Available on line www.jocpr.com

Journal of Chemical and Pharmaceutical Research

J. Chem. Pharm. Res., 2011, 3(4): 542-550

ISSN No: 0975-7384
CODEN(USA): JCPRC5

In vitro evaluation of antifungal activity of *Psoralea corylifolia* L. (seeds) and its different fractions on seed borne fungi of maize

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

[1]*PG Department of Biosciences, CMR Institute of Management Studies (Autonomous), Bangalore, Karnataka State, India*
[2]*Department of Studies in Botany and Microbiology, Maharanis Science College for Women, Palace Road, Bangalore, Karnataka State, India*
[3]*Department of Studies in Botany, Manasagangotri, University of Mysore, Mysore, Karnataka State, India*

ABSTRACT

Antifungal activity of five solvent extract viz., Petroleum ether , Benzene, chloroform, methanol and ethanol extract of seeds of Psoralea corylifolia tested at 0.5, 2.0 and 3.0% concentration against five Fusarium species viz., F. equiseti, F. graminearum, F. moniliforme, F. oxysporum, and F. solani was evaluated in vitro condition. Among five solvents tested, petroleum ether extract recorded a significant antifungal activity compared to other four solvent extract . The Fraction I, II and III of all the five solvent extracts of seeds of P corylifolia revealed that Fraction II which consists of three bands recorded significant antifungal activity against all the five Fusarium species tested at 250, 500 and 700ppm concentration.

Key words: *Psoralea coryifolia*, Antifungal, *Fusarium*, Solvent extract.

INTRODUCTION

Post harvest loss of grains are a significant factor in the worlds food supply. Estimates of total losses as high as 50% have been reported by some countries. In India 30% of agriculture production almost 15000 cores is lost due to storage diseases [1]. A smaller but quite significant proportion of the total losses by fungi and insects results from respiration and gradual deterioration of viability, nutritive quality and end use properties during storage under commercial conditions. Fungal deterioration of grains is a dynamic process that involves a

succession of microorganisms which produces different mycotoxins linked to increased incidence of cancer in human begins and loss of quality of grains[2]. To overcome these problems in agricultural field, the common procedure is to use synthetic pesticides. India ranks third in world in terms of pesticide consumption. These synthetic pesticides cause several side effects which include carcinogenecity, teratogenicity and residue toxicity [3]. To avoid synthetic pesticides and its effect on agriculture system, from the last decades medicinal plants are used as a novel source to avoid the use of synthetic fungicides to manage fungal contamination during storage. Herbal medicines represent one of the most important fields of traditional medicine all over the world. Medicinal plants represents a rich source of antimicrobial agents[4]. To promote the proper use of herbal medicine and to determine their potential as sources for new drugs, it is essential to study medicinal plants which have folklore reputation in a more intensified way [5]. Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanism of action contrary to synthetic drugs. Antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases [5]. Medicinal plants are widely used for treatment of diseases all over the world. According to world health organization report about 80% of the world population are taking interest in indigenous medicinal plants remedies[6]. Plants provide a rich source of novel biologically active compounds. Biological and chemical screenings are complementary approaches for the detection and isolation of interesting new plant constituents [7]. Plant metabolites were mainly investigated from a phytochemical and chemotaxonomic viewpoint. The interest in drugs of plant origin has been growing steadily over the last decade. Pure compounds are generally employed when the active principles of a medicinal plant exhibit strong, specific activity and have a small therapeutic index requiring accurate and reproducible dosage [8]. In the present investigation , different solvent extracts of *Psoralea corylifolia* L. (Seeds) were evaluated for antifungal activity and it was subjected for phytochemical analysis to identify the different fractions responsible for antifungal activity *in vitro* condition.

EXPERIMENTAL SECTION

Test Plant: 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, and used for preparation of extracts [9].

Solvent extraction: The dried seeds of *P. corylifolia* were powdered with the help of waring blender. 25grams of fine powder of *P. corylifolia* was filled in the thimble and extracted successively with petroleum ether, benzene, chloroform, methanol and ethanol for 48 hours. All the solvent extracts were concentrated using rotary flash evaporator under reduced pressure. The extracts were preserved in airtight brown bottle until further use [10, 11].

Test fungi: Five species of *Fusarium* viz., *F. equiseti, F. graminearum, F. moniliforme, F. oxysporum,* and *F. solani* isolated from maize seeds were used as test fungi for antifungal activity assay.

Antifungal activity assay by poisoned food technique

Solvent extract: One gram of each of the solvent extract was dissolved in 10ml of respective solvents, which served as the mother solvent extracts. Czapek Dox Agar(CDA) medium with

different concentration of each of the solvent extracts viz., 0.5%, 1.0% and 2.0% were prepared. CDA medium amended with the same concentrations of these respective solvents served as control. Five mm mycelial discs from the margins of seven day old cultures of *Fusarium* species were placed in the center of CDA medium. The plates were incubated at 25 ± 1^{o} C for seven days and ten replicates were maintained for each treatment. The percent inhibition of mycelial growth was determined by the formulae PI = C-T/CX100 Where C= Diameter of control colony, T=Diameter of treated colony [12,13]. The data were subjected to statistical analysis by ANOVA and Tukey's HSD.

Synthetic fungicide: Two synthetic chemicals viz., Dithane M-45 and Bavistin at 2 grams per liter of recommended dose were used for comparison against plant extract. CDA medium with 2 percent concentration of Dithane M-45 and Bavistin were prepared. Eighteen ml of CDA media with synthetic fungicide was poured into petri plates. Five mm mycelial discs from the margins of seven day old cultures of *Fusarium* species were placed in the center of the CDA medium. The inoculated plates were incubated at 25 ± 1^{o} C for seven days and ten replicates were maintained for each treatment. The percent inhibition of mycelial growth was determined by the formulae PI = C-T/CX100 Where C= Diameter of control colony, T=Diameter of treated colony [12,13]. The data were subjected to statistical analysis by ANOVA and Tukey's HSD.

Separation of different fractions by Thin Layer Chromatography (TLC):

***Preparation of TLC plates and separation of fractions*:** Five 20cm x 20cm glass plates were taken for coating with silica gel. Plates are thoroughly washed with detergent and water, rinse with distilled water and allow to drain. Plates were wiped with acetone soaked tissue to remove grease and dirt. Plates were mounted on plate spreader and plates were clamped to provide an even spreading surface. 25 grams of silica gel adsorbant was mixed with 60 to 70ml of distilled water. The gap of the TLC applicator was adjusted to 0.25mm using feeler gauge provided. The applicator was placed on the end. Silica gel slurry was poured into spreader and with a single constant motion, the slurry was drawn along the plates. After spreading, the plates were incubated at 110° to 120°C overnight and cooled in a desiccators before use. On thin layer plates, gently mark the intended positions of samples with a clean pointed glass rod at one horizontal edge of the plate. The obtained concentrated solvents of Petroleum ether, Benzene, Chloroform, Methanol and Ethanol were dissolved in 10 micro liter of their respective solvents and used for loading it into prepared TLC plates. All the loaded samples were eluted with methanol: chloroform extract In the ratio 9:1. After the elutent were run for more then $3/4^{th}$ of the TLC plates, the plates were removed and examined under normal, short wavelength UV (254 nm) and long wavelength UV (366 nm) light in UV chamber. The obtained bands were divided into three fractions and each bands consists of three bands. R_f value of each band were calculated using the formulae Distance moved by compound / Distance moved by solvent system and used further for antifungal activity[14].

Separation of different fractions: After obtaining different fractions and identification of R_f value, each fractions consists of three bands were divided and carefully the bands along with silica gel was scraped and transferred into beaker. The silica and bioactive compound was dissolved in chloroform and passed through Whatman No.1 filter paper. The collected filterate was evaporated and total yield was determined. The procedure was followed for all the fractions of different solvent.

Antifungal activity of different fractions: The obtained yield of different bands was dissolved in 1 Micro liter of chloroform and different concentrations viz., 250ppm, 500ppm and 750ppm were made. Czapek Dox Agar(CDA) medium with different concentration of each of the fractions were prepared. CDA medium amended with the same concentrations of these respective solvents served as control. Five mm mycelial discs from the margins of seven day old cultures of *Fusarium* species were placed in CDA medium. The plates were incubated at 25 ± 1^{o} C for seven days and ten replicates were maintained for each treatment. The percent inhibition of mycelial growth was determined by the formulae PI = C-T/CX100 Where C= Diameter of control colony, T=Diameter of treated colony [12,13]. The data were subjected to statistical analysis by ANOVA and Tukey's HSD.

RESULTS AND DISCUSSION

Antifungal activity assay by poisoned food technique

Solvent extract: Among the five solvents tested , petroleum ether extract recorded a maximum inhibition of all the test fungi at 0.5%, 1.0% and 2.0% concentration. *F. graminearum* was completely (100%) inhibited at 2.0% concentration and 89.3% at 0.5% concentration. *F.graminearum* is followed by *F.solani* and recorded 86.6% inhibition. *F.moniliforme* showed 85.1% inhibition , *F.oxysporum* recorded 78.4% inhibition and least inhibition of 74.5% was recorded in *F.equiseti* at 2.0% concentration(Table 1). In Benzene extract, *F.graminearum* recorded 90.10% inhibition at 2.0% concentration followed by *F.moniliforme*(82.40%), *F.solani*(80.73%), *F.equiseti*(80.20%) and *F.oxysporum*(76.0%) respectively(Table 1). In Chloroform extract, *F.solani* recorded a highest inhibition of 71.40% at 2.0% concentration tested. *F.oxysporum* recorded 70.86%, *F.moniliforme* recorded 70.70%, *F.graminearum* recorded 65.63% and *F.equiseti* recorded 59.30% inhibition (Table 1). In methanol extract, *F.moniliforme* recorded 84.10% inhibition followed by *F.solani*(80.20%), *F.oxysporum*(73.30%), *F.graminearum*(59.46%) and *F.equiseti*(41.93%) at 2.0% concentration tested(Table 2). Least inhibition was observed in Ethanol extract and recorded highest inhibition in *F.moniliforme*(85.40%) followed by *F.oxysporum*(82.76%), *F.solani*(77.20%), *F.graminearum*(60.0%) and *F.equiseti*(54.0%) at 2.0% concentration(Table 2). At 0.5% and 1.0% concentration tested, all the *Fusarium* species was moderately inhibited in all the solvents tested.

Synthetic fungicide: Compared to synthetic fungicide Dithane M-45 and Bavistin at 2.0% recommended dosage, *F.solani* recorded 93.1% and 100% inhibition in Dithane M-45 and Bavistin followed by *F.graminearum* (91.3 and 100%), *F.moniliforme*(90.0 and 100%), *F.oxysporum*(85.5 and 100%) and *F.equiseti*(80.2 and 100%) respectively(Table 1 and 2).

Separation of different fractions by Thin Layer Chromatography: Among the five solvent extracts , in each solvent, three bands were considered as one fractions and three fractions viz., Fraction I, Fraction II and Fraction III were isolated for further assay. In petroleum ether extract, Fraction I recorded a bands of R_f value 0.30, 0.32 and 0.35. Fraction II recorded 0.43, 045 and 0.47 R_f bands and Fraction III recorded 0.51, 0.53 and 0.56 R_f value bands. In benzene extract, Fraction I recorded 0.31, 0.33 and 037 R_f value bands, Fraction II (0.40, 0.43 and 0.49 R_f bands), Fraction III(0.51, 0.54 and 0.57 R_f bands). In chloroform extract , Fraction I (0.34, 0.39 and 0.40 R_f band), Fraction II (0.41, 0.43 and 0.46 R_f bands), Fraction III (0.51, 0.54 and 0.57 R_f bands).

In Methanol extract, Fraction I recorded 0.21, 0.23 and 0.30 R_f bands followed by Fraction III(0.31, 0.33 and 0.35 R_f bands)and Fraction III (0.31, 0.33 and 0.36 R_f bands) respectively(Table 3).

Antifungal activity of different fractions: Among the five solvent tested with three fractions, in petroleum ether extract, Fraction II recorded a maximum inhibition in all the test *Fusarium* species tested at 250, 500 and 750ppm concentration. At 750ppm concentration , *F.graminearum* recorded 100% inhibition followed by *F.solani*(96.3%), *F.equiseti*(90.8%), *F.oxysporum* (90.0%) and *F.moniliforme*(89.1%). Moderate activity was observed in fraction III against all the test *Fusarium* species and Least activity was observed in Fraction I(Table 4).

In Benzene extract, Fraction III recorded a moderate inhibition at 750ppm concentration against all the test *Fusarium* species tested. *F.equiseti* recorded 53.0% inhibition followed by *F.graminearum*(41.0%), *F.oxysporum*(39.6%), *F.moniliforme*(29.0%) and *F.solani*(26.6%) respectively. In Fraction I and Fraction III, no significant activity was observed (Table 5). In Chloroform extract, Frcation II recorded a maximum of 20.0% inhibition in *F.graminearum* and Least inhibition in *F. equiseti*(11.5%) tested at 750ppm concentration(Table 6). In Methanol extract, Fraction II recorded a mioderate activity . *F.solani* recorded 33.0%, *F.equiseti*(31.11%), *F.graminearum*(26.3%), *F.oxysporum*(26.0%) and *F.moniliforme*(23.3%) inhibition tested at 750ppm concentration (Table 7). In Ethanol extract, no activity was observed in all the three fractions isolated(Table 8).

Table 1: Antifungal activity of petroleum ether, benzene and chloroform extract of seeds of *P.corylifolia*

Fungi	Solvent extract									Dithane M-45	Bavistin
	Petroleum ether			Benzene			Chloroform				
	Concentration										
	0.5%	1.0%	2.0%	0.5%	1.0%	2.0%	0.5%	1.0%	2.0%	2.0%	2.0%
	Percent Inhibition (%)										
Fusarium equiseti	62.8^a ±0.7	73.7^b ±0.7	74.5^c ±0.2	68.00^c ±0.3	68.20^a ±0.2	80.20^b ±0.5	50.50^a ±0.5	57.30^b ±0.2	59.30^c ±0.5	80.2^a ±0.1	100^b ±0.0
F. graminearum	89.3^a ±0.3	89.7^b ±0.0	100^c ±0.2	71.30^c ±0.5	90.10^a ±0.1	90.10^b ±0.5	59.73^a ±0.2	60.63^b ±0.2	65.63^c ±0.1	91.3^a ±0.2	100^b ±0.1
F. moniliforme	79.1^a ±0.3	81.8^b ±0.3	85.1^c ±0.5	65.00^c ±0.0	80.80^a ±0.0	82.40^b ±0.3	67.50^a ±0.1	67.90^b ±0.2	70.70^c ±0.2	90.0^a ±0.0	100^b ±0.1
F. oxysporum	72.5^a ±0.4	75.4^b ±0.4	78.4^c ±0.2	63.50^c ±0.0	71.80^a ±0.0	76.00^b ±0.2	66.03^a ±0.1	68.43^b ±0.1	70.86^c ±0.1	85.5^a ±0.0	100^b ±0.2
F. solani	74.4^a ±0.5	84.5^b ±0.4	86.6^c ±0.4	70.30^c ±0.5	77.50^a ±0.0	80.73^b ±0.2	69.33^a ±0.1	70.56^b ±0.1	71.40^c ±0.1	93.1^a ±0.0	100^b ±0.2

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 percent Inhibition increase is not uniform for all the microorganisms.

Several pressures have accelerated for the search of more environmentally and toxicologically safe and more selective and efficacious pesticides. Most commercial successful pesticides have been discovered by screening compounds synthesized in the laboratory for pesticides properties. The number of compounds that must be screened to discover commercially viable pesticides has increased dramatically. The increasing incidence of pesticides resistance is also fueling the need to search for new pesticides. Furthermore, most synthetic chemicals that have been commercialized as pesticides are with relatively long environmental half-lives and more suspect toxicological properties than the natural compounds [15].

Table 2: Antifungal activity of methanol and ethanol extract of seeds of *P.corylifolia*

Fungi	Solvent extract						Dithane M-45	Bavistin
	Methanol			Ethanol				
	Concentration							
	0.5%	1.0%	2.0%	0.5%	1.0%	2.0%	2.0%	2.0%
	Percent Inhibition (%)							
Fusarium equiseti	27.03^{a} ±0.1	37.30^{b} ±1.0	41.93^{c} ±0.3	45.16^{a} ±0.1	53.23^{b} ±0.3	54.00^{c} ±0.1	80.2^{a} ±0.1	100^{b} ±0.0
F. graminearum	48.56^{a} ±0.0	59.10^{b} ±0.1	59.46^{c} ±0.2	71.6^{a} ±0.2	58.46^{b} ±0.2	60.00^{c} ±0.1	91.3^{a} ±0.2	100^{b} ±0.1
F. moniliforme	76.10^{a} ±0.0	77.26^{b} ±0.6	84.10^{c} ±0.0	76.70^{a} ±0.1	79.10^{b} ±0.2	85.40^{c} ±0.1	90.0^{a} ±0.0	100^{b} ±0.1
F. oxysporum	65.83^{a} ±0.1	67.70^{b} ±0.5	73.30^{c} ±0.0	73.76^{a} ± 0.1	74.20^{b} ±0.3	82.76^{c} ±0.0	85.5^{a} ±0.0	100^{b} ±0.2
F. solani	59.60^{a} ±0.3	64.40^{b} ±0.4	80.20^{c} ±0.2	69.00^{a} ±0.1	73.70^{b} ±0.1	77.20^{c} ±0.0	93.1^{a} ±0.0	100^{b} ±0.2

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 percent Inhibition increase is not uniform for all the microorganisms.

Table 3: Separation of fractions and determination of R_f value of different solvent extracts of seeds of *P.corylifolia*

Fractions	**Petroleum ether extract**	**Benzene extract**	**Chloroform extract**	**Methanol extract**	**Ethanol extract**
	R_f value				
Fraction I	0.30	0.31	0.34	0.21	0.18
	0.32	0.33	0.39	0.23	0.21
	0.35	0.37	0.40	0.30	0.22
Fraction II	0.43	0.40	0.41	0.31	0.25
	0.45	0.43	0.43	0.33	0.27
	0.47	0.49	0.46	0.35	0.30
Fraction III	0.51	0.52	0.51	0.39	0.31
	0.53	0.54	0.54	0.41	0.33
	0.56	0.59	0.57	0.43	0.36

Table 4: Antifungal activity of different fractions of petroleum ether extract of seeds of *P.corylifolia*

Fungi	Petroleum ether extract									Dithane M-45	Bavistin
	Fraction I			Fraction II			Fraction III				
	Concentration										
	250 ppm	500 ppm	750 ppm	250 ppm	500 ppm	750 ppm	250 ppm	500 ppm	750 ppm	2000 ppm	2000 ppm
Fusarium equiseti	30.1^{a} ±0.1	43.6^{b} ±0.0	50.1^{c} ±0.0	70.3^{a} ±0.1	80.1^{b} ±0.0	90.8^{c} ±0.0	50.1^{a} ±0.0	56.3^{b} ±0.0	60.0^{c} ±0.0	80.2^{a} ±0.1	100^{b} ±0.0
F. graminearum	56.3^{a} ±0.2	60.8^{b} ±0.1	69.1^{c} ±0.0	80.5^{a} ±0.1	91.1^{b} ±0.0	100^{c} ±0.1	61.6^{a} ±0.0	65.5^{b} ±0.0	72.1^{c} ±0.0	91.3^{a} ±0.2	100^{b} ±0.1
F. moniliforme	45.5^{a} ±0.2	50.0^{b} ±0.0	63.1^{c} ±0.1	69.1^{a} ±0.0	78.3^{b} ±0.1	89.1^{c} ±0.2	50.0^{a} ±0.1	55.0^{b} ±0.1	60.0^{c} ±0.2	90.0^{a} ±0.0	100^{b} ±0.1
F. oxysporum	50.1^{a} ±0.1	58.3^{b} ±0.0	67.8^{c} ±0.0	80.1^{a} ±0.0	86.3^{b} ±0.1	90.0^{c} ±0.1	65.0^{a} ±0.1	69.3^{b} ±0.2	73.1^{c} ±0.1	85.5^{a} ±0.0	100^{b} ±0.2
F. solani	52.3^{a} ±0.1	60.8^{b} ±0.0	65.6^{c} ±0.1	83.3^{a} ±0.0	90.0^{b} ±0.0	96.3^{c} ±0.0	68.0^{a} ±0.0	71.3^{b} ±0.0	79.8^{c} ±0.2	93.1^{a} ±0.0	100^{b} ±0.2

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 percent Inhibition increase is not uniform for all the microorganisms

Tens of thousands of secondary products of plants have been identified and there are estimates that hundreds of thousands of these compounds exist. There is growing evidence that most of

these compounds are involved in the interaction of plants with other species primarily the defense of the plant from plant pests. Thus, secondary compounds represent a large reservoir of chemical structures with biological activity. This resource is largely untapped for use of pesticides [15]. In the present investigation it was identified that, petroleum ether extract is a potent solvent which showed highest antifungal activity and moderate activity was observed in benzene and chloroform extract. In different fractions isolated, Fraction II showed a maximum and complete inhibition among all the test *Fusarium* species tested. From the result, it can be concluded that the bioactive compound was present in Fraction II which consists of 0.43, 0.45 and 0.47 R_f bands. Further isolation , identification ,purification and characterization of bioactive compound is needed

Table 5: Antifungal activity of different fractions of Benzene extract of seeds of *P.corylifolia*

Fungi	Benzene									Dithane M-45	Bavistin
	Fraction I			Fraction II			Fraction III				
	Concentration										
	250 ppm	500 ppm	750 ppm	250 ppm	500 ppm	750 ppm	250 ppm	500 ppm	750 ppm	2000 ppm	2000 ppm
Fusarium equiseti	10.0^a ±0.0	15.6^b ±0.0	20.1^c ±0.0	30.1^a ±0.0	43.1^b ±0.0	53.1^c ±0.0	5.0^a ±0.0	7.3^b ±0.0	11.3^c ±0.0	80.2^a ±0.1	100^b ±0.0
F. graminearum	-	-	-	25.5^a ±0.0	36.3^b ±0.0	41.0^c ±0.2	8.9^a ±0.1	10.5^b ±0.1	15.0^c ±0.0	91.3^a ±0.2	100^b ±0.1
F. moniliforme	5.0^a ±0.1	8.2^b ±0.0	10.1^c ±0.0	18.0^a ±0.0	23.6^b ±0.1	29.0^c ±0.0	10.3^a ±0.0	14.7^b ±0.0	18.3^c ±0.0	90.0^a ±0.0	100^b ±0.1
F. oxysporum	15.3^a ±0.2	19.0^b ±0.0	22.5^c ±0.1	21.1^a ±0.2	30.0^b ±0.1	39.6^c ±0.1	5.0^a ±0.1	8.0^b ±0.0	10.0^c ±0.2	85.5^a ±0.0	100^b ±0.2
F. solani	10.1^a ±0.0	14.8^b ±0.1	17.8^c ±0.1	18.0^a ±0.0	23.1^b ±0.0	26.6^c ±0.0	2.0^a ±0.2	6.0^b ±0.0	9.0^c ±0.1	93.1^a ±0.0	100^b ±0.2

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 percent Inhibition increase is not uniform for all the microorganisms.

Table 6: Antifungal activity of different fractions of Chloroform extract of seeds of *P.corylifolia*

Fungi	Chloroform									Dithane M-45	Bavistin
	Fraction I			Fraction II			Fraction III				
	Concentration										
	250 ppm	500 ppm	750 ppm	250 ppm	500 ppm	750 ppm	250 ppm	500 ppm	750 ppm	2000 ppm	2000 ppm
Fusarium equiseti	1.0^a ±0.0	3.5^b ±0.0	7.1^c ±0.0	6.1^a ±0.1	7.3^b ±0.0	11.5^c ±0.0	-	-	-	80.2^a ±0.1	100^b ±0.0
F. graminearum	8.9^a ±0.0	11.1^b ±0.1	15.2^c ±0.0	11.3^a ±0.0	16.3^b ±0.0	20.1^c ±0.1	-	-	-	91.3^a ±0.2	100^b ±0.1
F. moniliforme	7.0^a ±0.0	10.6^b ±0.0	16.1^c ±0.0	10.3^a ±0.1	14.1^b ±0.0	19.3^c ±0.1	-	-	-	90.0^a ±0.0	100^b ±0.1
F. oxysporum	3.0^a ±0.1	5.8^b ±0.0	6.1^c ±0.0	6.3^a ±0.0	9.1^b ±0.0	17.3^c ±0.1	-	-	-	85.5^a ±0.0	100^b ±0.2
F. solani	4.5^a ±0.0	8.6^b ±0.0	12.5^c ±0.1	6.0^a ±0.0	10.0^b ±0.0	15.0^c ±0.0	-	-	-	93.1^a ±0.0	100^b ±0.2

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 percent Inhibition increase is not uniform for all the microorganisms.

Table 7: Antifungal activity of different fractions of Methanol extract of seeds of *P.corylifolia*

Fungi	Methanol									Dithane M-45	Bavistin
	Fraction I			Fraction II			Fraction III				
	Concentration										
	250 ppm	500 ppm	750 ppm	250 ppm	500 ppm	750 ppm	250 ppm	500 ppm	750 ppm	2000 ppm	2000 ppm
Fusarium equiseti	-	-	-	15.8^{a} ±0.0	22.3^{b} ±0.0	31.1^{c} ±0.0	-	-	-	80.2^{a} ±0.1	100^{b} ±0.0
F. graminearum	-	-	-	10.8^{a} ±0.1	17.0^{b} ±0.0	26.3^{c} ±0.1	-	-	-	91.3^{a} ±0.2	100^{b} ±0.1
F. moniliforme	-	-	-	9.0^{a} ±0.0	16.0^{b} ±0.1	23.3^{c} ±0.0	-	-	-	90.0^{a} ±0.0	100^{b} ±0.1
F. oxysporum	-	-	-	8.5^{a} ±0.1	19.1^{b} ±0.0	26.0^{c} ±0.1	-	-	-	85.5^{a} ±0.0	100^{b} ±0.2
F. solani	-	-	-	18.3^{a} ±0.2	26.6^{b} ±0.2	33.0^{c} ±0.0	-	-	-	93.1^{a} ±0.0	100^{b} ±0.2

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 percent Inhibition increase is not uniform for all the microorganisms.

Table 8: Antifungal activity of different fractions of Ethanol extract of seeds of *P.corylifolia*

Fungi	Ethanol									Dithane M-45	Bavistin
	Fraction I			Fraction II			Fraction III				
	Concentration										
	250 ppm	500 ppm	750 ppm	250 ppm	500 ppm	750 ppm	250 ppm	500 ppm	750 ppm	2000 ppm	2000 ppm
Fusarium equiseti	-	-	-	-	-	-	-	-	-	80.2^{a} ±0.1	100^{b} ±0.0
F. graminearum	-	-	-	-	-	-	-	-	-	91.3^{a} ±0.2	100^{b} ±0.1
F. moniliforme	-	-	-	-	-	-	-	-	-	90.0^{a} ±0.0	100^{b} ±0.1
F. oxysporum	-	-	-	-	-	-	-	-	-	85.5^{a} ±0.0	100^{b} ±0.2
F. solani	-	-	-	-	-	-	-	-	-	93.1^{a} ±0.0	100^{b} ±0.2

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 percent Inhibition increase is not uniform for all the microorganisms.

Acknowledgement

The authors are thankful to the Department of Studies in Botany and Department of Studies in Microbiology, University of Mysore, Mysore and Department of Studies in Botany and Microbiology, Maharanis science college for women, Palace road, Bangalore, and CMR institute of Management Studies(Autonomous), PG Department of Biosciences, Kalyan Nagar, Bangalore for providing facilities

REFERENCES

[1] SC Mathur; SK Tannan. *Pesticides information.*, **1999**,24 (4) , 9-18.
[2] BJ Jacobsen;KL Bowen;RA Shelby; VL Diener; BW Kemppainen. *Alabama*., **2000**, 2-12.
[3] RR Dalvi;MB Whittiker. *Journal of Environmental Biology.*, **1995** ,16, 333-338.
[4] S Balakumar; S Rajan ;T Thirunalasundari ;S Jeeva. *Asian Pacific Journal of Tropical Biomedicine* .,**2011**,309-312.
[5] K Rajandeep; K Harpreet. *Archives of Applied Science Research.*,**2010**, 2 (1), 302-309.

[6] AJ Pirzada; W Shaikh; KU Ghani; KA Laghari. *Sindh Univ. Res. Jour. (Sci. Ser.).,* **2009**, 41 (2),15-20.
[7] K Hostettmann; JL Wolfender. *Pesticide science.,* **1997**,51, 471-482.
[8] M Hamburger;K Hostettmann. *Phytochemistry.,* **1991**,30(12), 3864-3874.
[9] V Lalitha; B Kiran; KA Raveesha, *Journal of Phytology.,* **2011**, 3(5),4-8.
[10] A Nostro; MP Germano; VD Angelo;A Marino; MA Cannatelli. *Planta Medica.,* **2000**,25, 20-24.
[11] D Srinivasan;S Nathan;T Suresh P Lakshmanaperumalsamy *Asian . J. of microbiology. Biotechnology and Env.Sc.*, **2001**,3(1-2), 67-70.
[12] CMF Pinto;LA Maffia;VWD Casali; AA Cardoso. *J. phytopathology* **1998**, 146, 421-425.
[13] RR Bansal; RK Guptha. *Indian Phytopathology.*, **2000**, 53(1), 107-108.
[14] S Sadasivan; A Manickam. Biochemical methods. 3rd edition, New Age International Publications, New Delhi, **2003**, 87-88.
[15] SO Duke. Natural Pesticides from plants In : J. Janick and J. E. Simon (eds) Advances in new crops : Timber press, Portland, OR **1990**: 511-517.