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Identification of potentiality of essential oils of *Piper nigrum* L.(Berry) and *Santalum album* L.(Wood) for antifungal activity against seed borne fungi of paddy

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Abstract

In vitro evaluation of essential oils of *P. nigrum* (Berry) and *S. album*(Wood) against ten seed borne fungi were tested at 500 to 3500ppm concentration. In *P. nigrum* oil, at 2000ppm concentration, complete inhibition was observed in *Pyricularia oryzae , Tricoconis padwickii* and *Drechslera tetramera.* At 2500 ppm concentration, *Bipolaris oryzae, Drechslera halodes, Fusarium moniliforme* were recorded 100% inhibition. At 3000ppm concentration, *Alternaria alternate, Curvularia lunata, F. oxysprorum* and *F. solani* recorded complete inhibition. In *S.album*, at 2500-3500 all the test pathogens were completely inhibited and recorded 100% inhibition.

Keywords: *P. nigrum, S. album,* Paddy seeds, antifungal activity, essential oils.

Contents

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1. Introduction

Medicinal plants have the ability to inhibit the growth of wide range of pathogenic microorganisms due to presence of essential oils. The antimicrobial impact of essential oils and its various components extracted from medicinal plants has been well documented (Hammer et al., 2002) . The majority of essential oils are composed of terpenes

and terpenoids and other aromatic and aliphatic constituents, all characterized by low molecular weight. Terpenes are the major group of plant natural products haracterized by an extensive variety of structural types and the most valuable compounds (Degenhardt et al., 2009). Plant diseases is an om -going limiting factor in crop production.Diseases of crops lead to yield losses and are of increasing importance as world population increases .Plant diseases are of paramount significance to humans because they damage plants and plant products on which humans depend for food, clothing and fumiture . Fungi are the most important common cause of plant disease (Aisha, 2014.). Many synthetic chemicals are widely used for the management of seed borne fungi which are both efficient and effective. Thiram and bavistin are some of the most commonly used synthetic chemicals for the seed treatment of maize. Many of these synthetic fungicides are known for their non-biodegradable nature and residual toxicity (Pak, 2003). Pesticide pollution of soil and water bodies is well documented in the literature (Nostro et al. 2000). This has driven plant pathologists to search for alternative eco-friendly methods for the management of plant diseases. Traditionally, plants have been exploited by man for the treatment of human diseases (Kitula, 2007).

A special feature of higher plants is their capacity to produce a large number of organic chemicals of high structural diversity generally referred to as secondary metabolites. These secondary metabolites are broadly divided into three different categories based on their mechanism of action i.e., Chemotherapeutic, bacteriostatic/ bactericidal and antimicrobial (Singh et al., 1993). It has been reported that about 60% of the essential oils posses antifungal and about 35% antibacterial properties (Gangrade et al., 1991). A variety of synthetic chemicals are currently being used as pesticide to protect agricultural crops from various pests and insects. If we consider Indian agricultural scenario, there is an estimated loss of Rs.5000 crore every year due to pests and insects. The biological means of pest and insect control are receiving much attention these days throughout the world. The isolation of natural compounds and preparation of extracts to use effectively is a common practice. Considering all this negative effect of synthetic pesticides, in the present investigation, essential oils from *Piper nigrum* L.(Berry) and *Santalum album* L.(Wood) were tested for antifungal activity against ten seed borne fungi of paddy.

2. Materials and Method

Test Fungi

Important and frequently occurring seed borne viz., *Pyricularia oryzae, Bipolaris oryzae, Alternaria alternata, Tricoconis padwickii, Drechslera tetramera, Drechslera halodes, Curvularia lunata, Fusarium moniliforme, F. oxysprorum* and *F. solani* were isolated from the infected seeds of paddy by Standard Blotter Method (ISTA, 2003) and the pure culture maintained on CDA agar slants which served as test fungi.

Test essential oils

Two essential oils viz., *Piper nigrum* L.(Berry) and *Santalum album* L.(Wood) were obtained from Messrs Flavour and Essence Company, Mysore, Karnataka, India. The two oils were diluted (v/v) with absolute alcohol to achieve the desired concentrations of 500, 1000, 1500, 2000, 2500, 3000 and 3500ppm.

Antifungal activity assay

The evaluation of antifungal effects of essential oils on the mycelial growth of test pathogens was studied by poisoned food technique (Dhingra and Sinclair, 1995). The Czapek Dox Agar (CDA) containing 500, 1000, 1500, 2000, 2500, 3000 and 3500ppm concentrations of the each test oils were prepared. The CDA medium without any oil but with same quantity of absolute alcohol served as control. The test oils were added to CDA medium before sterilization. The oil amended medium was poured into sterile petriplates (9 cms diameter). Twenty ml of the medium was poured to each petriplate. The mycelial agar discs (5mm diameter) of the test pathogen were obtained from the margin of the seven-day-old culture of the test fungi and the disc was inoculated to the centre of petriplates. The inoculated petriplates were incubated for seven days at 22 ± 2^{o} C and the colony diameter was measured in millimeters. The percent mycelial growth inhibition (P) if any with respect to the control was computed from the formula. P=C-T X100/C. Where C is the colony diameter of the control and T is the colony diameter of the treated ones. Three replicates were maintained for each treatment and repeated three times (Pinto et al., 1998; Nidiry, 1998; Lisbalchin et al., 1998; Sarala et al., 2002). The MIC of each essential oil was also determined.

3. Results and Discussion

Antifungal activity assay: The antifungal efficacy of *P. nigrum* oil on different test fungi is presented in Table 1. All the test fungi were completely inhibited in *P. nigrum* oil treatment at 3000ppm concentration. However total inhibiton of *P. oryzae, T. padwickii* and *D. tetramera* were observed at 2000ppm concentration itself. Similarly *B. oryzae, D. halodes* and *F. moniliforme* were also completely inhibited at 2500ppm concentration. Highly significant mycelial growth inhibition was observed at 1000ppm concentration against all the test fungi and the percentage of inhibition was more than 70 against all the test fungi except *A. alternata*. Percent inhibition of mycelial growth of the test fungi due to *S. album* oil has determined by poison food technique is presented in Table 2. The antifungal efficacy was less than 30% against many test fungi at 500 ppm concentration. However with increasing concentration the percent inhibition also increased. Total inhibition was observed at 2500ppm

concentration against *P. oryzae, A. alternata , T. padwickii, C. lunata* and *F. moniliforme* while total inhibition of *D. tetramera* and *D. halodes* was observed only at 3000ppm concentration. All the test fungi including *B. oryzae* and *F. oxysporum* were completely inhibited at 3000ppm concentration.

Discussion

The use of many synthetic fungicides has been cautioned due to their pollutive effects, non-biodegradability and residual toxicities. Most of these fungicides have become a popular target of conservationists and are treated to be one of the most vital man-made pollutants (Athukoralage et al., 2001). Search is on for the development of plant disease control agents, which are non-toxic, biodegradable and eco-friendly. During recent years, many essential oils have been found as potent antifungal agents (Dubey et al., 2000; Tripathi et al., 2004). Since such antifungal essential oils have penetration action, these may especially be used to control seed-borne pathogens. The volatility, ephemeral nature and biodegradability of these compounds of angiosperms will be especially advantageous if they are developed as pesticides.

This finding of the present study reveals that this essential oil may constitute an ideal fungi toxicant for the protection of seeds from infestation by fungi. Since these plants are widely cultivated, its essential oil will be an indigenous and renewable source in plant protection in place of synthetic chemicals. During recent years, the antimicrobial principles of higher plants variously referred to as plant antibiotics, pseudo antibody, phytocide and their possible use in plant disease management has been advocated often and again (Dikshit et al., 1992; Mishra et al., 2003). The resurgence of interest in natural therapies and increasing consumer demand for effective, safe, natural products means that quantitative data on plant oils are required (Hammer et al., 1999). Thus the oil constitutes an ideal, indigenous and effective fumigant preservative for protection of paddy in particular and other food commodities in general.

The results of the present investigation suggests that *P. nigrum* and *S. album* have high antifungal degree of potency against important phytopathogens of paddy with very low MIC. The result of the present investigation also suggests that eventhough these oils do not possess broad spectrum of activity at lower MIC, they have shown the broad spectrum of activity at higher MIC of 3000ppm and more. *S. album* have shown broad spectrum of activity beyond 2500ppm concentration against all the test fungi while pepper have shown higher degree of activity at lower MICs. Further the results of the present investigation clearly suggests that essential oil obtained from *P. nigrum* and *S. album* could be evaluated for further investigations to find out the potentiality of these oils in seed treatment experiments. Blast disease caused by *P. oryzae* is a serious disease in this region caused by *P. oryzae* which is seed transmitted. This pathogen has been effectively controlled by Pepper oil at a very low MIC of 500ppm. Thus the results of the present investigation suggest that, this oil could be exploited for seed treatment to control *P. oryzae*. Similarly *B. oryzae* and *A. alternata* are also important phytopathogenic fungi known to be transmitted through seed which cause severe loss in yield and quality of paddy in this region. The mycelial growth of these pathogens have also been completely inhibited by pepper suggesting that these oil could also be exploited for managing these pathogens by seed treatment procedures.

4. Conclusion

In the present investigation, it can be concluded that, the essential oils of *P.nigrum* and *S.album* showed a promising result against ten seed borne pathogen of paddy. A further investigation is necessary to test *P.nigrum* and *S.album* plant parts to identify the potentiality of antimicrobial activity against different crops. Also , isolation and identification of the bioactive compound is essential to check the efficacy of antimicrobial activity.

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