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ANTIMYCOTIC ACTIVITY OF WEED EXTRACTS ON THE MYCELIAL GROWTH OF *BOTRYODIPLODIA PALMARUM*

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Abstract

The extracts of five common weeds (Ageratum conyzoides, Datura stramonium, Lantana camara, Parthenium hysterophorus and Ricinus communis) were evaluated for their antimycotic activity against Botryodiplodia palmarum and screened for important phytochemicals. Crude extract was prepared by macerating plant material in water (1:1 ratio) and evaluated for antimycotic efficiency at the concentration of 10, 20 and 30% in media. The solvent extracts (acetone, aqueous and ethanol) were evaluated for antimycotic activity at the concentration of 1500, 2000, 2500ppm. All extracts exhibited moderate to good antimycotic activity on test fungus, the extracts of Parthenium hysterophorus were most effective in supressing the mycelial growth of B. palmarum. Growth inhibition of B. palmarum was recorded in the range of 2.77- 85.71%. The phytochemical analysis revealed that the carbohydrates, flavonoids, phenolics, steroids, Tannins and terpenoids were detected in all of selected weed's species.

Keywords*: Phytoextract; Botanical; Weed; Antimycotic activity; Botryodiplodia palmarum*

Introduction

Botryodiplodia palmarum (Cooke) Petr. & Syd. cause set rot in the poplar nursery in northern states of India [1-3]. Poplars can be easily cultivated, and they constitute an important component of forestry and agroforestry systems for the livelihood of small-scale farmers [4]. Invasion by fungal pathogens in nurseries as well in plantations, reduce its productivity and result in substantial economic loss to the farmers. Although application of fungicides in disease management is a common practice, but they have negative impact on the environment, non-target organisms and human health. Moreover, their prolonged use can result in pollution of natural resources and development of resistance in pathogens. Therefore, it is desirable to explore eco-friendly alternatives like application of biocontrol agents and botanicals for disease management. The biopesticides have significant advantage on crop production and have minimum residues on fruits and vegetables [5].

During last few decades, researchers are evaluating plant extracts and oils to control pathogens by eco-friendly means. Some of secondary metabolites synthesized by plant are toxic

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to phytopathogens. Plants synthesize variety of secondary metabolites like phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins, which play an important role in plant defense mechanisms during pathogens invasion and some of them also exhibit antimicrobial activity [6-7]. The popularity of botanicals is increasing day by day for their use as green pesticides [8]. Plant derived pesticides are biodegradable and safe to human as well to wildlife. Initial screenings of plants for possible antimicrobial activities usually begin with crude aqueous or alcohol extractions, followed by various organic fractionation methods [9]. Many unwanted plants (weeds) are growing everywhere and causing economic and ecological loss. They can be exploited for the possible antimycotic activity against fungal pathogens. It will serve dual purpose of weed eradication and disease management.

Plant extracts of many higher plants including weeds have been reported to exhibit antimicrobial properties under laboratory trails. Antimicrobial properties of the extracts of selected weeds have been reported earlier by many researchers against variety of phytopathogens viz. *Parthenium hysterophorus* [10, 11], *Lantana camara* [12, 13], *Riccinus communis* [14], *Datura stramonium* [15], *Ageratum conyzoides* [16]. But little information is available for the management of *Botryodiplodia palmarum*. Therefore, crude, acetone, ethanol, and aqueous extracts of these species were evaluated at different concentrations for their antimycotic activity under laboratory conditions. The selected species were also screened for the presence of important phytochemicals.

Experimental part

Materials

Botryodiplodia palmarum culture was procured from Forest Pathology Division, Forest Research Institute, Dehradun and weeds (*Ageratum conyzoides, Datura stramonium, Lantana camara, Parthenium hysterophorus* and *Ricinus communis*) were collected from the surrounding areas of Forest Research Institute, Dehradun.

Methods

A. Phytochemical screening of plant extracts. The shade dried leaves of selected weed species were screened for the presence/absence of phytochemical constituents like carbohydrates, proteins, alkaloids, cholesterols, flavonoids, saponins, terpenoids, glycosides, tannins, phenols etc., by following standard procedure:

i. Test for alkaloids. Dragendroff's reagent test– Few mL of filtrate was taken in a test tube and 1-2mL of Dragendroff's reagent was added. Appearance of yellow coloured precipitate confirmed the presence of alkaloids.

ii. Test for phenolics and tannins. Ferric Chloride test - Extract was dissolved in the distilled water and few drops of 5% ferric chloride solution were added. A dark green color indicated the presence of Phenolics and Tannins.

iii. Test for flavonoids. 5mL of dilute ammonia solution was added to the portion of extract, followed by the addition of few drops of concentrated sulphuric acid. Appearance of yellow colour confirmed the presence of flavonoids.

iv. Test for carbohydrates and glycosides. Molisch test - 2mL of filtrate was taken in the test tube followed by addition of 1-2 drops of alcoholic of α-napthol, mixture shaken well and concentrated sulphuric acid was added along sides of the test tube. Formation of a violet colour ring confirmed the presence of carbohydrates. Fehling's solution test – 1.0mL of filtrate was boiled in water followed by addition of 1.0mL of Fehling solution. Red coloured precipitate indicated the presence of reducing sugars.

v. Test for terpenoids. Salkowski test - 5mL of extract was mixed with 2mL of chloroform and 3mL of sulphuric acid was added from the sides of the test tube. Formation of reddish-brown coloration at interface indicated the presence of terpenoids.

vi. Test for steroids. Libermann burchard's test - Extract was dissolved in 2mL of acetic anhydride and 1-2 drops of sulphuric acid was added along sides of the test tube. Blue green ring appears or the array of color changes indicate the presence of steroids.

vii. Test for amino acids. 1-2 drops of phenolphthalein were added to the extract and dilute sodium hydroxide solution was added drop by drop. Appearance of pink colour confirmed the presence of amino acids.

viii. Test for protein: Biuret test - 2 mL of filtrate was treated with one drop of 2% copper sulphate solution. To this, 1mL of ethanol (95%) and excess of potassium hydroxide pellets were added. A pink colour appears in the ethanolic layer confirmed the presence of proteins.

B. Evaluation of plant extracts against pathogen. Crude extract and solvent extracts (acetone, ethanol, aqueous) were tested against *B. palmarum* at different concentrations under laboratory conditions:

i. Preparation of crude extract. Leaf samples (100g) of plants were thoroughly washed, blot dried and macerated with 100mL sterile distilled water, ratio 1:1 w/v in a blender for 10 min. The macerate was first filtered through double layered muslin cloth and then centrifuged at 4000*g* for 30 min. The supernatant was filtered through Whatmann No. 1 filter paper and stored at 4°C until further use.

*ii. Preparation of solvent extract***.** Leaf samples were air dried, powdered and soaked separately in acetone, ethanol, water and boiled for 5 hours. The extract was filtered through Whatman No. 1 paper and solvents were evaporated at appropriate temperature to get final volume of 30mL. The extracts were stored at 4°C till further use.

iii. Determining dry content of extract. 0.5mL of extracts were pipetted out into the Petri plate weighed previously and kept in oven overnight at 80°C and weighed to get dry content of extract. Based on the dry content, the requisite amount of extract was mixed with PDA to get desired concentration in the media.

iv. In-vitro evaluation of antifungal activity of plant extracts against B. palmarum. Plant extracts were tested for their efficacy against the pathogen by following poisoned food technique [17]. The PDA was amended with the required volume of extracts (aqueous, ethanol and acetone extract) to get desired concentrations (1500, 2000 and 2500ppm), autoclaved and poured in Petri plates. For the evaluation of efficacy of crude extracts, PDA was amended just before pouring with crude extract to get 10, 20 and 30% concentration in media. Mycelial discs of 5mm diameter were cut from the periphery of actively growing culture of the pathogen and inoculated. PDA without amendment served as control. Inoculated plates were incubated at 25±1°C and the radial growth was recorded on 5th day and compared with control. Percent growth inhibition was calculated by the formula:

$$\text{Growth Inhibition (\%)} = \frac{\text{Colony diameter in control} - \text{Colony diameter in treatment}}{\text{Colony diameter in control}} \times 100$$

Results and discussion

Botryodiplodia species are opportunistic plant pathogens that cause variety of diseases in tropical and subtropical regions of world [18]. It has wide host range and exhibit varied pathological effects on its hosts. Although the management of fungal pathogens by applying

fungicides is still popular in developing countries, but their ill effect on human, wildlife and environment are well known. Therefore, eco-friendly practices like application of biocontrol agents or botanicals for the management of phytopathogens is the need of hour. Among botanicals, extracts of different plant species need to be screened under laboratory conditions before nursery and field application. In this field, weeds are most desired species because of their allelopathic and environment degrading nature. The species selected in present study are *A. conyzoides*, *P. hysterophorus*, *D. stramonium*, *L. camara* and *R. communis*, representing the family of Asteraceae, Solanaceae, Verbenaceae and Euphorbiaceae, respectively. The analysis of plant samples for the detection of important phytochemicals revealed that all of tested phytochemicals viz. alkaloids, amino acids, carbohydrates, flavonoids, phenolics, proteins, steroids, tannins and terpenoids were detected in the extracts of *P. hysterophorus* and *D. stramonium*, while proteins were not detected in *A. conyzoides*, alkaloids in *R. communis* and in case of *L. camera* alkaloids, amino acids and proteins were not detected (Table 1). The composition phytochemical in plants is influenced variety of biotic and abiotic factors [19-21].

Table 1. Phytochemical screening of selected weed species

S. No.	Phyto-chemical	Presence/ absence of phytochemicals in weed species				
		A. conyzoides	***D. stramonium***	***L. camara***	***P. hysterophorus***	***R. communis***
1.	Alkaloids	+	+	_	+	_
2.	Amino acids	+	+	_	+	+
3.	Carbohydrates	+	+	+	+	+
4.	Flavonoids	+	+	+	+	+
5.	Phenolics	+	+	+	+	+
6.	Proteins	_	+	_	+	+
7.	Steroids	+	+	+	+	+
8.	Tannins	+	+	+	+	+
9.	Terpenoids	+	+	+	+	+

Evaluation of crude extracts for antifungal activity

The crude extract of selected weeds restricted the growth of *B. palmarum* in the range of 16.66-51.81%. The rate of growth inhibition increased with increase in the concentration of extract in media (Table 2). At 30% concentration of crude extract in media, maximum growth inhibition (51.81%) was exhibited by the extract of *P. hysterophorus*, followed by *D. stramonium* (44.44%), *A. conyzoides* (40.54%), *L. camara* (38.88%) and minimum 30.55% by the extracts of *R. communis*.

Table 2. Growth inhibition of *B. palmarum* by crude extracts of weeds at different concentrations

S. No.	Name of Species	Growth inhibition (%) of *B. palmarum* at different concentrations of crude extracts		
		10%	**20%**	**30%**
1.	*A. conyzoides*	18.91	32.43	40.54
2.	*D. stramonium*	30.55	38.88	44.44
3.	*L. camara*	19.44	33.33	38.88
4.	*P. hysterophorus*	29.72	37.83	51.81
5.	*R. communis*	16.66	25.00	30.55

Dry content of extract

Dry content of aqueous, ethanolic and acetone extracts ranged between 0.01-0.16g/0.5mL. Maximum solid content was recorded in aqueous extract followed by ethanolic

and acetone extract. Highest solid content was observed for *P. hysterophorus* followed by *L. camara* and *R. communis* (Table 3).

Table 3. Dry content obtained after different solvent extractions

S. No.	Name of Species	Dry content in extract (g)		
		Aqueous	Ethanolic	Acetone
1.	*A. conyzoides*	0.10	0.03	0.01
2.	*D. stramonium*	0.14	0.03	0.01
3.	*L. camara*	0.15	0.09	0.02
4.	*P. hysterophorus*	0.16	0.04	0.01
5.	*R. communis*	0.15	0.05	0.01

Evaluation of solvent extracts for antifungal activity

The solvent extract of selected weeds has inhibited the growth of *B. palmarum* and the rate of growth inhibition increased with increase in the concentration of extract in media (Table 4). The extracts of *P. hysterophorus* were most effective in inhibiting the growth of *B. Palmarum*. The acetone extract of selected weeds restricted the growth of *B. palmarum* in the range of 14.28-55.88%. At the 2500ppm concentration of extract in media, maximum growth inhibition (55.88%) was exhibited by the extract of *P. hysterophorus*, followed by *A. conyzoides* (45.71%), *D. stramonium* (35.29%) and minimum 14.28% by the extracts of *R. communis*. The aqueous extract of restricted the growth of *B. palmarum* in the range of 2.77-40.00%. At the 2500 ppm concentration of extract in media, maximum growth inhibition (40.00%) was exhibited by the extract of *P. hysterophorus*, followed by *L. camara* (38.88%), *R. communis* (33.33%) and minimum 19.44% by the extracts of *D. stramonium* and *A. conyzoides*. The ethanolic extract inhibited the growth of *B. palmarum* with greater extent in the range of 47.06-85.71%. At the 2500 ppm concentration of extract, maximum growth inhibition (85.71%) was exhibited by the extract of *P. hysterophorus*, followed by *R. communis* (82.85%), *A. conyzoides* (80.00%), *L. camara* (76.47%) and *D. stramonium* (75.80%).

Table 4. Growth inhibition of *B. palmarum* by extracts of weeds at different concentrations

Solvent	Species	Growth inhibition (%) at different concentrations of extract		
		1500 ppm	2000 ppm	2500 ppm
Acetone	*A. conyzoides*	31.42	40.00	45.71
	D. stramonium	17.64	23.52	35.29
	L. camara	ND	ND	ND
	P. hysterophorus	35.29	44.11	55.88
	R. communis	14.28	22.85	31.42
Aqueous	*A. conyzoides*	2.77	5.55	19.44
	D. stramonium	8.33	13.88	19.44
	L. camara	22.22	30.55	38.88
	P. hysterophorus	34.28	37.14	40.00
	R. communis	25.00	27.77	33.33
Ethanol	*A. conyzoides*	71.42	74.28	80.00
	D. stramonium	58.60	68.70	75.80
	L. camara	47.06	61.76	76.47
	P. hysterophorus	80.00	85.71	85.71
	R. communis	57.14	65.71	82.85

Moderate to high mycelial growth inhibition of *B. palmarum* was recorded on PDA plates. It may be due to variety of antimicrobial compounds present in these species. The

presence of antifungal compounds in higher plants is well recognised and considered valuable for plant disease control [22]. Several authors have also reported the fungicidal activity in wide variety of taxa. *N. Sharma and P.C. Trivedi* [23] evaluated leaf extracts of fifteen plant species on *Fusarium oxysporum* and recorded growth inhibition in the range of 8.87-72.23%.

V. Jalander and B.D. Gachande [15] recorded antifungal activity of leaf extracts of *Datura stramonium* and *D. innoxia* on *Fusarium oxysporum* and *Alternaria solani*. Variety of plant extracts have been evaluated by researchers for their antimycotic property on different pathogens [11, 24-29].

Conclusions

The antimycotic effects of the extracts indicate the potential of these weeds species as a natural source of fungicidal material. Antifungal activity was recorded in all species with varied effectiveness in inhibiting the mycelial growth of *B. palmarum* at different concentrations. The efficacy of needs to be further evaluated under nursery and field conditions for the development of commercial formulatios.

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