International Journal of Multidisciplinary Research and Development
Online ISSN: 2349-4182, Print ISSN: 2349-5979, Impact Factor: RJIF 5.72
www.allsubjectjournal.com
Volume 3; Issue 7; July 2016; Page No. 188-192

Effect of plant extracts against early blight of potato (*Solanum tuberosum* L.) *in vitro* and field condition

Abdul Sami Ariafar, Sunil Zacharia

Department of Plant Pathology, SHIATS, Allahabad, Uttar Pardesh, India

Abstract
Early blight is one of the most common and devastating disease of potato plant which is caused by the fungus, *Alternaria solani*. The antifungal activity of five plants extracts namely *Azadirachta indica* (Neem*)*, *Eucalyptus chamadulonsis* (Eucalyptus), *Ocimum tenuiflorum* (Tulsi), *Nerium oleander* (Kaner), and *Calotropis procera* (Madar) at 10 % concentration and Carbendazim 0.1% concentration as a treated check, was tested for management of *Alternaria solani in vitro* and *in vivo*. In the present investigation the highest inhibition of mycelial growth of *Alternaria solani* was achieved by fresh aqueous extract of *Eucalyptus chamadulonsis, Ocimum tenuiflorum, and Azadirachta indica* caused the highest reduction of mycelial growth of *A. solani* (57.35, 50.0 and 44.12%, respectively), while *Nerium oleander*, and *Calotropis procera* caused the lowest inhibition of mycelial growth of the pathogen as compare to treated check and untreated check. In field experiments, the highest reduction of disease severity was achieved by applying the extracts of *Eucalyptus chamadulonsis* and *Ocimum tenuiflorum*, at 10% concentration (35.80%, 45.68%) as compare to treated check carbendazim at 0.1% concentration (25.93%). and treated check T_0 (95.06%). All treatments significantly reduced the early blight disease severity as well as increased the plant height number of branches and yield of potato compared to the infected control under field conditions, Thus the present study revealed that plant extracts have shown significant inhibition and proved to be cost effective and eco-friendly for the management of *A. solani* and were comparable with fungicides.

Keywords: *Alternaria solani*, plant extracts, Carbendazim, potato early blight management

1. Introduction
P Potato (*Solanum tuberosum L*.) is the fourth most important world crop, after rice, wheat, and maize (Spooner and Bamberg, 1994) [14]. Potato has been planted 18.2 million ha and total yield of that reached 364,808,768 tons in the world. India is the second biggest potato producer after China which produced 45,000,000 tons in 2012 (FAOSTAT, 2014) [4]. In Afghanistan, potato is the second most important staple food crop after wheat. It is grown in an area of 21,900 ha producing 333,600 t at an average productivity of 15.23 t/ha (FAOSTAT, 2012) [4].

Potato plants are susceptible to a wide variety of diseases that can severely reduce yield, quality and storability of tubers. Diseases can occur in the field or in storage and are caused by infections such as fungi, bacteria, viruses and other related organisms.

Early blight is one of the most important disease which is caused by the fungus *Alternaria solani* (Ellis and Martin) Jones and Grout, that occurs in most potato growing regions world-wide (Shtienberg *et al*. 1990; Vanderwalls *et al.*, 2001) [13, 15]. In recent years, increasing of early blight disease on potato foliage has been reported in various potato growing areas (Vloutoglou and Kalogerakis, 2000) [18]. Primary damage by early blight is attributed to premature defoliation of the potato plants, resulting in tuber yield reduction. Yield loss estimates resulting from foliar damage incited by early blight on potato vary by location, cropping season, cultivar, and the stage of potato maturity.

Early blight is a major foliar disease of potato and causes 20-50% yield loses, it produces small, darkened lesions on the plants that spread into growing black spots of dead tissue. *A. solani* overwinters as mycelium or conidia in plant debris, soil, infected tubers or on other host plants of the same family. The disease is controlled primarily through the use of cultural practices such as, crop rotation, tillage, removal and burning of infected plant debris, and eradication of weed hosts helps reduce the inoculums level for subsequent plantings, resistant cultivars and foliar fungicides. The most common and effective method for the control of early blight is through the application of foliar fungicides, but the fungicides treatment is not protected as chemicals pollute environment, effect health vulnerability in humans and when these harmful chemicals enter into the food chain become hazardous to all living entities. Botanical derivatives are environmentally safe and may be used as an alternative to commercial fungicides for controlling pathogenic fungi. The present study is designed to evaluate the antifungal activity of plant extracts such as, *Azadirachta indica (*Neem*)*, *Eucalyptus chamadulonsis* (Eucalyptus), *Ocimum tenuiflorum* (Tulsi), *Nerium oleander* (Kaner), and *Calotropis procera* (Madar) against destructive plant pathogenic fungus *Alternaria solani*.

2. Materials and Methods
The experiment was carried out during 2015-2016 at the field of Department of Plant Pathology, Sam Higginbottom Institute of Agriculture, Technology & Sciences (deemed- to- be university) Allahabad, Uttar Pradesh, India. The soil of the experimental field was sandy loam with pH 5.6. The present experiment was carried out under randomized block design (RBD) with three replications. The unit plot size was 2 m × 1.5 m. Row to row and plant - to-plant distances were 60 cm and 25 cm, respectively. The soil was raised and drains were

made to remove excess water. The symptoms appeared after 30 days of sowing

2.1. Isolation and identification of the pathogen

Leaves were collected from infected potato plants bearing characteristic symptoms of early blight. These leaves symptoms after mounting on slide were examined under microscope to confirm the presence of *Alternaria* spp. The infected leaf parts were cut into small pieces of two to three mm dimension in a manner so that pieces had some healthy portion also. Such leaf bits were surface sterilized with 0.1 per cent mercuric chloride ($HgCl_2$) 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 sterilized Petri plates with the help of forceps. These Petri plates were incubated at 27 ˚C± 2 ˚C, after 3 days mycelia growth was observed around leaf bits. With the help of cork borer from this colony growth a portion from the periphery having single hyphal tip was separated and transferred to other Petri plates having medium to get pure culture and identification of the pathogen were confirmed by observing the morphological features of colony, spore characteristics and referring the relevant literature (Aneja., 2004) [2].

2.2. Preparation of plant extracts

Extracts from leaves of five plants, namely *Azadirachta indica (*Neem*)*, *Eucalyptus chamadulonsis* (Eucalyptus), *Ocimum tenuiflorum* (Tulsi), *Nerium oleander* (Kaner), and *Calotropis procera* (Madar), were collected and tested for their efficacy in reducing the mycelia growth of *A. solani* using the poisoned food technique (Schmitz 1930) [12]. The fresh leaf material of each plant species was collected washed with water and surface sterilized with 0.1 per cent mercuric chloride ($HgCl_2$) solution for 30 seconds then washed with distilled water. Aqueous plant extracts was prepared by grinding 100 g fresh leaves with 100 ml distilled water (w/v) using a blender and filtrate through a double layered muslin cloth, all the extracts obtained and finally centrifuged at 10, 000 rpm for 10 minutes.

2.3. Determination of effect of plant extracts against the target pathogen by poison food technique

Efficacy of plant extracts against *A. solani, in vitro* was determined by poison food technique. 10mL of Plant extracts was added to 100mL of sterilized PDA medium in a conical flask, mixed thoroughly, sterilized in autoclave and poured in Petri plates to make up 10% of extract in the plated media. A 5mm diameter of actively growing mycelium disc of the pathogen of 6–7-day-old culture was placed in the centre of the Petri plates. Plates containing medium with fungicide Carbendazim 0.1% served as a positive control and plates with medium served as negative control. Plates were incubated at 27°C. Three replicates were maintained for each treatment. Radial growth of mycelium was measured after inoculation. The results were compared with negative control. The percent inhibition of the fungus in treatments was calculated using following formula given by Vincent (1947) [17].

$$I = \frac{C-T}{C} \times 100$$

Where:

I= Per cent inhibition of mycelia growth

C = Growth of mycelium in control (mm)

T = Growth of mycelium in treatment (mm)

3. Results and Discussion

In present study, we tested aqueous extracts of five plants at 10% concentration and carbendazim at 0.1% concentration in PDA,

3.1 Inhibition of Mycelial growth (%) as affected by different treatments on *Alternaria solani* by poison food technique

The table No.1 shows that At 24 hr, all the treatments showed significant inhibition in the mycelial growth as compared to control (T_0). Maximum inhibition was recorded from T_5-Eucalyptus leaf extract (fs) (54.84), followed by T4-Tulsi leaf extracts (fs) (43.55), Neem leaf extracts (fs) (20.03) as compared to treated check (100%) and untreated check (0). At 48, hr all the treatments showed significant inhibition in the mycelial growth as compared to control (T_0) Maximum inhibition was recorded from T_5-Eucalyptus leaf extract (fs) (59.70), followed by T4-Tulsi leaf extracts (fs) (47.76), Neem leaf extracts (fs) (46.27) as compared to treated check (100%) and untreated check (0). At 72, hr all the treatments showed significant inhibition in the mycelial growth as compared to control (T_0). Maximum inhibition was recorded from T_5-Eucalyptus leaf extract (fs) (65.55), followed by T4-Tulsi leaf extracts (fs) (57.90), Neem leaf extracts (fs) (52.15) as compared to treated check (100%) and untreated check (0). At 96, hr all the treatments showed significant inhibition in the mycelial growth as compared to control (T_0). Maximum inhibition was recorded from T_5-Eucalyptus leaf extract (fs) (57.35), followed by T4-Tulsi leaf extracts (fs) (50.00), Neem leaf extracts (fs) (44.12) as compared to treated check (100%) and untreated check (0).

3.2 Effect of treatments on disease intensity of early blight of potato (%) at different days intervals

All treatments differed in respect of early blight disease intensity (%) at different growth stages (Table1). At 30 DAS, The minimum disease intensity were recorded in T_5 Eucalyptus leaf extract (fs) (1.23), Followed by T_4 Tulsi leaf extract (fs) (1.25), T_1 Neem leaf extract (fs) (1.43) as compared to treated check (2.47) and untreated check (3.70). At 60 DAS, The minimum disease intensity were recorded in T_5 Eucalyptus leaf extract (fs) (33.33), Followed by T_4 Tulsi leaf extract (fs) (36.21), T_1 Neem leaf extract (fs) (37.27) as compared to treated check (25.89) and untreated check (43.21). At 90 DAS, The minimum disease intensity were recorded in T_5 Eucalyptus leaf extract (fs) (35.80), Followed by T_4 Tulsi leaf extract (fs) (45.68), T_1 Neem leaf extract (fs) (45.80) as compared to treated check (25.93) and untreated check (95.06).

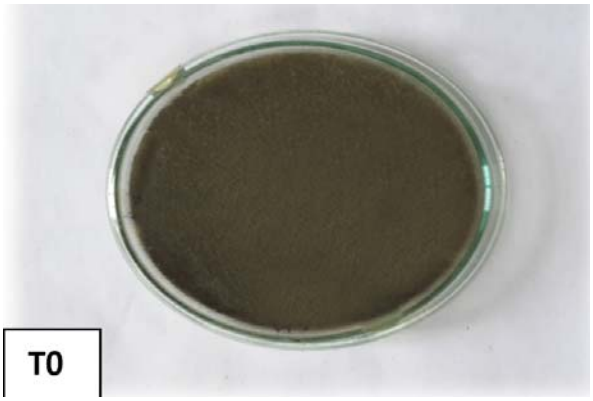
Table 1: Inhibition of Mycelial growth (%) as affected by different treatments on *Alternaria solani* by poison food technique

Treatments	Mycelial growth percentage			
	24 hr	**48 hr**	**72 hrs**	**96 hrs**
T_0-Control	0.00	0.00	0.00	0.00
T_1-Neem leaf extract (fs)	29.03	46.27a	52.15	44.12
T_2-Madar leaf extract (fs)	25.81a	38.51a	35.41a	20.59
T_3-Kaner leaf extract (fs)	25.70a	37.31a	33.97a	14.71
T_4-Tulsi leaf extract (fs)	43.55	47.76	57.90	50.00
T_5-Eucalyptus leaf extract (fs)	54.84	59.70	65.55	57.35
T_6-Carbendazim (fs)	100.00	100.00	100.00	100.00
F- test	S	S	S	S
S. Ed. (±)	0.814	1.148	1.215	1.190
C. D. (P = 0.05)	1.628	2.295	2.430	2.380

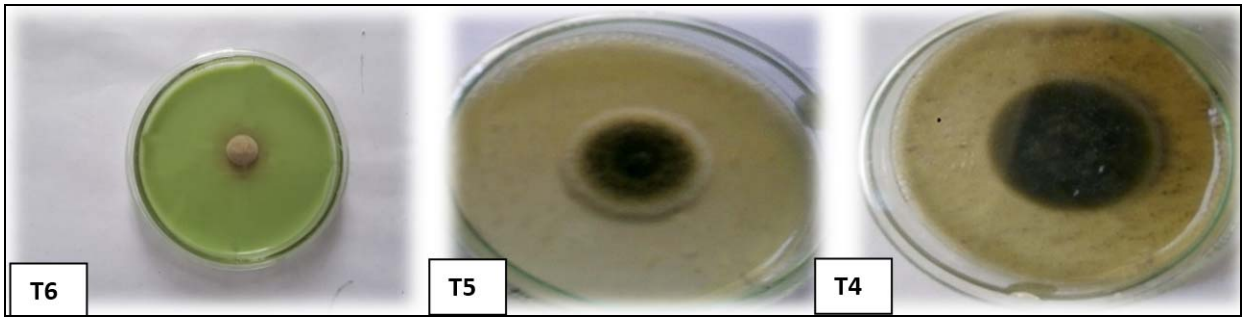
Table 2: Effect of treatments on disease intensity of early blight of potato (%) at different days intervals

Treatments		disease intensity percentage		
		30 DAS	**60 DAS**	**90 DAS**
T_0	Control	3.70	43.21	95.06
T_1	Neem leaf extract (fs)	1.43	37.27	45.80
T_2	Madar leaf extract (fs)	2.23	38.27	53.09
T_3	Kaner leaf extract (fs)	2.16	35.80	53.19
T_4	Tulsi leaf extract (fs)	1.25	38.21	45.68
T_5	Eucalyptus leaf extract (fs)	1.23	33.33	35.80
T_6	Carbendazim (fs)	2.47	25.89	25.93
Overal Mean		0.39	2.46	3.13
F- test		NS	NS	NS
S. Ed. (±)		0.228	0.605	0.946
C. D. (P = 0.05)		0.465	1.263	1.986

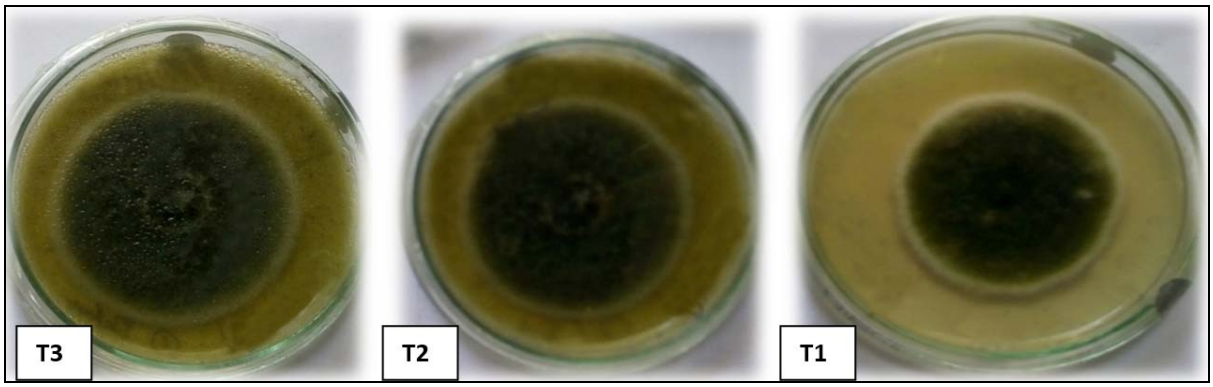
Plate 1: Conidium of *Alternaria solani*



Control



Carbendazim **Eucalyptus leaf extract** **Tulsi leaf extract**



Kaner leaf extract **Madar leaf extract** **Neem leaf extract**

Discussion

Our results indicated that all tested plant extracts, *Azadirachta indica (*Neem*), Eucalyptus chamadulonsis* (Eucalyptus), *Ocimum tenuiflorum* (Tulsi), *Nerium oleander* (Kaner), and *Calotropis procera* (Madar), at 10% concentration and carbendazim at 0.1% concentration caused a significant reduction in the linear growth of *A. solani*. This reduction was gradually increased by increasing the concentration of extracts in the growth medium. Similar effects of various other plant products effective against *Alternaria* spp were reported by several authors (Latha *et al.*, 2009; Goussous *et al*. 2010) [10, 6]. Vijayan (1989) [16] reported that the bulb extract of *A. sativum*, leaf extract of *Aegle marmelos* and flower extract of *Catharanthus roseus* inhibited the spore germination and mycelial growth of *A. solani*. The inhibitory effect of the tested plant extracts may be due to their direct toxic effect on the pathogen as reported by (Vijayan 1989) [16]. Investigations on the mechanisms of disease suppression by plant products have suggested that the active principles present in plant extracts may either act on the pathogen directly (Amadioha 2000) [1] or induce systemic resistance in host plants resulting in a reduction of the disease development (Kagale *et al.*, 2004) [7]. The greenhouse and field experiments indicated that the foliar sprays of tomato plants with plant extracts resulted in a significant reduction in early blight infection. These results were similar to previous work on the role of plant extracts in the fungal disease control. Several authors including Curtis *et al.*, (2004) [3] Krebs *et al.*, (2006) [8, 9] and Latha *et al.*, (2009) [10] reported that plant extracts from 20 non-host plant species caused a reduction of the early blight disease and suppressed the mycelial growth of *A. solani.* All treatments with tested plant extracts improved the yield of tomato plants compared to the infected control.

In conclusion, our study demonstrated that many plant extracts, e.g. from *Azadirachta indica (*Neem*), Eucalyptus chamadulonsis* (Eucalyptus), *Ocimum tenuiflorum* (Tulsi), *Nerium oleander* (Kaner), and *Calotropis procera* (Madar), can be used as an alternative of fungicides against early blight disease. Thus, this method of control can contribute to minimising the risks and hazards of toxic fungicides, especially on vegetables produced for fresh consumption. Further research into these extracts will identify the active compounds responsible for their fungicidal activity.

Acknowledgment

The authors are grateful for providing the necessary facilities to carry out the research from Head of Plant Pathology Department, SHIATS, Allahabad, UP, and AAIP (Afghanistan Agriculture Input Project) which supported us financially to complete this research successfully.

References

1. Amadioha AC. Controlling rice blast *in vitr*o and *in vivo* with extracts of Azadirachta indica. Crop Protection, 2000; 19:287-290.
2. Aneja KR. Experiment in microbiology, Plant pathology and bacteriology (4th Ed) New age International P (Ltd). Publisher, New Delhi, 2004, 437-450.
3. Curtis H, Noll U, Stormann J, Slusarenko AJ. Broad-spectrum activity of the volatile phytoanticipin allicin in extracts of garlic (*Allium sativum* L.) against plant pathogenic bacteria, fungi and Oomycetes, Physiological and Molecular Plant Pathology. 2004; 65:79-89.
4. FAO. Food and agricultural commodities production, 2014. In: http/ /www. Faostat. Fao. Org /site /339/default.aspx downloaded on 12 Apr.

5. Ganie SA, Ghani MY, Anjum Q, Nissar Q, Shabir-U-Rehman, Dar WA. Integrated management of early blight of potato under Kashmir valley conditions African Journal of Agricultural Research. 2013; 8(32):4318-4325.
6. Goussous SJ, Abu-El-Samen FM, Tahhan RA. Antifungal activity of several medicinal plants extracts against the early blight pathogen (*Alternaria solani*). Archives of Phytopathology and Plant Protection. 2010; 43:1746-1758.
7. Kagale S, Marimuthu T, Thayumanavan B, Nandakumar R, Samiyappan R. Antimicrobial activity and induction of systemic resistance in rice by leaf extract of Datura metel against Rhizoctonia solani and Xanthomonas oryzae pv. oryzae. Physiological and Molecular Plant Pathology. 2004; 65:91-100.
8. Krebs H, Dorn B, Forrer HR. Control of late blight of potato with medicinal plant suspensions. Agrarforschung. 2006; 13:16-21.
9. Krebs H, Dorn B, Forrer HR. Control of late blight of potato with medicinal plant suspensions. Agrarforschung. 2006; 13:16-21.
10. Latha P, Anand T, Ragupathi N, Prakasam V, Samiyappan R. Antimicrobial activity of plant extracts and induction of systemic resistance in tomato plants by mixtures of PGPR strains and Zimmu leaf extract against Alternaria solani. Journal of Biological Control. 2009; 50:85-93.
11. Nashwa SMA, Abo-Elyousr KAM. Evaluation of various plant extracts against the early blight disease of tomato plants under greenhouse and field conditions. Plant Protection and Science. 2012; 48:74-79.
12. Schmitz H. Poisoned food technique Industrial and Engineering Chemistry Analytical Editon, 1930; 2:361-363.
13. Shtienberg D, Bergeron SN, Nicholson AG, Fry WE, Ewing EE. Development and evaluation of a general model for yield loss assessment in Potatoes Phytopathology 1990; 80:466-472.
14. Spooner DM, Bamberg GB. Potato Genetic Resources, American Potato Journal. 1994; 71:325-337.
15. Vanderwalls JE, Korsen L, Aveling TAS. A review of early blight of potato, African journal of Plant Protection. 2001; 70:91-102.
16. Vijayan M. Studies on early blight of tomato caused by *Alternaria solani* (Ellis and Martin) Jones and Grout. [M.Sc. (Ag.) Thesis] Tamil Nadu Agricultural University, Coimbatore, India, 1989.
17. Vincent JM. Distortion of fungal hyphe in the presence of certain inhibitors. *Nature*, 1947; 159:239-241.
18. Vloutoglou I, Kalogerakis SN. Effects of inoculum concentration, wetness duration and plant age on development of early blight (*Alternaria solani*) and on shedding of leaves in tomato plants. Plant Pathology. 2000; 49:339-345.