In vitro evaluation of the effects of botanical extract on mycelial radial growth of *Alternaria solani*.

Abstract

A field experiment was conducted at the research plot of the Department of Plant pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, Uttar Pradesh in *Rabi* season of 2022-23 to evaluate different Plant extracts for management of early blight of Tomato caused by *Alternaria solani* and also to increase the growth parameters. A total of eight treatments namely Neem (*Azadirachta indica*) seed kernel extract @ 5% followed by Henna (*lawsonia inermis*) leaf extract @ 10%, Amaltas (*Cassia fistula*) leaf extract @ 10%,Castor (*Ricinus communis*) leaf extract @ 10%, Madar (*Calotropis gigantea*) leaf extract @ 10%, Onion (*Allium cepa*) extract @ 10 %, Garlic (*Allium sativum*) extract @ 10 % including control were replicated three times. During evaluation, all the seven treatments were found significantly superior over control in managing early blight and also in increasing the growth parameters and yield as well. Among all the tested treatments, Neem (*Azadirachta indica*) seed kernel Extract was found significantly superior over control along with all other treatments which recorded minimum disease intensity and maximum fruit yield followed by Henna (*Lawsonia inermis*). Plant disease intensity decreases with the application of different treatments over untreated control.

Comment [A1]: This abstract is not even related to the manuscript.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is the most popular in-home garden and the second most consumed vegetable after potato (*Solanum tuberosum* L.) in the world. The species is native to South America, possibly Peru and Ecuador, but was first domesticated in Mexico (Benton, 2007). In the mid-16th century, tomato was introduced into Europe, primarily featured in early herbals. It was grown for the beauty of its fruit but was not often eaten, except in Italy and Spain. The fruit was considered poisonous and it was not recognized as a useful vegetable until 1800. Today, tomato is widely grown in the world because of its taste, color, flavor, and nutrient contents. It may be eaten fresh or processed. Early blight of tomato caused

by the imperfect fungus *Alternaria solani* (Ellis & Martin) affects both foliage and fruits causing necrotic spots that vary in size and shape and usually takes the shape of concentric rings (Rotem 1994; Chaerani and Voorrips, 2006). This disease, in severe cases, can lead to complete defoliation of plants. The disease affects both tomato and potato and is most damaging on tomatoes in regions with heavy rainfall, high humidity and high temperatures (24–29 °C). Initial symptoms on leaves appear as small 1-2 mm black or brown lesions and under favourable environmental conditions the lesions will enlarge and are often surrounded by a yellow halo. It has become a limiting factor with increasing severity (49.5%) in successful cultivation (Abhinandan *et al*., 2004)and responsible for 47.60–79.00% fruit yield loss (Saha and Das 2012; Adhikari *et al*., 2017)in world wide. A fungus that lives in soil and may transfer to new hosts through the air and splashing rain is the responsible organism. Additionally produced conidia are spread by wind, splashing rain, and other methods once *Alternaria solani* spores germinate in delicate tissue or through wounds (Agrios, 2005). Although the disease can manifest itself in a range of environmental conditions, it is more common in regions that see extensive dew deposition, large rainfall, and high relative humidity.

1.MATERIALS AND METHODS

Comment [A2]: Materials methods can be given in different sections
1.Isolation of Pathogen
2.Preparation of aqueous extract of plant
3.*In vitro* evaluation of plant extract against *A. solani*

The present investigations were carried out in the laboratory, Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, Uttar Pradesh (Year 2022-23) to test the compatibility of different treatments with *Alternaria solani*under *in vitro* conditions. To find out the compatibility of various plants extracts *viz*., Neem (*Azadirachta indica*) seed kernel extract, Henna (*lawsonia inermis*),Amaltas (*Cassia fistula*), Castor (*Ricinus communis*) leaf extract, (Madar (*Calotropis gigantea*),Onion (*Allium cepa*),Garlic (*Allium sativum*)with *Alternaria solani*were used. Leaves were collected in a clean polythene bag and brought to the laboratory from infected tomato plants having characteristic symptoms of disease. The slide was prepared using lactophenol and cotton blue and observed under microscope to confirm the presence of *Alternaria solani.* Potato Dextrose Agar (PDA) were prepared and 80 mg of streptomycin, an antibiotic was added to each 500 ml preparation of the PDA to inhibit probable bacterial growth. The infected leaf parts were cut into small pieces of two to three mm dimension in a manner so that pieces may have some green portion also. Such

Comment [A3]: Remove the open bracket

leaf bits were surface sterilized with 0.1 per cent mercuric chloride (HgCl2) solution for 30 seconds and washed three times with sterile distilled water to remove any traces of mercuric chloride adhered with leaf bits. 2-3 leaf bits were transferred on PDA medium contained in petri Plates aseptically with the help of sterilized forceps. These petri Plates were incubated at 25±2° C. After 3 days mycelia growth was observed around leaf bits from this colony growth, a portion from the periphery that is, single hyphal tip was separated and transferred to other. The culture of *Alternaria solani* were purified by hyphal tip method and maintained by periodic sub-culturing on PDA petri Plates and slants. These were incubated at 25±2°C temperature. The fresh leaf extracts were gently washed under running tap water and finally in sterile distilled water. They were separately grinded in sterile water at the rate 1 **mlg-1**of plant material in pestle and mortar. Then it was filtered through double layer of muslin cloth and finally through sterilized Whatman no.1 filter paper. This forms 100% standard plant extract solution. Further its dilution was performed of required concentration with sterilized water. Five mm diameter of culture disc of *Alternaria solani* were kept at the centre of each petri Plate containing the **fungicides**of required concentration dissolved in PDA. Three replications were maintained. The Plates were incubated at for ten days and **colon** diameter were recorded (Vincent, 1947).

Mycelial inhibition =Radial growth in control – Radial growth in treatmentRadial growth in control×100

S. No	Treatment details	Mode of application
1	Neem (*Azadirachta indica*) seed kernel extract	Foliar
2	Onion (*Allium cepa*) extract	Foliar
3	Garlic (*Allium sativum*) extract	Foliar
4	Castor (*Ricinus communis*) leaf extract	Foliar
5	Amaltas (*Cassia fistula*) leaf extract	Foliar
6	Henna (*lawsonia inermis*) leaf extract	Foliar

Comment [A4]: Unit is wrong. It should be 1g/ml or $1gml^{-1}$

Comment [A5]: Change as plant extract

Comment [A6]: Colony

Comment [A7]: The present investigation only concentrated on in vitro evaluation of plant extract against *A. solani*
Then this details are not required.

7	Madar (*Calotropis gigantea*) leaf extract	**Foliar**

Table 1. List of Plant extracts and their scientific names

3.RESULT AND DISCUSSION

3.1*In vitro* evaluation of plant extracts on *Alternaria solani*:

Comment [A8]: The contents are repeated, It can be reduced.

Plant extracts were evaluated on the growth of pathogen by poison food technique. The results of the effect of different treatments i.e., plant extracts on inhibition growth of *Alternaria solani* are presented in Table-2, Figure-1 and Plate-1.

3.2 Effect of plant extracts on *Alternaria solani* by poison food technique:

In present investigation, effect of plant extracts were screened on the growth of pathogen by poison food technique.The percent inhibition of growth of the test fungus over control were calculate. The result is presented below in Table-2 and the Figure-1 and Plate-1.

3.3 Radial growth of *Alternaria solani* after 168 hrs of inoculation:

The data presented in table-2, depicted in figure-1 and Plate-1represents that the radial growth was found minimum in (T1)- Neem (*Azadirachta indica*) seed kernel extract @ 5% (14.8mm) followed by (T6) Henna (*lawsonia inermis*) leaf extract @ 10% (43.36mm), (T5) Amaltas (*Cassia fistula*) leaf extract @ 10% (67.13mm), (T4) Castor (*Ricinus communis*) leaf extract @ 10% (67.6mm), (T7) Madar (*Calotropis gigantea*) leaf extract @ 10% (69.6mm), (T2) Onion (*Allium cepa*) extract @ 10 % (70.8mm), (T3) Garlic (*Allium sativum*) extract @ 10 % (72.2mm) as compared to T0- control (89.4mm) untreated check. All the treatments are statistically significant over control. Among the treatments, (T3). (T2), (T7), (T6) and (T1) are statistically significant over the treatments and (T4 and T5) are statistically non-significant with each other.

3.4Percent inhibition of *Alternaria solani* after 168 hrs of inoculation:

The data presented in table-2, depicted in figure-1 and Plate-1 represents that the percent inhibition was found maximum in (T1)- Neem (*Azadirachta indica*) seed kernel extract @ 5% (83.44 %) followed by (T6) Henna (*lawsonia inermis*) leaf extract @ 10% (51.49 %), (T5) Amaltas (*Cassia fistula*) leaf extract @ 10% (24.91 %), (T4) Castor (*Ricinus communis*) leaf extract @ 10% (24.38 %) , (T7) Madar (*Calotropis gigantea*) leaf extract @

10% (22.41 %), (T2) Onion (*Allium cepa*) extract @ 10 % (20.80 %) (T3) Garlic (*Allium*

Treatments	Concentration	Radial growth of pathogen (mm)	Per cent inhibition (%)

sativum) extract @ 10 % (19.23 %) , as compared to T0- control (0 %) untreated check.

Similar findings were evaluated by Nashwa and Abo-Elyousr (2012)in *in vitro* study the leaf extract of *A. indica* cost the highest reduction of mycelial growth of *Alternaria solani***,** Phalisteen *et al.* (2008)concluded that the *Azadirachta indica* showed maximum inhibition of fungus (*Alternaria solani).* Similar findings were evaluated by Nashwa and Abo-Elyousr (2012)in *in vitro* study the leaf extract of *A. indica* cost the highest reduction of mycelialgrowth of *Alternaria solani*, Phalisteen *et al.* (2008)concluded that the *Azadirachta indica* showed maximum inhibition of fungus (*Alternaria solani*). Bhanage *et al*. (2019)was examined in *in vitro* and had the highest *in vitro* reduction of *Alternaria solani* mycelial growth**,** Sharma *et al*. (2021)were evaluated *in vitro* by poisoned food technique and showed reduction in mycelial growth of the fungus *Alternaria solani***,** Dhaka *et al*. (2022)where Neem (*Azadirachta indica*) seed kernel extract showed minimum radial growth under *in vitro* conditions.

Table 2. Effect of plant extracts on *Alternaria solani* by poison food technique:

Control	-	89.4	0
Neem seed kernel extract	5%	14.8	83.44
Onion extract	10%	70.8	20.80
Garlic extract	10%	72.2	19.23
Castor leaf extract	10%	67.6[a]	24.38
Amaltas leaf extract	10%	67.13[a]	24.91
Henna leaf extract	10%	43.36	51.49
Madar leaf extract	10%	69.6	22.14
SEm (±)		0.20	
C.D(5%)		1.04	

*****Average of three replications
*Data followed by same letter in a column are non-significant to each other at 5% level

Figure 1. Effect of treatments by poison food technique against early leaf blight of tomato

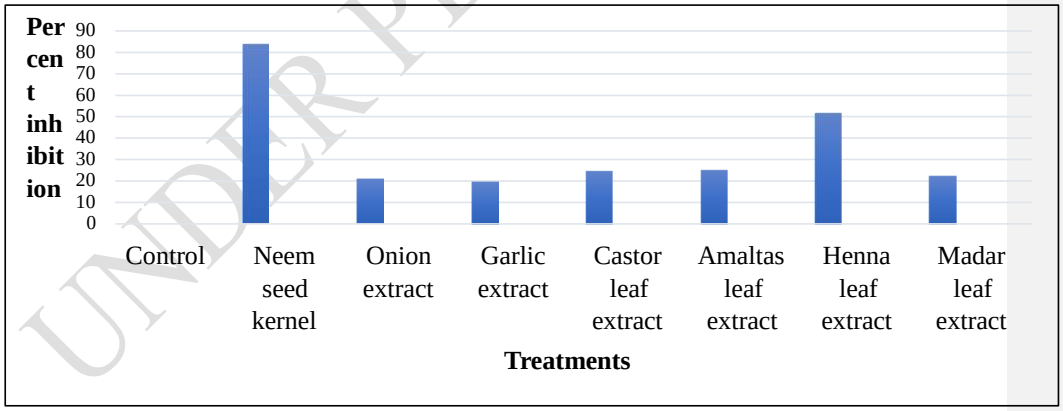
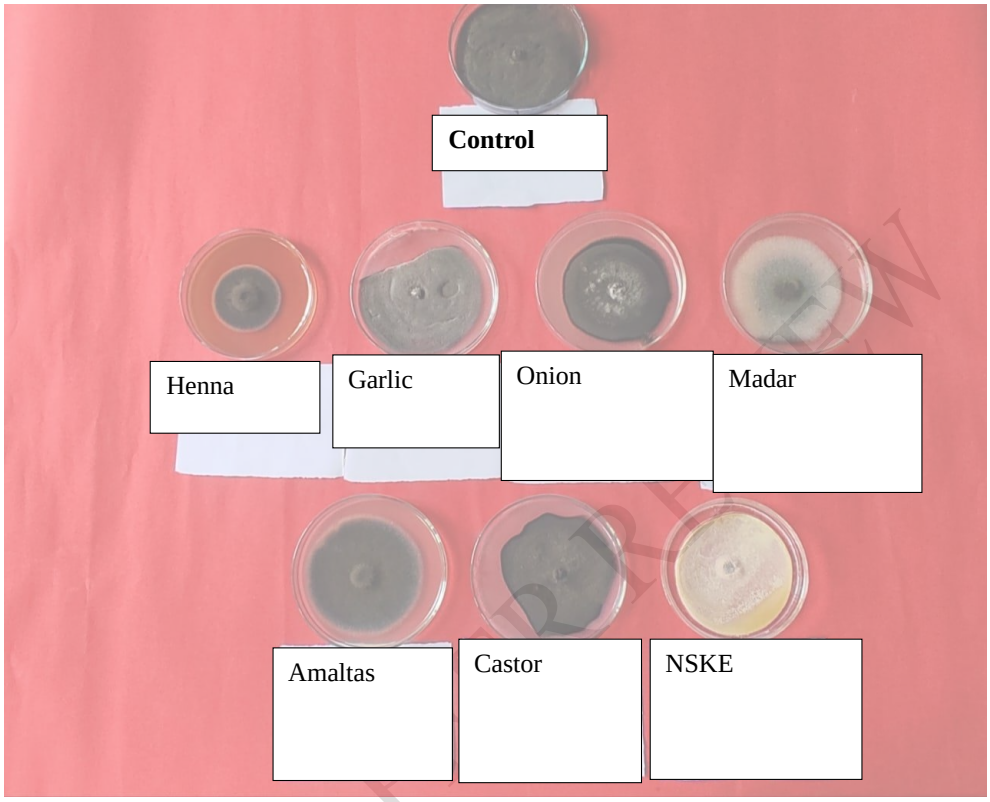


Plate 1. Effect of treatments by poison food technique against early leaf blight of tomato



CONCLUSION

Among the tested botanicals, it was found that, under *In vitro* condition among the treatments, maximum percent inhibition was found in the treatment Neem seed kernel over the control and in case of growth parameters the treatment Neem seed kernel proved to be the best one. The current experiment proved that without using any chemical, management of early blight disease of tomato can be done by the use of plant extracts.

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