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INVESTIGATION OF ANTIFUNGAL POTENTIALITY OF AQUEOUS EXTRACT OF *MILLINGTONIA HORTENSIS* LINN LEAVES AGAINST *ASPERGILLUS* AND *FUSARIUM* SPECIES OF MAIZE

B. Kiran[1]*, V. Lalitha[2], K.A.Raveesha[3]

1. CMR Institute of Management Studies (Autonomous), C.A. #2, 3rd 'C' Cross, 6th 'A' Main, HRBR layout, 2nd Block, Kalyana Nagar,Bangalore -560043, Karnataka, India
2. Department of Studies in Botany and Microbiology, Maharanis Science College for Women, Palace Road, Bangalore-560001, Karnataka, India
3. Department of Studies in Botany, Manasagangotri, University of Mysore, Mysore- 570 006, Karnataka, India

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For Correspondence:

Dr. B. Kiran

CMR Institute of Management Studies (Autonomous), C.A. #2, 3rd 'C' Cross, 6th 'A' Main, HRBR layout, 2nd Block, Kalyana Nagar,Bangalore - 560043, Karnataka, India

E-mail:

bkiran2702@gmail.com

ABSTRACT

Antifungal activity of aqueous extract of leaves of *Millingtonia hortensis* Linn when tested against eight fungal species of maize at 10, 20, 30, 40 and 50% concentration showed maximum activity against *A.flavus* at 50% concentration followed by *F.oxysporum* (90.2%), *F.solani* (89.5%), *F.moniliforme* (87.7%), *A.candidus* (78.9%), *A.niger* (78.0%), *A.flavipes* (73.2%) and *F.graminearum* (52.1%) at 50% concentration tested. Moderate activity was also observed in 20, 30 and 40% concentration and least activity was observed in 10% concentration tested. Compared to synthetic fungicide bavistin and thiram, both the fungicide recorded 100% inhibition.

INTRODUCTION

The use of medicinal plants as a source for aid from illness can be traced back over five millennia to written documents of the early culture in China, India and the Near east, but it is, without a doubt, an art as old as mankind [1]. Plants have been classified as an essential source of medicinal agents for centuries and a huge number of novel drug components have been isolated from natural plant sources. Many of these plants and their extracts were used in traditional medicine both as antibacterial and antifungal agent. Medicinal plants play a key role in health care with about 80% of the worlds populations relying on the use of traditional medicine which is predominantly based on plants [2,3]. Herbal products prepared either from single or multiple botanical ingredients are usually complex and variable in nature. Undoubtedly, the plant kingdom still holds many species of plants containing substances of medicinal value that have yet to be discovered [4]. Medicinal plants are of great importance to the health of individual and communities. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on human body. The most important of these chemically active constituents of plants are alkaloids, tannin, flavonoid and phenolic compounds. Many of these indigenous medicinal plants are also used for medicinal purposes [5]. In recent years, use of antimicrobial drugs in the treatment of infectious disease has developed multiple drug resistance and with increases in production of new antibiotics, by pharmaceutical industry, resistance to these drugs has also increased [6]. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity . India is a varietal emporium of medicinal plants and is one of the richest countries in the world in regard to genetic resources of medicinal plants. Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections[7]. Herbal extracts were showing a promising result in controlling both plant diseases particularly fungal and bacterial diseases. In the present study, an attempt was made to test antifungal activity of aqueous extract of leaves of *Millingtonia hortensis* Linn. belongs to family Bignoniaceae commonly called cork tree against seed borne fungi of maize.

MATERIALS AND METHODS

Test plant: Fresh and healthy leaves of *M. hortensis* collected from Mysore. The leaves were shade dried and washed thoroughly two to three times with running tap water and once with sterile distilled water, air dried at room temperature on a sterile blotter, and used for the preparation of extracts[7].

EXTRACTION

Aqueous extract: One hundred grams of the thoroughly washed and air dried healthy leaves of *M. hortensis* were macerated with 100 ml of sterile distilled water in a waring blender (Waring International, New Hartford, CT, USA) for five minutes. The macerate was filtered through double-layered muslin cloth, and then centrifuged at 4000g for 30 minutes. The supernatant was filtered through Whatman No.1 filter paper and sterilized at 120 ^{0} C for 10 min, which served as 100% aqueous mother extract. The extract was preserved aseptically in a sterile brown bottle at 5^0 C until further use [8].

Test fungi: Four species of *Fusarium* viz., *F. graminearum*, *F. moniliforme*, *F. oxysporum* and *F. solani* . Four species of *Aspergillus* viz., *A.niger*, *A.flavus* and *A. flavipes* and *A.candidus* isolated from maize seeds were used as test fungi for antifungal activity assay.

Antifungal activity assay by poisoned food technique: Czapek Dox Agar (CDA) medium with different concentrations of the aqueous extract of leaves of *M. hortensis* viz., 10, 20, 30, 40, and 50% were prepared and poured into sterile petriplates, and allowed to cool and solidify. Five mm mycelial discs of seven-day-old cultures of species of *Fusarium* and *Aspergillus* were placed at the centre of the Petri plates and incubated at 25 ±1^{0}C for seven days. The CDA medium without the aqueous extract but with the same concentration of sterile distilled water served as a control. The colony diameter was measured in mm. For each treatment three replicates were maintained. The percentage inhibition of mycelial growth, if any, was determined by the formula PI = C-T/ C x 100, where C = diameter of control colony and T = diameter of treated colony [9]. The minimal inhibitory concentration (MIC) for each of the test fungi was determined following the procedure of [10]. The data was subjected to statistical analysis by ANOVA and Tukey's HSD.

Chemical fungicides: Two chemical fungicides viz., Bavistin, and Thiram were evaluated for antifungal activity by poisoned food technique for comparison.

STATISTICAL ANALYSIS

The data were subjected to Tukey's HSD analysis. Data on percentages were transformed to arcsine and analysis of variance (Anova) was carried out with transformed values. The means were compared for significance using Tukey's HSD (P=0.05).

RESULTS

Among the eight fungal species tested for antifungal activity, Maximum activity was observed in *A.flavus* and recorded 93.4% inhibition at 50% concentration, 81.3% inhibition in 40% concentration, 68.9% inhibition at 30% concentration and 39.2% and 19.2% inhibition at 20 and

10% concentration tested. *A.flavus* is followed by *F.oxysporum* and recorded 90.2% inhibition and 18.1, 38.0, 51.3 and 72.9% inhibition at 10, 20, 30 and 40% respectively. *F.solani* showed maximum activity at 50% concentration and recorded 89.5% inhibition and at 10% concentration it was recorded 16.2% inhibition. Moderate activity was observed in 20, 30 and 40% concentration. *F.moniliforme* showed 87.7% inhibition in 50% concentration and 16.2% inhibition at 10% concentration. Moderate activity was observed in *A. candidus* and recorded 78.9% inhibition and 11.2% inhibition in 10% concentration. *A. niger* recorded 78.0 % inhibition in 50%concentration and 10.0% inhibition in 10% concentration. Least activity was observed in *A. flavipes* and *F. graminerum* and recorded 73.2% and 52.1% inhibition at 50%concentration. The percentage of inhibition goes on increasing with increasing the concentration. Compared to synthetic fungicides, Bavistin and Thiram at 2.0% recommended concentration,both fungicides recorded 100% inhibition against all the test fungi tested(Table 1).

Table 1: Antifungal activity of aqueous extract of seeds of leaves of *M. hortensis* L. against seed borne fungi of maize

Fungi	Mycelial Growth Inhibition(%)						
	Concentration of Aqueous Extract					Bavistin	Thiram
	10%	20%	30%	40%	50%	2%	2%
F. graminearum	10.0^{a} ±0.1	18.9^{b} ±0.1	28.3^{c} ±0.1	36.1^{d} ±0.1	52.1^{e} ±0.1	100.0^{f} ±0.0	100.0^{f} ±0.0
F. moniliforme	16.2^{a} ±0.0	29.3^{b} ±0.1	42.7^{c} ±0.2	65.2^{d} ±0.1	87.7^{e} ±0.0	100.0^{f} ±0.0	100.0^{f} ±0.0
F. oxysporum	18.1^{a} ±0.1	38.0^{b} ±0.0	51.3^{c} ±0.0	72.9^{d} ±0.0	90.2^{e} ±0.1	100.0^{f} ±0.1	100.0^{f} ±0.1
F. solani	16.2^{a} ±0.0	28.9^{b} ±0.0	45.9^{c} ±0.1	68.9^{d} ±0.0	89.5^{e} ±0.2	100.0^{f} ±0.0	100.0^{f} ±0.2
A.niger	10.0^{a} ±0.2	23.9^{b} ±0.0	43.9^{c} ±0.0	61.1^{d} ±0.0	78.0^{e} ±0.1	100.0^{f} ±0.2	100.0^{f} ±0.1
A.flavus	19.2^{a} ±0.0	39.2^{b} ±0.0	68.9^{c} ±0.0	81.3^{d} ±0.0	93.4^{e} ±0.0	100.0^{f} ±0.0	100.0^{f} ±0.0
A. flavipes	12.4^{a} ±0.0	21.0^{b} ±0.0	38.4^{c} ±0.0	56.8^{d} ±0.1	73.2^{e} ±0.1	100.0^{f} ±0.0	100.0^{f} ±0.0
A.candidus	11.2^{a} ±0.0	23.9^{b} ±0.0	43.1^{c} ±0.0	60.1^{d} ±0.0	78.9^{e} ±0.1	100.0^{f} ±0.1	100.0^{f} ±0.1

- Values are the mean of three replicates, ±standard error
- The means followed by the same letter (s) are not significantly different at P 0.05 when subjected to Tukey's HSD
- Pattern of percentage inhibition increase is not uniform for all the microorganisms

DISCUSSION

The control of post harvest fungal contamination of food commodities, are generally achieved by synthetic chemicals but such chemicals can create side effects including carcinogenecity, teratogenicity and residue toxicity. Moreover, the use of many of the synthetic fungicides has now been restricted because of their undesirable attributes, such as high and acute toxicity, long

degradation periods and accumulation in the food chain and an undesirable extension of their power to destroy useful and harmful pests [11,12,13,14]. Less than 0.1% of toxic pesticide applied to crop protection reaches the target pests. Thus remaining 99.9% moves into natural ecosystem [15]. To overcome the ill effects of costly but effective synthetic pesticides, scientists and researchers are looking for biological cultural and other ecologically safer, non-hazardous and non-polluting means to control pests [16]. Of late in different parts of the world attention has been paid to exploiting higher order plant products as novel chemotherpeutants in plant protection, because of non-phytotoxicity, systemacity, easy biodegradability and the stimulatory nature of host metabolism. Plant products possess the potential to be of value in pest management [17]. In the present study, leaves of leaves of *M. hortensis* proved strong antifungal activity against all the test fungi *Fusarium* and *Aspergillus* species. Further investigation is necessary to test the antifungal activity of different solvent extracts and also to isolate bioactive compound which shows strong antifungal activity.

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