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IN VITRO EVALUATION OF BOTANICALS AGAINST THE BROWN LEAF SPOT CAUSING FUNGUS, *Alternaria alternata* (Fr.) Kiessler IN TOBACCO

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ABSTRACT

Plant based pesticides which are relatively cheaper, safe and non-hazardous can be used successfully against the plant pathogenic fungi. The present investigation was aimed to study the antifungal activity of six botanicals like NSKE (*Azadirachta indica* A. Juss), Neem leaf extract (*Azadirachta indica* A. Juss), Clerodendron leaf extract (*Clerodendron inerme* (L.) Gaerth.), Congress grass leaf extract (*Parthenium hysterophorus* L.), Prosopis (*Prosopis juliflora* (Sw) Dc.) and Tridex (*Tridex procumbens* L.) against the fungus *Alternaria alternata* (Fr.) Kiessler causing brown leaf spot of tobacco. The botanicals were studied *in vitro* at two concentrations (5% and 10%) for their efficacy against *A. alternata,* by poison food technique. All the six botanicals evaluated against *A. alternata* were found to be significant. Prosopis (49.05 per cent inhibition of mycelia growth) was significantly superior over all other plant extracts evaluated. The next best treatment was parthenium (22.53 per cent inhibition of mycelia growth), which was on par with NSKE (20.83 per cent inhibition of mycelia growth). Least inhibition was noticed in case of clerodendron (11.05 per cent inhibition of mycelia growth). Irrespective of the plant species, botanicals were found to be the most effective at 10 per cent (24.79%). Interactions between botanicals and concentrations were significant. All the plant extracts reduced the mycelial growth with increase in concentrations. Maximum reduction of mycelial growth (53.26%) was noticed in case of prosopis followed by parthenium (25.06%) at 10 per cent and prosopis (44.83%) at 5 per cent concentration. Least reduction of mycelial growth was noticed in case of Clerodendron (9.84%) at 5 per cent concentration.

(Key words: Botanicals, brown leaf spot, bidi tobacco, management)

INTRODUCTION

Tobacco (*Nicotiana tabacum* L.), which belongs to the family solanaceae, is believed to have been introduced into India from its native Central America by Portuguese in 1603. It is a major commercial crop of India, grown throughout the country. India is one of the principal tobacco producing countries of the world, and tobacco has attained its commercial importance in India. It has a significant contribution to Indian economy through its earning by way of central excise and foreign exchange. Bidi tobacco suffers from many abnormalities caused by a wide range of pathogens, *viz*., fungi, nematodes, bacteria, viruses, flowering plant parasites and phytoplasma (Lucas, 1975). The losses due to these diseases are estimated to be in range of 5 to 15 per cent depending on their intensity. Among all foliar diseases, brown leaf spot of tobacco, has become a major threat in recent years.

The brown leaf spot of tobacco caused by *Alternaria alternata* (Fr.) Kiessler is a very important disease of the crop, which causes both qualitative and quantitative loss in the leaf yield. The disease is characterized by circular spots, ranging from 0.25 inch to 1.25 inches in diameter on the leaves. Spots are found primarily on the lower leaves of the plant. It produces target-like spots having a yellow or yellowish-green halo around them. The fungus causes the leaf tissue in the area of the spot to age prematurely. Each of the dark rings in the target spots are made of thousands of tiny spores (seed-like structures). The spots enlarge and coalesce and the dead tissues often tear and fall out of the leaf making the entire leaf ragged and worthless. Management of diseases by using chemical fungicides has hazardous effect on environment and also it enhances risk of development of new virulent races. To overcome these problems, effective botanicals must be used as a component of integrated disease management. Keeping these in view, six botanicals were studied *in vitro* at two concentrations (5% and 10%) for evaluating their efficacy against *A. alternata,* by poison food technique.

MATERIALS AND METHODS

Plant based pesticides which are relatively cheaper, safe and non-hazardous can be used successfully against the plant pathogenic fungi. The present investigation was

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aimed to study the antifungal activity of some plant extracts against *Alternaria alternata* (Fr.) Kiessler causing brown leaf spot of tobacco. The following plant extracts were selected.

Botanical Name	Common Name	Family	Plant parts used
Azadirachta indica A. Juss	NSKE	Meliaceae	Seed
Azadirachta indica A. Juss	Neem	Meliaceae	Leaf
Clerodendron inerme (L.) Gaerth.	Clerodendron	Lamiaceae	Leaf
Parthenium hysterophorus L.	Congress grass	Asteraceae	Leaf
Prosopis juliflora (Sw) Dc.	Prosopis	Mimosaceae	Leaf
Tridex procumbens L.	Tridex	Compositae	Leaf

Collection, isolation and identification of the pathogen

The leaves of tobacco showing typical symptoms of the disease were collected from the field. The standard tissue isolation procedure was followed to isolate the pathogen. The infected leaf bits were surface sterilized with two per cent $NaOCl_2$ for 2 minutes and repeatedly washed separately in sterilized distilled water to remove the traces of $NaOCl_2$ if any and then, it was transferred to sterilized Petri plates (1-2 leaf bits per Petri dish) containing potato dextrose agar (PDA). The Petri plates were incubated at room temperature (27±1°C) and observed periodically for the growth of the fungus. Bit of fungal growth developed from the infected tissue was transferred to PDA slants and incubated at 27°±1°C for 12 days. Then such slants with pure culture were used for further studies.

Single spore isolation

Ten ml of clear, sterilized water agar of two per cent strength was poured into Petri-plates and allowed to solidify. Dilute spore suspension was prepared using sterile distilled water from 12 days old culture. One ml of such suspension was spread uniformly on two per cent water agar plates. The plates was incubated at 27±1°C for eight hours. Then, such plates was examined under microscope to locate germinated conidia. Single isolated and germinated conidia was marked under the microscopic field with ink on the surface of the plate. These marked agar areas was cut and transferred to PDA slants with the help of cork-borer under aseptic conditions and incubated at a temperature of 27±1°C. Pure culture derived from such slants was used for further studies.

Maintenance of the culture

The pathogen was sub-cultured on PDA slants and it was allowed to grow at 27 ± 1°C for ten days and such slants were preserved in a refrigerator at 5°C and it was renewed once in 30 days.

Identification of the pathogen

The study was undertaken to confirm the identity of the isolated pathogen. Identification of the fungus was made after examining one hundred conidia under microscope (under100x) from mature pure culture of the fungus obtained from infected leaves of tobacco. Stage and ocular micrometer was be used to measure the length, breadth, beak length and number of septa of the fungus. The average length and breadth of the conidial body, beak and septal number was recorded. These observations were compared with those of the standard measurements given by Ellis (1971) to identify the pathogen. The pathogenic isolate of *Alternaria alternata* was used for further studies.

Preparation of aqueous extract

Fresh plant materials were collected and washed first in tap water and then in distilled water. Hundred grams of fresh sample was chopped and then crushed in a surface sterilized pestle and morter by adding 100 ml sterile distilled water (1:1 w/v). The extract was filtered through two layers of muslin cloth. Finally filtrate thus obtained was used as stock solution.

To study the antifungal mechanism of plant extracts, the poisoned food technique was used (Nene and Thapliyal, 1982).Ten and five ml of stock solution were mixed with 90 and 95 ml of sterilized molten PDA media, respectively so as to get 10 and 5 per cent concentrations. The medium was thoroughly shaken for uniform mixing of extract.

Twenty ml of medium was poured into sterile Petri plates, mycelium of five mm size discs form periphery of actively growing culture were cut out by sterile cork borer and one such disc was placed on the centre of each agar plate. Controls were also maintained by growing the pathogen on PDA plates. Then such plates were incubated at 27±1°C till growth of the fungus touched the periphery in control plate and the diameter of the colony was measured in two directions and average was worked out. The per cent inhibition of growth was calculated by using the formula given by Vincent (1947).

$$I = \frac{C-T}{C} \times 100$$

where,

I = Per cent inhibition of mycelium

C = Growth of mycelium in control

T = Growth of mycelium in treatment

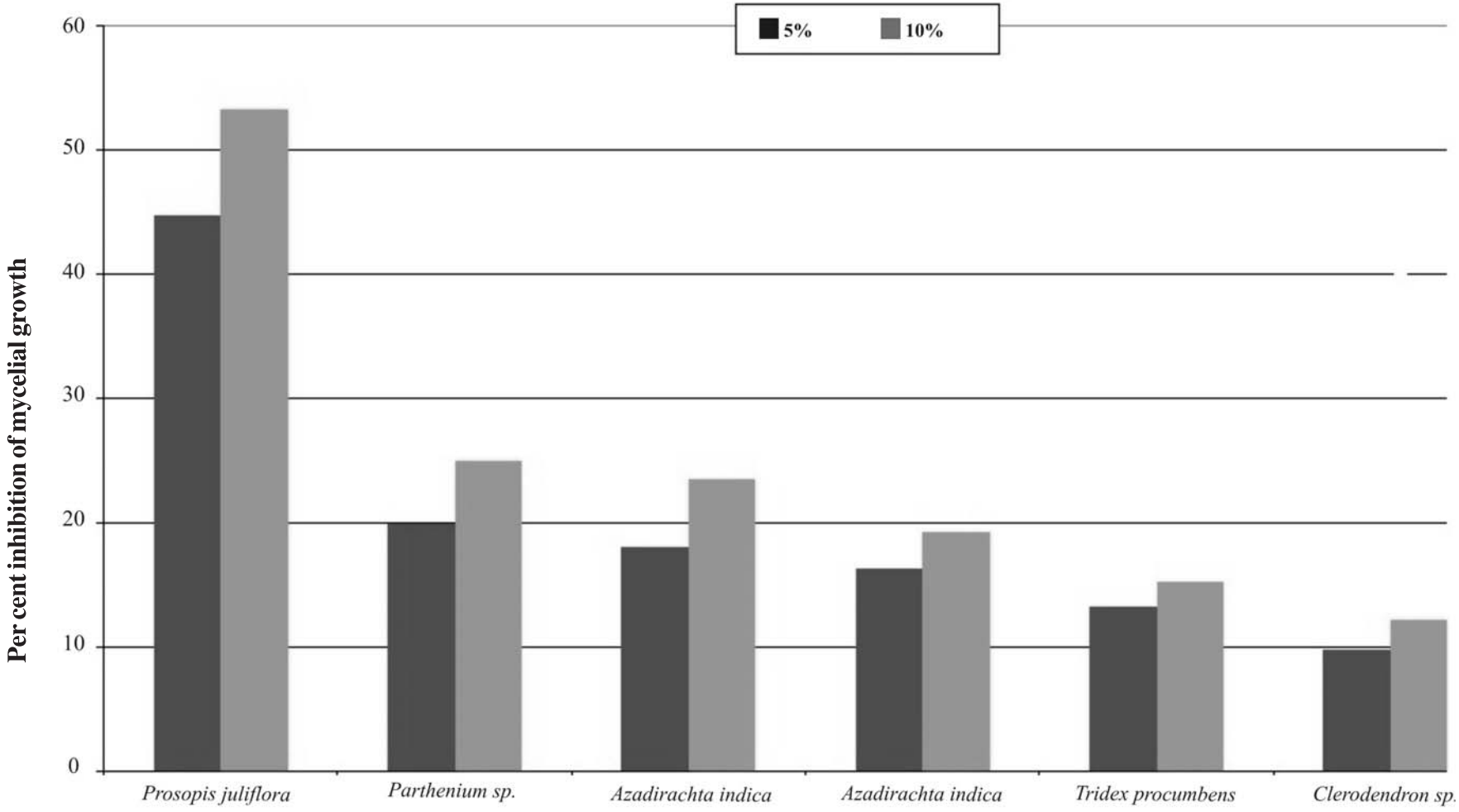
RESULTS AND DISCUSSION

Six botanicals were studied *in vitro* at two concentrations (5% and 10%) for their efficacy against *A. alternata,* by poison food technique. The data are presented in table 1, fig. 1 and plate 1. Six botanicals evaluated against *A. alternata* were found to be significant. Prosopis (49.05%) was significantly superior over all other plant extracts evaluated. The next best treatment was parthenium (22.53%), which was on par with NSKE (20.83%). Least inhibition was noticed in case of clerodendron (11.05%). Irrespective of the plant species, botanicals were found to be most effective

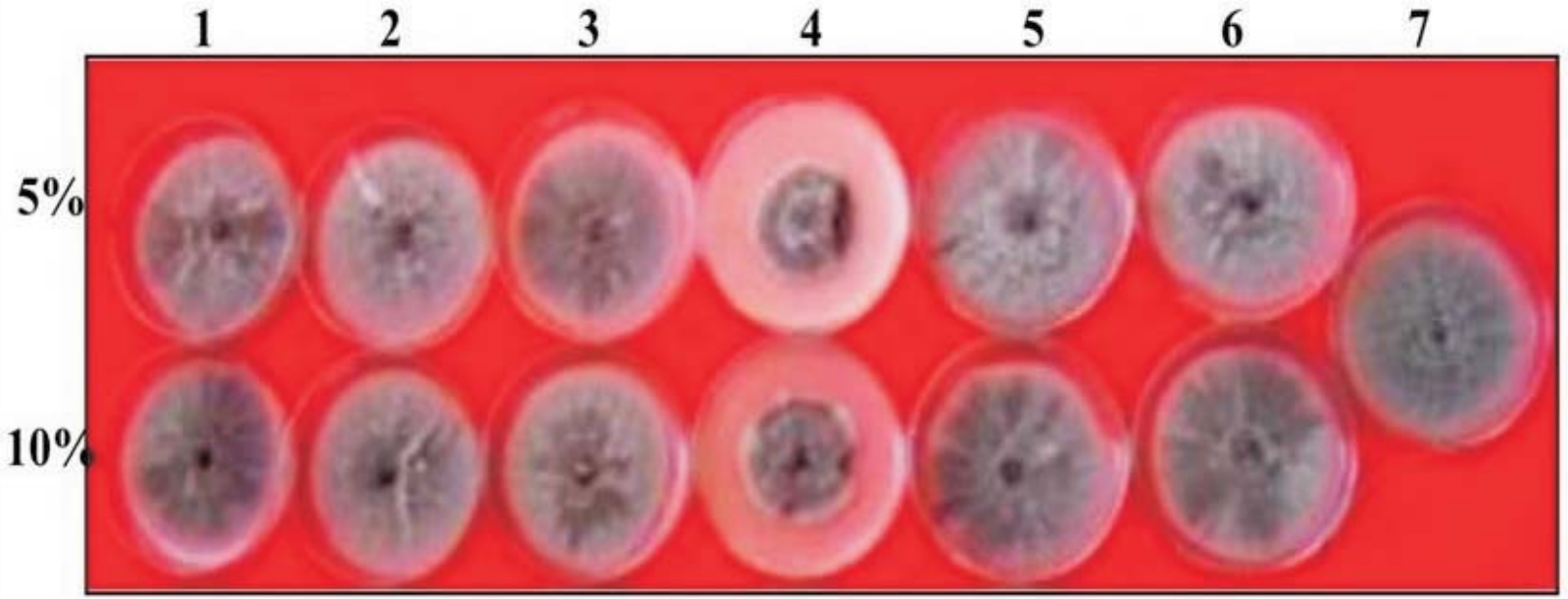
Table 1. *In vitro* evaluation of botanicals against Alternaria alternata

Common name	Botanical name	Plant part used	Per cent Inhibition of mycelial growth		
			Concentrations (%)		Mean
			5	10	
Prosopis	*Prasopis juliflora*	Leaf	44.83	53.26	**49.05**
			(42.03)	(46.87)	**(44.45)**
Congress grass	*Parthenium ageratum*	Leaf	20.00	25.06	**22.53**
			(26.56)	(30.04)	**(28.30)**
NSKE	*Azadirachta indica*	Seed	18.13	23.53	**20.83**
			(25.20)	(29.01)	**(27.10)**
Neem	*Azadirachta indica*	Leaf	16.32	19.31	**17.81**
			(23.83)	(26.06)	**(24.96)**
Tridex	*Tridex procumbens*	Leaf	13.3	15.34	**14.32**
			(21.38)	(23.06)	**(22.22)**
Clerodendron	*Clerodendron sp.*	Leaf	9.84	12.26	**11.05**
			(18.28)	(20.49)	**(19.39)**
Mean			**20.40**	**24.79**	
			(26.85)	**(29.86)**	

	SEm±	CD (1%)
Botanicals (B)	0.38	1.56
Concentrations (C)	0.21	0.90
B x C	0.56	2,21



In vitro Evaluation of botanicals against Alternaria laternata



1 - Clerodendron
2 - Tridex
3 - Congress grass
4 - Prosopis
5 - Neem
6 - NSKE
7 - Control

Plate 1 . *In vitro* evaluation botanicals against *Alternaria alternata*

at 10 per cent (24.79%). Interactions between botanicals and concentrations were significant. All the plant extracts reduced the mycelial growth with increase in concentrations. Maximum reduction of mycelial growth (53.26%) was noticed in case of prosopis followed by parthenium (25.06%) at 10 per cent and prosopis (44.83%) at 5 per cent concentration. Least reduction of mycelial growth was noticed in case of Clerodendron (9.84) at 5 per cent concentration.

The fungicidal spectrum of *Prosopis juliflora* has been attributed to alkaloids such as juliflorine, julifloricine and julifloridine (Raghavendra *et al.*, 2009). Inhibition of *A. alternata* in the present investigation by prosopis leaf extract may be due to production of these alkaloids. The present investigation of various botanicals inhibiting the growth of *A. alternata* is in line with the earlier finding by Raghavendra *et al.* (2009).

Contrary to the problems associated with the use of synthetic chemicals, botanicals are environmentally non pollutive, indigenously available, easily accessible, non-phytotoxic, systemic ephemeral, readily biodegradable, relatively cost effective and hence, constitute a suitable plant protection in the strategy of biological management of diseases. Hence, screening of plant products for its effective antifungal activity against the pathogen is essentially required to minimize the use of fungicides and to consider as one of the components in the integrated disease management

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