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Sanwani V
Department of Plant Pathology, Sam Higginbottom University of Agriculture Technology and Sciences. Allahabad, Uttar Pradesh, India

Nag K
Department of FLA, CoA, IGKV, Raipur, Chhattisgarh, India

Abhilasha A Lal
Department of Plant Pathology, Sam Higginbottom University of Agriculture Technology and Sciences. Allahabad, Uttar Pradesh, India

Corresponding Author:
Sanwani V
Department of Plant Pathology, Sam Higginbottom University of Agriculture Technology and Sciences. Allahabad, Uttar Pradesh, India

In-vitro evaluation of botanicals against pathogen isolated from diseased potato tubers

Sanwani V, Nag K and Abhilasha A Lal

Abstract
The experiment was conducted as a completely randomized design with seven treatments and three replications. The treatments were 10% concentrations of leaf extract. The treated plates were then incubated at 26 °C in the dark. The average diameter of the mycelia growth inhibition zone around the paper discs loaded with each treatment was measured seven days post incubation. The growth inhibition percent was calculated using the formula: IP=c-t/c×100, where IP was the growth inhibition percent, c and t were the diameter of growth inhibition zone in negative control and each of the other treatments. *Saraca asoca* leaf extract (10.00%), *Jatropha curcas* leaf extract (10.00%) *Lantana camara* leaf extract (10.00%), *Mentha spicata* leaf extract (10.00%), *Ocimum sanctum* leaf extract (10.00%), *Cymbopogon citratus* L. leaf extract (10.00%) and *Aloe barbadensis* Mill. leaf extract (10.00%) leaf extract (10.00%) were tested against *Fusarium solani* using poison food technique using Potato Dextrose Agar (PDA) which used as basal medium. Both the treatments tested were significantly effective in inhibiting the growth of pathogen over control. *Saraca asoca* leaf extract (10.00%) showed minimum inhibition percent (39.63%) as compared to *Jatropha curcas* leaf extract (10.00%) showed maximum inhibition percent (75.93%).

Keywords: Evaluation of botanicals, pathogen, isolated & potato tubers

Introduction
Potato (*Solanum tuberosum* L.) popularly known as 'The king of vegetables', has emerged as fourth most important food crop in India after rice, wheat and maize. Indian vegetable basket is incomplete without Potato. Because, the dry matter, edible energy and edible protein content of potato makes it nutritionally superior vegetable as well as staple food not only in our country but also throughout the world. Potato is a major food crop, grown more than 100 countries in world. The potato (*Solanum tuberosum*) crop is generally harvested during February and March in most regions of India. This is a time when temperature starts increasing to between 30 °C and 40 °C in the month of June followed by rains in July and August. The high temperature and humid conditions during this time favour dry rot and other types of rots of potatoes stored in heaps and country stores. This can lead to huge losses. Therefore, it becomes essential to store potatoes in cold stores for about five to six months. Nevertheless, the potatoes may still develop dry or soft rots if they have been mechanically damaged during a period of high temperatures. Sagar *et al.* (2011) [3]. Secor and Salas, (2001) [4] however, average annual crop losses attributed to dry rot have been estimated at 6 to25% and found that more than 60% of tubers in storage can be affected. Fusarium sp. that causes dry rot and spread readily among tubers during handling and planting which results in seed tuber rots and poor plants stand. The crop is mainly grown in rabi season, both under field as well as riverbed conditions and is generally harvested during February and March in most regions of India. Crop suffers from many diseases in field conditions and also at storage conditions. Fusarium dry rot of potato is a devastating postharvest disease worldwide and is caused by *Fusarium spp.* Fusarium species are common in most soils where potatoes are grown and can survive as resistant spores free in the soil. *F. sambucinum* and *F. solani* are commonly found on seed tubers in the spring and when temperature starts increasing to between 30°C and 40°C in the month of June followed by rains in July and August. The high temperature and humid conditions during this time favour dry rot and other types of rots of potatoes stored in heaps and country stores. This can lead to huge losses.

Materials and Methods

The *in-vitro* studies on management dry rot of potato carried out at Department of Plant Pathology, Sam Higginbottom University of Agriculture, and Technology & Science Allahabad. 2016-2017. The details of the materials used and methods followed during the course of the present investigation is collection of samples-The samples of dry rotted potato collected for isolation from local vegetable market of Naini, Allahabad and brought to the department of Plant Pathology laboratory for isolation of pathogen. Procedure - Dry rot-infected potato tubers were washed thoroughly with water, surface sterilized for 3 min. with 1.5% sodium hypochlorite, and washed twice with sterile distilled water. Tubers were cut and infected tissue was excised from the edge of the lesion or from the inside of potato dry cavity. The infected tissue was then cultured on potato dextrose agar supplemented with 100 mg/ml ampicillin plates were incubated at 25 ℃, 60% relative humidity and under a 12-h alternation of light and dark. Single spore cultures were obtained by dilution series after 4 days of single spore culture colony diameter was measured and morphological characters were recorded. All isolates were identified using morphological characteristics of colony and conidia including growth ratio and pigment of colony size and shape of conidia, and other morphological structures according to published descriptions (Booth, 1971: Leslie and Summerell, 2006) [2].

Culture medium

In this experimental study, standard Potato Dextrose Agar (PDA) medium used. The composition of PDA was as follows:

Peeled potatoes: 200 g
Dextrose: 20g
Agar agar: 20g
Distilled water: 1000ml

For the preparation of PDA media, 200 gm peeled and sliced potatoes boiled in one liter of water until potatoes became soft. Then it filtered through muslin cloth and adjust the filtrate to one litre and mixed with dextrose 20 gm and agar agar 20 gm and autoclaved for sterilization at 121.6 ^{O}C under pressure 1.54 kg/cm^2 for 15 minutes.

Isolation and purification

Dry rot-infected tubers washed thoroughly with water surface-sterilized for 3 min with 1.5% sodium hypochlorite, and washed twice with sterile distilled water. Tubers cut and infected tissue excised from the age of the lesion which is from the inside of potato dry cavity. The infected tissue then cultured on PDA supplemented with 100 mg par ampicillin. Plates incubated in 25, 60% relative humidity, and under a 12-h alternation of light and dark. Single spore culture obtained by dilution series. After 4 days of single spore culture. All isolates identified using morphological characteristics of colony and conidia, including growth ratio and pigment of colony, size and shape of conidia and other morphological structures, according to published description.

Results

The results of study entitled *"in-vitro* evaluation of botanicals against pathogen isolated from diseased potato tubers lab condition were conducted at the Department of Plant Pathology, Sam Higginbottom University of Agricultural, Technology and Sciences (SHUATS), Allahabad.

Table 1: Effect of plant extracts on the radial growth (mm) of *Fusarium solani* at 24 hrs. of the incubation.

Treatments		Conc.	R_1	R_2	R_3	24 hr
T_1	*Lantana camara*	10.00%	82.56	80.23	81.25	81.35
T_2	*Mentha spicata*	10.00%	81.34	81.40	81.88	81.54
T_3	*Ocimum sanctum*	10.00%	81.07	80.74	82.50	81.44
T_4	*Jatropha curcas*	10.00%	79.23	81.56	78.75	79.85
T_5	*Cymbopogon citratus* L.	10.00%	75.12	70.35	76.25	73.91
T_6	*Saraca asoca*	10.00%	97.30	85.71	83.75	88.92
T_7	*Aloe barbadensis* Mill.	10.00%	83.66	83.14	80.75	82.52
T_0			0.00	0.00	0.00	0.00
Mean						71.19
F- test						S
S. Ed. (±)						2.348
C. D. (P = 0.05)						4.977
C.V.						0.168

Table 2; Effect of plant extracts on the radial growth (mm) of *Fusarium solani* at 48 hrs. of the incubation.

Treatments		Conc.	R_1	R_2	R_3	48 hr
T_1	*Lantana camara*	10.00%	72.20	78.26	76.21	75.56
T_2	*Mentha spicata*	10.00%	78.85	83.15	77.20	79.73
T_3	*Ocimum sanctum*	10.00%	74.40	73.26	77.75	75.14
T_4	*Jatropha curcas*	10.00%	78.02	85.60	78.02	80.55
T_5	*Cymbopogon citratus* L.	10.00%	77.14	72.17	75.55	74.95
T_6	*Saraca asoca*	10.00%	1.10	2.17	79.67	27.65
T_7	*Aloe barbadensis* Mill.	10.00%	77.47	76.09	76.87	76.81
T_0			0.00	0.00	0.00	0.00
Mean						61.30
F- test						S
S. Ed. (±)						13.087
C. D. (P = 0.05)						27.745
C.V.						1.090

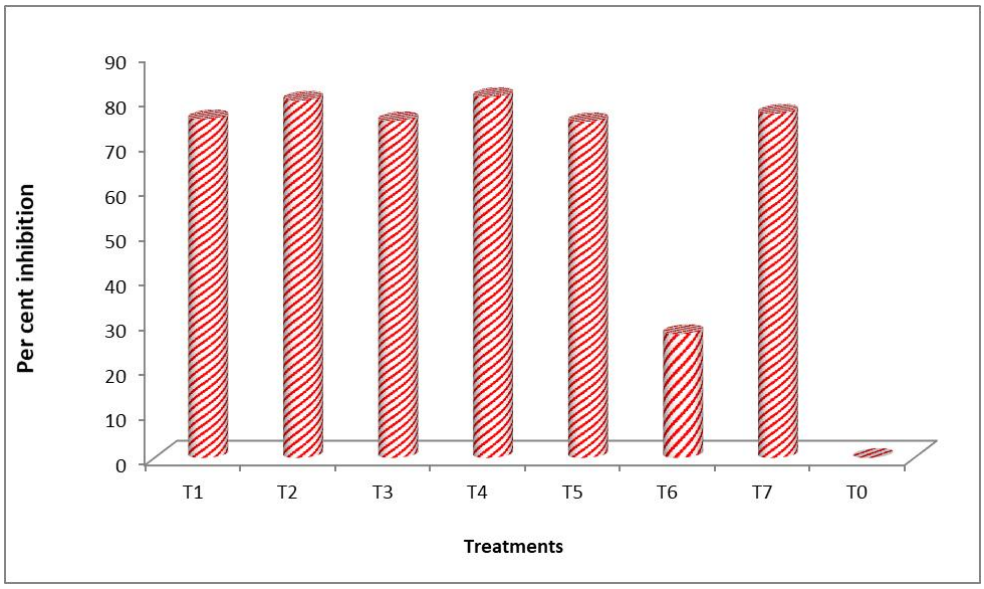

Fig 1: Percent inhibition mycelial growth of *Fusarium solani* as affected by treatments after 48 hrs.

Table 3: Effect of plant extracts on the radial growth (mm) of *Fusarium solani* at 72 hrs. of the incubation

Treatments		Conc.	R_1	R_2	R_3	72 hr
T_1	*Lantana camara*	10.00%	85.71	82.44	81.74	83.30
T_2	*Mentha spicata*	10.00%	90.18	84.82	82.93	85.98
T_3	*Ocimum sanctum*	10.00%	82.50	85.36	82.90	83.59
T_4	*Jatropha curcas*	10.00%	88.99	87.50	83.23	86.57
T_5	*Cymbopogon citratus* L.	10.00%	83.57	81.31	79.94	81.61
T_6	*Saraca asoca*	10.00%	81.20	81.90	83.23	82.11
T_7	*Aloe sbarbadensis* Mill.	10.00%	80.88	82.74	81.44	81.69
T_0			0.00	0.00	0.00	0.00
Mean						73.10
F- test						S
S. Ed. (±)						1.514
C. D. (P = 0.05)						3.209
C.V.						0.106

Table 4: Effect of plant extracts on the radial growth (mm) *Fusarium solani* at 96 hrs. of the incubation

Treatments		Conc.	R_1	R_2	R_3	96 hr
T_1	*Lantana camara*	10.00%	71.11	74.44	71.67	72.41
T_2	*Mentha spicata*	10.00%	71.11	75.56	73.89	73.52
T_3	*Ocimum sanctum*	10.00%	71.11	71.11	73.33	71.85
T_4	*Jatropha curcas*	10.00%	71.67	81.67	74.44	75.93
T_5	*Cymbopogon citratus* L.	10.00%	61.11	60.00	69.44	63.52
T_6	*Saraca asoca*	10.00%	0.00	45.00	73.89	39.63
T_7	*Aloe barbadensis* Mill.	10.00%	74.44	74.44	76.11	75.00
T_0			0.00	0.00	0.00	0.00
Mean						58.98
F- test						S
S. Ed. (±)						10.399
C. D. (P = 0.05)						22.045
C.V.						0.900

Summary and Conclusion

The treatments were 10% concentrations of leaf extract. The treated plates were then incubated at 26 °C in the dark. The average diameter of the mycelia growth inhibition zone around the paper discs loaded with each treatment was measured seven days post incubation (before the plates were completely covered with mycelia of the fungus).The growth inhibition percent was calculated using the formula: IP=c-t/c×100, where IP was the growth inhibition percent, c and t were the diameter of growth inhibition zone in negative control and each of the other treatments. *Saraca asoca* leaf extract (10.00%), *Jatropha curcas* leaf extracts (10.00%) *Lantana camara* leaf extract (10.00%), *Mentha spicata* leaf extract (10.00%), *Ocimum sanctum* leaf extract (10.00%), *Cymbopogon citratus* L. leaf extract (10.00%) and *Aloe barbadensis* Mill. leaf extract (10.00%) leaf extract (10.00%) were tested against *Fusarium solani* using poison food technique using Potato Dextrose Agar (PDA) which used as basal medium. Both the treatments tested were significantly effective in inhibiting the growth of pathogen over control. *Saraca asoca* leaf extract (10.00%) showed minimum inhibition percent (39.63%) as compared to *Jatropha curcas* leaf extract (10.00%) showed maximum inhibition percent (75.93%). Ashoka leaf extracts (10%) were found the most effective against *Fusarium solani*, after at 96 hours inoculation. Which were found minimum mycelium growth

than Lemon grass leaf extracts (10%) was found effective in mycelium growth as compare to other treatments except lemon grass which was taken as treated control. The results of the present study are *in- vitro* condition as such for validation of the results more such trials should be carried out in future.

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