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Disease severity and yield loss management of tomato early blight through botanical exudates

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Abstract
Tomato (*Lycopersicon esculentum* Mill) is one of the most important vegetable crop which belongs to solanaceae family and is known as protective food because of its special nutritive value and wide spread production. Early blight is hugely occurred and devastating disease of tomato plant which incited by *Alternaria solani.* The anti pathogenic activity of five plants extracts viz., Neem oil, Harad powder, Sanay powder, Castor oil and Aonla powder were tested against early blight of tomato incited by *A. solani* inhibited the mycelial growth, inhibition increased with increased concentration of botanicals. Significantly minimum growth was noticed at 20 per cent concentration followed by 15 per cent and 10 per cent. Among the botanicals minimum growth were obtained with Neem oil followed by Harad powder, Sanay powder, Castor oil and Aonla powder as compared to control. PDI of early blight was recorded periodically from 45 to 105 days after planting (DAP) with an interval of 15 days. The per cent disease severity in different treatments at 105 days after planting (DAP) evaluated that minimum disease severity (14.56%) with 73.08% PDI reduction and increased yield 82.03% was recorded in Neem oil treated plot which was at par with Harad powder (20.10%) with 62.83% PDI reduction and yield increased 64.44% both treatments were followed by Sanay powder, Castor oil and Aonla powder treated treatments respectively with PDI of 25.66%, 29.82% and 32.06%.

Keywords: Early blight of tomato, Alternaria solani, botanicals, disease severity, yield

Introduction
Tomato is native of tropical America and it spread to word wide in 16th century beside, it became popular in India within the last six decades. In India, tomato has occupy an area 7.91 lakh hectares with total estimated 193.97 Lakh Tonnes production in the year 2018-19 [1]. Tomato is cultivated over 65.72 thousand hectares with production of about 1937.37mt and productivity about 29 tonnes/ha in the year of 2014 of M.P. [2]. Madhya Pradesh rank second in total tomato production of India with 12.95% share [1].

The symptom of early blight appears on tomato fruits, stem and foliage. Primary symptoms appear on leaves as 1-2 mm black to brown lesions and in favorable environmental conditions the lesions enlarged often surrounded by a yellow halo. The concentric rings appeared in greater than10 mm lesions with dark pigments. These kinds of symptoms called as “bulls eye” type lesion is highly characteristic of early blight. If the lesions expanded and new lesions develop on entire leaves turn chlorosis and dehisce which leading to significant defoliation. Lesions occurring on stems are often sunken canker and lens-shaped with a light center. The young seedlings lesions may completely girdle on the stem, which may lead to reduced plant vigor (Gleason and Edmonds, 2006; Kemmitt, 2002) [7, 12].

The tomato early blight disease incited by *Alternaria solani* occurred in all the areas where the crop taken year after year. Severity of the disease in leaf and stem varied between 12.0 to 34.6% and 14.2 to 26.6% respectively depending on place to place however, highest intensity 34.6% in leaf was recorded and disease intensity 26.6% at Nigari in stem followed by Pithoria 32.6% in leaf and 21.2% in stem (Ramesh and Praveen 2019) [21]. The worldwide trend to managing diseases towards environmentally safe methods in sustainable agriculture calls for reducing the use of these synthetic chemical fungicides. Management of the disease can be achieved through sanitation, crop rotation and fungicide application during humid environment and rainy season (Jayaraj and Punja, 2007) [8]. The continuous use of chemicals/fungicides considerably increases the cost of production and is hazardous to animals, humans and the environment (Nasr, 2018) [15]. The losses caused by the disease and hazard produced due to chemicals can be reduce by use of botanical exudates or its preparations the better alternative.

Material and Methods

The following material and methods used for experiments and related studies were conducted in the Department of Plant Pathology, B.M. College of Agriculture Khandwa under RVSKVV, Gwalior.

Isolation and Identification of the Pathogen

A. solani was isolated from infected tomato fruit showing early blight symptoms. The fruit were thoroughly washed under running tap water and small portions (approx 2 mm) of tissue from the advancing margin of a lesion were cut out with a sterile scissor. The cut portions were surface sterilized in 0.1% sodium hypo chloride solution for 2 min. (Arunakumara, 2006) [4] and serially washed by dipping into sterile water 3 times. The specimen was then plated on PDA medium at 27°C for 7 days and sub cultured until pure isolate of *A. solani* was obtained and identified as *A. solani* (Simmsons, 2007) [24].

The growing edges of the hyphal mycelium developing from the disease tissue discs were then transferred aseptically to potato dextrose agar. The fungus was identified following sporulation. The identification of *Alternaria solani* was done based on the morphological and colony characters. Spore morphology and colony characters were studied (Koley and Mahapatra, 2015) [13]. Jones and Grout (1986) [10] compared morphological characters of isolated pathogens for identification of isolated *Alternaria solani* (Ellis & Marts.)

The fungus isolated from the infected leaves was identified when suitable growth and sporulation had occurred. Identification was made depending on the visual characteristics of the fungus which was the culture growth pattern and pigmentation. Further investigation was made by using a Stereoscopic binocular microscope to view the slide prepared from the fungus added to it drops of lactophenol for pigmentation. The microscope was used to study the conidiophores formation, arrangement of the conidia on conidiophores, the beaks of the conidia, the transverse and longitudinal septation as well as the length and width of the conidia and the colour. The fungus growth was measured in the Petri-dishes by taking the radius growth for 10 days.

Pathogenecity test

The pathogenecity was conducted on ten healthy tomato fruit (TO-1389 cultivar) by the pin prick method as described by pandey and pandey (2002) [19]. Jones & Grout (1897) [9] that involved Pin-pricking the tomato fruit surface to a 1-mm depth and then dipping the fruit in the conidial suspension. The fruit surface sterilized with mercuric chloride (1:1000 w/v) and subsequently rinsed in sterile distilled water. These fruit were pin pricked with a sterile needle. Separately, spore suspension was prepared from an actively growing 10 day old culture of *Alternaria solani* by flooding with sterile water. The harvested spores were then applied to the pricked area of the fruit. The inoculated fruit were incubated at room temperature in a Petri dish lined with moist blotter paper. The inoculated fruit were observed regularly for development of no. of spots, size of lesions and typically symptoms induced and Isolations were made again from these lesions and the identity of the pathogen was confirmed.

In order to investigate the pathogenecity of isolated pathogen, a small injury was made on the surface of sterilized tomato fruit. Then an agar disc, taken from the margin of the freshly grown colony of isolated pathogen was placed on injured surface. These inoculated fruits were kept in a suitable place at room temperature in laboratory conditions. As soon as the disease symptoms were evident, the pathogen was again isolated from the artificially inoculated diseased fruits. Then reisolated culture was compared with original isolated pathogen (Nikam *et al*., 2015) [16].

In-vitro evaluation of herbal decoction on growth of *Alternaria solani*

Six different species of plants, namely: Aonla powder, Castor oil, Sanay powder, Neem oil and Harad powder (Table-1) were tested for their antimicrobial activities against *A. solani*. The plants were selected on the basis of traditional medicinal values and previous studies that have demonstrated antifungal properties using different kinds of extracts.

The bioassay studies were carried out by employing the Poisoned Food Technique as reported earlier (Nene, 1971; Nene and Thapliyal, 1973) [17, 18].

Preparation of Crude Extracts

Once identified, each of the selected plant materials were air dried and ground until a fine powder is obtained. The powdered plant materials were extracted at room temperature by dissolving 50g plant powder in 250ml pure methanol for 2 hours and shaking the mixture using a shaker. The extracts were filtered using filter paper and then concentrated using a rotary evaporator to remove methanol. Then, the extracts were stored in a refrigerator until used in antifungal bioassay (Fazli *et al*., 2012) [6].

In vitro evolution of Plant Extracts

The efficacy of six antagonists was evaluated against *A. solani* for radial growth inhibition on the potato dextrose agar medium using dual culture technique under *in vitro* condition (Table 2).

Nintee milimeter diameter petri dish was used in the antifungal assay. The Petri dishes were divided into four equal sections by drawing two perpendicular lines on the bottom of plates, the point of intersection indicating the center of the plate (Amadioha and Obi, 1999) [3]. Different concentrations of the extracts were prepared by diluting the crude extracts with methanol to get 1%, 5% and 10% concentration. All concentrations from each extract were introduced into each of the different plates containing molten growth medium (potato dextrose agar) and gently mixed. Four mm diameter mycelial disk taken from the advancing edge of a 7 day old pure culture of *A. solani* was cut out using sterile cork borer and placed into the well prepared at the middle of the solidified medium-extract mixture just at the point of intersection of the two lines drawn at the bottom of the plates. The resulting inoculated medium was then incubated at a temperature of 27 °C. The experiment was arranged in completely randomized design (CRD) with three replications.

Table 1: List of botanicals used in the experiment

No	Common name	Symbol	Scientific name	Site of collection	Part used
1	Aonla	H1	*Emblica officinalis*	SAK	Fruit powder
2	Castor	H2	*Ricinus communis*	SAK	Oil
3	Sanay	H3	*Cassia angustifolia*	SAK	Leaf powder

4	Neem	H4	*Azadirachta indica*	SAK	Oil
5	Harad	H5	*Terminalia chebula*	SAK	Fruit powder
6	Control	H6			

SAK: Sanjivani Ayurvadic Kendra Khandwa

Management of Early blight of tomato through botanicals in field conditions: Five plants were selected randomly in each plot and observation were recorded on disease severity individually using 0-5 rating scale (Table-2) based on leaf area, stem and fruit covered by blight symptoms as the rating scale described by Pandey *et al*. (2003) [20].

Table 2: Scale for rating of early blight disease in tomato

Rating	Reaction description
0	Free from infection
1	< 10% surface area covering leaf, stem and fruit infected by early blight
2	11-25% foliage of plant covered with a few isolated spot
3	Many spot coalesced on the fruit, covering 26-50%surface area of plant
4	51-75% area of the plants infected fruits also infected at peduncle end defoliation and blightening started. Sunken lesions with prominent concentric ring on stem, petioles and fruits
5	> 75% area of plant part blighted, severe lesion on stem and fruit rotting on peduncle end

Disease incidence was calculated on the basis of per cent of infected fruit and stem. Percentage disease incidence (PDI) was calculated as follows:

$$\text{PDI} = \frac{\text{Sum of all rating}}{\text{Total no. of observtions} \times \text{maximum rating grade}} \times 100$$

Data collected from *in vivo* experiments included PDI were followed the local standard assessment procedure for disease in tomato and record observation at 7 days intervals. Total fruit weight/plot was recorded and taken as yield/plant and calculated Yield per plot. The fruit yield in each plot was recorded. The per cent yield increase over untreated control was calculated by given formula:

$$\text{Yield over control} = \frac{\text{Yield in treatment} - \text{Yield in control}}{\text{Yield in control}} \times 100$$

Results and Discussion

In the present investigation, efficacy of five different botanicals Viz., Aonla powder, Castor oil, Sanay powder, Neem oil and Harad powder was evaluated on the growth of *A. solani.* Data presented in table-3, revealed that all the botanicals significantly inhibited the growth of *A. solani.*

The results regarding efficacy of different botanicals against *A. solani* presented in table-3. According to the result, inhibition of mycelial growth of *A. solani* increased with increase in concentration of botanicals. Significantly minimum growth was noticed at 20 per cent concentration followed by 15 per cent and 10 per cent. In absence of botanical *A. solani* showed the radial growth of 75.88 mm. Among the botanicals minimum growth (37.58 mm) were obtained in treatment Neem oil followed by Harad powder (45.59 mm), Sanay powder (48.67 mm), Castor oil (52.36 mm) and Aonla powder (54.46 mm) as compared to control (75.88 mm).

At 10 per cent concentration, all tested botanicals significantly reduced mycelial colony diameter over the control (75.50 mm). Minimum mycelial growth was recorded in Neem oil (49.37 mm) followed by Harad powder, Sanay powder, castor oil and Aonla powder (54.89 mm, 58.26 mm, 61.33 mm and 65.00 mm, respectively).

The results obtained are presented in table-3. Significantly minimum mycelial growth was recorded in Neem oil (37.20 mm) followed by Harad powder (47.15 mm), Sanay powder (51.68 mm), castor oil (53.44 mm) and Aonla powder (54.59 mm) as compared to control (76.35 mm) at 15 per cent concentration.

It was perusal from the data significantly minimum mycelial growth was recorded in Neem oil (26.16 mm) followed by Harad powder, Sanay powder, castor oil and Aonla powder (34.74 mm, 36.08 mm, 42.30 mm and 43.79 mm, respectively) at 20 per cent concentration. In absence of botanical A. solani showed the radial growth of 75.80 mm.

Efficacy of five different botanicals *Viz.,* Aonla powder, Castor oil, Sanay powder, Neem oil and Harad powder was evaluated on the growth of *A. solani.* All the botanicals significantly inhibited the growth of *A. solani.*

The result was revealed that inhibition of mycelial growth of *A. solani* increased with increase in concentration of botanicals. Significantly minimum growth was noticed at 20 per cent concentration followed by 15 per cent and 10 per cent. In absence of botanical *A. solani* showed maximum radial growth. Among the botanicals minimum growth were obtained in treatment Neem oil followed by Harad powder, Sanay powder, Castor oil and Aonla powder as compared to control at all concentrations. Roy *et al*. (2019) [23] also tested efficacy of botainicals against *A. solani in vitro* and they were observed that the inhibition of the *A. solani* increases with the increase of the concentration of the plant extracts in culture. It is also cleared that *A. sativum* extract was the most effective on the inhibition of the radial growth of *A. solani* (74.07%).

Rahman *et al*. (2015) [22] evaluated some botanical against *A. porri*. *Adhatoda vasica* extract @ 5% showed the maximum (91.11%) inhibition of mycelial growth of *A. porri* followed by *A. indica* (60%) and *Ocimum sanctum* (55.33%). These findings are in accordance to the present findings and supported that botanical extracts are useful for the management of fungal pathogens.

Table 3: Radial growth of Alternaria solani in botanicals amended medium

Botanicals	Mean radial growth (mm)*			
	10%	15%	20%	Mean A
Aonla powder	65.00	54.59	43.79	54.46
Castor oil	61.33	53.44	42.30	52.36
Sanay powder	58.26	51.68	36.08	48.67
Neem oil	49.37	37.20	26.16	37.58
Harad powder	54.89	47.15	34.74	45.59
Control	75.50	76.35	75.80	75.88
Mean B	60.73	53.40	43.15	
Factors	SE(m)±		C.D. at 5%	
Botanicals(B)	0.37		1.05	
Concentration(C)	0.52		1.49	
(B X C)	0.89		2.58	

Efficacy of botanicals on early blight of tomato and their effect on yield

The results regarding efficacy of different botanicals against early blight of tomato presented in table-4. According to the result, all botanicals were found significantly superior over control in reducing per cent disease control.

The data on PDI of early blight was recorded periodically from 45 to 105 days after planting (DAP) with an interval of 15 days. It has been observed that in all treatments PDI increased with age of the plants. Data on disease severity revealed that all botanicals tested reduced the disease intensity significantly compared to control. It is evident from data that all the treatments, there was increases in disease index from 45 to 105 DAP. However, the rate of increase in PDI was slow in case of botanicals treated plots compared to control.

The percent disease severity in different treatments at 105 days after planting (DAP) evaluated that minimum disease severity (14.56%) was recorded in Neem oil treated treatment which was at par with Harad powder (20.10%) and both treatments were followed by Sanay powder, Castor oil and Aonla powder treated treatments with PDI of 25.66%, 29.82% and 32.06%, respectively.

All the botanicals tested *viz*., Aonla powder, Castor oil, Sanay powder, Neem oil and Harad powder could significantly reduce the early blight disease and increase the yield also compared to control. The percentage reduction in disease ranged from 40.72 to 73.08% and increase in yield ranged from 26.70 to 82.03%. Results of the present study indicated that all fungicide treatments significantly controlled the early blight infection on tomato as compared to untreated control. There was a significant difference in all the treatments. In our finding pristine at both the concentration controlling the disease significantly, and increasing yield. Our study clearly indicates that Neem oil when applied give maximum protection against *A. solani*. Other botanicals such as Aonla powder, Castor oil, Sanay powder and Harad powder also found effective against *A. solani*. Application of Neem oil and Harad powder showed best results followed by Sanay powder, Castor oil and Aonla powder. Thus, it can be said that botanicals tested in the present study may be effectively used not only to manage early blight but increase the yield of tomato as well as Lengai *et al.* (2017) [14] reported that plant extracts reduced disease infections by more than 65%. Majority of the yield fell under the grade 3 category with the highest being from the garlic extracts.

Joseph *et al.* (2017) [11] investigated the efficacy of leaf extracts of *Chromolaena odorata*, *Euphorbia heterophylla*, *Tithonia diversifolia*, *Azadiractha indica* and *Carica papaya* in the management of early blight on tomato. The results showed that application of the extracts had a significant influence on early blight severity, number of fruits produced, fruit length and fruit weight. Bhanage *et al*. (2019) [5]. also reported the devastating disease of tomato plant caused by *Alternaria solani* under *in-vivo* condition, minimum disease intensity was recorded with *Azadirachta indica* and Eucalyptus globulus (26.99% and 27.25%, respectively)these reports are in accordance to the present findings.

Table 4: Efficacy of botanicals tested on early blight of tomato and their effect on yield

Treatment	Diseases incidence per cent					Reduction PDI (%)	Yield (t/ha)	Increase in yield (%)
	45 DAP	60 DAP	75 DAP	90 DAP	105 DAP			
Aonla powder	6.13 (13.85)	10.24 (18.06)	18.10 (24.45)	25.60 (29.64)	32.06 (33.81)	40.72	19.88	26.70
Castor oil	4.86 (12.67)	9.02 (17.39)	15.94 (23.42)	22.94 (28.49)	29.82 (32.97)	44.86	21.37	36.20
Sanay powder	4.16 (11.66)	7.62 (15.88)	13.86 (21.68)	19.43 (25.95)	25.66 (30.22)	52.55	24.18	54.11
Neem oil	2.08 (8.22)	4.16 (11.66)	8.32 (16.62)	11.45 (19.61)	14.56 (22.25)	73.08	28.56	82.03
Harad powder	3.46 (10.63)	6.94 (15.15)	11.10 (19.31)	16.58 (23.85)	20.10 (26.45)	62.83	25.80	64.44
Control	11.10 (19.45)	17.34 (24.59)	32.78 (34.91)	43.64 (41.35)	54.08 (47.35)		15.69	
SE(m)±	1.33	1.80	2.51	3.14	3.71		1.67	
C.D. at 5%	2.79	3.78	5.28	6.59	7.80		3.50	

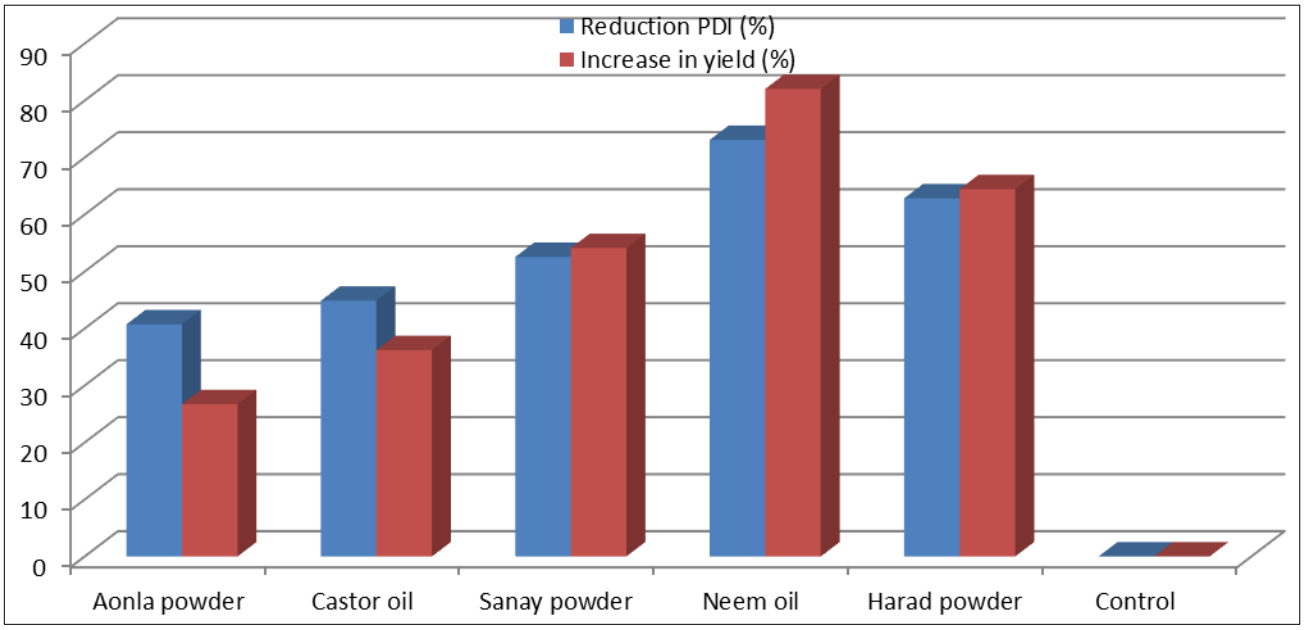

Fig 1: Efficacy of botanicals tested on severity of early blight disease and their effect on yield of tomato

Conclusion

Among the botanicals minimum growth were obtained in treatment Neem oil followed by Harad powder**,** Sanay powder**,** Castor oil and Aonla powder as compared to control at all concentrations *in vitro* conditions**.** Application of Neem oil and Harad powder showed best results followed by Sanay powder in field conditions and these treatments have not only potential to manage early blight of tomato but increase the yield of crop.

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