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Screening of Different Solvent Extracts for Antifungal Activity of Seeds of *Psoralea corylifolia* L. against Important Seed borne *Aspergillus* species of Maize

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ABSTRACT
Petroleum ether extract of seeds of *Psoralea corylifolia* was recorded a maximum antifungal activity in *Aspergillus flavus oryzae* (93.5%) and *A. tamarii* (92.43%); Benzene extract recorded a maximum inhibition in *A. niger* (88.10%), *A. flavus oryzae* (83.80%) and *A. tamarii* (81.10%). In chloroform extract *A. tamarii* recorded 74.60% and *A. niger* showed 74.16% inhibition. In methanol extract *A. tamarii* and *A. flavus oryzae* recorded 69.50% and in ethanol extract *A. flavus oryzae* showed 74.63% and *A. flavus* recorded 67.10% inhibition in 2.0% concentration of extract. Compared to synthetic fungicides Captan and Thiram, Captan recorded 85.0% to 95.0% inhibition and Thiram showed 100% inhibition against all the test fungi. Among the three different fractions I, II and III, Fraction II showed a significant activity against all test fungi compared to fraction I and fraction III.

Keywords: *P. corylifolia*, *Aspergillus*, Solvent extract, Antifungal activity.

INTRODUCTION

Seed borne pathogens play an important role in biodeterioration of seeds during storage. 30% of the potential food production valued at Rs. 15,000 crore per annum is lost in India due to insect's pests, plant pathogens, weeds, rodents, birds and in storage. [1] To manage biodeterioration causing fungi, the regular practice in agriculture is to use 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 problem. There is an urgent need to search for alternative strategies for the management of pre and post harvest crop diseases. Medicinal plants are the local heritage with global importance and world is endowed with a rich wealth of medicinal plants. [2] 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 is taking interest in indigenous medicinal plants remedies. [3] Medicinal plants are considerably useful and economically essential. They contain active constituents that are used in the treatment of many human diseases. [4] Plants contain hundreds or thousands of metabolites. Medicinal and aromatic plants a gift of nature are being used against various infections and diseases in the world since past history. [5] Among the estimated 2, 50, 000 to 5, 00, 000 plants species, only a small percentage has been investigated phytochemically and the fraction submitted to biological screening is even smaller. Plant kingdom represents an extraordinary reservoir of novel molecules. Plant derived products have been used for medicinal purposes for centuries. [6] In the present investigation, different solvent extracts of *P. 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.

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MATERIALS AND METHODS

Test Plant: Seeds of *P. corylifolia* were collected from the seed market, Mysore. The seeds were washed thoroughly two to three times with running tap water and once with sterile distilled water, air dried and further used for the preparation of extracts.

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

Fungi	Solvent extract									Captan	Thiram
	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 (%)										
Aspergillus flavus	68.80^{a}±0.0	73.07^{b}±0.9	79.03^{c}±0.0	44.90^{a}±0.0	59.70^{b}±0.2	70.40^{c}±0.4	28.90^{a}±0.0	44.30^{b}±0.0	55.56^{c}±0.0	95.0^{a}±0.1	100^{b}±0.1
A. niger	72.70^{a}±0.0	84.06^{b}±0.2	87.10^{c}±0.3	68.40^{a} ±0.1	85.60^{b}±0.8	88.10^{c}±0.6	63.06^{a}±0.0	68.40^{b}±0.6	74.16^{c}±0.0	91.2^{a}±0.2	100^{b}±0.1
A. terreus	63.60^{a}±0.5	68.90^{b}±0.0	77.80^{c}±0.3	64.30^{a} ±0.0	73.90^{b}±0.5	76.70^{c}±0.5	55.50^{a}±0.0	62.93^{b}±0.5	66.13^{c}±0.2	85.3^{a}±0.0	100^{b}±0.1
A. tamarii	85.30^{a}±0.0	88.68^{b}±0.5	92.43^{c}±0.6	74.10^{a} ±0.2	79.80^{b}±0.1	81.10^{c}±0.3	71.80^{a}±0.0	69.30^{b}±0.0	74.60^{c}±0.0	87.1^{a}±0.1	100^{b}±0.1
A. flavus oryzae	87.86^{a}±0.6	87.86^{b}±0.3	93.50^{c}±0.1	70.70^{a} ±0.0	77.10^{b}±0.9	83.80^{c}±0.5	66.86^{a}±0.1	69.56^{b}±0.5	73.60^{c}±0.0	92.3^{a}±0.1	100^{b}±0.2

- Values are the mean of five 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 Methanol and Ethanol extract of seeds of *P. corylifolia*

Fungi	Solvent extract						Captan	Thiram
	Methanol			Ethanol				
	Concentration							
	0.5%	1.0%	2.0%	0.5%	1.0%	2.0%	2.0%	2.0%
	Percent Inhibition (%)							
Aspergillus flavus	47.00^{a}±0.2	60.33^{b}±0.9	65.13^{c}±0.0	50.70^{a} ±0.0	58.20^{b} ±0.2	67.10^{c} ±0.0	95.0^{a} ±0.1	100^{b}±0.1
A. niger	59.73^{a}±0.5	68.46^{b}±0.9	75.50^{c}±0.2	22.36^{a} ±0.2	47.50^{b} ±0.4	50.80^{c} ±0.0	91.2^{a}±0.2	100^{b}±0.1
A. terreus	58.46^{a}±0.5	62.03^{b}±0.0	70.60^{c}±0.3	33.70^{a} ±0.0	44.30^{b} ±0.6	47.80^{c} ±0.0	85.3^{a}±0.0	100^{b}±0.1
A. tamari	66.46^{a}±0.6	69.53^{b}±0.3	78.36^{c}±0.5	59.20^{a} ±0.0	66.00^{b} ±0.0	46.53^{c} ±0.0	87.1^{a} ±0.1	100^{b}±0.1
A. flavus oryzae	66.46^{a}±0.2	69.50^{b}±0.5	78.36^{c}±0.6	58.03^{a} ±0.5	64.60^{b} ±0.3	74.63^{c} ±0.0	92.3^{a}±0.1	100^{b}±0.2

- Values are the mean of five 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 3: Separation of fractions and determination of R_f value of different solvents 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

Solvent extraction: The dried seeds of *P. corylifolia* were powdered. 25grams of fine powder of seeds 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 .The extracts were preserved in airtight brown bottle until further use. [7]

Test Fungi: Five species of *Aspergillus* viz., *A. flavus, A. niger, A. terreus, A. tamarii* and *A. flavus oryzae* isolated from maize seeds employing standard blotter method. [8] All the fungi were identified using standard manual and pure cultures were preserved in lower temperature for further use.

Antifungal activity assay by poisoned food technique

Solvent extract: One gram of each of the solvent extract was dissolved in 1ml of Dimethyl sulfoxide (DMSO) solvents, 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 *Aspergillus* species were placed in the center of medium. The plates were incubated at 36 ±1°C for seven days and five replicates were maintained for each treatment. The percent inhibition of mycelial growth was determined by the formulae PI = C-T/C×100, where C= Diameter of control colony, T=Diameter of treated colony. [9] The same procedure was followed for two synthetic fungicides Captan and Thiram at 2.0% standard recommended dosage. All the data obtained were subjected for statistical analysis.

Separation of different fractions by Thin Layer Chromatography (TLC)

Preparation of TLC plates and separation of fractions: Five 20cm × 20cm glass plates were taken for coating with silica gel. Plates are thoroughly washed with detergent and water, rinsed with distilled water and allow draining. Plates were wiped with acetone soaked tissue paper 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 adsorbent was mixed with 60 to 70 ml of distilled water. The gap of the TLC applicator was adjusted to 0.25 mm 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°C to 120°C overnight and cooled in 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 liters 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 3/4th 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 band 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. [10]

Separation of different fractions: After obtaining different fractions, the R_f value was identified and carefully each bands were scraped and dissolved in chloroform and passed through Whatman No.1 filter paper and the collected filtrate was subjected for evaporation. After complete evaporation the obtained bioactive compound was collected and yield was calculated. The same procedure was followed for all the solvents.

Antifungal activity of different fractions: The bioactive compound after collection was dissolved in 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 with the same concentrations of these respective solvents served as control. Five mm mycelial discs from the margins of seven day old cultures of Aspergillus species were placed in CDA medium. The plates were incubated at 35±1°C for seven days and five replicates were maintained for each treatment. The percent inhibition of mycelial growth was determined by the formulae PI = C-T/C×100, where C = Diameter of control colony, T=Diameter of treated colony. [11] The data were subjected to statistical analysis by ANOVA and Tukey's HSD.

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

Fungi	Petroleum ether extract									Captan	Thiram
	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	2000ppm
Aspergillus flavus	30.1^{a} ±0.1	40.8^{b}±0.0	46.7^{c}±0.1	86.3^{a}±0.1	96.0^{b}±0.1	100^{c} ±0.1	45.5^{a}±0.0	49.6^{b}±0.0	53.1^{c}±0.1	95.0^{a} ±0.1	100^{b}±0.1
A. niger	38.0^{a}±0.1	46.5^{b}±0.1	51.7^{c}±0.1	75.5^{a}±0.0	81.1^{b}±0.1	85.5^{c}±0.2	52.1^{a}±0.1	58.8^{b}±0.0	65.1^{c}±0.1	91.2^{a}±0.2	100^{b}±0.1
A. terreus	43.3^{a}±0.0	51.6^{b}±0.1	58.0^{c}±0.0	65.5^{a}±0.0	70.1^{b} ±0.0	76.6^{c}±0.1	56.0^{a}±0.0	65.1^{b}±0.1	70.0^{c}±0.1	85.3^{a} ±0.0	100^{b}±0.1
A. tamarii	40.0^{a}±0.0	47.6^{b}±0.3	53.3^{c}±0.1	78.0^{a}±0.0	83.0^{b}±0.1	89.1^{c}±0.1	45.1^{a}±0.0	50.6^{b}±0.0	57.1^{c}±0.1	87.1^{a} ±0.1	100^{b}±0.1
A. flavus oryzae	50.0^{a}±0.0	56.1^{b}±0.1	63.8^{c}±0.0	80.0^{a}±0.1	83.3^{b}±0.0	86.8^{c}±0.0	56.0^{a}±0.0	63.7^{b}±0.0	70.0^{c}±0.0	92.3^{a} ±0.1	100^{b}±0.2

- Values are the mean of five 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 5: Antifungal activity of different fractions of Benzene extract of seeds of *P. corylifolia*

Fungi	Benzene									Captan	Thiram
	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	2000ppm
Aspergillus flavus	5.0^{a}±0.0	8.0^{b}±0.0	10.0^{c}±0.0	12.5^{a}±0.1	17.1^{b}±0.0	22.5^{c}±0.1	4.5^{a}±0.0	6.6^{b}±0.1	8.5^{c}±0.0	95.0^{a}±0.1	100^{b}±0.1
A. niger	-	2.0^{b}±0.1	5.0^{c}±0.0	6.5^{a}±0.1	12.0^{b}±0.1	14.6^{c}±0.0	-	-	-	91.2^{a}±0.2	100^{b}±0.1
A. terreus	-	-	-	5.0^{a}±02	8.0^{b}±0.0	11.6^{c}±0.0	-	-	-	85.3^{a}±0.0	100^{b}±0.1
A. tamari	-	-	-	12.1^{a}±0.1	16.5^{b}±0.1	24.5^{c}±0.1	-	-	-	87.1^{a}±0.1	100^{b}±0.1
A. flavus oryzae	-	-	-	15.0^{a}±0.1	22.6^{b}±0.0	30.6^{c}±0.0	-	-	-	92.3^{a}±0.1	100^{b}±0.2

- Values are the mean of five 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 6: Antifungal activity of different fractions of Chloroform extract of seeds of *P. corylifolia*

Fungi	Chloroform									Captan	Thiram
	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
Aspergillus flavus	8.3^{a}±0.0	12.6^{b}±0.1	16.5^{c}±0.0	11.3^{a}±0.0	13.2^{b}±0.0	21.8^{c}±0.2	-	-	-	95.0^{a}±0.1	100^{b}±0.1
A. niger	6.0^{a}±0.0	9.0^{b}±0.0	11.1^{c}±0.0	9.0^{a}±0.0	13.0^{b}±0.0	18.0^{c}±0.1	-	-	-	100^{b}±0.2	100^{b}±0.1
A. terreus	4.5^{a}±0.1	6.6^{b}±0.0	9.0^{c}±0.0	6.3^{a}±0.0	10.0^{b}±0.0	14.8^{c}±0.0	-	-	-	100^{b}±0.0	100^{b}±0.1
A. tamarii	-	-	-	-	-	-	-	-	-	100^{b}±0.1	100^{b}±0.1
A. flavus oryzae	-	-	-	-	-	-	-	-	-	95.0^{a}±0.1	100^{b}±0.2

- Values are the mean of five 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

RESULTS

Antifungal activity assay by poisoned food technique

Solvent extract: Among the five solvents tested, petroleum ether extract recorded a maximum inhibition of 93.50% in *A. flavus oryzae* followed by *A. tamarii* (92.43%), *A. niger* (87.10%), *A. flavus* (79.03%) and *A. terreus* (77.80%) at 2.0% concentration tested. Significant activity was also observed in 0.5% and 1.0% concentration tested in all the test fungi. In benzene extract, maximum inhibition was observed in *A. niger* (88.10%) followed by *A. flavus oryzae* (83.80%) and *A. tamarii* (81.10%). Moderate activity was observed in *A. terreus* and recorded an inhibition of 76.70% and 70.40% in *A. flavus* at 20% concentration tested. In chloroform extract, maximum inhibition was observed in *A. tamarii* (74.60%) followed by *A. niger* (74.16%), *A. flavus oryzae* (73.60%), *A. terreus* (66.13%) and *A. flavus* (55.56%) respectively at 2.0% concentration. In methanol extract, significant activity was observed in *A. tamarii* and *A. flavus*

oryzae which recorded 59.20% and 59.20% inhibition and least activity was observed in *A. flavus* which showed 65.13% inhibition at 2.0% concentration tested. In ethanol extract, highest activity was observed in *A. flavus oryzae* (74.63%) and least activity was observed in *A. tamarii* which recorded 46.53% inhibition at 2.0% concentration.

Compared to synthetic fungicides Captan and Thiram, Captan recorded 95.0% inhibition in *A.flavus* followed by *A. flavus oryzae* (92.3%), *A. niger* (91.2%), *A. tamarii* (87.1%) and *A. terreus* (85.3%) respectively at 2.0% recommended concentration. At 2.0% concentration, thiram recorded a complete inhibition (100%) against all the test fungi (Table 1 and 2).

Separation of different fractions by Thin Layer Chromatography*:* Among all the five solvent extracts tested, three bands were considered as one fraction and three fractions viz., Fraction I, Fraction II and Fraction III were isolated and used 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 recorded 0.40, 0.43 and 0.49 R_f bands, Fraction III recorded 0.51, 0.54 and 0.57 R_f bands. In chloroform extract, Fraction I recorded 0.34, 0.39 and 0.40 R_f band, Fraction II recorded 0.41, 0.43 and 0.46 R_f bands, Fraction III recorded 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 solvents tested and three fractions isolated, in all the solvent tested at 250, 500 and 750ppm concentration, fraction II recorded a maximum antifungal activity. Complete inhibition (100%) was observed in *A. flavus* followed by *A. tamarii* (89.1%), *A. flavus oryzae* (86.8%), *A. niger* (85.5%) and *A. terreus* (76.6%) respectively at 750ppm concentration. Significant activity was also observed in 250ppm and 500ppm concentration. In fraction III, maximum activity was observed in *A. flavus oryzae* and *A.terreus* which recorded 70.0% and 70.0% inhibition. Least activity was observed in *A. flavus* which showed 53.1% inhibition at 750ppm concentration (Table 4).

In benzene extract, fraction II recorded a maximum inhibition in *A. flavus* and *recorded* 30.6% inhibition followed by *A. tamarii* (24.5%), *A flavus* (22.5%). *A. niger* (14.6%) and *A. terreus* (11.6%) respectively at 750ppm concentration. In fraction I, least activity was observed in *A. flavus* (10.0%) and in fraction II, 8.5% inhibition was observed in *A. flavus*. No antifungal activity was observed in *A. terreus*, *A. tamarii*, *A. niger* and *A. flavus oryzae* in fraction I and II (Table 5). In chloroform extract, fraction II recorded moderate activity and showed 21.8% inhibition in *A.flavus,* followed by *A. niger* (18.0%) and *A. terreus* (14.8%) at 750ppm concentration. No activity was observed in *A. tamarii* and *A. flavus oryzae* in all the three fractions (Table 6). In methanol extract, fraction II recorded highest inhibition in *A. niger* (27.3%) and least activity was observed in *A. flavus oryzae* (19.0%) at 750ppm concentration tested. No activity was observed in fraction I and II (Table 7). In ethanol extract antimicrobial activity was not observed in all the three fractions tested (Table 8).

DISCUSSION

Development of synthetic products to control plant diseases has become difficult because of strict requirements of their efficacy, selectivity, toxicology and general impact on the environment. Consequently, there is an increasing interest in evaluating other mechanisms of control including the effects of plant metabolites on plant pathogens. [9] Several higher plants and their constituents have shown success in plant disease control and proved to be harmless and non phytotoxic unlike chemical fungicides. [12] Indiscriminate use of chemical not only hazardous to living beings but adversely affects the microbioal population present in the ecosystem. [13]

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

Fungi	Methanol									Captan	Thiram
	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
Aspergillus flavus	-	-	-	9.0^{a}±0.0	14.3^{b}±0.1	21.1^{c}±0.0	-	-	-	95.0^{a}±0.1	100^{b}±0.1
A. niger	-	-	-	11.1^{a}±0.1	19.8^{b}±0.2	27.3^{c}±0.0	-	-	-	100^{b}±0.2	100^{b}±0.1
A. terreus	-	-	-	12.0^{a}±0.2	18.0^{b}±0.0	23.0^{c}±0.0	-	-	-	100^{b}±0.0	100^{b}±0.1
A. tamarii	-	-	-	8.0^{a}±0.1	14.0^{b}±0.0	21.1^{c}±0.0	-	-	-	100^{b}±0.1	100^{b}±0.1
A. flavus oryzae	-	-	-	6.0^{a}±0.0	15.5^{b}±0.2	19.0^{c}±0.0	-	-	-	95.0^{a}±0.1	100^{b}±0.2

- Values are the mean of five 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 8: Antifungal activity of different fractions of Ethanol extract of seeds of *P. corylifolia*

Fungi	Ethanol									Captan	Thiram
	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
Aspergillus flavus	-	-	-	-	-	-	-	-	-	95.0^{a}±0.1	100^{b}±0.1
A. niger	-	-	-	-	-	-	-	-	-	100^{b}±0.2	100^{b}±0.1
A. terreus	-	-	-	-	-	-	-	-	-	100^{b}±0.0	100^{b}±0.1
A. tamarii	-	-	-	-	-	-	-	-	-	100^{b}±0.1	100^{b}±0.1
A. flavus oryzae	-	-	-	-	-	-	-	-	-	95.0^{a}±0.1	100^{b}±0.2

- Values are the mean of five 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

Alternative to this effect, to control plant diseases, plant products are gaining prominence as fungicides and bacteriocides. [14] Antifungal compounds from higher plants are advantageous over synthetic fungicides due to their easily biodegradable nature. [15] In the present investigation, among the five solvent extracts, petroleum ether extract showed a maximum antifungal activity against all the test fungi. When all the solvents were subjected to TLC for isolation of bioactive compound, petroleum ether extract fraction II showed a maximum antifungal activity compared to fraction I and III. In benzene extract, chloroform extract and methanol extract, fraction II recorded significant activity against all the test fungi. In ethanol extract, no activity was observed against all the test fungi. From the result, maximum antifungal activity was observed in 2.0% concentration in solvent extract and in different fractions maximum activity was observed in 750ppm concentration compared to synthetic fungicide Captan and Thiram at recommended dosage of 2.0% concentration. Hence it can be concluded that, Seeds of *P. corylifolia* is a potent plant against seed borne fungi of maize and further work is necessary to isolate the bioactive compound which is an eco friendly approach.

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