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Effect Of Leaf Extracts Of Lawsonia Inermis Linn. On Curvularia Lunata, Caused Leaf Spot Disease Of Maize

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Abstract: ***In the present study the effect of alcoholic, hydroalcoholic and aqueous crude extracts of Lawsonia inermis Linn. leaf on Curvularia lunata which caused leaf spot disease of maize was studied. For the in vitro control of curvularia lunata each extract used as 10mg/ml concentrate with best selectively solvents. The extract that most effectively inhibited growth was found to be alcoholic leaf extract. The results indicated that this plant can be used to develop the plant extract based bio-formulation for effectively control of leaf spot disease of maize in the way of eco-friendly.***

Keywords: Plant extracts, Bio-formulation, Antimicrobial activity, Crop plant.

I. INTRODUCTION

Plant parasitic fungi play direct role in the decreasing of productivity of several economically important crop plants [21]. Wheat, rice, maize and barley are foremost cereal crops in world [6]. Maize is an essential crop and it can be used to prepared large variety of food and non food product [7, 15, 23]. It is vulnerable to several bacterial and fungal diseases, out of them leaf spot disease of maize caused by *Curvularia lunata* is one of the most disparaging disease, spreading worldwide and causes up to 60% losses [2, 14, 16] and Li-yan *et al*. [17] also reported the range of yield losses is 10.10% ~ 48.62. According to International Maize and Wheat Improvement Center, Mexico it is caused significant damages in maize growing region. For controlling plant diseases human used waste amount of chemical fungicides because of its diverse use and ease of synthesis. But it has now been prove that chemically synthesized fungicides create serious environmental problems and also poisonous to non-target organisms [3, 11, 12, 18, 20, 4, 8].

So now a day's center of attention is changing towards biological methods to manage plant diseases as they have no adverse consequence on humans as well as environment [1, 24]. Use of plant based fungicides in control plant disease is progressively gaining importance as they are eco-friendly and cost effective [9, 19].

Hence in the present study effort will be made to develop the bio formulation using leaf extract of *Lawsonia inermis* for systematic control of leaf spot disease of maize caused by *Curvularia lunata.*

II. MATERRIALS AND METHODS

TEST MATERIAL

PATHOGEN: Collection of disease plant materials, isolation and purification of the pathogen:

Infected leaves were collected from the agriculture field of RCA, Udaipur. The fungus was isolated from infected leaf by Agar plate method [13]. For this leaves showing typical lesion of *Curvularia* leaf spot were cut into small pieces, surface sterilized with 0.1% mercuric chloride ($HgCl_2$) for two minutes, and there after rinsed thrice in distilled water and transferred on potato dextrose agar (PDA) plates and incubated at 28 ± 2^0 C for 6-7 days. Further subculture was made from periphery of mycelia growth. And cultures are being maintained on media i.e. Potato dextrose agar at 4^0C.

IDENTIFICATION OF ISOLATED PLANT PATHOGEN: The culture isolated from the maize was identified as fungus *Curvularia lunata by* the senior maize pathologist (AICRP-Maize New Delhi) Professor S. S. Sharma, Department of Plant Pathology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and plant technology.

III. PREPARATION OF CRUDE EXTRACT

The fresh leaves of *Lawsonia inermis* were collected from the campus of University College of science M.L.S.U. Udaipur. The plant was submitted and identified at Herbarium of Department of Botany, University of Rajasthan, Jaipur, India as *Lawsonia inermis* Linn. and given voucher specimen number RUBL211447.The collected plant material was shade dried at room temperature and then ground in an electrical grinder. The ground material was passed through a sieve of mesh size 60 to obtain a fine powder which was used to prepare the extract. Crude extract was prepared according to the cold extraction method suggested by Shadomy and Ingraff [22]. Cold extraction was done in water, 50% hydro alcohol as well as absolute alcohol. 20 gm dried and powdered plant material was suspended in 100 ml of solvent (Alcohol/water and 50% hydro alcohol) for 48 hrs. The suspension was filtered through Whatman filter paper no.1 then vacuum dried with the help of rotary vacuum evaporator. The dried residue was used as extract and solvent was recycled.

PERCENT EXTRACTIVE VALUE

The dry extracts were weighed and their percentage in term of the dry weight of the plant material was estimated by following formula:

$$\text{Percent extractive} = \frac{\text{Weight of dried extract}}{\text{Weight of dried plant material}} \times 100$$

IV. SELECTION OF SUITABLE SOLVENT FOR ANTIFUNGAL ASSAY

A. SOLUBILITY TEST OF PLANT EXTRACT

Solubility test plays an important role in the selection of suitable solvent for antifungal assay. Most of the test molecules are insoluble in water; solubility of test compound is generally enhanced by use of suitable organic solvent (methanol, acetone, DMF, DMSO, T20 and T80). To test the solubility, extract was dissolved in 10mg/ml concentration of respective solvents. After 24 hrs the extracts were filtered through a pre-weighed filter paper.

B. INHIBITORY ACTIVITY OF SOLVENT AGAINST TEST PATHOGEN

Besides the solubility, inhibitory activity of solvent is also subject of selection of suitable solvent. Best solvent may have good solubility as well as less toxic. To find out the less toxic solvent all the solvent preferred in solubility testing were examined for the inhibitory effect on test pathogen using agar well diffusion method [5]. In agar well diffusion method 12 mm wells bored in PDA plates were filled with 250 µl of 10%, 50% and 100% concentration of respective solvent and plates were inoculated with 10 µl spore suspension (1.0×10^{-7} spore ml^{-1}) of 7 day old culture and inhibitory activity was measured as appearance of inhibition growth zone without any kind of mycelia growth.

ANTIFUNGAL ACTIVITY OF CRUDE EXTRACTS OF LAWSONIA INERMIS LINN.: The inhibitory activity of crude alcoholic, 50% alcoholic and aqueous extract was done using poison food technique [10]. In respect 100 mg of extract was dissolved in 10 ml acetone to prepare stock solution of 10mg/ml concentration. 1 ml of stock solution was mixed with 9 ml molten sterile PDA culture medium and further this mixture was poured into pre-sterilized petri-plates (9 cm diameters) and allowed to solidify at room temperature. Thus prepared petri-plates were inoculated aseptically with 6mm disc of test pathogen's cultures. The petri-plates were then incubated at 28±2C for seven days. Bavistin, mancozeb and only PDA culture media are used as control series along with test samples. Antifungal activity of extract was measured as a function of increasing in growth of 6 mm disc of inoculums.

After seven day of incubation the Average diameter of the fungal colonies was measured and mycelia growth in percentage was calculated by the following formula given below:

$$\text{Mycelial growth inhibition} = \frac{gc - gt}{gc} \times 100$$

gc= growth of mycelia colony after 7days incubation period in control set subtracting the diameter of inoculums disc.

gt= growth of mycelia colony after 7days incubation period in treatment set subtracting the diameter of inoculums disc.

V. RESULTS AND DISCUSSION

In the present study preparation of crude extract, selection of suitable solvent for antifungal activity, and assay of antifungal activity of different type of crude extract were studied. The percentage extract values of all extracts are depicted in table 1.

The highest percentage extractive value was found in 50% Alcoholic extract followed by 100% Alcohol extract and Aqueous extract.

There are two parameters i.e. solubility of extract and toxicity of solvent, play important role in selection of suitable solvent for antifungal activity.

The solubility of extract and toxicity of solvents are depicted in table no 1.1 and 1.2 respectively. On the basis of solubility of extract and inhibitory activity of solvent, acetone is the best solvent for further evaluation of antifungal activity of extracts. The results of antifungal activity of crude extract of leaf of *Lawsonia inermis* are depicted in table no. 1.3. The best activity was observed with 100% alcoholic extract.

Result: Following percentage extractive values of extracts found

100% Alcohol extract	50% Alcoholic	Aqueous
13.65	14.95	12.67

Table 1

Result: Following results were obtained of solubility

Extracts / Solubilty	100% Alcohol extract	50% Alcohol extract	Aqueous extract
Highest	Acetone	Acetone	Acetone
Lowest	Tween 20	Tween 80	Tween 20

Table 1.1

Solvents	Concentration	Zone of inhibition (mm)			
		R1	R2	R3	Mean±SD
Control	100%	**NI**	**NI**	**NI**	--
methanol	10	**NI**	**NI**	**NI**	--
	50	**NI**	**NI**	**NI**	--
	100	**2**	**3**	**3**	**2.67±0.58**
Acetone	10	**NI**	**NI**	**NI**	--
	50	**NI**	**NI**	**NI**	--
	100	**NI**	**NI**	**NI**	--
DMF	10	**NI**	**NI**	**NI**	--
	50	**2**	**3**	**2**	**2.33±0.58**
	100	**4**	**4**	**5**	**4.33±0.58**
DMSO	10	**NI**	**NI**	**NI**	--
	50	**NI**	**NI**	**NI**	--
	100	**NI**	**NI**	**NI**	--
T20	10	**NI**	**NI**	**NI**	--
	50	**NI**	**NI**	**NI**	--
	100	**3**	**3**	**4**	**3.33±0.58**
T80	10	**NI**	**NI**	**NI**	--
	50	**3**	**2**	**5**	**3.33±1.53**
	100	**4**	**5**	**5**	**4.67±0.58**

NI- No Inhibition

Table 1.2: Inhibitory activity of various concentrations of organic solvent against test pathogen

S. No.	Treatment		Growth Zone (mm) 7 days Mean±SD	%Mycelial growth inhibition
1.	**Bavistin**		**52.67±1.52**	**36.28**
2.	**Mancozeb**		**22.67±1.52**	**72.57**
3.	**Control only PDA**		**82.67±0.57**	**NI**
4.	**Extracts**	100% alcoholic	**35.33±0.57**	**57.26**
		50% alcoholic	**54.67±2.08**	**33.86**
		Aqueous	**65.00±1.00**	**21.37**

NI- No Inhibition

Table 1.3: Effect of crude extract of leaves of Lawsonia inermis

VI. CONCLUSION

On the basis of results obtained it can be concluded that *Lawsonia inermis* leaf extract possess significant antifungal activity against *Curvularia lunata.* The extract can be exploited in the management of seed-borne pathogenic fungus *Curvularia lunata* to prevent loss of production of maize in an eco-friendly way.

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