Research Article ISSN:2321-2624

International Journal of Medicine and Pharmaceutical Research
www.pharmaresearchlibrary.com/ijmpr

In vitro evaluation of antifungal, seed germination and seedling vigour of aqueous seed extract of *Psoralea corylifolia* L. against maize seeds

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

[1]*Department of Biosciences CMR Institute of Management Studies (Autonomous), Kalyana Nagar, Bangalore -560043, Karnataka State, India*
[2]*Department of Studies in Botany and Microbiology, Maharanis Science College for Women, Palace Road, Bangalore-560001*
[3]*Department of Studies in Botany, Manasagangotri University of Mysore, Mysore-570 006*
Received: 16 April 2014, Accepted: 25 May 2014, Published Online: 10 June 2014

Abstract

Aqueous seed extract of *P.corylifolia* when tested at 10,20,30,40 and 50% concentration for 3, 6,12 and 24 hours of duration against seventeen seed borne of fungi of maize, *A. flavus oryzae* were completely inhibited at 12 hours of duration in all the concentration tested. *A.tamarii* was completely inhibited in 6 hours of duration in 10% concentration tested. *A. terreus* were completely inhibited at 20% concentration in 12 hours of treatment. *A.flavus columnaris, Macrophomina phaseolina, Trichoderma viride, Curvularia lunata and Cladosporium cladosporoides* were completely inhibited at 10 concentrations in 12 hours of treatment. *Phoma lingam, Rhizopus nigricans* and *Drechslera halodes* were inhibited completely at 30% concentration in 3 hours of duration. In seed germination and seedling vigour of maize, maximum germination was observed in 12 hours of treatment at 20% concentration and recorded 88% germination and 1398.5 vigour index. Compared to control, it was recorded 72% germination and 1010.0 vigour index. Significant activity was also observed in 3 and 6 hours of treatment tested at 10,20,30,40 and 50% concentration. In 24 hours of treatment at 40% and 50% concentration, seed deterioration was observed in all the concentration tested.

Keywords: *P.corylifolia*, maize, aqueous, fungi, germination, seedling vigour

Contents

***Corresponding author**
Kiran.B
Department of Biosciences
CMR Institute of Management Studies
E-mail: bkiran2702@gmail.com
Manuscript ID: IJMPR2079

PAPER-QR CODE

1. Introduction

Agriculture helps to meet the basic needs of human and their civilization by providing food, clothing, shelters, medicine and recreation. Hence, agriculture is the most important enterprise in the world. In India 30% of agriculture production almost 15000 cores is lost due to storage diseases. Plants have played an important role in the discovery of novel and useful drugs used in modern medicine (Njeru et al., 2014). To overcome these problems, 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 residual toxicity (Kiran et al., 2011). 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). Thus alternative approaches are preferred which are ecofriendly. To avoid the use of synthetic pesticides, *In vitro* evaluation for antifungal potency of plants against phytopathogenic fungi in general and biodeterioration causing fungi in particular is the first step towards developing plant based fungicides. Natural products perform various functions, and many of them have interesting and useful biological activities. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many plant based on their use in traditional medicine.

The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization (Joshi et al., 2011). Various medicinal plants have been used for years in daily life to treat plant and human diseases all over the world. The use of traditional plant extracts as well as other alternative forms of medical treatments have been getting momentum since 1990 (Abbas et al., 2008). The use of medicinal plants to treat human diseases has its roots in pre-historical times. Medicinal plants are used by 80% of the world population as the only available medicines especially in developing countries (Hatil and Mohammed,2010). At present, nearly 30% or more of the modern pharmacological drugs are derived directly or indirectly from plants and their extracts dominate in homeopathic or ayurvedic medicines (Murugesan et al., 2011). Natural products of higher plants may provide a new source of antimicrobial agents with possibly novel mechanisms of action (Adenisa et al. 2000). Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country(Joshi et al., 2009). Herbal medicine is still the mainstay of about 75-80% of the whole population, and the major part of traditional therapy involves the use of plant extract and their active constituents (Akerele, 1993). In the present investigation, antifungal and growth promoting potentiality of aqueous seed extract of *Psoralea corylifolia* L. belongs to family Fabaceae against maize seeds were investigated.

2. Materials and Method

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.

Extraction

Aqueous extraction

One hundred grams of the thoroughly washed and air dried healthy seeds of *P. corylifolia* were macerated with 100ml sterile distilled water in a waring blender (Waring international, new hart-ford, CT, USA) for 5 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 10minutes, 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).

Test Seeds:

Maize seeds collected from Farmers, Seed market, APMC yard and Retail shop were used to isolate seed borne fungi.

Isolation and identification of biodeterioration causing fungi in 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 ± 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. The diversity of the fungal species were recorded and the percentage of infection of each of the fungi were determined (ISTA, 1999).

All the fungi associated with the seeds were isolated and their pure cultures maintained on specific media. Species of field fungi were maintained on Czapek Dox Agar (CDA) medium, where as species of storage fungi were maintained on Malt Extract Salt Agar (MESA) medium. The fungi were subcultured periodically.

Effect of the aqueous extract treatment on seed mycoflora, seed germination and seedling vigour of Maize

Seed treatment

Maize seeds (Local) were soaked in 10, 20, 30, 40 and 50% concentration of the aqueous seed extract for 3, 6, 12 and 24 hours duration. Seeds treated with sterile distilled water and soaked for 3, 6, 12 and 24 hours served as control.

Seed germination and seedling vigour

Effect of aqueous seed extract of P. corylifolia on seed germination and seedling vigour of maize under laboratory condition was studied by treating the seeds and subjecting them to germination test and vigour index analysis. Seeds treated with extract and untreated seeds were subjected to germination test following the procedure of paper towel method (ISTA, 1999). Seedling vigour was determined at the end of fourteen days of incubation following the method of Abdul Baki and Anderson (1973). The experiment was carried out with four replicates of 100 seeds each and repeated three times.

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)

3. Results and Discussion

Isolation and identification of biodeterioration causing fungi in maize:

Seventeen fungi viz., *Aspergillus flavus A. flavus oryzae A. tamarii A. niger A. terreus A.flavus columnar is Fusarium moniliforme F. solani Penicillium chrysogenum Trichoderma viride Macrophomina phaseolina Curvularia lunata Cladosporium cladosporoides Mucor* sp. *Phoma lingam Rhizopus nigricans Drechslera halodes* were isolated from maize and preserved in low temperature for further use.

Effect of the aqueous extract treatment on seed mycoflora, seed germination and seedling vigour of maize

Seed Mycoflora

The percent incidence of different fungi observed in the untreated and treated seeds with different concentrations of the aqueous extract for different periods. Treating seeds with different concentrations of aqueous extract for 3hours revealed no significant control of the percent incidence of different species of fungi. Total elimination of *Phoma lingam*, *Rhizopus nigricans* and *Drechslera halodes* was observed. Rotting of seeds was not observed in any of the concentrations tested (Table 1).

At 6 hours of soaking with different concentrations of the extract, significant decrease in percent incidence of species of *Aspergillus, Fusarium, Penicillium, Phoma, Rhizopus* and *Drechslera* were observed at 20% concentration and above. Total elimination of all the fungi were observed at 50% concentration. However, in this concentration total rotting of seeds was also observed (Table 1). During 12 hours of soaking, total elimination of majority of both field and storage fungi were observed. Highly significant control of *A. flavus, A. niger, F. moniliforme*, *P. chrysogenum* were also observed at 20% concentration and above. Total elimination of all the fungi were observed at 50% concentration. However, at this concentration rotting of seeds was also observed(Table 1). At 24hours duration of soaking, at 10% concentration, decreased incidence of species of *Aspergillus*, *Fusarium*, *Trichoderma*, *Macrophomina*, *Phoma*, *Rhizopus* and *Drechslera* were observed. Rest of the fungi were totally eliminated. At 20% and 30% concentration, highly significant decrease in the percent incidence of all the fungi compared with control were observed. Total elimination of all the fungi were also observed at 40 and 50% concentration treatment. However, in these concentrations, total rotting of seeds were observed(Table 1).

Seed germination and seedling vigour

Total germination failure was observed at 6,12 and 24hours of treatment with 50% concentration of the extract and at 24hours treatment with 40% concentration of the extract. Slight increase in seed germination and seedling vigour was observed in seeds treated with 10% concentration of the extract in all the duration of treatments tested. Highly significant increase in seed germination and seedling vigour was observed in the seeds treated with 20% concentration of the extract for the different durations tested. The seedings vigour increased with increased period of soaking in this concentration upto 12hours. No significant increase in seed germination or seedling vigour at 24hours treatment in this concentration over 12hours treatment. At 30% concentration treatment, marginal increase in seed germination and seedling vigour was observed at 3,6 and 12hours of treatment over control. However, total germination failure of seeds was observed in this concentration at 24hours treatment(Table 2).At 40% concentration,

significant decrease in seed germination and vigour index were observed in all the duration of treatment tested compared with control. At 24hours period of soaking, total germination failure was observed in this concentration (Table 2). At 50% concentration , highly significant reduction in seed germination and vigour index was observed at 3hours treatment, total germination failure was observed in this concentration at 6, 12 and 24hours treatment compared with control (Table 2).

Discussion:

In vitro antifungal efficacy of the aqueous extract of seeds of *P. corylifolia* in maize seeds system were also done in the present investigation employing standard blotter method. Maize seeds were soaked for 3,6,12 and 24 hours in 10,20,30,40 and 50% concentration of the aqueous extract. The remarks of the present investigation revealed highly significant control of wide variety of seed borne fungi in the maize seeds treated with 20% concentration of the extract for 12 hours. Three hours and six hours treatment did not show significant control of the seed borne fungi of maize. Twenty four hours of soaking in the different concentrations revealed total control of all the seed borne fungi at 50% concentration of the aqueous extract also totally eliminated all the fungi. However, evaluation of different concentration of seed extract on seed germination and seedling vigour by paper towel method revealed that these concentration (40 and 50%) and duration of treatment (12 and 24 hours) resulted in total germination failure suggesting that higher concentrations with longer duration of treatment is highly phytotoxic to maize seeds.

This is evident from the fact that at 24 hours periods of treatment with 30 and 40% of aqueous extract and 6,12 and 24 hours treatment of 50% aqueous extracts resulted in 100% loss in seed germination compared with control. The result of the present investigations of standard blotter method and seed germination and seedling vigour test suggests that 20% concentration of the extract is the highly suitable treatment for maize seeds to control seed borne fungi of maize coupled with increased seed germination. Thus, in the present investigation, appropriate / optimum dosage and period of soaking of maize seeds for the control of seed borne fungi has been standardized for the first time.

Table 1A. Effect of aqueous extract of seeds of *P. corylifolia* L. on seed mycoflora of maize seed

Fungi (%)	Control	Percent Incidence									
		Duration of Soaking									
		3 hours					6 hours				
		Concentration (%)					Concentration (%)				
		10	20	10	20	10	20	10	20	10	20
Aspergillus flavus	57	56	56	56	56	56	56	56	56	56	56
A. flavus oryzae	5.0	5	5	5	5	5	5	5	5	5	5
A. tamarii	2.0	2	2	2	2	2	2	2	2	2	2
A. niger	50	45	45	45	45	45	45	45	45	45	45
A. terreus	5	5	4.5	5	4.5	5	4.5	5	4.5	5	4.5
A.flavus columnaris	19.5	18	18	18	18	18	18	18	18	18	18
Fusarium moniliforme	7	7	6	7	6	7	6	7	6	7	6
F. solani	21.5	20	20	20	20	20	20	20	20	20	20
Penicillium chrysogenum	19	18	18	18	18	18	18	18	18	18	18
Trichoderma viride	18.5	14	13.5	14	13.5	14	13.5	14	13.5	14	13.5
Macrophomina phaseolina	14.5	18	18	18	18	18	18	18	18	18	18
Curvularia lunata	18	15	15	15	15	15	15	15	15	15	15
Cladosporium cladosporoides	13	13	13	13	13	13	13	13	13	13	13
Mucor sp.	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Phoma lingam	10.5	10	10	10	10	10	10	10	10	10	10
Rhizopus nigricans	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Drechslera halodes	16.5	15	15	15	15	15	15	15	15	15	15

*Values are the mean of four replicates with 100 seeds each.

Table 1B. Effect of aqueous extract of seeds of *P. corylifolia* L. on seed mycoflora of maize seed

Fungi (%)	Control	Percent Incidence									
		Duration of Soaking									
		12 hours					24 hours				
		Concentration (%)					Concentration (%)				
		10	20	30	40	50	10	20	30	40	50
Aspergillus flavus	57	52	8	7.5	7.5	0.0	52	8	7.5	0.0	0.0
A. flavus oryzae	5.0	-	-	-	-	0.0	-	-	-	0.0	0.0
A. tamarii	2.0	-	-	-	-	0.0	-	-	-	0.0	0.0
A. niger	50	15	11.5	11.5	11.5	0.0	15	11.5	11.5	0.0	0.0
A. terreus	5	5	-	-	-	0.0	5	-	-	0.0	0.0
A.flavus columnaris	19.5	-	-	-	-	0.0	-	-	-	0.0	0.0
Fusarium moniliforme	7	3.5	1.5	1.5	1.5	0.0	3.5	1.5	1.5	0.0	0.0
F. solani	21.5	16	-	-	-	0.0	16	-	-	0.0	0.0
Penicillium chrysogenum	19	19	5.0	5.0	5.0	0.0	19	5.0	5.0	0.0	0.0
Trichoderma viride	18.5	11.5	-	-	-	0.0	11.5	-	-	0.0	0.0
Macrophomina phaseolina	14.5	-	-	-	-	0.0	-	-	-	0.0	0.0
Curvularia lunata	18	-	-	-	-	0.0	-	-	-	0.0	0.0
Cladosporium cladosporoides	13	-	-	-	-	0.0	-	-	-	0.0	0.0
Mucor sp.	2.5	-	-	-	-	0.0	-	-	-	0.0	0.0
Phoma lingam	10.5	-	7	7	7	0.0	-	7	7	0.0	0.0
Rhizopus nigricans	2.5	2.5	-	-	-	0.0	2.5	-	-	0.0	0.0
Drechslera halodes	16.5	16.5	-	-	-	0.0	16.5	-	-	0.0	0.0

*Values are the mean of four replicates with 100 seeds each.

Table 2. Effect of aqueous seed extract of *P. corylifolia* L. on seed germination and seedling vigour of maize

Duration of seed treatment (hrs.)	Concentration (%)	Germination (%)	Vigour index (MRL + MSL) x germination %
3 hours	10	74.00^{e} ±0.0	1017.5^{g}±0.0
	20	76.00^{g} ±.5	1029.8^{i}±0.3
	30	73.00^{d} ±0.1	1010.7^{d}±0.0
	40	71.00^{b} ±0.3	1004.6^{b}±0.1
	50	71.00^{b} ±0.3	1004.6^{b}±0.1
	Control	71.00^{b} ±0.2	1015.2^{f}±0.1
6 hours	10	74.00^{e} ±0.0	1036.0^{k}±0.0
	20	77.00^{h} ±0.5	1031.9^{j}±0.5
	30	72.00^{c} ±0.1	1029.6^{i}±0.1
	40	72.00^{c} ±0.5	1017.5^{g}±0.0
	50	0.0^{a} ±0.0	0.0^{a}±0.0
	Control	72.00^{c} ±0.2	1036.0^{k}±0.1
12 hours	10	75.00^{f} ±0.1	1008.5^{c}±0.2
	20	88.00^{i} ±0.2	1398.5^{l}±0.3
	30	74.00^{e}±1.0	1021.2^{h}±0.1
	40	73.00^{d}±0.5	1011.6^{e}±0.0
	50	0.0^{a}±0.0	0.0^{a}±0.0
	Control	72.00^{c}±0.2	1010.1^{d}±0.1
24 hours	10	75.00^{f}±0.1	1008.5^{c} ±0.2
	20	88.00^{i}±0.2	1398.5^{l}±0.3
	30	0.0^{a}± 1.0	0.0^{a}±1.0
	40	0.0^{a}±0.0	0.0^{a} ±0.0
	50	0.0^{a}±0.0	0.0^{a}±0.0
	Control	72.00^{c}±0.2	1010.1^{d}±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.

4. Conclusion

From the above experiments it can be concluded that, the seeds of *P.corylifolia* is a potent medicinal plant which showed a significant activity in inhibiting the seed borne pathogens of maize. It also showed a promising result in seed germination and seedling vigour of maize seeds in aqueous extract. Further investigation is necessary to test the aqueous extract of seeds of *P.corylifolia* on vegetative growth of maize plant and also to isolate the bioactive compound and test against seed mycoflora, potentiality of vegetative growth and yield.

5. Acknowledgement

The authors are thankful to the CMR Institute of Management Studies (Autonomous), PG Department of Biosciences, Kalyan Nagar, Bangalore, Department of Studies in Botany and Microbiology, Maharanis Science college for women, Palace road, Bangalore and Herbal Drug Technology laboratory. Department of Studies in Botany , University of Mysore, Mysore for providing facilities.

6. References

1. AM Abbas, MM Abdul, Y Sarmina, MK Astaq, Sayeed MA. *Research Journal of Agriculture and Biological Sciences*, **2008**, 4(6), 871-874.
2. HK Hatil, Mohammed YA. *Current Research Journal of Biological Sciences*, **2010**, 2(2): 143-146.
3. SK Adenisa, O Idowu, AO Ogundaini, H Oladimeji, TA Olugbade, GO Onawunmi, Pais M., *Phytotherapy Research*, **2000**,14: 371-374.
4. B Joshi, S Lekhak , Sharma A., *Kathmandu University Journal of Science, Engineering and Technology*, **2009**, 5(1): 143-150.
5. Akerele O . *Herbal Gram*, **1993**, 22,13-28.
6. B Kiran, V Lalitha, Raveesha KA. *Journal of Chemical and Pharmaceutical Research*, **2011**, 3(4): 542-550.
7. MZ Basilico, Basilico JC., *Letters in Applied Microbiology*,**1999**, 29(4), 238-241.
8. B Joshi, PS Govind, BB Buddha, RB Megh;S Dinita, S Krishna, P Janardhan, Rajani M. *Journal of Microbiology and Antimicrobials*, **2011**, 3(1), 1-7.
9. S Murugesan, A Pannerselvam; Tangavelou AC. *Journal of Applied Pharmaceutical Sciences*, **2011**, 1: 42-45.
10. AA Abdul – Balki, Anderson JD., *Crop science*, **1973**, 13: 630-633.
11. ISTA. *Seed Science and Technology*, **1999**, 76: 481-484.
12. S Verma, Dohroo NP. *Plant Disease Research*, **2003**, 18(2): 131-134.
13. SN Njeru, R Musa, S Mwaniki, Ngari OPL. *European Journal of Medicinal Plants*, **2014**, 4(1): 45-51.