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EVALUATION OF ANTIFUNGAL ACTIVITY OF AQUEOUS EXTRACT OF *PICRORHIZA KURROA* (RHIZOME) AGAINST TEN FUNGI OF MAIZE

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Abstract: Antifungal activity of aqueous extract of *Picrorhiza kurroa* (Rhizome) showed complete inhibition of *Fusarium graminearum* at 100% concentration, *Aspergillus flavus* at 80% concentration, *A. terreus* at 100% concentration, *Curvularia lunata* at 100% concentration, *Alternaria alternata* at 100% concentration and *Cladosporium cladosporides* at 80% concentration. Maximum inhibition of *F. oxysporum, A niger, Penicillium* sp and *Drechslera halodes* showed upto 75 to 93% inhibition tested at 70 to 100 percent concentration of *P. kurroa* rhizome extract. All the test fungi were compared with synthetic fungicides viz., Dithane M 45 and Thiram at recommended concentration of 2% and recorded 100% inhibition against all the test fungi.

Keywords: *Picrorhiza kurroa,* Maize, Fungi, Antifungal activity

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INTRODUCTION

Synthetic chemicals are most commonly used for the management of both pre harvest and post harvest pathogens. Many of these synthetic fungicides are known for their non-biodegradable nature and residual toxicity (Pak, 2003). The ill effects associated with the use of chemical fungicides like carcinogenicity, teratogenicity a health assureds necessitated the search for alternative strategies for the management of pre and post harvest crop diseases. Further extensive use of chemicals leads to biohazordous effects on ecosystem, and their persistent applications lead to resistance in pathogens against these fungicides (Basilico and Basilico, 1999). To avoid the problems of synthetic pesticides, traditionally medicinal plants have been exploited for the management of plant diseases. Medicinal plants have been used for treatment of diseases since the early civilizations of the Middle East, India, China and the New World (Ferrence and Bendersky, 2004). Ayurveda is a medical system primarily practiced in India that has been known for nearly 5000 years. It includes diet and herbal remedies, while emphasizing the body, mind and spirit in disease prevention and treatment (Morgan, 2002). The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. The earliest mention of medicinal use of plant in Hindu culture is founds in “Rig veda”, which is said to have been written between 4500-1600 B.C. and is supposed to be the oldest repository of human knowledge. It is Ayurveda, the foundation of medicinal science of Hindu culture, in its eight division deals with specific properties of drugs and various aspects of science of life and the art of healing (Rastogi and Mehrotra, 2002, Saranraj and Sivasakthi , 2014]. Plants have provided a source of inspiration for novel drug compounds, as plant-derived medicines have made large contributions to human health and well being. Plant extracts have been used for a wide variety of purposes for many thousands of years (Jones, 1996). The use of traditional medicine is wide spread through- out the world. The term, traditional medicine, is inter-changeably used with herbal medicine and natural medicine (Anyanwu and Nwosu, 2014, Hazan and Atta, 2005). Hence in the present study, *Picrorhiza kurroa* rhizome belongs to family Scrofulariaceae was investigated for the control of some important fungi of Maize.

MATERIALS AND METHODS

Collection of plant materials

Test plant: Healthy rhizome of *P. kurroa* collected from Mysore market. The rhizome 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 (Kiran *et al* 2010).

Extraction

Aqueous extract: One hundred grams of the thoroughly washed and air dried healthy rhizomes of *P. kurroa* 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 (Verma and Dohroo, 2003).

Isolation of seed borne mycoflora from maize: Standard blotter method was employed for isolation of seed borne biodeterioration causing fungi. Three layers of blotters equivalent to the size of the petridish were soaked in distilled water, the surplus water is drained from the blotters and placed in the lower lid of the petridish. Four hundred seeds of each of the samples were placed on the blotters at the rate of ten seeds per plate. These plates were incubated for seven days at 22$\pm$2^{o} C under alternating cycles of 12/12 hours of NUV light and darkness. After the period of incubation the seeds were observed under stereobinocular microscope and the fungi associated with these seeds were identified based on their growth habit, mycelial structure and spore morphology using standard manuals (ISTA, 1999). All the fungi associated with the seeds were isolated and their pure cultures maintained on specific media The fungi were subcultured periodically.

Test fungi: Ten species of fungi viz., *Fusarium graminearum*, *F. oxysporum, Aspergillus flavus, A niger, A. terreus, Penicillium* sp, *Curvularia lunata, Drechslera halodes, Alternaria alternata* and *Cladosporium cladosporides* 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 rhizome of *P. kurroa* viz., 10, 20, 30, 40, 50, 60,70,80,90 and 100% were prepared and poured into sterile petriplates, and allowed to cool and solidify. Five mm mycelial discs of seven-day-old cultures of all the test fungi were placed at the centre of the Petri plates and incubated at 25 $\pm$1^{0} C for seven days. The CDA medium without the aqueous extract but with the same volume 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 (Pinto *et al* 1998). The minimal inhibitory concentration (MIC) for each of the test fungi was determined following the procedure of Bansal and Guptha , 2000.

Chemical fungicides: Two chemical fungicides viz., Di-Thane M45 and Bavistin were evaluated for antifungal activity by poisoned food technique for comparison with plant extract

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).

RESULT: Among the ten fungi tested at different concentration from 10-100%, six fungi recorded complete inhibition and four fungi showed maximum inhibition. *F. graminearum* recorded 100% inhibition in 100 percent concentration of rhizome extract, 92.0% in 90 percent concentration and 83.0% inhibition in 80 percent concentration. *A. flavus* recorded complete inhibition at 80 percent concentration, *F. oxysporum* recorded 78-93% inhibition from 80-100 percent concentration of the extract. *A niger* recorded a maximum inhibition of 94.0% in 100 percent concentration and 86.0% in 90 percent concentration. Significant activity was also observed from 10-70 percent concentration tested. *A. terreus* recorded 100% inhibition in 90 percent concentration of the extract, 93.0%inhibition in 80 percent concentration and 81.0% in 70 percent concentration. *Penicillium* sp showed highly significant activity from 80-100 percent concentration and recorded 71.0 to 87.0% inhibition. *C. lunata* recorded complete inhibition in 100 percent concentration and 78.0, 86.0 and 94.0% percent inhibition at 70, 80 and 90 percent concentration of the rhizome extract. *D. halodes* recorded moderate activity and showed 63-75% inhibition tested at 80-100 percent concentration. *A. alternata* recorded complete inhibition at 100 percent concentration and 78-96% inhibition at 70-90 percent concentration of the rhizome extract. *C. cladosporides* recorded complete inhibition at 80% concentration and recorded highly significant activity from 50-70 percent concentration and showed 71.0, 86.0 and 94.0% inhibition respectively. All the result was compared with two synthetic fungicides viz., Dithane M 45 and Thiram at 2% recommended concentration. All the test fungi were completely inhibited (Table 1).

DISCUSSION:

In agriculture, majority of the crop is protected from diseases by the application of several doses of synthetic pesticides which becomes responsible for generating new pathological races due to accumulation of pesticides resistances and this will pose a problems to many other cultivated crops which results in huge loss in agriculture. To avoid the effect of synthetic pesticides to plants and also to concentrate on human health, the alternate source is the use of medicinal plants. Plants have been the basis of traditional medicines throughout the world for thousands of years and continue to provide new remedies to mankind (Kaewseejan *et al* 2012). Since ancient times, people have been exploring the nature particularly plants in search of new drugs. This has resulted in the use of large number of medicinal plants with curative properties

to treat various diseases.(Verpoorte, 1998). 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 (Duschatzky *et al* 2005). In the present study, rhizome extract of *P. kurroa* were tested against ten different species of field and storage fungi isolated from maize. Six fungi were completely inhibited and four fungi was moderately inhibited tested at 10 to 100 percent concentration of the rhizome extract. Hence the *P. kurroa* plant showed a promising result in inhibiting different species of fungi.

CONCLUSION: Form the above observation, it can be concluded that, a further research is necessary to isolate and identify the active principles and tested against different species of fungi of different crops and also against different species of bacteria.

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Table 1: Antifungal activity of aqueous extract of *Picrorhiza kurroa* (rhizome) against seed borne fungi of maize

Fungi	Mycelium Growth Inhibition (%)												
	Concentration of Aqueous Extract										MIC	Di-Thane M 45 2%	Thiram 2%
	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%			
Fusarium graminearum	5.0^{a} ±0.0	12.0^{b} ±0.0	17.0^{c} ±0.2	26.0^{d} ±0.0	48.0^{e} ±0.1	54.0^{f} ±0.0	71.0^{g} ±0.0	83.0^{h} ±0.1	92.0^{i} ±0.1	100.0^{j} ±0.0	100%	100.0^{j} ±0.0	100.0^{j} ±0.0
F. oxysporum	7.0^{a} ±0.0	15.0^{b} ±0.0	26.0^{c} ±0.0	32.0^{d} ±0.1	45.0^{e} ±0.0	57.0^{f} ±0.0	69.0^{g} ±0.1	78.0^{h} ±0.0	85.0^{i} ±0.0	93.0^{j} ±0.0	-	100.0^{h} ±0.0	100.0^{h} ±0.0
Aspergillus flavus	10.0^{a} ±0.1	28.0^{b} ±0.1	40.0^{c} ±0.0	57.0^{d} ±0.0	75.0^{e} ±0.0	83.0^{f} ±0.1	92.0^{g} ±0.0	100.0^{h} ±0.0	100.0^{h} ±0.0	100.0^{h} ±0.0	100%	100.0^{h} ±0.1	100.0^{h} ±0.1
A niger	8.0^{a} ±0.0	18.0^{b} ±0.0	29.0^{c} ±0.0	38.0^{d} ±0.1	47.0^{e} ±0.0	56.0^{f} ±0.0	66.0^{g} ±0.0	78.0^{h} ±0.1	86.0^{i} ±0.0	94.0^{j} ±0.0	-	100.0^{h} ±0.0	100.0^{h} ±0.2
A. terreus	5.0^{a} ±0.2	11.0^{b} ±0.0	18.0^{c} ±0.2	31.0^{d} ±0.0	48.0^{e} ±0.1	59.0^{f} ±0.0	81.0^{g} ±0.0	93.0^{h} ±0.0	100.0^{i} ±0.0	100.0^{i} ±0.0	100%	100.0^{j} ±0.2	100.0^{j} ±0.1
Penicillium sp.	3.0^{a} ±0.0	10.0^{b} ±0.2	21.0^{c} ±0.0	33.0^{d} ±0.1	47.0^{e} ±0.2	55.0^{f} ±0.1	64.0^{g} ±0.0	71.0^{h} ±0.0	80.0^{i} ±0.0	87.0^{j} ±0.0	-	100.0^{k} ±0.0	100.0^{k} ±0.0
Curvularia lunata	6.0^{a} ±0.0	15.0^{b} ±0.0	20.0^{c} ±0.0	36.0^{d} ±0.2	49.0^{e} ±0.0	60.0^{f} ±0.0	78.0^{g} ±0.1	86.0^{h} ±0.1	94.0^{i} ±0.1	100.0^{j} ±0.0	100%	100.0^{j} ±0.0	100.0^{j} ±0.0

Drechslera halodes	3.0[a] ±0.0	12.0[b] ±0.1	19.0[c] ±0.0	29.0[d] ±0.0	37.0[e] ±0.1	46.0[f] ±0.0	55.0[g] ±0.0	63.0[h] ±0.0	71.0[i] ±0.0	75.0[j] ±0.0	-	100.0[k] ±0.1	100.0[k] ±0.1
Alternaria alternata	6.0[a] ±0.0	17.0[b] ±0.0	27.0[c] ±0.0	38.0[d] ±0.2	50.0[e] ±0.1	63.0[f] ±0.0	78.0[g] ±0.0	89.0[h] ±0.0	96.0[i] ±0.0	100.0[j] ±0.0	100%	100.0[k] ±0.0	100.0[k] ±0.1
Cladosporium cladosporides	10.0[a] ±0.1	25.0[b] ±0.0	38.0[c] ±0.1	50.0[d] ±0.1	71.0[e] ±0.0	86.0[f] ±0.1	94.0[g] ±0.0	100.0[h] ±0.0	100.0[h] ±0.0	100.0[h] ±0.0	100%	100.0[i] ±0.1	100.0[i] ±0.0

- 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

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