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Research Article

BIOCONTROL POTENTIAL OF *PARMOTREMA* SPECIES AGAINST *COLLETOTRICHUM CAPSICI* ISOLATED FROM ANTHRACNOSE OF CHILLI

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ABSTRACT

Chilli is an important commercial crop and is consumed as spice as well as vegetable. *Colletotrichum capsici* is an important fungal pathogen causing anthracnose in chilli. The objective of the present study was to determine inhibitory activity against *C. capsici* of extracts of three foliose macrolichen species of the genus *Parmotrema viz*., *P. tinctorum*, *P. grayanum* and *P. praesorediosum* from Western Ghats of Karnataka, India. The antifungal effect was checked in terms of mycelial growth inhibition by Poisoned food technique. The spore suspension of *C. capsici* was inoculated on the centre of Potato dextrose agar plates (control and poisoned). The colony diameter of fungus in control and poisoned plates was recorded on 5th day of incubation. The lichen extracts caused marked inhibition of test fungus as revealed by reduced colony diameter of test fungus in poisoned plates. Among lichens, *P. tinctorum* showed potent inhibitory activity and caused > 50 % of inhibition of test fungus. The lichens of the present study appeared promising as bio control agents against *C. capsici*.

Keywords: Western Ghats, *Parmotrema*, Anthracnose of chilli, *Colletotrichum capsici*, Poisoned food technique

INTRODUCTION

Plants serve mankind as an important source of food, timber, fodder and medicine from ancient time. However, plants are also very vulnerable for diseases caused by pathogens such as bacteria, fungi, mycoplasma, actinomycetes and nematodes. Phytopathogens, in particular fungi are responsible for poor establishment and stand loss in a variety of commercial crops. Chemicals are widely used to prevent and control plant diseases. However, the control of the plant diseases by chemical method is not beneficial in many respects such as high cost, breakdown of resistance, residual problem and deleterious effect on non-target organisms including humans[1,2]. Hence, search for alternatives for control of fungal diseases is necessary. Natural products such as plant based formulations, cow urine, cow urine extracts of plants, lichens and their metabolites etc have been shown to possess marked antifungal effect against several phytopathogenic fungi. Moreover, these agents are non-toxic and are easily decomposed[3-8]. Lichens are symbiotic organisms comprising of a fungal partner (a mycobiont) and a photosynthetic partner (a photobiont). The photobiont can be an alga or a *cyanobacterium*. Lichens occur in various growth forms namely crustose, foliose and fruticose and are known to grow on rocks, non-fertile ground, as well as epiphytes on the trees and leaves. They constitute dominant components in some ecosystems while in others their presence is scarce. Lichens have been used as traditional medicine for treating various kinds of ailments such as stomach diseases, diabetes, cough, pulmonary tuberculosis, wounds curing and dermatological diseases in various parts of the world. They have been used as food and added in the preparation of foods in certain cultures. Besides, lichens are one of the best indicators of air pollution and are also considered as sources of colors, perfumes, alcohols etc. Lichens are able to synthesize a vast number of bioactive compounds called lichen substances (most are phenolic derivatives) which sometimes make even more than 30 % of the dry mass of thallus. Lichen and their bioactive compounds are known to exhibit bioactivities such as antimicrobial, antioxidant and enzyme inhibitory, cytotoxic, antiprotozoal, antiviral, analgesic, anti-inflammatory and others[9-15]. Western Ghats of India constitute one of the global biodiversity hotspots and houses > 30 % of all plant, fish, herpeto-fauna, birds, and mammal species of India. The mountain ranges of Western Ghats runs through Gujarat, Maharashtra, Goa, Karnataka and Kerala, India. Numerous species of plants, animals and microbes including a number of globally threatened and endemic species of plants and animals are found in Western Ghats. Several studies have been undertaken on distribution and bioactivities of macro lichens of Western Ghats of Karnataka, India[12-21]. The members of the lichen genus *Parmotrema* belongs to Parmeliaceae and are characterized by large foliose thalli with broad rotund lobe apices, the absence of pseudocyphellae, broad erhizinate marginal zone on the lower surface, marginal cilia, simple rhizines and thick walled ellipsoid ascospores. The species of *Parmotrema* are best developed in tropical regions[22-24]. The aim of the present study was to determine antifungal activity of three *Parmotrema* species *viz*., *P. tinctorum*, *P. grayanum* and *P. praesorediosum* from Maragalale and Guliguli Shankara,

Western Ghats of Karnataka, India against *Colletotrichum capsici* isolated from anthracnose of chilli.

MATERIALS AND METHODS

Collection and identification of lichens

The lichens of this study were collected during September 2013 from two areas of Western Ghat belt of Shivamogga district, Karnataka, India. *P. grayanum* (saxicolous) was collected at Guliguli Shankara, Hosanagara taluk. *P. tinctorum* (corticolous) and *P. praesorediosum* (saxicolous) were collected at Maragalale, Thirthahalli taluk. The lichens were identified on the basis of morphological, anatomical and chemical tests. Color tests were carried out on cortex and medulla of lichens by using 10 % potassium hydroxide (K), Steiner's stable paraphenylenediamine solution (P) and calcium hypochlorite solution (C). Secondary metabolites were detected by Thin layer chromatography (TLC) using solvent system A (Benzene: 1, 4-Dioxane: Acetic acid in the ratio 90:25:4)[25-27].

Extraction

The dried and powdered lichen materials (15 g) were extracted by using methanol (Hi Media, Mumbai, India) in a Soxhlet assembly. The contents were filtered through sterile Whatman No. 1 filter paper after completion of extraction. The methanol extracts were concentrated in vacuum under reduced pressure[14].

Antifungal activity of lichen extracts

The test fungus *C. capsici* was isolated from anthracnose of chilli in our previous study[5]. We employed Poisoned food technique to determine antifungal potential of lichen extracts in terms of inhibition of mycelial growth of test fungus. Here, petriplates containing sterile Potato dextrose agar medium (poisoned with lichen extracts, 1 mg/ml of medium) were inoculated at the centre with the spore suspension of test fungus by point inoculation. The plates were incubated at 28^{o}C for 5 days. Later, colony diameters were measured in mutual perpendicular directions. The antifungal effect of lichen extracts was recorded in terms of inhibition of mycelial growth (%) and was calculated using the formula:

Inhibition of mycelial growth (%) = (C – T / C) x 100,
Where C is colony diameter in control plates and
T is colony diameter in poisoned plates[5].

Statistical analysis

The experiment was done in triplicates. Results are recorded as Mean ± Standard deviation (SD).

RESULTS

On the basis of morphological, anatomical and color tests, the foliose lichens of this study were identified as species of *Parmotrema viz*., *P. tinctorum*, *P. grayanum* and *P. praesorediosum*. The details of color tests and TLC of *Parmotrema* species are given in Table 1.

Table 1: Details of Color tests and TLC of *Parmotrema* species

Lichen	Color test	TLC
P. tinctorum	Cortex K+ yellow; Medulla K-, C +red, KC +red, Pd -	Lecanoric acid, Atranorin, Orsellinic acid
P. grayanum	Cortex K+ yellow; Medulla K-, C -, KC -, Pd -	Atranorin, Protolichesterinic acid
P. praesorediosum	Cortex K+ yellow; Medulla K-, C -, KC -, Pd -	Atranorin, Chloroatranorin, Protopraesorediosic acid, Praesorediosic acid

The antifungal effect of extract of *Parmotrema* species in terms of mycelial growth inhibition of *C. capsici* is presented in Table 2 and Figure 1. The lichen extracts exhibited marked inhibitory activity against test fungus as indicated by reduction in the colony diameter of test fungus in poisoned plates when compared to control plates. Among lichens, *P. tinctorum* exhibited stronger inhibitory activity. An inhibition of > 50 % was observed in case of extract of *P. tinctorum*. *P. grayanum* and *P. praesorediosum* exhibited more or less similar inhibitory activity against *C. capsici* (Figure 2).

Table 2: Colony diameter (CD) of *C. capsici* in control and poisoned plates

Treatment	CD in cm
Control	3.1 ± 0.1
P. tinctorum	1.3 ± 0.0
P. grayanum	1.8 ± 0.0
P. praesorediosum	1.9 ± 0.1

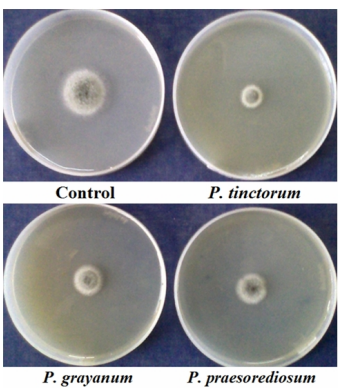

Figure 1: Mycelial growth of *C. capsici* in control and poisoned plates

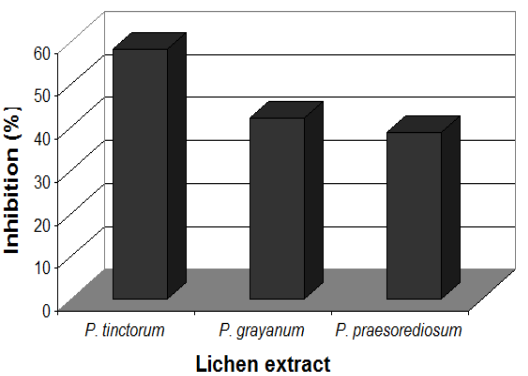

Figure 2: Inhibition (%) of *C. capsici* by extracts of *Parmotrema* species

DISCUSSION

Chilli belongs to the genus *Capsicum* (Solanaceae). It is an herbaceous, annual, dicotyledonous flowering plant grown worldwide in tropical and subtropical regions. It is a commercial crop and is grown extensively for consumption, nutritional and economy purposes. It is used as both as spice (ripe and dried form) and vegetable (green fruit). In terms of international trade, India is known to be the largest producer of chilli. Chilli is known to contain a number of chemicals such as steam-volatile oils, fatty oils, capsaicinoids, carotenoids, vitamins, protein, fiber and minerals. The production of chilli is greatly affected by various diseases caused by fungi, bacteria and viruses. These diseases account for marked reduction in productivity. Anthracnose (both pre-harvest and post-harvest) is the most important disease among various diseases of chilli. It results in yield loss and deterioration of fruit quality. Anthracnose symptoms on chilli fruit include sunken necrotic tissues with concentric rings of acervuli. The anthracnose may result in yield loss up to 50 %. The disease is known to be caused by *Colletotrichum* species such as *C. capsici, C. acutatum, C. gloeosporioides, C. coccodes* and *C. dematium*. Among these, *C. capsici* is the most important pathogen[28-35]. In the present study, we determined the inhibitory effect of three *Parmotrema* species *viz*., *P. tinctorum*, *P. grayanum* and *P. praesorediosum* from Western Ghats of Karnataka, India against *C. capsici* isolated from anthracnose of chilli. These lichens were able to inhibit the growth of *C. capsici* but to a varied extent. *P. tinctorum* was more effective in inhibiting the mycelial growth followed by *P. grayanum* and *P. praesorediosum*. It has been found that solvent extracts and purified secondary metabolites from lichens exhibit inhibitory activity against phytopathogenic fungi. Aqueous extract of *Heterodermia leucomela* was found to exhibit significant inhibition of germination of spores of some phytopathogenic fungi[9]. A lichen forming fungus isolated from *Heterodermia* sp. was found to exhibit strong inhibitory activity against *Pythium* spp.[10]. Acetone extracts of *Evernia prunastri*, *Hypogymnia physodes* and *Cladonia portentosa* were found to possess antifungal activity against phytopathogenic fungi. *E. prunastri* and *H. physodes* exhibited marked inhibition of *Pythium ultimum*, *Ustilago maydis* and *Phytophthora infestans*. Lichenic acids *viz*., evernic acid and usnic acid were also active against some of the fungi tested[3]. It has been observed that hexane and ethyl acetate extracts of *Parmelia reticulata* exhibit inhibitory activity against some of the phytopathogenic fungi. Lichen substances *viz*., (±)-protolichesterinic acid and atranorin were inhibitory against *Rhizoctonia solani* and *Sclerotium rolfsii*[4]. Methanol extract of *Parmelia sulcata*, *Flavoparmelia caperata* and *Evernia prunastri* were shown to possess antifungal activity against a panel of human and plant pathogenic fungi[36]. It has been shown that hexane and dichloromethane extracts from *Parmelia reticulata, Ramalina roesleri, Usnea longissima* and *Stereocaulon himalayense* were found most active against some phytopathogenic fungi[37]. Solvent extracts of *Lecanora atra*, *Lecanora muralis, Parmelia saxatilis*, *Parmelia sulcata* and *Parmeliopsis ambigua* were shown to exhibit antifungal activity against a panel of fungi which included plant pathogenic fungi[11].

CONCLUSION

The extracts of *Parmotrema* species have shown inhibitory potential against mycelial growth of *C. capsici*. To the best of our knowledge, it is the first report on inhibitory effect of *Parmotrema* species from Western Ghats of Karnataka, India against *C. capsici* from chilli anthracnose. The lichen based formulations can be effective agents for the control of anthracnose of chilli.

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