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Eco-friendly management of *Pyricularia oryzae* - The causal agent of blast of paddy

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Abstract

Integrated management of *Pyricularia oryzae* by eco-friendly approaches is promising. In this study we isolated and tested the essential oils and oleoresin from *Piper nigrum*, *Coriander sativum and Curcuma domestica* for their effect on *Pyricularia oryzae* in rice. These were evaluated for their antifungal activity against *P. oryzae in vitro* by poison food technique. Seed health testing was performed using the standard blotter method. We isolated *P. oryzae* and cultured on potato dextrose agar (PDA) medium to maintain pure culture for our future experiments. The pure culture of *P. oryzae* was tested against different concentration of essential oils and oleoresin on PDA medium. Incubated for several days and plates were checked for mycelial growth inhibition and percentages of inhibitions were calculated. Maximum inhibition of mycelial growth was observed in pepper oil which showed 5.9%, 5.5%, 4.4% and 7.0% of inhibition at 100ppm, 200ppm, 300ppm and control, respectively. Mycelial growth was completely inhibited at 500ppm and 1000ppm concentration. This indicated that pepper oil is most effective against *P. oryzae* pathogen. Oleoresin and coriander oil is less effective. The results of the current study indicated that each of the three essential oils tested, reduced the natural infection frequency in *P. oryzae.*

Keywords: *Pyricularia oryzae, Piper nigrum*, *Coriander sativum, Curcuma domestica*, Disease management

INTRODUCTION

Rice is an important crop contributing approximately 23% of the per capita energy for six billion people worldwide. It is not just a grain; it is the lifeline and the second most important crop next to wheat at global level [1]. It has been under cultivation from time immemorial, being grown under varying climatic conditions. It is widely affected by many diseases caused by fungi, bacteria, viruses and mycoplasma that results in significant yield losses [2]. The blast disease alone is estimated to cause more than US$55 million production losses at each year in South and Southeast Asia. The losses are even higher in East Asia and other more temperate rice growing regions around the world.

Magnaporthe grisea (anamorph: *Pyricularia oryzae*), a filamentous ascomycetes fungus, parasitizes many grasses, including economically important crops like wheat, rice, barley and millet [3]. But the pathogen is best known as the casual agent of the rice blast disease because of its wide distribution and its destructiveness under favorable environments [4]. The disease occurs in almost all rice growing areas of the world and is the most serious in temperate and tropical area of non irrigated environments. In temperate regions, the blast problem is perpetuated by the high pathogenic variability of the fungus while in the upland environments blast exacerbated with drought.

Among the fungal diseases, blast is causing yield loss of up to 90 per cent [5] despite decades of research towards its management. Therefore, controlling this disease is one of the main goals of rice breeding and improvement programs. The management of blast disease was done by integrated approach like using fungicides, growing resistant varieties, application of organic amendments, balanced nutrition, biological agents and resistance inducing chemicals. The excess use of chemicals resulted in environmental pollution and ill health to biotic community as a whole. Therefore, the biological method of plant disease management seems to be a better alternative to chemical fungicides in managing the blast disease. In addition, the biological control of plant pathogens is an attractive preposition as it mimics the nature's own way of balancing the population of living organisms [6].

P. oryzae can cause damage to any aerial part of a rice plant, although the leaves and panicles (necks) are the most commonly affected. Leaf infection reduces the photosynthetic area of the plant, and panicle infection reduces the yield [7]. Dharam *et al*. (1971) [8], Bateman *et al*. (1986) [9] and De Waard *et al*. (1993) [10] have reported the use of fungicides in controlling diseases of rice. Nevertheless, agrochemicals are not widely used by small-scale farmers due to the non availability and high cost [11, 12]. Furthermore, obvious pollution of the environment and the toxic effects of synthetic chemicals on non-target organisms including humans have prompted investigations on pesticides of plant origin [13].

The practical use of natural compounds as control agents is receiving increased attention and this is partly due to their non-toxicity and biodegradability [14]. Volatile compounds from plants, especially essential oils have been demonstrated to possess potent antifungal, antibacterial, insecticidal and nematocidal activity [15, 16, 17, 18 and 19]. Nevertheless, the use of essential oils as seed treatments for controlling seed-borne infection is promising [20, 21]. Therefore, the main objective of the present study was to assess the

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effect of essential oils and oleoresin on *P. oryzae* in infected rice seeds. Further, we studied the impact of essential oils and oleoresin which inhibits the mycelial effect of *P. oryzae*.

MATERIALS AND METHODS

Rice seed samples

Seed samples of different rice cultivars growing in the Karnataka state, India were used. Seeds were collected mainly from Mandya, Mysore, Raichur, Tumukur, Pandavapura, Nanjanagud and T. Narasipura in southern Karnataka, India. The collected seeds were washed with running tap water, thus washed seeds are surface sterilized with Sodium hypochlorite and they were repeatedly rinsed with sterile distilled water. Later sterile seeds were used for further experiments.

Essential oils

The two types of essential oils from fresh fruit of *Piper nigrum*, *Coriander sativum* and one oleoresin freshly harvested rhizome of a plant *Curcuma domestica* were used for our study (Table1). We followed the extraction method suggested by Nguefack et al (2007) [15]. They extracted by Clevenger's apparatus as recommended by Amvam Zollo *et al* (1998) [17]. The essential oils recovered from each extraction was dried over anhydrous sodium sulphate and stored. The yield of the essential oils was 0.48%, 0.52% and 0.46% weight of the fresh plant material of *P. nigrum*, *C. sativum and C. domestica,* respectively. These plant species were selected on the basis of the previous knowledge on their antifungal activities [2].The plants and its products were used as food flavors and medicinal purposes.

Seed health testing

Seed health testing was performed using the standard blotter method described by the International rules for seed testing (International Seed Testing Association (ISTA), 1985) [22]. Four hundred seeds were tested from each cultivar and for each treatment eight replicates of 50 seeds. Seed-borne fungi were identified on incubated seeds. *P. oryzae* was isolated and cultured on potato dextrose agar (PDA) medium, kept for incubation and observed for the growth characters. The subcultures were repeatedly carried out and pure culture was maintained.

Effect of essential oil and oleoresin on P. oryzae

Two Essential oils from *P. nigrum* (Pepper), *C. sativum* (Coriander) and one oleoresin namely *C. domestica* (Turmeric) (Table 1) were considered for studying their effect on *P. oryzae.* Essential oils and oleoresins were dissolved separately in acetone (100mg oil in 1ml of acetone). The PDA containing 100, 200, 300, 500 and 1000 ppm concentration of each sample were prepared. The PDA medium without oil served as control. Each plate of different concentration of essential oil and oleoresin was inoculated with seven day old culture of *P. oryzae*. Each mycelial disc was inoculated in the center and incubated for seven days. The plates were observed for the mycelia growth. After seven days of incubation the percentage of mycelial growth inhibition was calculated with respect to the control with empherical formula $P=(C-T) \times 100/C$ Where P = percentage of mycelial growth, C=colony diameter, T=treated. Three replicates of the petri-plates were maintained and the experiment was repeated thrice.

RESULTS

The incidence of *Alternaria, Pyricularia oryzae, Cladospoorium, Curvularia, A. nidulans, Aspergillus niger, Fusarium moniliforme, Helminthosporium* and others were recorded according to the standard blotter method described by ISTA and separated from our study on *P. oryzae*. The pathogen *P. oryzae* obtained from blotter method was taken and cultured on PDA medium. After incubation growth characters of the *P. oryzae* was observed. It showed very good mycelial growth on PDA media.

The *in vitro* efficacy of two essential oils of *P. nigrum*, *C. sativum* and one oleoresin from *C. domestica* on *P. oryzae* showed varied level of mycelial inhibition when we compared with control when we had done by poison food technique (Table 2). Among them maximum inhibition of mycelial growth was observed for treatment with pepper oil which showed 5.9%, 5.5%, 4.4% and 7.0% of inhibition at 100ppm, 200ppm, 300ppm and control, respectively. Mycelial growth was completely inhibited at 500ppm and 1000ppm concentration. This indicated that pepper oil is effective against *P. oryzae* pathogen. Whereas with coriander oil and oleoresin showed complete inhibition of the mycelial growth at 1000ppm. For coriander oil, maximum mycelial growth inhibition was observed upto 5.5%(100ppm), 4.5%(200ppm), 2.6%(300ppm), 1.2%(400ppm) and 8.5%(control). With oleoresin 6.7%(100ppm), 6.4%(200ppm), 4.5%(300ppm), 3.1%(400ppm) and 8.5%(control) of mycelia growth inhibition was observed.

Table1. Essential oil/oleoresin tested against *Pyricularia oryzae*

Sl. No.	Essential oil	Plant source	Family	Plant part
1	Pepper oil	*Piper nigrum*	Piperaceae	Fruit
2	Coriander oil	*Coriander sativum*	Apiaceae	Fruit
3	Turmeric oleoresin	*Curcuma domestica*	Zingiberaceae	Rhizome

Table 2. Effect of essential oils/ oleoresins on *Pyricularia oryzae* (seven days cultures isolated from paddy)

Sl. No	Essential oil/ Oleoresin	Control	Concentration in PPM				
			100	200	300	500	1000
1	Pepper oil	7.00±0.01	5.99±0.10	5.50±0.00	4.44±0.10	-	-
2	Coriander oil	8.50±0.00	5.55±0.04	4.54±0.10	2.68±0.13	1.23±3.2	-
3	Turmeric oil	8.50±0.00	6.70±0.00	6.45±1.2	4.55±0.09	3.12±0.07	-

DISCUSSION

Effective management of plant diseases is essential in rice which is economically important food crop. Fungal diseases are primarily controlled by the application of fungicides [23]. However, developing resistance against chemicals is dangerous and not an eco-friendly. Synthetic fungicides are reported to have carcinogenic,

teratogenic, oncogenic and genotoxic properties. These fungicides are bio-hazardous and adversely affect the components of ecosystem. Further the cost of these fungicides is relatively high. Presently blast disease is controlled by the application of some systemic fungicides such as Hinosan, Kitazin, Bavistin, Contaf-5EC, till-25 and Beam-75 [24]. The non systemic fungicides were also used to control the disease like Kavach-75, Blitox-45, Indofil-45, Rovaral-50 and also certain Neem based formulations like Neem gold, Nimbicidin and wanis were also reported to be used to control *P. oryzae* [25].The constant and excess use of fungicides results in development of resistance in the pathogens [26], thus creates hazardous effects on flora and fauna. To overcome these problems researchers have put forth the introduction of certain derivatives which are supposed to have an antibacterial and antifungal properties [27], biodegradable, stimulatory in function and environmental friendly.

The use of essential oils has taken a new dimension in controlling certain viruses and nematodes [28]. These essential oils are known to posses certain antifungal and antibacterial properties [29]. These essential oils have got high rate of penetration action which especially inhibits seed borne pathogens. Antifungal activity of eucalyptus and clove oil was tested against certain dermatophytic fungi [30]. Pundir and Jain (1994) [31] reported the antifungal properties of essential oils of the turmeric, pepper and coriander against *Staphylococcus aureus*. Essential oils represent very complex mixture of aromatic compounds mainly monoterpenes and sesquiterpenes some related species of the plants consists of predominant oils in the form of resins, where oleoresin is the complex mixture of essential oils and resin. On the other hand these essential oils are nontoxic, non-polluting and biodegradable.

In the present investigation paddy seeds of different cultivars from different paddy growing areas were included and studied the antifungal effect of the essential oil on the growth of *P. oryzae* by poisoned food technique. The effect of these essential oils presented in Table 2 indicates the effectiveness of these essential oils on control of mycelial growth of *P. oryzae* pathogen at different concentrations. From our current investigation we stated that when the concentration of the essential oil and oleoresin increases and the mycelial growth of the fungus decreases. Essential oil of coriander was found to be less effective in inhibiting the mycelial growth at lower concentrations than pepper oil. At 500ppm concentration, pepper oil completely inhibited the mycelial growth of *P. oryzae.* The turmeric oleoresin showed the complete inhibition at 1000ppm. These results show the antimicrobial activity of different essential oils which control the pathogen *P. oryzae*. These essential oils can open up new ways and act as supplement to reduce the application of pesticide and systemic fungicides but further detail study is required to know the activity and mechanism for the suppression of pathogen particularly at the field level if we want to use other than seed treatment. Present study reveals that *P. nigrum, C. sativum* and *C. domestica* has a potential and promising plants that could be successfully exploited for the bio control management of *P. oryzae* and possibly other seed borne fungi of paddy.

The *in vitro* efficacy of essential oils particularly with *P. nigrum, C. sativum* and *C. domestica* against phytopathogenic fungi such as *P. oryzae* causal agent of blast of paddy was studied for the first time. The results of the current study indicated that each of the three essential oils tested, reduced the natural infection frequency in *P. oryzae*. This pathogen was well known important seed borne fungus of rice, found in seed samples of the different cultivars. The results showed that the antifungal activity of essential oils can be use as an alternative source to the systemic fungicides. However, further detailed study is required to know the activity and mechanism of the suppression of growth of the pathogen. The results of the present research work will be useful for devising effective eco-friendly strategies to manage the blast disease of paddy. The knowledge gained during the present investigations will serve as a foundation for further research work on the biology of the pathogen and epidemiology and management of the disease.

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