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Antifungal Activities (*In vitro*) of Some Plant Extracts and Smoke on Four Fungal Pathogens of Different Hosts

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Abstract: Inhibition of spore/conidial germination of four fungi viz., *Bipolaris sorokiniana*, *Fusarium oxysporum* f. sp. *vasinfectum*, *Rhizopus artocarpi* and *Botryodiplodia theobromae* was tested using the extracts of different parts of *Vinca rosea* and *Azadirachta indica* and smoke of rice straw, wheat straw, tobacco leaf and 'dhup' (incense) and showed good results in their inhibition. *Vinca rosea* root extract inhibited 100% spore germination of *Bipolaris sorokiniana* and *Rhizopus artocarpi* when it was immersed from 5-30 minutes at 5:1.25 (w/v) concentration. *A. indica* (leaf, root and seed) extracts showed good (100%) inhibition results on *B. sorokiniana*, and *R. artocarpi*. Smoke of rice straw, wheat straw, tobacco leaf and 'dhup' had a great antifungal effect against these fungi.

Key words: Plant extracts, antifungal activities, fungitoxicity of smoke, fungi

Introduction

Several higher plants and their constituents have shown success in plant disease control and are proved to be harmless and non-phytotoxic unlike chemical fungicides (Spencer *et al.*, 1957; Shekhawat and Prasad, 1971; Appleton and Tansey, 1975; Misra and Dixit, 1976; Singh *et al.*, 1986 and Dubey, 1991). The extracts of plants also exhibited marked effect on germination of fungal spores as well (Singh and Singh, 1981; Singh *et al.*, 1983 and Dubey, 1991). The extracts of plant parts are recommended to control the disease (Dixit *et al.*, 1983; Pandey *et al.*, 1983; Chary *et al.*, 1984 and Singh and Dwivedi, 1990). Smoke has also antifungal activities (Alam *et al.*, 1999). In the present study, an attempt has been made to observe the effect of different plant extracts and smoke as fungicides on *Bipolaris sorokiniana*, *Fusarium oxysporum* f. sp. *vasinfectum*, *Rhizopus artocarpi* and *Botryodiplodia theobromae*.

Materials and Methods

Bipolaris sorokiniana, *Rhizopus artocarpi*, *Fusarium oxysporum* f. sp. *vasinfectum* and *Botryodiplodia theobromae* were isolated from leaf blight disease of wheat, fruit rot disease of jackfruit, wilt disease of cotton and bud rot disease of coconut respectively. Fungi were cultured on PDA medium. Extraction of root, seed and leaf tissues of different plants viz., *Vinca rosea* and *Azadirachta indica* in alcohol was done following the method described by Mahadevan and Sridhar (1982). Five gram tissues were cut into pieces and immediately plunged in boiling ethyl alcohol (80%) in a beaker and allowed to boil for 5-10 minutes using 5-10 ml of alcohol for each gram of tissue. The extraction was done on top of a steam bath. Then these extracts, were cooled in a pan of cold water. The tissues were crushed thoroughly in a mortar with a pestle and then passed through two layers of cheese-cloth. Re-extracted the ground tissues for 3 minutes in hot 80 per cent alcohol and 2-3 ml of alcohol were used for every gm of tissues. The volume (10 ml) of the extract was evaporated on a steam bath to dryness and 1.25 ml of sterilized distilled water was added for five gm of tissues and the extracts were used as fungicides. Conidia/spore from 10 days old culture on PDA plates were taken and conidial/spore suspensions were made separately with different plant extracts (Leaf and root of *V. rosea* and leaf, root and seed of *A. indica*). These suspensions (1.25-ml) were taken in small sterilized petri dishes (65 mm) and were kept at 30 ± 2°C for 5-30 minutes. After that period, a drop of treated conidial/spore suspension (from different plant extracts) was taken on separate slides for 24 hours of incubation. Then a drop of lactophenol cotton blue was placed on the conidial/spore suspension in the slides. The slides were examined under high power (×40) for recording the percentage of conidial/spore germination.

The evaluation of fungitoxicity of smoke in the laboratory was made by Parmeter's technique (1975) with a modification. Rice straw, wheat straw, tobacco leaf and 'dhup' were burnt in a metal pot with a cover fitted with rubber tube, the resulting smoke was cooled to ambient temperature by passing through the rubber tube. The cooled smoke was introduced into petri plates containing PDA with fungal colony. The petri plates containing cultures were placed for varying lengths of time (5, 10 and 15 minutes) in the smoke chamber (a wooden box of 0.5 m × 0.5 m × 0.5 m, where smokes are passed through by rubber tube) and exposed to dense rice straw, wheat straw, tobacco leaf and 'dhup' smoke. Aqueous spore/conidial suspension of the pathogen was placed on slides previously exposed to rice straw, wheat straw, tobacco leaf and 'dhup' smoke and was incubated in a moist chamber for 24 hours. After that period the slides were examined under high power (×40) microscope for recording the percentage of spore/conidial germination. Statistical analysis of data given as percentage was carried out from angular transformed values and performed using Microsoft Excel software. LSD were determined, whenever, the calculated 'F' values were significant at 5% level (Snedecor and Cochran, 1980).

Results and Discussion

Vinca rosea root extracts inhibited 100% spore/conidial germination of *Bipolaris sorokiniana* and *Rhizopus artocarpi*, when immersed from 5-30 minutes at 5:1.25 (w/v) concentration (Table 1). It has moderate to good effect on the inhibition of *Botryodiplodia theobromae* and *Fusarium oxysporum* f. sp. *vasinfectum* (81 and 74%) at 5:1.25 (w/v) concentration and immersion after 30 minutes. *Vinca rosea* leaf extracts, showed moderate types of inhibition effect on *B. sorokiniana*, *F. oxysporum* f. sp. *vasinfectum*, *R. artocarpi* and *B. theobromae* (57, 62, 40 and 59%) with immersion after 30 minutes. Leaf, root and seed extracts of *A. indica*, showed good results against the inhibition of these fungi. Hundred per cent spore germination inhibited on root, leaf and seed extracts of *A. indica* against *R. artocarpi*. The extracts of leaf and root of *A. indica* inhibited 100% spore germination of *Bipolaris sorokiniana* after 5-30 minutes of immersion. Moderate to good effect showed on the inhibition of spore/conidial germination (67, 56 and 75%) of *F. oxysporum* f. sp. *vasinfectum*, immersion after 30 minutes in leaf, root and seed extracts of *A. indica*. Correlation (r1) value (0.958-0.997) indicates that there was a highly significant relationship between immersion period (5-30 minutes) in plant extracts and inhibition of spore/conidial germination, except *R. artocarpi* in root, leaf and seed extracts of *A. indica* and *B. sorokiniana* in leaf and root extracts of *A. indica* and root extracts of *V. rosea*. Calculated F

Table 1: Effect of different plant extracts as fungicides on the inhibition of spore/conidial germination of four fungi (after immersing 5 - 30 minutes).

Name of plant extracts	Name of Fungi	Percentage of spore germination* inhibition.						Correlation (r1)	Calculated F value (5%)		$LSD_{[0.05]}$
		5	10	15	20	25	30		Fungi	Immersion period	
Leaf of *Vinca rosea*	*Bipolaris sorokiniana*	20(80)	33(67)	37(63)	49(51)	54(46)	57(43)	0.974	878.88	637.30	2.03041
	Fusarium oxysporum f. sp. *vasinfectum*	21(79)	26(74)	31(69)	37(63)	48(52)	62(38)	0.978			
	Rhizopus artocarpi	5(95)	9(91)	12(88)	17(83)	21(79)	40(60)	0.962			
	Botryodiplodia theobromae	27(73)	30(70)	34(66)	46(54)	51(49)	59(41)	0.982			
Root of *Vinca rosea*	*Bipolaris sorokiniana*	100(0)	100(0)	100(0)	100(0)	100(0)	100(0)	0	259.27	4.887	10.3972
	Fusarium oxysporum f. sp. *vasinfectum*	37(63)	45(55)	51(49)	58(42)	62(38)	74(26)	0.989			
	Rhizopus artocarpi	100(0)	100(0)	100(0)	100(0)	100(0)	100(0)	0			
	Botryodiplodia theobromae	40(60)	51(49)	54(46)	69(31)	74(26)	81(19)	0.989			
Leaf of *Azadirachta indica*	*Bipolaris sorokiniana*	100(0)	100(0)	100(0)	100(0)	100(0)	100(0)	0	921.28	190.14	1.8711
	Fusarium oxysporum f. sp. *vasinfectum*	19(81)	27(73)	35(65)	41(59)	53(47)	67(33)	0.991			
	Rhizopus artocarpi	100(0)	100(0)	100(0)	100(0)	100(0)	100(0)	0			
	Botryodiplodia theobromae	41(59)	48(52)	53(47)	61(39)	64(36)	77(23)	0.983			
Root of *Azadirachta indica*	*Bipolaris sorokiniana*	100(0)	100(0)	100(0)	100(0)	100(0)	100(0)	0	6598.18	230.97	2.229
	Fusarium oxysporum f. sp. *vasinfectum*	13(87)	21(79)	25(75)	39(61)	45(55)	56(44)	0.993			
	Rhizopus artocarpi	100(0)	100(0)	100(0)	100(0)	100(0)	100(0)	0			
	Botryodiplodia theobromae	62(38)	67(33)	71(29)	79(21)	85(15)	90(10)	0.993			
Seed of *Azadirachta indica*	*Bipolaris sorokiniana*	36(64)	40(60)	41(59)	67(33)	72(28)	82(18)	0.958	3967.16	266.85	2.3299
	Fusarium oxysporum f. sp. *vasinfectum*	31(69)	36(64)	48(52)	60(40)	69(31)	75(25)	0.994			
	Rhizopus artocarpi	100(0)	100(0)	100(0)	100(0)	100(0)	100(0)	0			
	Botryodiplodia theobromae	45(55)	48(52)	52(48)	57(43)	61(39)	66(34)	0.997			

* Mean of three replications. ( ) Parenthesis show the percentage of spore/conidial germination.
r1 = Correlation value between immersion period and germination inhibition of spore/conidia.

Table 2: Effect of smoke on the inhibition of spore/conidial germination of different fungi grown on PDA.

Name of Treatment	Name of Fungi	Percentage of inhibition of spore germination*.			Correlation (r2)	Calculated F value (5%)		$LSD_{[0.05]}$
		5	10	15		Fungi	Exposed period	
Rice straw	*Bipolaris sorokiniana*	95 (5)	96 (4)	97 (3)	0.999	55.725	24.394	5.8279
	Fusarium oxysporum f. sp. *vasinfectum*	91 (9)	100 (0)	100(0)	0.866			
	Rhizopus artocarpi	100 (0)	100 (0)	100 (0)	0			
	Botryodiplodia theobromae	80 (20)	82 (18)	96 (4)	0.907			
Wheat straw	*Bipolaris sorokiniana*	85 (15)	95 (5)	97 (3)	0.955	134.745	55.503	4.1104
	Fusarium oxysporum f. sp. *vasinfectum*	93 (7)	100 (0)	100 (0)	0.866			
	Rhizopus artocarpi	100 (0)	100 (0)	100 (0)	0			
	Botryodiplodia theobromae	80 (20)	89 (11)	91 (9)	0.948			
Tobacco leaf	*Bipolaris sorokiniana*	93 (7)	94 (6)	95 (5)	0.999	648.774	4.2906	6.0987
	Fusarium oxysporum f. sp. *vasinfectum*	11 (89)	15 (85)	17 (83)	0.977			
	Rhizopus artocarpi	100 (0)	100 (0)	100 (0)	0			
	Botryodiplodia theobromae	51 (49)	55 (45)	78 (22)	0.923			
Dhup (incense)	*Bipolaris sorokiniana*	23 (77)	29 (71)	54 (46)	0.948	8994.473	779.094	1.4541
	Fusarium oxysporum f. sp. *vasinfectum*	89 (11)	100 (0)	100 (0)	0.866			
	Rhizopus artocarpi	100 (0)	100 (0)	100 (0)	0			
	Botryodiplodia theobromae	32 (68)	52 (48)	61 (39)	0.976			

* Mean of three replications. ( ) Parenthesis show the percentage of spore/conidial germination.
r2 = Correlation value between smoke exposed period and germination inhibition of spore/conidia.

value of spore/conidial germination of selected pathogens in different types of plant extracts and immersion period is grater than table value. It indicates a significant role of plant extracts and immersion period on spore/ conidial germination (Table 1). Alam *et al.* (1999) reported the antifungal effects of leaf and root extracts of *Vinca rosea* and leaf, root and seed extracts of *Azadirachta indica* against chilli fruit rot pathogen *Alternaria tenuis*. Singh *et al.* (1993) reported the antifungal activities of leaf extracts against *Botryodiplodia theobromae, Fusarium oxysporum, Helminthosporium spiciferum, Curvularia lunata, Aspergillus flavus* and *Trichothecium roseum*. They used some medicinal plants such as, *Calotropis procera, Vitex negundo, Lantana camara, Azadirachta indica, Ficus religiosa, Ocimum sanctum, Thuja orientalis, Argemone mexicana, Achyranthes aspera, Datura fastuosa* and *Ricinus communis* and observed good control against these pathogens. Of the 11 leaf extracts, those of *A. indica* and *O. sanctum* were the most effective in controlling the fungi. The present study indicates the presence of some antifungal compounds in *A. indica* and *V. rosea*.

Smoke of rice straw, wheat straw, tobacco leaf and 'dhup' were effective for the inhibition of fungi. Hundred per cent spore germination inhibition of *R. artocarpi* occurred, when this fungus was smoked using wheat straw, rice straw, tobacco leaf and 'dhup' smoke for 5-15 minutes in a smoked chamber. Effect of smoke is more or less equal on *F. oxysporum* f. sp. *vasinfectum*, but 100% inhibition occurred within 10 – 15 minutes of smoke using rice straw, wheat straw, tobacco leaf and 'dhup' smoke. Not only that, Table 2 also indicates the effectiveness of smoke against all the tested fungi. Correlation (r2) value (0.866-0.999) indicates that there was a highly significant relationship between smoke exposed period (5-15 minutes) and inhibition of spore germination, except *R. artocarpi*. Calculated F value of germination inhibition of tested fungi was greater than table value in all the cases. This result indicates a significant role of smoke from different sources and their exposure period on the inhibition of selected fungi (Table 2). Alam *et al.* (1999) reported similar results against chilli fruit rot pathogen *Alternaria tenuis*. They observed that the growth of *A. tenuis* was totally inhibited when inoculated on the medium exposed to rice straw and 'dhup' smoke for 5 to 15 minutes. Tobacco leaf smoke was ineffective against *A. tenuis* as

fungitoxicide.

Application of chemical fungicides is a conventional method to control the diseases caused by fungal pathogens. Tremendous health hazards are reported to occur during the application of fungicides in field conditions (Alam *et al.*, 1999). Present experiment was carried out for the protection of environment and save the health hazards of animal kingdom. This study suggested that all the tested plant extracts and smoke of rice straw, wheat straw, tobacco leaf and 'dhup' has antifungal effect and their application in field condition will reduce severity of diseases.

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