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## Effect of Leaf Exudates and Leaf Tissue Sap of *Barleria prionitis L.* on Spore Germination of Some Spices of Anamorphic Fungi

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### ABSTRACT

The extracts of plants part were recommended to control the disease. In the present investigation, the leaf exudates and leaf tissue sap of *Barleria prionitis L.* has been evaluated for its antifungal properties against some species of anamorphic fungi i.e *Curvularia lunata*, *Curvularia clavata*, *Alternaria alternata*, *Nigrospora oryzae*, *Cladosporium oxysporum* isolated from leaf surface of *Barleria prionitis L.* The percentage inhibition of spore germination was calculated and result revealed that 40%-85% inhibition of all anamorphic species.

**Key word:** Spore germination, anamorphic species, leaf exudates, leaf tissue sap

### INTRODUCTION :

*Barleria prionitis L.* (Family Acanthaceae; commonly known as Vajradanti), is an annual shrub, 3-4 feet high, found throughout tropical South Africa and in Asia. This plant parts use for boils glandular swellings cleaning wounds, whooping cough, dropsy and anacsarcs[1-4]. The plant extract has also shown its potential applications as diaphoretic and expectorant. The plant has also shown anti-respiratory syncytial virus, anti-arthritis, anti-inflammatory and anti-fertility activities and strength the gum in toothache [5-7]. In all fungi spore germination is a key process and most common fungal infection structures. They have tendency to germinate on the surface of a putative host were they come in contact. The extracts of 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 [8].The extracts of plants also exhibited marked effect on germination of fungal spores [9]. In early

studied shown that the chemicals found in leaf induce wheat stem rust to differentiate the appressoria. The compounds related to nicotine in plant leaf having stimulatory effect on germination of urediospores of *Uromyces* species [10-11]. The leaves exudates consist of stimuli which intensifies the establishment of fungi on their surfaces. Keeping this in view the present investigation, the leaf exudates and leaf tissue sap of *Barleria prionitis* L. has been evaluated for its antifungal properties against some selected anamorphic fungi isolated from leaf surface of *Barleria prionitis* L. and the percentage inhibition of spore germination was calculated.

## MATERIAL AND METHODS

Antifungal activity of water soluble exudates of *Barleria prionitis* L was assayed. Exudates of leaves were collected by dipping them in sterilized distilled water for different length of time and conidial germination of non-pathogenic to *Barleria prionitis* L

### A. Selection of leaf and fungi

*Barleria prionitis* L was selected for present investigations which were cultivated in Botanical Garden of Govt. Science College Raipur allowed to grow under regular conditions of moisture. For obtaining leaf exudates, potted plants of *Barleria prionitis* L. were cover in moist bags and sprayed three times with distilled water. At three hourly intervals for 12 hours, droplets were collected with the help of sterilized dropper from leaves which represented a solution of exudates. The conidial suspension of selected fungi sprayed over leaf of *Barleria prionitis* then the plants were covered by plastic bags for 4 days to keep the humidity high.

For obtaining tissue sap fresh leaves wash with distilled water are picked and drop into sterilized polythene bags than these leaves crush in mortar and finally sap of different concentration obtained by adding proper amount of distilled water.

The selected fungi were *Curvularia lunata*, *Curvularia clavata*, *Alternaria alternata*, *Nigrospora oryzae* and *Cladosporium oxysporum* form leaf surface of *Barleria prionitis*.

### B. Percentage Inhibition in spore Germination

Hanging drop technique was use to carried out spore germination. This technique permit fungi to grow in normal living condition. The suspension of exudates and fungal spore (200-300) from 5 day old culture were individual picked up with sterile inoculatio Needle and mixed in the exudate. Each fungal spofre is placed on coverslip and then inverted to produce a hanging drop in depression of the slide. The suspension of each fungi slides were incubated at 25+\_2 for 24 hours and then fixed with cotton blue in lactophenol .Three replication of wxperiment were made and spore mixed carried out by "Hanging Drop Technique" The suspension of exudates and fungal spores (200–300) from 5- day old cultures were prepared to study the germination of fungal spores. All the tested fungi were individually picked up with a sterile inoculation needle and mixed in the exudates. For the each experiment the suspension of fungal spores is placed on a cover slip, and then inverted over the concave depression of the slide to produce a hanging drop. Spores mixed in

distilled water without leaf exudates served as the control.. Germination was observed under a light microscope

$$\text{Percentage of Germination Spore (GS)} = \frac{\text{Number of spore germinated}}{\text{Total number of Spores Examined}} \times 100$$

$$\text{Percentage Inhibition in spore Germination} = \frac{\text{GS}}{\text{GS} - \text{TSG}} \times 100$$

Where,

SG - Number of Spore Germinated in Control

TSG- Total number of Spores Germinated in Treatment [12]

## RESULT AND DISCUSSION

The spore germination percentage of different fungi in different concentration of leaf exudates and tissue sap is shown in **Table No.1** form the data percentage for germination of spore of *Curvularia lunata* in control was 93.33%, while percentage germination was 40.00 in leaf exudates and 53.33 in leaf tissue sap. With increase in concentration of leaf exudates and leaf tissue sap decrease in percentage of germination of spore of *Curvularia lunata* in leaf exudates were 35.00 while percentage germination was 45.00 in leaf tissue sap.

The germination of spore of *Curvularia clavata* in distilled water was 93.75%, while percentage of germination was 43.75 in leaf exudates and 50.00 in leaf tissue sap. With increase in concentration of leaf exudates and leaf tissue sap decrease in percentage of germination of spore in leaf exudates were 36.10, while 35.00 in leaf tissue sap. Whereas germination of spore of *Cladosporium oxysporum* in control was 94.44%, while percentage of germination was 33.33 in leaf exudates and in 44.44 eaf tissue sap. With increase in concentration of leaf exudates and leaf tissue sap decrease in percentage of germination of spore in leaf exudates were 28.20 While 36.20 in leaf tissue sap.

In case of *Nigrospora sphaerica* germination of spore in distilled water was 95.75%, while percentage germination was 45.24 in leaf exudates and 50.00 in leaf tiwssue sap. With increase in concentration of leaf exudates and leaf tissue sap decrease in percentage of germination of spore of *Nigrospora sphaerica* in leaf exudates were 45.24 while percentage germination was 32.67 in leaf tissue sap, while in *Alternaria alternata* the spore germination was 87.50% in control, 37.50 in leaf exudates and 43.75 in leaf tissue sap, while increase in concentration in leaf exudates spore germination was 24.60 and 34.12 in leaf tissue sap.

The percentage inhibition of spore germination of different fungi in different concentration of leaf exudates and leaf tissue sap were show in **Table. No. 2** The percentage inhibition of spore germination in *Curvularia lunata* were, 60.00 in leaf exudates and 46.67 in leaf tissue sap, while increase in concentration in leaf exudates spore germination was 65.00 and 55.00 in leaf tissue sap. Whereas spore germination of *Curvularia clavata* inhibited to 56.25 in leaf exudates and in 50.00 leaf tissue sap. While increase in concentration of leaf exudates and leaf tissue sap decrease in percentage inhibition of spore germination in leaf exudates were 63.90 and 64.80 in leaf tissue sap.

The percentage inhibition of spore germination in *Cladosporium oxysporum* were, 66.66 in leaf exudates and 55.56 in leaf tissue sap, while increase in concentration in leaf exudates spore germination was 71.80 and 63.80 in leaf tissue sap. While in case of *Nigrospora sphaerica* percentage inhibition of spore germination was 54.75 in leaf exudates and 50.00 in leaf tissue sap. With increase in concentration of leaf exudates and leaf tissue sap increase in percentage inhibition of spore germination of *Nigrospora sphaerica* in leaf exudates were 65.35 while percentage inhibition was 67.33 in leaf tissue sap. The percentage inhibition of spore germination in *Alternaria alternata* were, 62.50 in leaf exudates and 56.25 in leaf tissue sap, while increase in concentration in leaf exudates spore germination was 75.4 and 65.88 in leaf tissue sap. From **figure No.1** the percentage inhibition of *Alternaria alternata* and *Cladosporium oxysporum* were maximum in both leaf tissue sap and leaf exudates, while increase in concentration of leaf tissue sap and leaf exudates the inhibition increase in *Nigrospora sphaerica* and *Cladosporium oxysporum* was maximum while *Alternaria alternata* was least affected.

As moisture is key factor for the growth of spore germination, so the relative humidity over leaf was increase by putting them in polythene bag [13]. The effect of relative humidity on spore germination in leaf exudates were show in **Table No.3**. Form data it show that the percentage spore germination of *Curvularia lunata* increase from 40.00 to 68.00 as RH increase 55 to 70 in leaf exudates Whereas in *Curvularia clavata* increase from 56.25 to 63.90 in leaf exudates, while spore germination of *Cladosporium oxysporum* increase from 33.33 to 52.23 in leaf exudates. This shows that moisture makes some favorable environment for the growth of spore germination.

The spore germination percentage of *Nigrospora sphaerica* increase from 45.24 to 68.70 in leaf exudates, while spore germination of *Alternaria alternata* increase from 37.50 to 67.00 as RH increase 55 to 70 in leaf exudates. Both the species mostly affected by moisture in environment that make favorable for its growth.

**Table No.1 Percentage of Germination of some dominant fungal spores in leaf exudates and leaf tissue sap.**

S.No	Fungi	Percentage Germination of Fungal Spores				
		In distilled water (control)	In leaf exudates		In leaf tissue sap	
			10µl	20µl	10µl	20µl
1	<i>Curvularia lunata</i>	93.33	40.00	35.00	53.33	45.00
2	<i>Curvularia clavata</i>	93.75	43.75	36.10	50.00	35.00
3	<i>Cladosporium oxysporum</i>	94.44	33.33	28.20	44.44	36.20
4	<i>Nigrospora sphaerica</i>	95.75	45.24	34.65	50.00	32.67
5	<i>Alternaria alternata</i>	87.50	37.50	24.60	43.75	34.12

**Table No.2 Percentage of Inhibition of some dominant fungal spores in leaf exudates and leaf tissue sap.**

S.No	Fungi	Percentage Inhibition of Fungal Spores			
		In leaf exudates		In leaf tissue sap	
		10µl	20µl	10µl	20µl
1	<i>Curvularia lunata</i>	60.00	65.00	46.67	55.00
2	<i>Curvularia clavata</i>	56.25	63.90	50.00	64.80
3	<i>Cladosporium oxysporum</i>	66.66	71.80	55.56	63.80
4	<i>Nigrospora sphaerica</i>	54.75	65.35	50.00	67.33
5	<i>Alternaria alternata</i>	62.50	75.4	56.25	65.88

Figure No.1 Show Percentage inhibition of different fungi at different con of Leaf exudates (LE), and Leaf Tissue sap(TS)

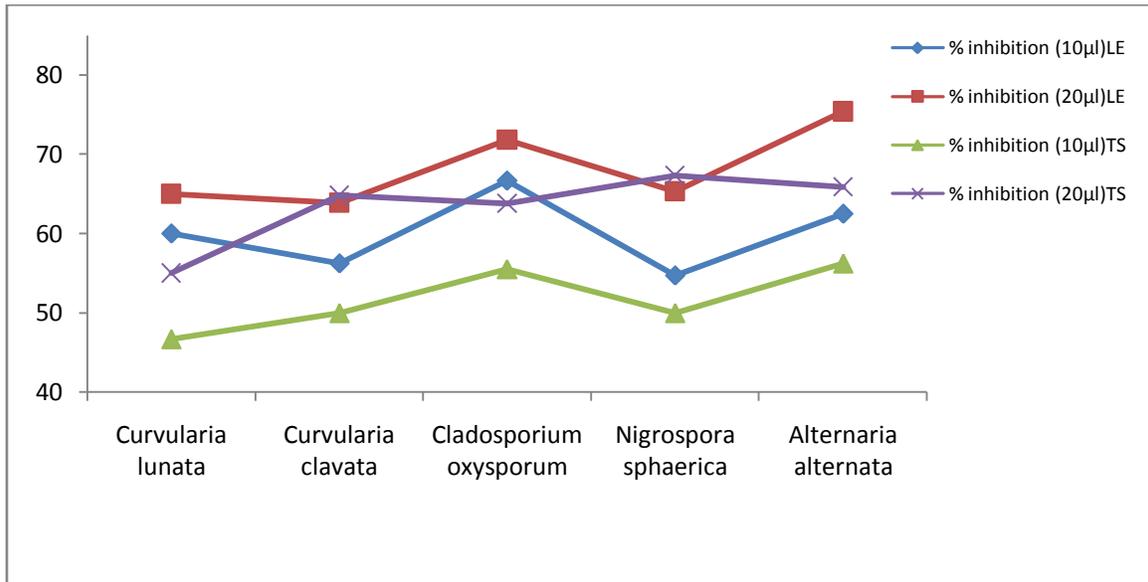


Table No.3 Effect of RH factor on spore germination

S.No	Fungi	Spore Germination of Fungal Spores			
		In distilled water (control)		In leaf exudates 10µl	
		RH 55.00	RH 70.00	RH 55.00	RH 70.00
1	<i>Curvularia lunata</i>	93.33	96.12	40.00	68.00
2	<i>Curvularia clavata</i>	93.75	96.78	43.75	58.97
3	<i>Cladosporium oxysporum</i>	94.44	98.14	33.33	52.23
4	<i>Nigrospora sphaerica</i>	95.75	98.80	45.24	68.70
5	<i>Alternaria alternata</i>	87.50	94.56	37.50	67.00

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