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## Screening of various organic treatments against leaf blight disease of tomato *in vitro* and green house conditions

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### ABSTRACT

Leaf blight disease caused by *Alternaria solani* is one of the prime diseases of tomato and is pervasive in almost all the tomato growing countries. The fungicidal phenomenon of the botanical extracts against leaf blight disease of tomato was evaluated under laboratory and greenhouse conditions. Extracts of *Eucalyptus obliqua*, *Calotropis procera*, deoiled cakes viz., Neem (*Azadirachta indica*), Mustard (*Sinapis arvensis*), groundnut (*Arachis hypogaea*), Karanj (*Millettia pinnata*), Mahua (*Madhuca longifolia*), Castor (*Ricinus communis*), Salmeal (*Shorea robusta*) and cow urine were evaluated singly or in combined form to examine the efficacy for inhibition of the fungal pathogen. The treatments were done on various isolates of *A. solani* in the different concentrations of 5%, 10%, 15%. Extracts in the combined form viz. *Eucalyptus obliqua* (10%) + Neem cake (*Azadirachta indica*) (10%) + cow urine (0.5%) showed the maximum inhibition of 85.3% in the laboratory experiments and 78 % in the greenhouse conditions as compared with untreated control. The minimum inhibition of 61.6% in percent disease index was exhibited by *Calotropis procera* + Karanj cake (*Millettia pinnata*) + cow urine (0.5%) in greenhouse conditions. Environmental suitable techniques could prove to be promising approach against the harmful and expensive chemical fungicides. The use of such crop yielding organic extracts can be suggested to farmers for inhibition of *A. solani*.

**Key Words:** *Alternaria solani*, botanical extract, leaf blight disease, tomato.

### INTRODUCTION

Tomato (*Lycopersicon esculentum*) is the second vegetable crop next to potato in the world. It is an excellent source of vitamins and minerals. Tomato crops are highly susceptible to plant pathogens viz. fungi, viruses and bacteria which collapse the yield and quality of tomato fruit [1]. Among the foliar diseases early blight caused by pathogen *Alternaria solani* is the most desecrate disease causing 78% losses in the yield [2]. Fungal pathogen diseases have always been a limitation on the efficacious production

of crops. The control of plant diseases and pests by synthetic chemicals cause serious intimidation to the environment and the health of humans. However, the plant pathologists are optimistic in developing ecofriendly alternatives to induce resistance in plants. It has been reported earlier that volatile constituents of aqueous extracts of medicinal plants inhibits the conidial germination of plant pathogens. The extracts of plants viz. Eucalyptus obliqua and Calotropis procera have especial antifungal effects on plant pathogens [3], [4]. In ancient content, several Agro based by products is specified and acknowledged to play vital role in the management of plant diseases due to their pharmacological significances. Cow urine has antimicrobial, growth suppressive constituents against plant pathogens. They induce resistance in plants and change the physicochemical characteristics of the soil by improving plant health [5]. The appliance of various deoiled cakes viz., Neem (Azadirachta indica), Mustard (Sinapis arvensis), groundnut (Arachis hypogaea), Karanj (Milletia pinnata), Mahua (Madhuca longifolia), Castor (Ricinus communis) and Salmeal (Shorea robusta) enhances the yield by increasing nitrogen and other growth enhancing substances. Several workers have reported about the integration of various plant extracts and cow urine serves as substrate media, thus improving the yield of plant and reducing the disease [7]. Therefore, the present study aims to develop effectual bio strategies for the management of early blight of tomato caused by *A. solani* with combination of different plant extracts, botanical cakes and cow urine.

## MATERIAL AND METHODS

### *Isolation and purification of A. solani*

Sample of diseased plants were collected from several fields in Faridabad district, India in August to September 2016. The diseased leaf bits were sterilized using 1:1000 mercuric chloride solution for 30 seconds and rinsed in sterile distilled water thrice. After drying between sterile filter paper infected bits were placed on PDA (potato dextrose medium) in Petri dishes. These plates were incubated at  $28 \pm 2^\circ\text{c}$  and the cultures were further purified by single spore isolation techniques and maintained at  $25 \pm 20^\circ\text{c}$ . [8]. Pathogen was identified on their morphological and cultural characters. Identification was further confirmed from Division of Plant Pathology, IARI, New Delhi.

### *Aqueous extracts of cow urine, botanical cakes*

Fresh cow urine was collected and filtered through Whatman No. 1 paper to get rid of unwanted material and stored at  $4^\circ\text{c}$  in sterile container. The three different concentrations of 5, 10 and 15% of cow urine and botanical cakes viz. Neem (Azadirachta indica), Mustard (Sinapis arvensis), groundnut (Arachis hypogaea), Karanj (Milletia pinnata), Mahua (Madhuca longifolia), Castor (Ricinus communis) and Salmeal (Shorea robusta) were obtained by dissolving sterilized dissolved water for further experimentation.

### *Preparation of plant extracts*

Extracts from the leaves of plants, namely Eucalyptus obliqua and Calotropis procera were collected from different parts of Faridabad. They were washed and crushed in sterile distilled water with the help of mortar and pestle [9]. Mixture obtained was filtered through muslin cloth to obtain extract solution which were further diluted to different concentrations of 5, 10 and 15%.

### ***Screening of plant extracts, cakes, and cow urine against A. solani in vitro***

Plant extracts, botanical deoiled cakes and cow urine were incorporated into melted PDA medium at different concentrations (5, 10 and 15%) and sterilized. Food poisoning technique was followed and PDA without treatments served as control [10]. The treated PDA medium was poured into sterilized petriplates and after solidification 7 days old culture of *Alternaria solani* was transmitted and incubated at 25±2°C. Percent inhibition of botanical cakes was calculated singly and in combination with cow urine separately. Selected plant extracts and deoiled cakes in combination with cow urine were analyzed against *Alternaria solani* at 2.5, 5 and 10% concentration. All the treatments were repeated thrice. After 7 days' percent inhibition was calculated by following formula [11].

$$I\% = 100(C-T)/C; \text{ I- Inhibition percent, C- colony diameter in control plate and T- colony diameter in treated plates.}$$

### ***Screening of plant extracts, cakes, and cow urine against A. solani in pot culture experiments***

The experiment of pot culture study was conducted to analyze the effect of different concentrations of selected plant extracts, deoiled cakes in combination with cow urine on the growth of tomato plants and in reduction of leaf blight disease. Seeds of tomato plants were soaked overnight and sown in different pots filled with field soil. Treatments with selected plant extracts, deoiled botanical cakes and cow urine at 2.5, 5 and 10% concentration were performed as foliar application to the tomato plant which was inoculated with suspension of 20 ml *Alternaria solani* containing  $5 \times 10^5$  CFU/ml. Treatments were repeated after every 15 days up to 2 months. Disease severity was recorded in each treatment following chart 0-9 scale [12]. {0= (Healthy), 1= (1-5%), 2= (6- 10%), 3= (11- 25%), 5= (6 -50%), 7= (51- 75%) and 9= (> 76% of the infected leaf area).

### ***Statistical analysis***

All the experiments were performed thrice. The data thus obtained was analyzed using SPSS computer package programme. LSD was employed to determine the significant difference between treatments at  $P \leq 0.05$  [13].

## **RESULTS**

### ***Identification of causal agent***

Five isolates were obtained from naturally diseased tomato plants. The identification of isolates of *Alternaria solani* was carried out based on morphological characters viz. septation, spore size and shape of conidia and conidiophores. The minor variations were observed in shape and size due to environmental factors [14].

### ***Pathogenicity tests***

All tested isolates were able to infect tomato plants producing symptoms of early blight disease as depicted in Figure I. The symptoms were similar to those observed in diseased field. Isolates of *A. solani* varied in their virulence isolate A3 proved to be more virulent and showed highest percentage of disease severity followed by isolate A1 whereas isolate A5 produced the lowest percentage of disease severity. Control plants showed no symptoms. According to these results isolate A3 was selected for further experimentation.

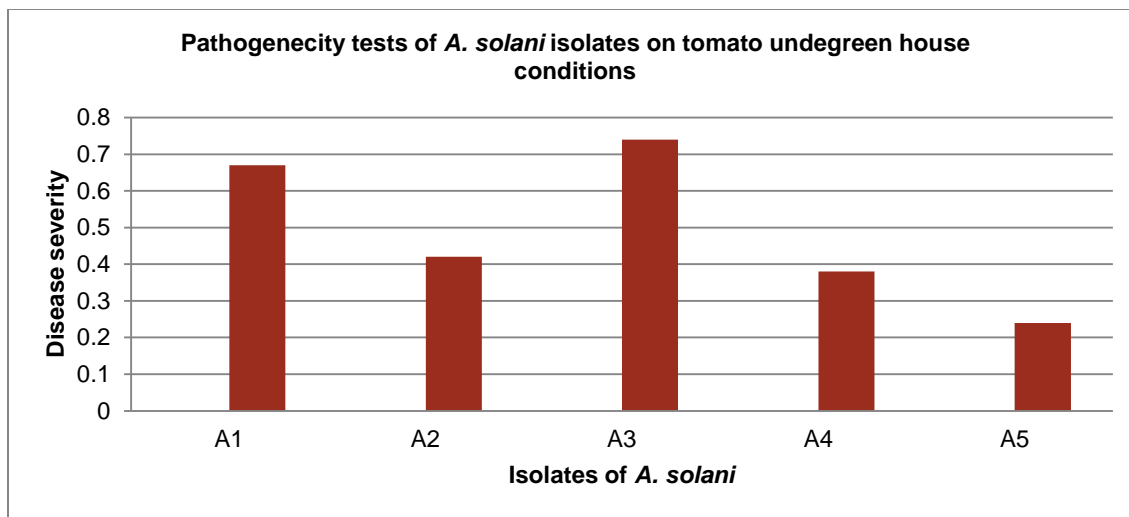


Figure-1: Pathogenicity tests of different isolates of A. solani on tomato plant under greenhouse conditions

**Screening of plant extracts, cakes, and cow urine against A. solani in vitro**

Antifungal efficacy of aqueous cake extracts of Neem (*Azadirachta indica*), Mustard (*Sinapis arvensis*), groundnut (*Arachis hypogaea*), Karanj (*Millettia pinnata*), Mahua (*Madhuca longifolia*), Castor (*Ricinus communis*) and Salmeal (*Shorea robusta*) were analyzed for calculating the percent inhibition of mycelium against A. solani (Table 1) and mycelium inhibition of various cake extracts in combination with cow urine were observed (Table 2). Among the different treatments Karanj cake extract was found to be most effective against A. solani where 58.4% mycelium inhibition was observed at 15% concentration followed by Neem cake which showed 56.2% inhibition at same concentration. Minimum inhibition of 21.7% was observed in groundnut cake. Efficacy of cakes in combination with cow urine was found to be maximum in Neem cake extract of 67.5% at 15% concentration followed by Karanj cake and cow urine of 63.3% at same concentration. Minimum inhibition was observed in Sal meal combination with cow urine of 33.1%. Overall, application of combined treatments gave the highest reduction of disease severity compared with the treatments applied singly. In the combined approach of various plant extracts, cake extracts and cow urine the maximum inhibition was observed in Neem cake extract, Eucalyptus obliqua extract and cow urine to be 85.3% in laboratory conditions followed by Karanj cake extract, Calotropis procera extract and cow urine of 78.0% at 2.5,5 and 10% concentration (Table 3).

Table-1: Efficacy of different deoiled cakes against *Alternaria solani*, causal organism of leaf blight of tomato in vitro

S no.	Treatments	Colony Diameter			Inhibition%		
		5%	10%	15%	5%	10%	15%
1	Neem cake	48.9	42.1	38.5	44.3	51.9	56.2
2	Castor cake	61.8	54.2	52.8	29.6	38.1	39.9
3	Karanj cake	61.3	49.1	36.6	30.3	43.9	58.4
4	Mustard cake	66.8	58.2	56.4	24.0	33.5	35.8

5	Groundnut cake	74.8	72.5	68.8	14.9	17.2	21.7
6	Salmeal cake	71.8	65.9	63.1	18.3	24.7	28.2
7	Mahua cake	61.2	54.8	47.8	30.4	37.4	45.6
8	Control	88.0	87.7	87.9	0.0	0.0	0.0
9	CD at 1%	0.117	0.195	0.183	0.147	0.212	0.196
Average of three replications, Figures in parenthesis are arc sine values.							

**Table-3: Efficacy of deoiled cakes in combination with cow urine and plant extracts against *Alternaria solani*, causal organism of leaf blight of tomato *in vitro***

S no.	Treatments	Percent inhibition		
		2.5%	5%	10%
1	Neem cake+ <i>Eucalyptus obliqua</i> +cow urine	71.7	78.4	85.3
2	Mahua cake+ <i>Eucalyptus obliqua</i> +cow urine	54.9	67.6	71.4
3	Karanj cake+ <i>Calotropis procera</i> +cow urine	55.1	67.9	78.0
4	Control	0.0	0.0	0.0
5	CD at 1%	0.186	0.237	0.151
Average of three replications Figures in parenthesis are arc sine values.				

#### **Screening of plant extracts, cakes, and cow urine against *A. solani* in pot culture experiments**

In pot experiment conditions the maximum inhibition of 78% was observed by Neem cake extract, *Eucalyptus obliqua* extract and cow urine followed by Mahua cake extract, *Eucalyptus obliqua* extract and cow urine of 67.8% (Table IV) and minimum inhibition of 61.6% was observed in Karanj cake extract, *Calotropis procera* extract and cow urine (Figure II).

On the other hand, suppression of *A. solani* was estimable when combined treatments were applied as foliar application every 15 days up to 2 months after planting. Based on the investigations during the experiments integrated approach of botanical cakes, cow urine and plant extracts promise for the organic and non-hazardous management of leaf blight disease caused by *A. solani*.

**Table-4: Efficacy of different deoiled cakes in combination with cow urine and plant extracts on mycelial growth of *Alternaria solani*, causal organism of leaf blight of tomato in pot experiments**

S no.	Treatments	Disease severity	Percent inhibition
1	Neem cake+ <i>Eucalyptus obliqua</i> +Cow urine	19.3	78
2	Mahua cake+ <i>Eucalyptus obliqua</i> +Cow urine	28.2	67.8
3	Karanj cake+ <i>Calotropis procera</i> +Cow urine	33.7	61.6
4	Control	87.7	0
5	CD at 1%	0.181	0.167
Average of three replications Figures in parenthesis are arc sine values.			

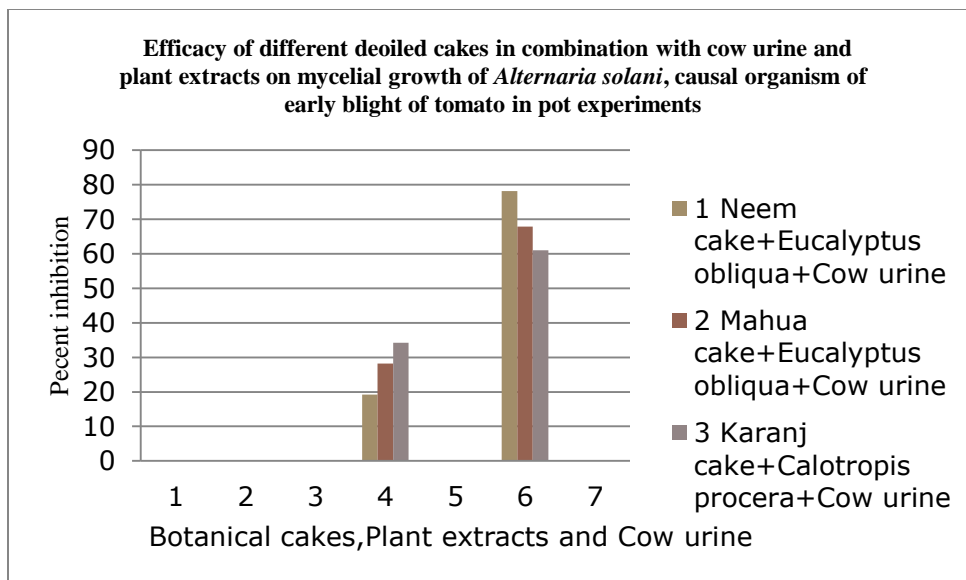


Figure-2: In pot experiments the efficacy of integrated management of different plant extracts cakes and cow urine against *A. solani*.

## DISCUSSION

The results of the present investigation clearly demonstrate that *A. solani* is the causal plant pathogen of leaf blight disease of tomato. All the obtained isolates of *A. solani* are virulent and cause blight disease in pathogenicity tests. *A. solani* induced blight disease on tomato has been reported worldwide. Tomato is the economically important vegetable. Management through integrated biological approach has the capability to induce resistance and increase yield of crop. Several researchers had observed the antifungal efficacy of aqueous extracts of Neem cake and Karanj cake to be most effective against phytopathogenic fungi [6]. Cow urine has magnificent antifungal power, growth enhancing and germicidal influence [15]. Investigations on plant extracts suggests about the presence of active metabolites which increase fungicidal activity thus reducing development of disease. Several workers have reported about the potential of plant extracts to inhibit *A. solani* and induce systemic resistance in tomato plant [16]. Botanical approach is less expensive, biodegradable and environment friendly.

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