa oil (1:2) 80 EC (0.1%) were effective which recorded the mycelial growth inhibition of 100 and 87.34% in S. *oryzae*. Several workers have reported the antifungal activity of essential oils against *S. oryzae* (Rajappan *et al.* 2001; Mahalakshmi and Yesu Raja 2013). The above results support to the present findings.

In modern Agriculture, diseases are managed by using fungicides. The ecological concern over the excessive

Table 4. Efficacy of essential oils on the radial growth of *Sarocladium oryzae* (Poisoned food technique)

	Radial growth of pathogen (mm)					
Essential oils	0.06%	0.07%	0.08%	0.09%	0.1%	0.2%
Cymbopogan citratus	12.0[a]	8.3[a]	5.0[a]	2.3[a]	0.0[a]	0.0[a]
Cymbopogan martinii	12.3[a]	9.6[b]	5.3[a]	2.6[a]	0.0[a]	0.0[a]
Cymbopogan nardus	30.3[e]	25.3[e]	21.6[d]	17.0[d]	12.3[d]	8.0[d]
Eucalyptus globulus	26.6[d]	22.3[d]	17.6[c]	11.0[b]	9.3[c]	6.6[c]
Ocimum sanctum	23.0[c]	21.3[c]	17.6[c]	12.3[c]	7.6[b]	5.3[b]
Pelargonium graveolens	13.6[b]	10.0[b]	5.6[ab]	2.6[a]	0.0[a]	0.0[a]
Control	88.3[f]	88.6[f]	89.0[e]	88.0[e]	88.6[e]	89.2[e]

Values are mean of three replications
In a column means followed by a common letter are not significantly different at 5 per cent (P=0.05) level by DMRT

use of these fungicides and their high cost have motivated the farmers to select methods that are eco-friendly and also relatively cheap. The last two decades have witnessed an increasing interest in investigations on plants as a source of disease control materials and as possible alternatives to synthetic fungicides. In the present investigation, essential oils from *C. citratus*, *C. martinii* and *P. graveolens* strongly inhibited the mycelial growth of *C. lunata*, *F. moniliforme*, *H. oryzae* and *S. oryzae* under *in vitro* conditions.

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