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Original Research

Antifungal Activity of *Parmotrema tinctorum* (Delise ex Nyl.) Hale and *Parmotrema cristiferum* (Taylor) Hale Against Seed Mycoflora - A Comparative Study

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Abstract	Article Information
<p>Lichens are composite organisms comprising of a photobiont and a mycobiont. Studies have shown that extracts and secondary metabolites from lichens exhibit various bioactivities. The present study evaluates antifungal potential of crude methanolic extract of two corticolous <i>Parmotrema</i> species viz. <i>Parmotrema tinctorum</i> (Delise ex Nyl.) Hale and <i>Parmotrema cristiferum</i> (Taylor) Hale against a panel of fungi isolated from seeds of maize and groundnut. Extraction of powdered lichens was carried out by maceration process using methanol. Antifungal activity was evaluated by poisoned food technique. Both extracts were effective in causing dose dependent inhibition of radial growth of test fungi in poisoned plates. Among lichens, marked inhibitory activity was shown by <i>P. cristiferum</i>. At 1mg/ml concentration, <i>P. cristiferum</i> displayed an inhibition of >50% of all test fungi. The antifungal activity of two <i>Parmotrema</i> species against seed mycoflora could be ascribed to the presence of secondary metabolites in extracts.</p> <p>Copyright©2016 STAR Journal, Wollega University. All Rights Reserved.</p>	<p>Article History: Received : 26-01-2016 Revised : 10-03-2016 Accepted : 15-03-2016</p> <p>Keywords: Lichens <i>Parmotrema</i> Antifungal Poisoned food technique Seed mycoflora</p> <p>*Corresponding Author: Vinayaka K.S E-mail: ks.vinayaka@gmail.com</p>

INTRODUCTION

Fungi cause a number of devastating diseases in crops leading to pre- and post-harvest losses. Fungi cause diseases in field as well as storage conditions. Farmers suffer from considerable economic loss due to fungal diseases which may account for >50% in severe disease incidences. Fungi that are associated with the seeds are known to cause seed rot, seed abortion, seedling damage and reduction in nutritive value and germination. Management of phytopathogenic fungi involves the use of synthetic fungicide. However, the use of chemicals is often associated with several drawbacks including resistance development in pathogens. Hence, in recent years much emphasis has been focused on natural products having antifungal activity. Lichens seems to be one of the promising alternatives for management of fungal pathogens (Punja and Utkhede, 2003; Halama and Van Haluvin, 2004; Suberu, 2004; Chang *et al.*, 2008; Al-Reza *et al.*, 2010; Kowalski *et al.*, 2011; Goel *et al.*, 2011; Al-Aksar, 2012; Chandra & Mahesh, 2013; Bahraminejad *et al.*, 2013). Lichens are composite organisms that comprise of a photosynthetic partner (a photobiont) and a fungal partner (a mycobiont). Lichens usually occur in one

of the three growth forms viz. crustose, foliose and fruticose. Worldwide, lichens are used as food, flavoring agent and to treat several diseases or disorders. Lichens are known to produce secondary metabolites (often referred to as lichen substances) that seldom occur in other organisms. Among the metabolites, depsides and depsidones are most common. Studies have shown that extracts and purified metabolites from lichens exhibit diverse biological activities (Karunaratne *et al.*, 2005; Molnár and Farkas, 2010; Goel *et al.*, 2011; Thadhani *et al.*, 2012; Shrestha and St. Clair, 2013; Babiah *et al.*, 2014). *Parmotrema* A. Massal. is one of the largest genus of lichens in the family Parmeliaceae comprising about 350 species. The genus name *Parmotrema* literally means perforate apothecia (Greek parmos - cup; trema - perforation). It belongs to the family Parmeliaceae and the species are characterized by large foliose thalli with broad lobes (Divakar and Upreti, 2005; Jayalal *et al.*, 2013; Michlig *et al.*, 2014; Vivek *et al.*, 2014). In the present study, we evaluated antifungal activity of two *Parmotrema* species viz. *P. tinctorum* and *P. cristiferum* against fungi isolated from seeds of maize and ground nut.

MATERIALS AND METHODS

Collection and Identification of Lichens

The corticolous macrolichens of this study viz. *P. tinctorum* (on *Areca catechu*) and *P. cristiferum* (on *Mangifera indica*) were collected at the outskirts of Shikaripura, Shivamogga district, Karnataka, India during January 2014. The intact thalli of lichens were carefully separated from the bark of host trees and were brought to

the laboratory in labeled pouches. Identification of lichens was carried out on the basis of morphological, anatomical, color tests (K, C, KC and P tests) and secondary metabolites detected by thin layer chromatography (Orange, 2001; Divakar and Upreti, 2005; Awasthi, 2007). Details on results of color tests and the secondary metabolites detected are shown in Table 1.

Table 1: Color test and TLC of lichens

Lichen	Color Test	Secondary Metabolites
<i>P. tinctorum</i>	Cortex K+ yellow; Medulla K-, C +red, KC +red, P -	Atranorin, Lecanoric acid
<i>P. cristiferum</i>	Cortex K+ yellow; Medulla K+ red; C-, P+ orange	Atranorin, Salazinic acid, con-salazinic acid

Extraction

The dried lichen materials were powdered and extracted by maceration process using methanol (HiMedia, Mumbai). A known quantity (10g) of each of the lichen powder was left in methanol (100ml) a stoppered container. The contents were mixed occasionally. After 48 hours, the contents were filtered through 4-fold muslin cloth followed by Whatman filter paper No: 1. The filtrates were dried at room temperatures to obtain crude extract of lichens (Agbor, 2015).

Test Fungi

Isolation of seed-borne fungi was carried out by standard blotter method. A total of 9 fungi viz. *Helminthosporium* sp., *Curvularia* sp., *Alternaria* sp., *Mucor* sp. and *Aspergillus fumigatus* were isolated from maize seeds. Fungi viz. *Aspergillus niger*, *A. flavus*, *Penicillium* sp. and *Rhizopus* sp. were isolated from ground nut seeds. Identification of the fungi was made on the basis of cultural and microscopic characteristics. The fungi were maintained on Potato dextrose agar (PDA; HiMedia, Mumbai) slants under refrigeration.

Antifungal Activity of Extract

The antifungal activity of crude extracts of selected lichens was evaluated by Poisoned food technique. PDA medium, sterilized by autoclaving, poisoned with the lichen extracts (0.5 and 1.0mg/ml of medium) and poured into sterile petri dishes. The test fungi were allowed to grow in control (without extract) and poisoned PDA plates for a period of 5 days at room temperature. Later, the diameter of fungal colonies was measured in mutual perpendicular directions. Antifungal effect of lichen extracts, in terms of inhibition of radial growth of test fungi, was determined using the formula:

$$\text{Inhibition of fungal growth (\%)} = (C - T / C) \times 100$$

Where 'C' and 'T' denotes the diameter of fungal colonies in control and poisoned plates respectively (Vivek *et al.*, 2014).

Statistical Analysis

The experiment was carried out in triplicates (n=3) and the results are presented as Mean±S.D (Standard deviation).

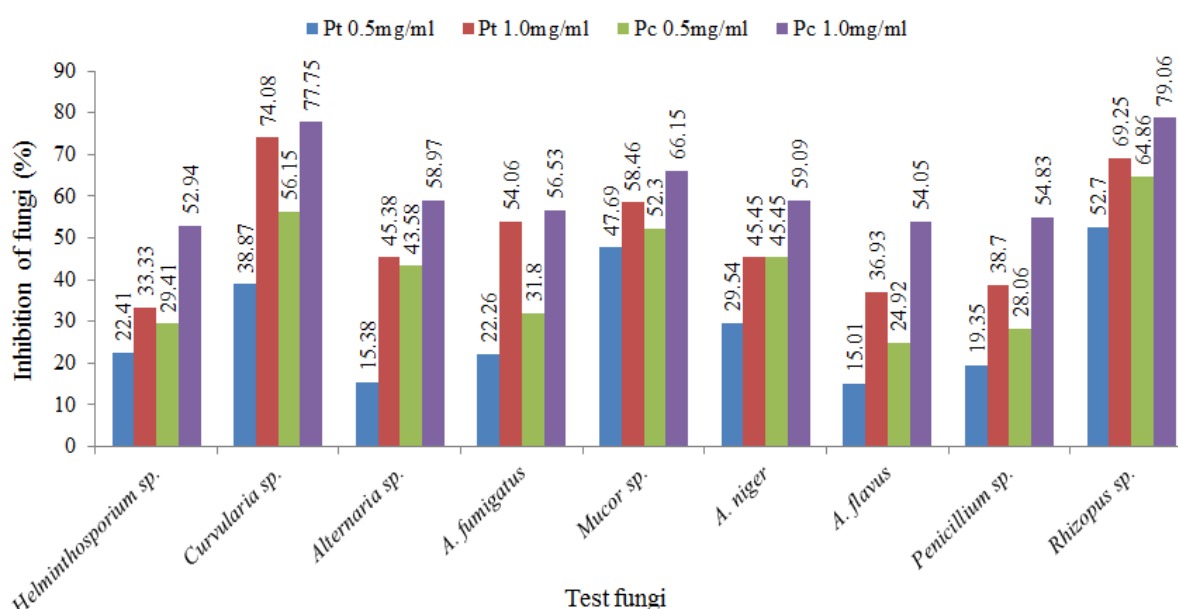
RESULTS AND DISCUSSION

Lichens and their metabolites are shown to be promising sources of antifungal agents against a range of fungi including plant pathogenic fungi (Halama and Van Haluvin, 2004; Goel *et al.*, 2011; Kowalski *et al.*, 2011; Karabulut and Ozturk, 2015). It is shown that *Parmotrema* species exhibit antifungal activity against wide range of phytopathogenic fungi including seed-borne fungi (Tiwari *et al.*, 2011; Kekuda *et al.*, 2014; Babiah *et al.*, 2014; Vivek *et al.*, 2014; Devi *et al.*, 2015; Shivanna and Garampalli, 2015). In the present study, we evaluated the antifungal potential of extract from *P. tinctorum* and *P. cristiferum* against fungi isolated from seeds of maize and ground nut by poisoned food technique. A reduction in mycelial growth of test fungi in poisoned plates is an indication of antifungal potential. Both lichens were effective in inhibiting the growth of test fungi in a dose dependent manner. Among lichens, *P. cristiferum* was found to inhibit test fungi to higher extent. At 1mg/ml concentration of *P. tinctorum* and *P. cristiferum* extract, the extent of inhibition of test fungi was in the range 33.33% to 74.08% and 52.94% to 79.06% respectively. An inhibition of >50% of all test fungi was observed at an extract concentration of 1mg/ml of *P. cristiferum*. In case of *P. tinctorum*, only 4 out of 9 test fungi were inhibited to >50% at 1mg/ml extract concentration. Fungi such as *Curvularia* sp., *Mucor* sp. and *Rhizopus* sp. were inhibited to appreciable extent when compared to other fungi (Table 2; Figure 1).

Few studies have been carried out to investigate antifungal activity of *Parmotrema* species against seed mycoflora. In an earlier study, Vivek *et al.* (2014) revealed the inhibitory potential of *P. tinctorum* against *Alternaria* sp., *Helminthosporium* sp. and *Aspergillus flaus* isolated from seeds of sorghum. Kekuda *et al.* (2015) showed the potential of *P. cristiferum* to inhibit mycelial growth of fungi viz. *Alternaria* sp., *Helminthosporium* sp., *Fusarium* sp. and *Curvularia* sp. isolated from moldy grains of sorghum. In the present study, thin layer chromatogram revealed the presence of atranorin in both lichens. Compounds viz. lecanoric acid and orsellinic acid were present in *P. tinctorum* while salazinic acid and con-salazinic acid were present in *P. cristiferum*. It is shown from earlier studies that lichen metabolites such as atranorin (Goel *et al.*, 2011), lecanoric acid (Thadhani *et al.*, 2012), salazinic acid (Candan *et al.*, 2007; Goel *et al.*, 2011) exhibit antimicrobial activity. In the present study, the observed antifungal potential of *P. tinctorum* and *P. cristiferum* to inhibit seed-borne fungi could be ascribed to the presence of these metabolites.

Table 2: Colony diameter of test fungi in control and poisoned plates

Test Fungi	Colony Diameter in cm (Mean±S.D)				
	Control	Pt* 0.5mg/ml	Pt* 1.0mg/ml	Pc** 0.5mg/ml	Pc** 1.0mg/ml
<i>Helminthosporium</i> sp.	5.10±0.00	3.80±0.10	3.40±0.00	3.60±0.00	2.4±0.00
<i>Curvularia</i> sp.	4.63±0.05	2.83±0.05	1.20±0.00	2.03±0.05	1.03±0.05
<i>Alternaria</i> sp.	3.90±0.00	3.30±0.00	2.13±0.05	2.20±0.10	1.60±0.00
<i>A. fumigatus</i>	2.83±0.05	2.20±0.00	1.30±0.00	1.93±0.05	1.23±0.05
<i>Mucor</i> sp.	6.50±0.10	3.40±0.10	2.70±0.17	3.10±0.00	2.20±0.00
<i>A. niger</i>	4.40±0.00	3.10±0.00	2.40±0.00	2.40±0.00	1.80±0.00
<i>A. flavus</i>	3.33±0.05	2.83±0.05	2.10±0.00	2.50±0.10	1.53±0.05
<i>Penicillium</i> sp.	3.10±0.10	2.50±0.10	1.90±0.00	2.23±0.05	1.40±0.10
<i>Rhizopus</i> sp.	6.83±0.05	3.23±0.05	2.10±0.10	2.40±0.00	1.43±0.05

Pt*- *P. tinctorum*; Pc**- *P. cristiferum***Figure 1:** Extent of inhibition of test fungi by extracts of *Parmotrema* species (Pt- *P. tinctorum*; Pc- *P. cristiferum*)

CONCLUSIONS

Both lichens were shown to display inhibitory activity against seed mycoflora of ground nut and sorghum. *P. cristiferum* was effective in inhibiting mycoflora to higher extent when compared to *P. tinctorum*. The antifungal activity of lichens observed in this study could be related to the presence of bioactive secondary metabolites in the extracts. Isolation of active principles from these lichens and their inhibitory activity against seed mycoflora are to be carried out.

Conflict of Interest

None declared.

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