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Antifungal activity of aqueous and solvent extracts of seeds of *Psoralea corylifolia* L. against seed borne fungi of maize

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Abstract

Antifungal activity of aqueous and solvent extract of seeds of *P.corylifolia* against five seed borne fungi of maize viz., *Curvularia lunata*, *Dreschslera halodes*, *Alternaria alternata*, *Cladosporium cladosporioides* and *Rhizopus* sp. were tested *in vitro*. In aqueous extract, maximum inhibition was observed in *A.alternata* and recorded 95.4% inhibition at 50% concentration followed by *C.lunata* (86.0%), *Rhizopus* sp.(82.3%), *D. halodes* (68.0%) and *C. cladosporioides* (57.7%). Significant activity was also observed in 10, 20, 30 and 40% concentration. In solvent extracts tested at 250,500, 750 and 1000µl concentration, maximum inhibition was observed in petroleum ether extract and moderate activity was observed in methanol extract. Compared to synthetic fungicide Bavistin and Thiram, complete inhibition was observed against all the test fungi tested at 2% recommended concentration.

Key-Words: *P.corylifolia*, Antifungal activity, Maize, Bavistin, Thiram

Introduction

Medicinal plants are used by 80% of the world population as the only available medicines especially in developing countries [1]. A wide range of medicinal plant parts extracts is used as raw drugs and they possess varied medicinal properties. The use of different parts of several medicinal parts to cure specific diseases has been in vogue from ancient times. The indigenous system of medicine namely ayurveda, siddha and unani have been in existence for several centuries. This system of medicine caters the needs of nearly 70% of the population residing in villages [2]. Plants have been placed at top among the sources of novel drugs with antimicrobial activity as traditional medicines based on plants and plant extracts have made considerable contributions to human health and plant health. Plant based antimicrobials represent a vast untapped source for medicines and they provide enormous therapeutic potential [3].

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Many potent drugs have been purified from medicinal plants having anti-rheumatic, antithrombotic, antimalarial, anticancer, antidiabetic and antimicrobial properties [4]. Scientists are engaged to achieve some plant derived compounds to control plant diseases. About thirty percent of the food was lost by storage fungi which is playing a dominant role in biodeterioration. To manage biodeterioration causing fungi, the regular practice of farmers is to use a large quantities of chemical fertilizers, chemical growth regulators and chemical pesticides. The ill effects associated with the use of chemical fungicides like carcinogenicity and teratogenicity which cause a serious health problems. There is a urgent need to search for alternative strategies for the management of pre and post harvest crop diseases. Natural plants products are biodegradable, exhibit structural diversity and rarely contain halogenated atoms. These can act directly as pesticides or may provide structure lead for pesticidal discovery [5]. Many recent studies have shown that both crude extracts and purified compounds isolated compounds from plants can effectively be used as natural fungicides for the management of plant diseases [6,7]. Hence in the present investigation

Psoralea corylifolia L. (Seed) belongs to family Fabaceae were subjected to aqueous and solvent extraction and further antimicrobial evaluation of these extracts against important biodeterioration causing fungi was conducted in *in vitro* condition.

Material and Methods

Plant Material: Shade dried, healthy seeds of *P. corylifolia* were collected from seed market, Mysore. The seeds were washed thoroughly 2-3 times with running tap water and once with sterile distilled water, air dried at room temperature on a sterile blotter. After complete drying seeds were used for preparation of aqueous and solvent extract [8].

Extraction

Aqueous extract: One hundred grams of the thoroughly washed and air dried healthy seeds of *P. corylifolia* were macerated with 100 ml of sterile distilled water in a waring blender (Waring International, New Hartford, CT, USA) for five min. 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 120^0C for 10 minutes, which served as 100% aqueous mother extract. The extract was preserved aseptically in a sterile brown bottle at 5^0 C until further use [9].

Solvent extract: The dried seeds of *P. corylifolia* were powdered with the help of the waring blender. Twenty five grams of the fine powder was filled in the thimble and extracted successively with petroleum ether and methanol for 48 hours and solvent extracts were concentrated separately using rotary flash evaporator under reduced pressure. The extracts were preserved in an airtight brown bottle until further use [10].

Test fungi: Five species of fungi viz., *Curvularia lunata, Dreschslera halodes, Alternaria alternata, Cladosporium cladosporioides* and *Rhizopus* sp. isolated from maize seeds were used as test fungi for antifungal activity assay.

Antifungal activity assay by poisoned food technique

Aqueous extract: Czapek Dox Agar (CDA) medium with different concentrations of the aqueous extract viz., 10, 20, 30, 40, and 50% of seeds of *P. corylifolia* 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 *C. lunata, D. halodes, A. alternata, C. cladosporioides* and *Rhizopus* sp were placed at the centre of the Petri plates and incubated at 25 ± 1^0C 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/Cx 100, where C = diameter of control colony and T = diameter of treated colony[11]. The data was subjected to statistical analysis by ANOVA and Tukey's HSD.

Solvent extract: One gram of petroleum ether and methanol solvent residue was dissolved in 10ml of Methanol. 250 µl ,500µl, 750 µl and 1000µl of each of the solvent extracts was amended with 15ml of Czapek Dox agar medium per plate before solidification of the medium. Pure Methanol and Pertoleum ether (250µl ,500µl, 750µl and 1000µl) amended with the medium served as control. 5mm discs of 7 day old culture of the test fungi were placed at the center of the petriplates and incubated at 22 ± 2^0 C for 7 days. The diameter of the colony was measured and percent inhibition of mycelial growth was calculated using the formula PI= C-T/Cx100, where C= Diameter of control colony and T= Diameter of treated colony[11].

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

Results and Conclusion

Antifungal activity of aqueous extract: Among the five fungi tested at 10,20,30,40 and 50% concentration, *A. alternate* recorded a maximum inhibition of 95.4% in 50% concentration, 81.9% in 40% concentration and 63.8% inhibition in 30% concentration. Significant activity was also observed in 10% and 20% concentration. *A.alternata* was followed by *C. lunata* and recorded 86.0% inhibition at 50% concentration and 64.3% in 40% concentration. *Rhizopus* sp recorded 82.3% inhibition at 50% and 68.7% in 40% concentration. In *D. halodes* at 50% concentration, it was recorded 68.0% inhibition and 47.6% inhibition in 40% concentration. Least inhibition was observed in *C. cladosporioides* and recorded 57.7% inhibition. Moderate and significant activity was observed in 10, 20 and 30% concentration in all the fungi tested. Compared to synthetic fungicides Bavistin and Thiram tested at recommended dosage of 2 grams/liter, all the test fungi were completely inhibited(Table1).

Antifungal activity of solvent extract: Among the two solvent tested, petroleum ether extract recorded a significant activity compared to methanol. In petroleum ether extract *A. alternata* recorded a complete inhibition at 1000 µl concentration and at 750 µl concentration, it was recorded 75.5% inhibition. In 250µl and 500µl concentration, it was recorded 37.2% and 60.9% inhibition. *C. lunata* recorded 96.4% inhibition at 1000 µl concentration and 73.0% inhibition at 750 µl concentration. *Rhizopus* sp.

Recorded 87.2% inhibition at1000 µl concentration and 70.3% inhibition at 750 µl concentration. Moderate activity was observed in *D. halodes* and recorded 70.7% inhibition at 1000 µl and 54.2% inhibition at 750 µl concentration. Least activity was observed in *C. cladosporioides* and recorded 61.3% inhibition at 1000 µl concentration. Significant activity was observed against all the fungi at 250 µl and 500 µl concentration. In Methanol extract, moderate activity was observed against all the fungi at 250, 500, 750 and 1000 µl concentration tested. *A. alternate* recorded 51.6% inhibition at 1000 µl concentration followed by *C. lunata* (41.1%), *Rhizopus* sp (32.1%), *D. halodes* (23.3%) and *C. cladosporioides* recorded 21.8% inhibition at 1000 µl concentration tested. No significant activity was observed in 250, 500 and 750 µl concentration tested against all the fungi (Table 2).

The use of higher plants and their preparations to treat infectious diseases is an age-old practice and in the past possibly the only method available. However, the systematic study of higher plants for detecting antimicrobial activity is of comparatively recent origin[12]. Based on results it can be concluded that *P.corylifolia* seed is a potent medicinal plants which showed a strong antimicrobial activity against many seed borne fungi both in aqueous and solvent extract. A further work is necessary to isolate an bioactive compound and to test its potentiality against all the storage fungi and also some important bacteria.

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Table 1: Antifungal activity of aqueous extract of seeds of *P.corylifolia* against seed borne fungi of maize

Fungi	Mycelial Growth Inhibition(%)						
	Concentration of Aqueous Extract					Bavistin	Thiram
	10%	20%	30%	40%	50%	2%	2%
C. lunata	15.1±0.1	33.4±0.0	47.2±0.2	64.3±0.0	86.0±0.0	100.0±0.0	100.0±0.0
D. halodes	17.2±0.0	25.7±0.1	35.3±0.0	47.6±0.0	68.0±0.0	100.0±0.0	100.0±0.0
A. alternata	23.6±0.0	41.9±0.1	63.8±0.2	81.9±0.2	95.4±0.0	100.0±0.1	100.0±0.1
C. cladosporioides	16.3±0.2	23.2±0.0	31.4±0.1	44.2±0.1	57.7±0.1	100.0±0.0	100.0±0.2
Rhizopus sp	20.6±0.0	37.4±0.0	49.6±0.2	68.7±0.0	82.3±0.2	100.0±0.2	100.0±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

Table 2: Antifungal activity of petroleum ether and methanol extract of seeds of *P.corylifolia* against seed borne fungi of maize

Fungi	Mycelial Growth Inhibition (%)									
	Concentration of the Extract								Bavistin	Thiram
	Petroleum ether extract in µl				Methanol extract in µl					
	250	500	750	1000	250	500	750	1000	2%	2%
C. lunata	33.1± 0.0	56.4± 0.0	73.0± 0.1	96.4± 0.1	12.1± 0.1	17.7± 0.1	29.2± 0.0	41.1± 0.0	100.0± 0.0	100.0±0. 0
D. halodes	23.4± 0.1	37.6± 0.0	54.2± 0.2	70.7± 0.2	7.0± 0.2	10.9± 0.1	17.6± 0.0	23.3± 0.0	100.0± 0.0	100.0±0. 0
A. alternata	37.2± 0.2	60.9± 0.0	75.5± 0.0	100.0± 0.0	13.2± 0.0	20.4± 0.0	33.1± 0.1	51.6± 0.1	100.0± 0.1	100.0±0. 1
C. cladosporioides	20.7± 0.1	36.2± 0.0	47.8± 0.0	61.3± 0.0	5.8± 0.0	10.7± 0.0	17.8± 0.0	21.8± 0.0	100.0± 0.0	100.0±0. 2
Rhizopus sp	31.8± 0.0	47.4± 0.1	70.3± 0.1	87.2± 0.0	9.9± 0.0	12.2± 0.0	20.0±0 .0	32.1±0. 0	100.0±0.2	100.0±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