Efficacy of plant oils against grain discolouration of rice under *in vitro* condition

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Abstract

Grain discolouration is an important constraint in reducing productivity of rice. Curvularia lunata and Exserohilum rostratum are two important pathogens responsible for it. An in vitro study was undertaken to test the efficacy of different plant oils against these pathogens. It revealed from the experiment using poison food technique that all the plant oils recorded a significant inhibition over control. Among the different concentration (1, 2 and 3%) of plant oils such as Eucalyptus, Neem Pungam, Coconut and Mahua oil were tested. The result revealed that eucalyptus oil (3%) recorded the complete mycelial inhibition of C. lunata (100 %) and E. rostratum (78%) followed by pungam oil (58.8 % of C. lunata), (52% of E. rostratum) and least mycelial growth were recorded by coconut oil (3%). Our findings suggest that the plant oils may be valuable as potential antifungal properties against grain discolouration of rice.

Keywords: Grain discolouration, Plant oils, *Curvularia lunata, Exserohilum rostratum*

Introduction

Rice (*Oryza sativa* L.) is an important staple food crop belongs to poaceae family which grown on large area every year. It provides food security to more than two-third populations and a means of livelihood to millions of rural households (Khamari, 2020). In world's population, estimated that will exceed 8 billion people by 2025 and need to increased the rice production by 40% more to meet the increasing food demands, by the year 2030 (Yadav *et al*., 2019; Jena *et al*., 2018). Grain discoloration has been an emerging disease which reduces yield and potent threat to rice crops (Schaad, 2008). It is otherwise known as "glume discolouration" or "dirty panicle". Baite *et al*., (2019) reported the incidence of grain discoloration ranged from 25 to 92% in different rice varieties. Many fungal microorganisms *viz*., *Alternaria* spp., *Curvularia* spp., *Fusarium* spp. *Drechslera oryzae, Pyricularia oryzae, Sarocladium oryzae*, *Sclerotium* spp., *Aspergillus* spp., *Penicillium* spp., *Phoma* spp., *Cercospora* spp. has been reported as causal agents of grain discolouration (Islam and Ahmed, (2017). Out of which *Curvularia lunata*, *Helminthosporium oryzae* are commonly found. Currently, *Curvularia* has been classified into family Pleosporaceae under Dothidiomycetes fungi (Ariyawansa *et al*. (2015)). The nomenclature of *Bipolaris* was taken priority over *Cochliobolus* because of the importance as economically phytopathogens (Rossman *et al*. 2013). So far, chemical and biological methods have been used to alleviate and control dirty panicle diseases of rice. Use of synthetic chemical fungicides are ample to control of the pathogen, but besides it existence to toxic the soil microflora, and hazardous to human and animal health. The recent efforts have focused on developing environmentally safe, long lasting and

effective essential oils for the control of plant diseases. Use of plant oils for the control of plant disease have been reported to be effective antimicrobial agents against several seed-borne, soil-borne and foliar pathogens (Muthukumar and Sanjeevkumar 2012). The main objective are the present study which are isolation and identification of fungi from discoloured collected samples and screening the antifungal activity of the five plant oils against the major seed borne fungi of rice.

Materials and Methods

Collection and isolation of pathogen

Samples were collected from fifteen districts of Tamilnadu to estimate the disease index. Random samples were collected from the fields during summer and rainy season. The samples were brought to the laboratory at Agricultural college and Research Institute, Madurai (9º58'20.0"N 78º12'11.1"E) and used for the analysis. The grains were separated as healthy (no discoloration) and discolored grains based on visual observation. Varieties such as ADT45, ADT43, CO51, ASD16, IR50 and TKM13 growing in different ecologies were selected for the study. All the samples (500 g each) were collected in a polyethylenebags and stored at room temperature until further use for mycological analysis. The disease incidence in the field was calculated by the following formula.

$$\text{PDI} = \frac{\text{sum of individual diseased grain rating} \times 100}{\text{Number of grains assesed} \times \text{Maximum disease grade}}$$

Isolation and identification of pathogen associated with rice seeds

The pathogen was isolated from an infected rice seeds by agar plate method. The infected seeds were surface sterilized with 1% sodium hopochlorite for 30 S followed by sterile water. After removing the moisture from seeds using a sterile filter paper, the seeds were placed onto potato dextrose agar (PDA) plates amended with streptomycin sulfate. After 5 days of incubation at 26 °C, the plates were observed for mycelial growth. The microscopic slides were prepared from mycelium emerged from the seeds plated into PDA. The spores observed using microscope were carefully separated and plated onto PDA. The pure culture obtained using single spore isolation technique was mass multiplied and stored at −4 °C for use in the current study.

Collection of plant oils

The locally available five plant oils viz., neem, pungam, castor, coconut and eucalyptus were purchased from local market from Madurai, Tamil Nadu, India. These essential oils were selected on the basis of the local availability and previous knowledge on their antifungal activities of essential oils against plant pathogens (Nguefack et al. 2008).

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 ($P< 0.05$) (Gomez and Gomez 1984).

Result

Pathogen associated with infected grains

A total of two fungal genera were found predominantly associated with the infected seeds. The associated fungi were *C. lunata* and *E. rostratum*. Among them, the most predominant one was *E. rostratum* which was associated with 39.9 per cent incidence of infected seed samples followed by *C. lunata* (37.1 %). Among these, *C. lunata* was the most frequently isolated seed-borne fungus irrespective of the source of the different rice varieties tested in the present study. A similar observation was made by Akila and Ebenezar (2009) reported that the pathogens *D. oryzae*, *F. moniliforme* and *C. lunata* when artificially inoculated individually on rice panicles were able to cause 77.39, 67.44 and 74.03 per cent infection respectively while the mixed inoculum of these three pathogens caused the highest infection (81.80%). Archana and Prakash (2013) stated that they collected 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 grain discolouration of rice

Curvularia lunata

The inhibitory effects of five essential oils were tested against *C. lunata* following poisoned food technique. All the five plant oils viz., eucalyptus, neem, pungam, coconut, mahua oils at 1 to 3 per cent concentrations were tested against *C. lunata*. Among them Eucalyptus oil (3%) recorded the total inhibition of mycelial growth inhibition of pathogen followed by pungam and neem oil which inhibited the mycelial growth upto 58 and 56 percent over control. The least growth observed in mahua oil at 44 % (fig.1). Similiarly, Naveen kumar *et al*. (2017) tested Three plant oils (*Cymbopogon citratus, Cymbopogon martini, and Pelargonium graveolens*) among that of *C. citratus* oil 30EC recorded 100% inhibition on the mycelial growth of test pathogens such as *C. lunata, F. moniliforme, B. oryzae and S. oryzae*.

Exserohilum rostratum

The experimental results revealed that eucalyptus oil (3%) recorded the 77 percent inhibition of mycelial growth of E. rostratum. This treatment was followed by pungam oil (3%), which inhibited the mycelial growth upto 33.1 per cent reduction over control. The minimum growth inhibition percentage was observed in mahua 22 % (Table 2, Fig. 1). 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.

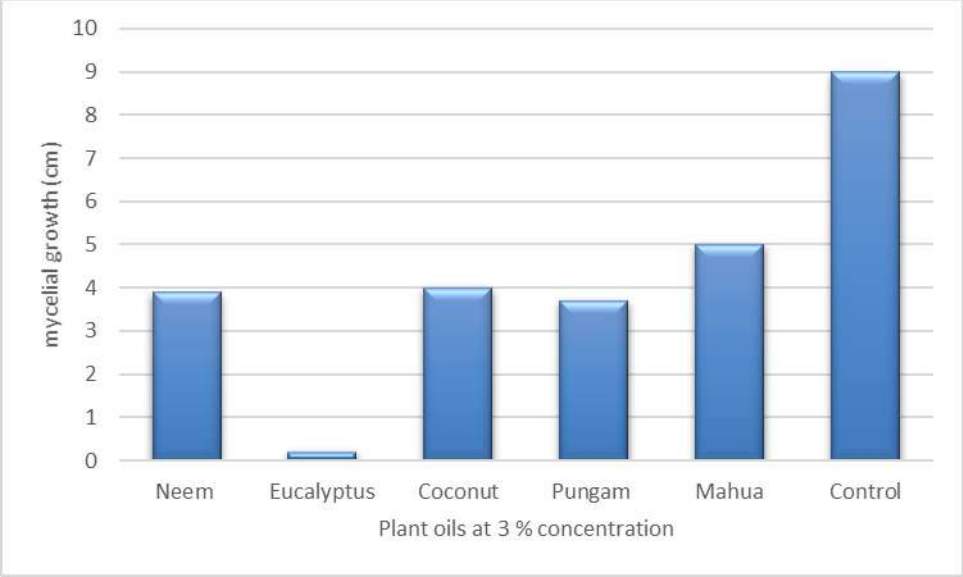
Tab.1. Efficacy of plant oil (3%) against the mycelial growth of *Curvularia lunata* in *in vitro*

S. No	Plant oil	Concentration (3%)	
		Mycelial growth (cm)*	Per cent reduction over control (%)
1.	Neem	3.9	56.6[c]
2.	Eucalyptus	0.0	100[a]
3.	Coconut	4.0	55.5[d]
4.	Pungam	3.7	58.8[b]
5.	Mahua	5.0	44.4[e]
6.	control	9.0	-

*Mean of three replications

*In a column, means followed by a common letter are not significantly different at 5% level by Duncan's multiple range test (DMRT)

Fig.1. Efficacy of plant oil (3%) against the mycelial growth of *Curvularia lunata* in *in vitro*



Conclusion

Use of these synthetic fungicides are hazardous to human health and high cost. So, the farmers are motivated to select ecofriendly and also relatively cheap. Present study was concluded that use of plant oils such as Eucalyptus, neem, pungam, coconut and mahua are easily available in local market. In the present investigation, essential oils eucalyptus, pungam and neem oils are strongly inhibited the mycelial

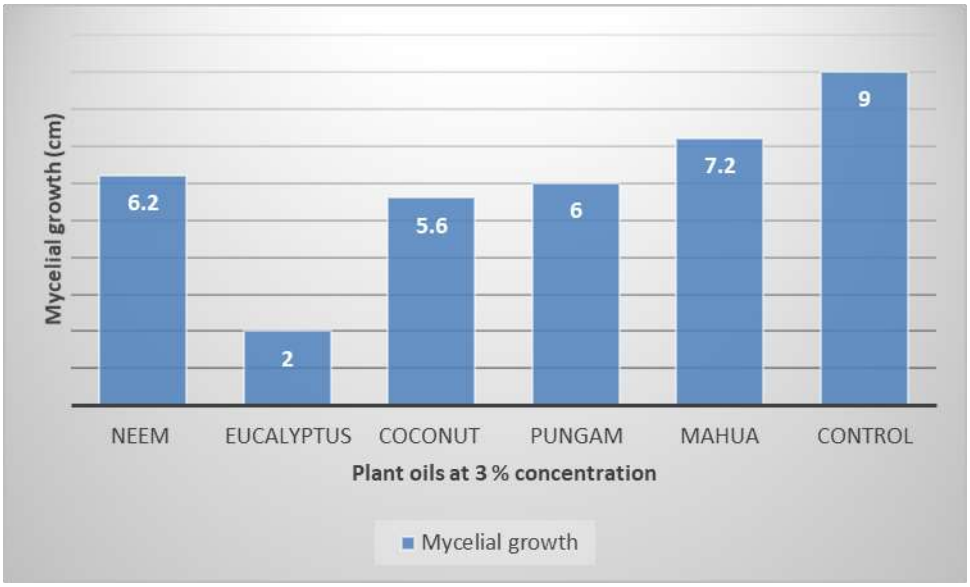
Tab.2. Efficacy of plant oil against the mycelial growth of *Exserohilum rostratum* in *in vitro*

S. No	Plant oil	Concentration (3%)	
		Mycelial growth (cm)*	Per cent reduction over control (%)
1.	Neem	6.2	31.1[d]
2.	Eucalyptus	2.0	77.7[a]
3.	Coconut	5.6	37.7[b]
4.	Pungam	6.0	33.3[c]
5.	Mahua	7.2	20.0[e]
6.	control	9.0	-

*Mean of three replications

*In a column, means followed by a common letter are not significantly different at 5% level by Duncan's multiple range test (DMRT)

Fig.2. Efficacy of plant oil against the mycelial growth of *Exserohilum rostratum* in *in vitro*



growth of *C. lunata* and *E. rostratum* which causes the grain discolouration of rice under *in vitro* condition.

References

R. Akila and E. G. Ebenezar, "Ecofriendly approaches for the management of grain discolouration in rice", Journal of Biological Control., vol. 23, no. 2, (2009), pp. 175-180.

B. Archana and H. S. Prakash, "Survey of seed-borne fungi associated with rice seeds in India", Internat. J. Res. Pure and Appl. Microbiol., vol. 3, no. 1, (2013), pp. 25-29.

H. A. Ariyawansa, K. M. Thambugala, D. S. Manamgoda, R. Jayawardena, E. Camporesi, S. Boonmee, " Towards a natural classification and backbone tree for Pleosporaceae", Fungal Divers., vol. 71, (2015), pp. 85–139.

M. S. Baite, S. Raghu, S. R. Prabhukarthikeyan, U. Keerthana, N. N. Jambulkar and P.C. Rath, "Disease incidence and yield loss in rice due to grain discolouration", J. Plant Dis. Prot., vol. 127, no.1, pp. 9-13.

W. Islam and M. Ahmed, "Identifcation of different fungi associated with grain discolouration complex disease of rice and management of infested grains through fungicides", Int J Sci Res Agric Sci., vol. 4, no.2, (2017), pp. 30–35.

M. Jena, G. Guru Pirasanna Pandi, T. Adak, P. C. Rath, G. Basana Gowda, "Paradigm shift of insect pests in rice ecosystem and their management strategy", Oryza., vol.55, (2018), pp. 82-89.

B. Khamari, "Grain Discolouration: An Emerging Threat to Rice Crop", Research Biotica., vol 2, no.2 (2020), pp. 080-087.

A. Muthukumar, and K. Sanjeekumar, "Chemical composition and antifungal activity of essential oils against die-back of hippeastrum", Indian Journal of Horticulture., vol. 69, no. 3, (2012), pp. 385-389.

R. Naveenkumar, A. Muthukumar, G. Sangeetha and R. Mohanapriya, "Developing eco-friendly biofungicide for the management of major seed borne diseases of rice and assessing their physical stability and storage life", Comptes rendus biologies, vol. 340, no. 4, (2017), pp. 214-225.

J. Nguefack, V. Leth, J.B. Lekagne, J. Dongmo, P. H. Torp, S. Amvam Zollo and Nyasse, "Use of three essential oils as seed treatments against seed borne fungi of rice (Orzae sativa L.)", American-Eurasian J. Agric. Environ. Sci., vol. 4, (2007), pp. 5-554.

G. J. Nguefack, G. E. Wulff, J.B.L. Dongmo, F. R. Fouelefack, F. Daniel, M. B.O. Joseph and Jan Torp, "Effect of plant extracts and an essential oil on the control brown spot disease, tillering, number of panicles and yield increase in rice", Eur.J. Pl. Pathol, vol. 137., (2013), pp. 871-882.

A. Y.Rossman, D.S. Manamgoda and K.D. Hyde, "Proposal to conserve the name Bipolaris against Cochliobolus (Ascomycota: Pleosporales: Pleosporaceae)", Taxon, vol. 62, (2013), pp. 1331–1332.

N. W. Schaad, "Emerging plant pathogenic bacteria and global warming. In: Pseudomonas Syringae Pathovars and Related Pathogens—Identification, Epidemiology and Genomics, (Eds.) Fatmi, M., Collmer, A., Iacobellis, N.S., Mansfield, J.W., Murillo, J., Schaad, N.W. and Ullrich, M., New York, USA, (2008) pp. 369-379.

M.K. Yadav, S. Aravindan, U. Ngangkham, S. Raghu and S. R. Prabhukarthikeyan, "Blast resistance in Indian rice landraces: Genetic dissection by gene specific markers", Plos one, vol. 14, no. 1, (2019) pp. 0211061.

K. A. Gomez and A.A. Gomez, Editor, "Statistical Procedure for Agricultural Research", John Wiley and Sons, Publishers, NewYork, USA, (1984).