In vitro evaluation of botanical extractsagainst mycelial growthof *Alternaria solani*

Abstract

An experiment was conducted at the laboratory of the Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, Uttar Pradesh.To test the efficacy of different treatments *viz*.,Neem (*Azadirachta indica*), Onion (*Allium cepa*), Garlic (*Allium sativum*), Castor (*Ricinus communis*), Amaltas (*Cassia fistula*), Henna (*lawsonia inermis*), Madar (*Calotropis gigantea*)against *Alternaria solani* under *in vitro* condition.Botanicals were tested through the poisoned food technique at 5% and 10 % concentrations and 168 hours of incubation. The Minimum radial growthof observed in Neem (*Azadirachta indica*) seed kernel extract @ 5% (14.8mm) followed by Henna (*lawsonia inermis*) leaf extract @ 10% (43.36mm),Amaltas (*Cassia fistula*) leaf extract @ 10% (67.13mm), Castor (*Ricinus communis*) leaf extract @ 10% (67.6mm), Madar (*Calotropis gigantea*) leaf extract @ 10% (69.6mm), Onion (*Allium cepa*) extract @ 10 % (70.8mm), Garlic (*Allium sativum*) extract @ 10 % (72.2mm) as compared to Control (89.4mm) untreated check.

Key Words: Plant disease, Solanum lycopersicum, Azadirachta indica, Solanum tuberosum

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). "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 plantsThe 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)worldwide. 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)

MATERIALS AND METHODS

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 efficacy of different treatments with *Alternaria solani* under *in vitro* conditions. To find out the efficacy 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*) against*Alternaria solani* were used.

Isolation of Pathogen

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) was 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 healthy portion also. Such 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 placed in BOD incubator at25±2°C. After 3 days mycelia growth was observed around leaf bits from this colony growth, a portion from the periphery, that is, a single hyphal tip was separated and transferred to another. The culture of *Alternaria solani* were purified by the hyphal tip method and maintained by periodic sub-culturing on PDA petri Plates and slants.

Preparation of aqueous extract of plant

The fresh plant parts were gently washed under running tap water and finally in sterile distilled water. Equal number of washed plant partswere grinded in mortar and pestle by adding same amount of sterilized water (1:1 w/v) and boiled at 80^0 C for 10 minutes in hot water bath. The extract was filtered by double layer muslin cloth followed by sterilized Whatman No.1 Filter paper (Bhaskar *et.al.*,2023) Aqueous extract of 5% and 10 % was prepared according to the treatment by mixing 5 and 10 ml of botanical extract with 95,90 ml PDA respectively in separate conical flask. The flasks were thoroughly shaken to ensure an even mix of the extract under aseptic conditions.

In vitro evaluation of plant extracts against *A. solani*

Twenty ml of sterilized melted PDA was aseptically poured in sterilized Petri dishes and allowed to solidify. After solidification of media 5mm disc of 7 days old subculture of *Alternaria solani*were placed in the centre of the Petri plates and one control plate which has only the PDA medium inoculated with culture disc and used as control. Each treatment and control were repeated three times to make three replications. Replicates were maintained for each test and those plates were incubated at 27 ± 1^0C at incubator. The Plates were incubated for 168 hours and colonydiameters were recorded (Vincent, 1947).

The radial growth of mycelium of each plate was measured by taking average of the two diameters taken right angles for each colony. Percent inhibition in growth was calculated in relation to growth in control using the following formula of Vincent (1947). The experiment was conducted in completely randomized block design (CRD) with three replications in each treatment. The conclusion was arrived at after statistical analysis of the data. The Completely Randomized Design (CRD) method recommended by Goon *et. al.* was used to conduct the statistical analysis of laboratory experiments (1931). The variance ratio test at the 5% level of probability was used to determine the significance of treatment differences. The observation of per cent inhibition of mycelial growth, were transformed in to "Arc sin Transformation" = sin-1 $\sqrt{p}/100$ used for statistical analysis.

Mycelial inhibition = (Radial growth in control – Radial growth in treatment) / (Radial growth in control) ×100

Table 1. List of Plant extracts and their scientific names

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

RESULT AND DISCUSSION

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

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

Radial growth of *Alternaria solani* after 168 hrs. of incubation:

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 significant over the treatments and (T4 and T5) are non-significant with each other.

Percent inhibition of *Alternaria solani* after 168 hrs of incubation:

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

Similar findings were reported 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 mycelial growth 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:

Treatments	Radial growth of pathogen (mm)	Percentinhibition (%)

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	24.38
Amaltas leaf extract @10%	67.13	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**

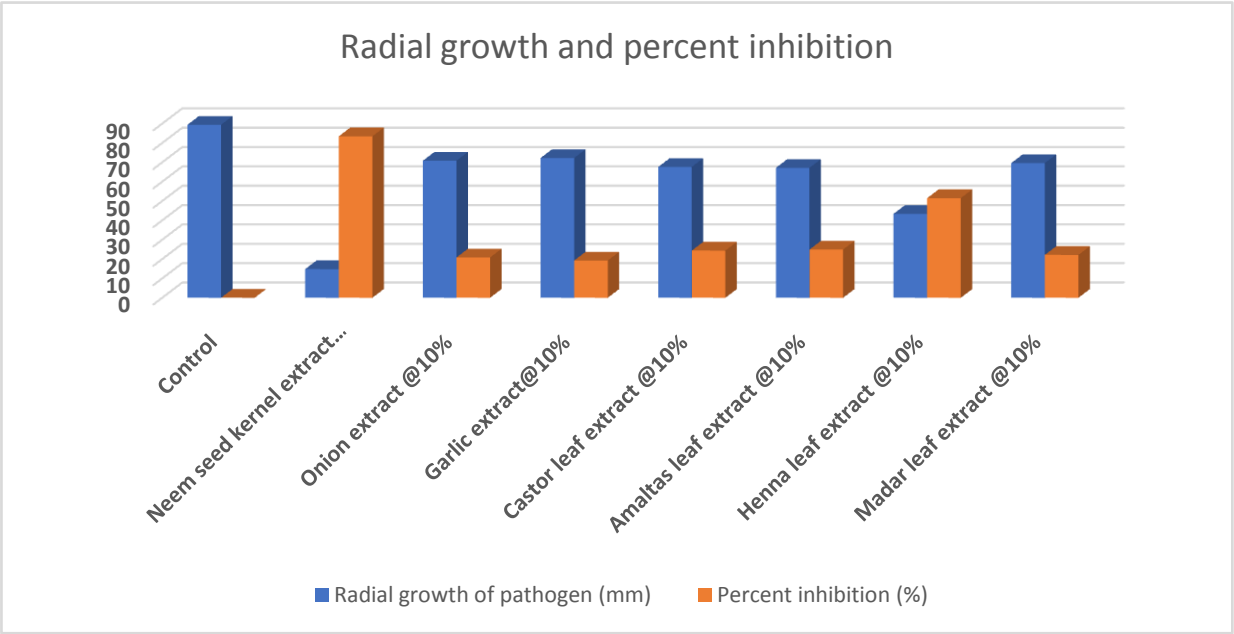

Figure 1. Effect of plant extracts on Alternaria solani on percent inhibition at 168 hours

Plate 1. Response of botanicals against *Alternaria solani* on mycelial growth

CONCLUSIONS

Among the tested botanicals, it was found that, under *in vitro* condition, 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 tomatoes can be done by the use of plant extracts.

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