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In vitro efficacy of plant extract (botanicals) against *Alternaria solani* (early blight of tomato)

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Abstract
Aqueous extracts of 5 plants selected from local flora were evaluated for antifungal potential against *Alternaria solani* by Poisoned Food Technique at 10% concentrations. In the experiment of *In vitro* efficacy of plant extracts against *Alternaria solani* by crude extraction method, the maximum inhibition was obtained by *Azadirachta indica* (60.49%), *Zingiber officinale* (54.73%) and *Ocimum sanctum* (53.09%) @10% concentration over all other plant extracts. In the experiment of *In vitro* efficacy of plant extracts against *Alternaria solani* by acetone extraction method, the maximum exhibition was obtained by *Azadirachta indica* (64.24%), *Ocimum sanctum* (58.62%) and *Zingiber officinale* (57.32%)@10% concentration over all other plant extracts. Out of these two methods, there was slight increase in inhibiting the mycelial growth of the pathogen *Alternaria solani* in acetone extract as compared to crude extract and therefore acetone extract is better than crude extract.

Keywords: Cherry tomato, antifungal, *Alternaria solani*, *in-vitro*, acetone extract, crude extract.

Introduction
There has been a tremendous increase in tomato (Lycopersicon esculentum. Mill, Family Solanaceae) production and consumption worldwide in recent years as an important vegetable and as an important raw material for many manufactured goods. Early blight caused by *Alternaria solani* is one of the most devastating tomato diseases in India. Processed tomato products have become an important dietary component in the developed world and also in developing countries. Each 1 percent increase in severity decreases yield by 1.36 percent, and when the illness is most serious, complete crop failure may occur (Pandey *et al*. 2003) [13]. In the U.S.A, yield losses of up to 79 percent are recorded, 20-40 percent of which are due to seedling losses throughout the field (Chaerani and Voorrips 2006) [9]. *Alternaria solani* is one of the various pathogens of tomatoes managed with the same substances, which makes it difficult to reliably estimate the total economic loss and the total expenditure on fungicides for early blight control. The best estimates indicate that the gross annual global fungicide control expenditure of *A. Solani* is around 77 million dollars, of which 32 million is for tomatoes (Kemmitt G). The use of resistant varieties against early blight is an environmentally friendly process, but most of the resistant varieties are not durable. Furthermore the method is expensive to implement and needs tremendous investment with great technological expertise. An significant eco-friendly and cost-effective production of herbal formulations for plant disease management is the development of approaching.
In the present research, for antifungal activity against *Alternaria solani*, higher plants were screened to select the effective aqueous plant extract that could be used for disease management.

Material and Methods
The laboratory experiment was conducted at Department of Plant Pathology and Agri. Microbiology, Rahuri during 2019 to evaluate the efficacy of plant extracts against *Alternaria solani.*

Methods of plant extraction
1.1 Crude extraction method
The crude extracts were obtained as per the method described by Bambode and Shukla (1973). In this method, 10g of the plant parts were weighed and thoroughly washed. The plant material was crushed in the mortar and pestle by adding 10 ml of sterilized water. After that the crushed material was strained through double layered muslin cloth and filter paper (Whatman No.40) and the filtrate obtained was used in the experiment.

1.2 Acetone extraction method

To extract the antifungal principle from the test plants in pure form, alcohol extracts of the plants were obtained by the method recommended by Shekhawat and Prasada (1971). In this method, the weighed quantity of plant material (10g) was washed in tap water and crushed in mortar and pestle. The pulp was taken in the flask and acetone (extra pure) was added to it in 1:4 proportion (w/v). A cork with glass tube in the center was fitted to the flask and they were made airtight with plaster of paris. The flasks were held in water bath for 1 hour at 60° C temperature for refluxing. At the end, the extracts obtained were passed through double layered muslin cloth and alcohol was evaporated by pouring the extracts in to the Petri dishes, having large surface area (150 mm). Within 10 to 15 minutes, the acetone in the extracts was evaporated and dense, acetone free extracts were obtained. These extracts were filtered through filter papers.

1.3 Screening of plant extracts for their antifungal properties against *Alternaria solani In vitro*

The plant extracts obtained by acetone extraction method was screened against the test fungus as follows

The plant extracts were tried against the test fungus. The plant extracts were supplemented to sterilized PDA in 1:2 proportion (Tripathi and Dixit, 1977) and the poisoned medium was poured in petri- dishes. A mycelial disc of the test fungus, 0.5 cm in diameter, was cut from the periphery of a 7 to 10 days old culture of fungus and aseptically inoculated into the medium. Each set was replicated thrice. The Petri plates poured with only PDA (without any plant extract) and inoculated served the control. The petri plates were incubated in incubator at temperature (28±1° C). Observations were recorded when the mycelial growth in control set touched the edges of the Petri plate. Percent inhibition of mycelial growth was calculated by the formula of Vincent (1927) [18].

Plate 1: Plant parts used for screening of their antifungal activities.

1.4 Experimental Details for plant extracts

Design : Completely Randomized Design, Replication: Three, Treatments : Six

Treatment details

T_1: *Ocimum sanctum*@ 10% concentration, T_2: *Azadirachta indica* @ 10% concentration, T_3: *Catharanthus roseus* @ 10% concentration, T_4: *Zingiber officinale* @ 10% concentration, T_5: *Lantana camera* @ 10% concentration, T_6: *Alternaria solani* Observations recorded for colony diameter of *Alternaria solani* in (mm) and percent growth inhibition due to plant extracts.

Result and Discussion

1. *In vitro* evaluation of plant extracts against *Alternaria solani*

1.1 Efficacy of plant extracts against *Alternaria solani* @10% concentration by crude extraction method

The data pertaining to *In vitro* evaluation of plant extracts against pathogen *Alternaria solani* causing early blight of Cherry tomato @10% concentration by crude extract method is presented in Table 1. (Plate II-a) revealed that the inhibition of pathogen was influenced significantly due to different plant extract treatments, percent inhibition ranged from 47.74 to 60.49 %.

Table 1: Efficacy of plant extracts against *Alternaria solani* @10% concentration by Crude extraction method.

Tr. No.	Plant extracts used	Mycelial growth of *Alternaria solani*	
		*Average colony diameter (mm)	**Growth inhibition (%)
T_1	Tulasi (*Ocimum sanctum*)	38.00	53.09
T_2	Neem (*Azadirachta indica*)	32.00	60.49
T_3	White Sadaphuli (*Catharanthus roseus*)	42.33	47.74
T_4	Ginger (*Zingiber officinalis*)	36.67	54.73
T_5	Ghaneri (*Latana camera*)	41.67	48.56
T_6	Control	81.00	00
S.E.±		0.36	
CD at 5%		0.79	

*Each value is the mean of three replications.

**The growth inhibition percent calculated on the basis of mean colony diameter (mm) recorded in

The maximum inhibition was obtained by T_2: *Azadirachta indica* (60.49%) over all other plant extracts which at par with T_4: *Zingiber officinal*e (54.73%) and T_1: *Ocimum sanctum* (53.09%). Plant extracts likeT_5: *Lantana camera (*48.52%) and T_3: *Catharanthus roseus* (47.78%) are significantly

different from T_2: *Azadirachtaindica* and showing least inhibition compared to control.

Similar results were proved by Waqas Raza *et al*., (2016) who proved that among five plant extract tested against for management of *Alternaria solani*, *Azadirachta indica* (69.65%) around significantly superior over the treatments.

1.2 Efficacy of plant extracts against *Alternaria solani* @10% concentration by acetone extraction method

The data pertaining the *In vitro* evaluation of plant extracts against pathogen *Alternaria solani* causing early blight of cherry tomato@ 10% concentration

Table 2: Efficacy of plant extracts against *Alternaria solani* @ 10% concentration by Acetone extraction method.

Tr. No.	Plant extracts used	Mycelial growth of *Alternaria solani*	
		*Average colony diameter (mm)	**Growth inhibition (%)
T_1	Tulasi (*Ocimum sanctum*)	35.70	58.82
T_2	Neem (*Azadirachta indica*)	31.00	64.24
T_3	White Sadaphuli (*Catharanthus roseus*)	46.33	47.78
T_4	Ginger (*Zingiber officinalis*)	37.00	57.32
T_5	Ghaneri (*Latana camera*)	49.70	42.68
T_6	Control	86.70	00
S.E.±		0.4005	
CD at 5%		0.8449	

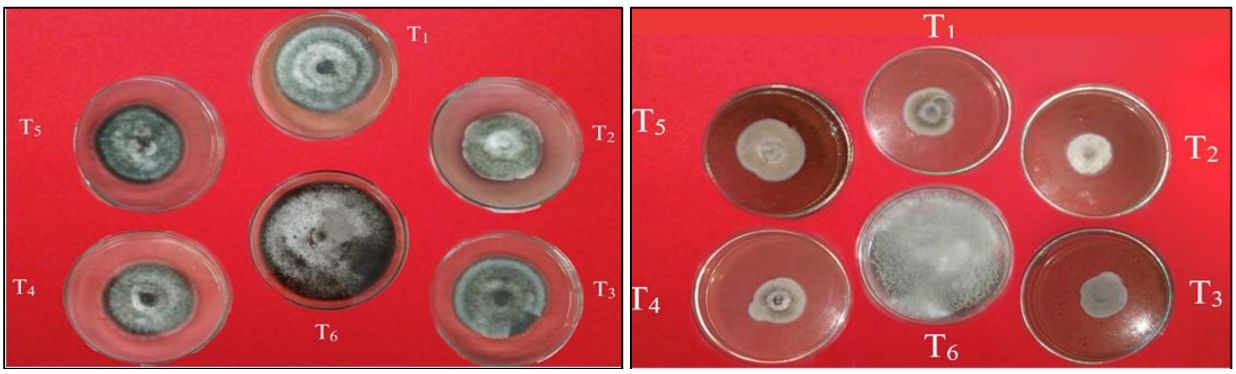
*Each value is the mean of the three replications.

**The growth inhibition percent calculated on the basis of mean colony diameter(mm) recorded in control showing 86.70mm growth by acetone extract method presented in (Table 2, Plate II-b) which revealed that the inhibition of pathogen was found significantly due to plant extract treatments. The overall percent inhibition was ranged from 42.68% to 64.24%. The maximum inhibition was obtained by T_2: *Azadirachta indica* (64.24%) followed by T_1: *Ocimum sanctum* (58.62%) and T_4: *Zingiber officinale* (57.32%). These three plant extracts are statistically at par witheach other. The least inhibition shown by T_3: *Catharanthus roseus* (47.78%) and T_5: *Lantana camera* (42.68%) compared to control, and both were at par with each other.

Similar results were obtained by Vaibhav Singh *et al*., (2018) [14] proved that among different plant extracts used, *Azadirachta indica* (Neem) was significantly inhibit the mycelial growth of the pathogen at all concentrations (5,10,15 and 20%). Sahar Murmu *et al.,* (2015) reported that for controlling early blight of potato only neem extract exhibited the percent reduction of disease (33.18%) over control treatment in field condition and inhibition of radial growth (59.85%) and spore germination (81.95%) in *In vitro* condition.

1.3 Comparative efficacy of crude and acetone extract of plant extracts against *Alternaria solani*.

Different researchers used crude or partially purified extract is the mixture of all secondary metabolites present in the plant part. The purpose of organic solvent like acetone for the separation of these metabolites according to their polarity and solubility. The result of comparative efficacy of the prominent plant extracts obtained by crude extraction and acetone extraction showed that, there was slight increase in inhibiting the mycelial growth of the pathogen *Alternaria solani* in acetone extract compared to crude extract. Neem, Ginger and Tulasi were found most effective plant extracts.



A. Efficacy of Crude Plant Extracts against *A. solani* **B.** Efficacy of Acetone Plant Extracts against *A. solani*

Plate 2: Efficacy of plant extracts on inhibition of mycelial growth of *Alternaria solani*

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