Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(4): 889-891
Received: 13-05-2020
Accepted: 15-06-2020

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Isolation and *in vitro* evaluation of different botanicals on mycelia growth of *Alternaria alternata* (Fr.) Keissler causing leaf spot of brinjal

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Abstract
Brinjal is subjected to several diseases at all the stages of crop. Among all the diseases infecting brinjal, the leaf spot of brinjal is the most severe threat among all the vegetable crops. This disease is incited by the number of pathogens but the major causal organism is *Alternaria alternata*. Four plant extracts (Turmeric, Neem, Tulasi and Alovera) were evaluated against *Alternaria alternate* under *in vitro* conditions by using the poison food technique method at different concentration levels of (2.5%, 5%, 7.5%). Among the four plant extracts tulasi and turmeric showed most efficacious and successful in inhibiting the mycelial growth of *Alternaria alternate*, where as alovera showed lowest inhibition of pathogen causing leaf spot of brinjal.

Keywords: Plant extracts, Mycelium, *Alternaria alternata*, Leaf spot of brinjal

Introduction
Brinjal (*Solanum melongena* L.) belongs to:

Kingdom	:	Plantae,
Division	:	Magnoliophyta,
Class	:	Magnoliopsida,
Order	:	Solanine's,
Family	:	Solanaceae,

Brinjal (*Solanum melongena* L.) (USDA,2008) [15] is also known as aubergine, the name eggplant derived from the shape of the fruit of some varieties, which are white and shaped very similarly to chicken eggs (Chen and Li,19998) [4]. Total brinjal production in the world is about 32 million tons where India is the second largest producer after China in the world [6]. (Choudhary and Gaur. 2009) The crop grows in India on about 5,66,000 hectares and production 95,96,000 metric tons of fruits (Anon, 2008) [1]. The country's major brinjal growing states are West Bengal, Orissa, Bihar, Karnataka, Maharashtra and Gujarat. In Gujarat, the total area under cultivation of brinjal is 55,800 hectares with a annual production of 9,87,700 metric tons with a productivity of 177 metric tons / ha. (Anon, 2008). South Gujarat is an important state growing area of 1,3005 hectares under cultivation with a total production of 2,26,123 metric tons (Anon, 2009) [2]. In India, brinjal is eaten in a variety of ways as cooked vegetables. It has nutritional value in human diet because it contains around 1.4g of protein, 4.0g of carbohydrates, 0.3 g of fat, 18mg of calcium, 2.0mg of potassium and 0.9mg of iron per 100g of edible part. The disease occurs in the appearance of leaf spot with concentric rings. The spots are mostly uneven and coalesced to cover a large leaf area. In view of the seriousness of the issue, the present work has been carried out on the assessment of various botanicals against *Alternaria alternata* (Fr.) Keissler. *In vitro* condition.

Materials and Methods
The research on brinjal leaf spot (*Alternaria alternata*) was carried out in the Plant Pathology Lab, School of Agricultural, Uttaranchal University, Dehradun, Uttarakhand, India. (2019-2020)

Isolation of *Alternaria alternata*
The causal organism, *Alternaria alternata* was isolated from brinjal leaves showing the typical leaf spot symptoms of the disease. The infected leaves were cut in to small leaf bits and surface sterilized with one per cent sodium hypochlorite solution for 2-3 minutes and 3 times

repeatedly washed in sterilized distilled water. Then the infected leaf bits were transferred on to petri dishes (1-2 leaf bits per petri plate) containing PDA with the help of a sterile forceps and incubated at 25 °C for 10 days. Further purification and sub culturing were done on PDA slants and petri plates by hyphal tip isolation method.

Hyphal Tip Isolation

The method was followed for obtaining pure culture of *Alternaria alternata* since the fungus is known to be highly heterozygous. Hyphal tip isolation was done on 2% water agar plates. Diluted hyphal suspensions were prepared in sterile distilled water. One ml of such suspension was spread uniformly on water agar plates and observed for hyphae under microscope. Single isolated hypha was allowed to germinate. Each plate was incubated at 25 ±2 °C and periodically observed for germination under the microscope. Germinating hyphae was marked using marker and were cut by using cork borer and transferred on to the PDA plates incubated at 25±2°C to get the pure culture. No sectoring was observed in any of the isolates and all of them were found identical in their growth and colony character, Hence it was taken as pure pathogenic culture and was maintained for further studies**.**

In vitro* evaluation of botanicals against *Alternaria alternata

The relative efficiency of four different plant extracts were evaluated under *in vitro* conditions i.e, Turmeric (*Curcuma longa*), Tulasi (*Ocimum tenuiflorum*), Neem (*Azadiracta indica*) and Alovera (*Aloe barbadensis miller)*. The collected plant samples are washed thoroughly and shade dried for 7 days. The dried plant parts were crushed as fine powder by pestle and mortar. This powder was soaked at 1:10 ratio of the organic solvent methanol for 24 hours. The content was then filtered through whattman filter paper no. 1 then filter was evaporated to dryness. This dried extract was further powdered and then dissolved in distilled water. For standardization of the concentration of the effective from the methanol extracts were used @2.5, 5, 7.5% concentration. After standardization, was significantly effective form of officinal extracts for respective pathogens was further compared with recommended chemicals to find out an alternate towards the ecofriendly. Management of the pathogens responsible of important plant diseases.

Evaluation of botanical extracts was done by poisoned food technique. Botanical extracts were tested at 2.5%,5%,7.5% concentration by incorporating desired quantity of the plant extract respectively in separate flask. The poisoned media was poured into 70mm pre-sterilized petri plates under aseptic condition and incubated at BOD incubator at15+2 c the radial mycelial growth was observed and recorded in alternative days until the control plate reaches full growth. The percentage growth inhibition was calculated.

$$I=100(C-T)/C$$

Where I - Per cent inhibition in mycelial growth C - Linear mycelial growth in control (mm) T - Linear mycelial growth in control (mm)

The experiment was carried out by CRD and data was analyzed after applying the applicable transformation.

Table 1: In vitro screening of following botanicals against Alternaria alternata

Roll No:	Common Name:	Botanical Name:	Plant Parts Used	Concentration (%):
1	Turmeric	*Curcuma longa*	Leaves	2.5, 5, 7.5
2	Tulasi	*Ocimum tenuiflorum*	Leaves	2.5, 5, 7.5
3	Neem	*Azadiracta indica*	Leaves	2.5, 5, 7.5
4	Alovera	*Aloe barbadensis miller*	Leaves	2.5, 5, 7.5

Experimental Results

In vitro* evaluation of botanical extracts against *Alterneria alternata

Four botanical extracts i.e. Turmeric, Tulasi, Neem, Alovera were tested against *Alterneria alternata* at three concentrations (2.5%, 5%, 7.5%) by poison food technique under *in vitro* condition.

Turmeric, Tulsi, Neem and Aloevera extracts were screened against *Alternaria alternata*, among these tulsi extract showed the maximum growth inhibition of 51.90%, 44.42%, and 38.09% at 7.5%, 5% and 2.5% concentration respectively. Followed by turmeric extract showed the growth inhibition of 50%, 44.28% and 38.09% at the concentration of 7.5%, 5% and 2.5% respectively, it was par with e coach other. However, neem extract showing growth inhibition at 7.5%, 5% and 2.5% concentration with the growth inhibition of 45.71%, 41.90% and 38.01% respectively. The result showed that the aloevera extract have poor ability to inhibit *Alterneria alternata* mycelial growth, which shows the growth inhibition of 34.28%, 30.95% and 23.33% at respective concentrations of 7.5%, 5% and 2.5%.

***In-vitro* evaluation of % Inhibition of different botanicals against leaf spot of brinjal caused by *Alternaria alternata* (Fr.) Keissler**

Table 2: *In-vitro* evaluation of % Inhibition of different botanicals against leaf spot of brinjal caused by *Alternaria alternata* (Fr.) Keissler.

Concentration (%)	Growth Inhibition (%) Table			
	Turmeric	Tulsi	Neem	Alovera
2.5	38.09	38.09	38.01	23.33
5	44.28	44.42	41.90	30.95
7.5	50	51.90	45.71	34.28
C.D.	7.45	3.21	5.65	3.21
SE(m)	2.11	0.91	1.60	0.91

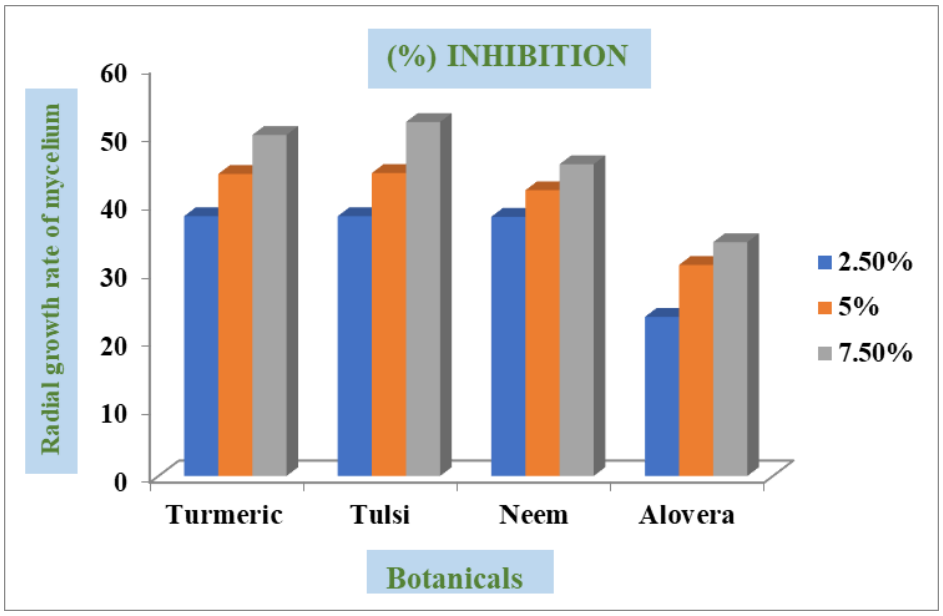

Fig 1: Graph showing % of inhibition of Alternaria alternata against different plant extracts

Discussion

Botanical are ecological safer, non-hazardous and non – polluting means of plant diseases management. Use of plant extracts is an alternative method of controlling the diseases of crop in the absence of resistant cultivars or when there is sudden outbreak of disease. Hence, they would continue to be one of the major tools of IDM. Evaluation of plant extracts *in vitro* is a handy tool to screen a large number of plant extracts and thus can serve as guide for testing. The results of *in vitro* studies revealed that out of four plant extracts the most effecting plant extracts was turmeric with 50% inhibition at all concentrations followed by tulasi with 51.90%, neem and alovera was the least effective. R. B. Rajput and S. R. Chaudhari reported that turmeric shows the maximum inhibiting growth.

Conclusion

In this study we revealed that four plant extracts i.e, Tumeric, Tulasi, Neem, Alovera gave best effective against mycelial growth inhibition of *Alterneria alternata* and it may be used for the control of leaf spot of brinjal.

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