

Original Research Article

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***In Vitro* Antifungal Activities of Common Weed Extracts on Mycelial Growth of *Alternaria solani* (Ell. and Martin) Jones and Grout**

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Herbal fungicides are mostly used to control plant disease because of their eco-friendly nature and cost effectiveness. The present investigation focuses on the antifungal activity of some weed extracts viz., *Salix sp.*, *Achyranthus aspera*, *Solanum nigrum*, *Parthenium hysterophorus*, *Datura fastusa*, *Melilotis alba*, *Lantana camara*, and *Achyranthus aspera*. Out of 8 weed tested, the extracts of *Datura*, *Parthenium*, *Achyranthus*, *Salix sp.* and *Physallis minima* were found most effective against phytopathogenic fungi, *Alternaria solani*. The antifungal activity of these plant extract was determined *in vitro* following Poison Food Technique. The results are showed that maximum inhibition was obtained by *Datura* extract (80.78 reduction) followed by *Parthenium* (76.48), *Achyranthus* (69.66), *Salix sp.* (65.14) and *Physallis minima* (49.53). The other treatments were also significantly controlled the mycelium growth of *Alternaria solani*.

Introduction

Tomato (*Lycopersicon esculentum* Mill). Belongs to the family Solanaceae and is one of the most remunerable and widely grown vegetables in the world. Among vegetable crop, tomato ranks second important vegetable in the world next to potato and first among the processing crops.

The productivity of tomato in India is 20.7 MT/ha during 2012-13 which is very low as compare to other country of the world like USA, 81.0 tones /ha. One of main reason of

low productivity of tomato in India is disease which is caused by fungi, bacteria, virus, nematode and abiotic factors (Balanchard, 1992). Among the fungal diseases, early blight also known as target spot disease incited by *Alternaria solani* (Ellis and Martin) Jones and Grout is one of the world's most catastrophic disease. The pathogen is air and soil borne in nature and is responsible to cause disease as early blight, collar rot and fruit rot etc (Datar and Mayee, 1981).

Spraying of broad spectrum fungicides like mancozeb, captan and Paraclostrobin (25%), copperoxychloride, chlorothalonil has been recommended for the control of early blight of tomato by several workers (Ramakrishnan *et al.*, 1971 and Stevenson, 1977) and (chethana, 2009 and sahu *et al.*, 2013).

Cultural practices like field sanitation, deep summer ploughing, soil solarization, soil amendment, crop rotation etc. can be minimized the possibility of disease but cannot completely control the disease on standing crop. Biological control is another important method of plant disease management. In this content, *Trichoderma* sp. *Gliochodium virens*, *Chaetomium globosum*, *Aspergillus niger* AN 27, *Pseudomonas fluorescens* etc. have been exploited for management of plant diseases (Biswas *et al.*, 2003, Girdhari lal *et al.*, 2008, Razic *et al.*, 2012, Butler, 1905). However, slight fluctuation of temperature, relative humidity, pH, moisture largely affects the efficacy of bioagents. Therefore, the use of chemicals is one of the best methods of plant disease management. Several fungicides Mancozeb, propineb, metalaxyl, foretyl-Al, aureofungin, streptocycline, phosphonic acid, and chlorthanil @ 0.2% and Iprovalicarb + propineb for seed treatment have been used for management of Alternaria blight of tomato (Chaudhari *et al.*, 2002 and Andreu and Coldiz 2006).

However, continuous use of same chemicals is not advisable for management of plant diseases, because of development of resistance strains among the pathogens.

It has also hazardous to human health and environment. Therefore, there is a need to search for on alternate methods for management of plant disease in sustainable agriculture. In this content use of plant products has been great attention in plant

disease management. (Arzoo *et al.* (2013). reported that use of plant extracts Neem, Dhatura, Zinger, Garlic, Mutha etc. as seed treatment increases. Plant growth and decreases seedling mortality of tomato causes by Fusarium wilt. Zarina *et al.*, (2003) also found that soil amendment with extract of neems and dhatura increases the growth of bringal plant and also controlled infestation of root knot nematodes.

Materials and Methods

The materials used and methods adopted in laboratory and glass house experiment was undertaken at Department of Plant Pathology, C.S.A. University of Agriculture and Technology, Kanpur.

Collection of plant materials for preparation of extract

The plant materials like. (*Salix sp.*, *Achyranthus aspera*, *Solanum nigrum*, *Parthenium hysterophorus*, *Datura fastusa*, *Melilotis Alba*, *Lantana camara* and *Achyranthus aspera*) were collected from vicinity area of University campus for extraction and further use in bioassay test against pathogen.

Preparation of plant extract

The fresh and mature leaves of collected plant materials were thoroughly washed under running clean tap water to remove dust and other surface contaminant. The extract was obtained from individual plant material by electric mixie in distilled water 1:1 (w/v). The resultant slurry was strained through a double layered muslin cloth to remove the uncrushed fibrous tissue of plant material. The procedure repeated twice and each time, the resultant slurry was filtered through two fold muslin cloth. The final volume of the filtrate thus obtained was made up to one liter by adding

water to maintain at ten percent concentration on fresh weight basis of plant material (w/v).

Antifungal activity of plant extracts

Anti-fungal activity of plant extracts was determined by Poison food technique (Kumar and Tyagi 2013; Mohanta and Raveesha 2007). The prepared plant extracts were poured in conical flask containing tomato extract media in 1:5 (v/v) ratios. The poisoned media was later autoclaved at 15 psi and 121.6°C temperature for 20 minutes.

Inoculation of pathogen

The autoclaved media containing plant extracts was poured in sterile Petri plates under aseptic conditions. After solidification of media 10 mm diameter mycelia discs cut from periphery of actively growing pure culture of the pathogen (*A. solani*), was placed at the centre of each Petri plate.

Three replications were maintained for each treatment and tomato extract media without any treatment served as control. Petri plates were incubated at 28 °C and mycelia growth was measured at every 24hrs interval upto 7 days.

In-vitro evaluation of plant extracts on radial growth of fungal colony

Two replicates of 5- μ L aliquots of the plant extracts were placed 90° apart on the perimeter of each of two Petri dishes each (9 cm in diameter) of potato dextrose media. Petri dishes were incubated overnight at room temperature. A 5-mm plug of *A. solani* was transferred to the center of each tomato extract medium Petri dish.

The Petri dishes were incubated at 20°C and duplicate diameters of fungal colonies were measured at 90° with a ruler when the control

colony reached full growth (85 mm). This occurred 7 days after incubation on potato dextrose media.

Results and Discussion

Effect of plant extracts on mycelial growth of *Alternaria solani* in vitro

Natural products from many plants are known to control plant pathogens. Antifungal activity testing of weeds remains an area of interest. However not many reports are available on the exploitation of antifungal property of weeds plants and even the data regarding use of weeds as an antifungal agents are scanty, (Shivastava and Singh, 2011).

The antimicrobial potency of plants is believed to be due to tannis, saponins, phenolic Compounds, essential oils and flavonoids, (Tapwal *et al.*, 2011). The antimicrobial activity of plant oils and extracts has formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies, (Lis-Balchin and Deans, 1997).

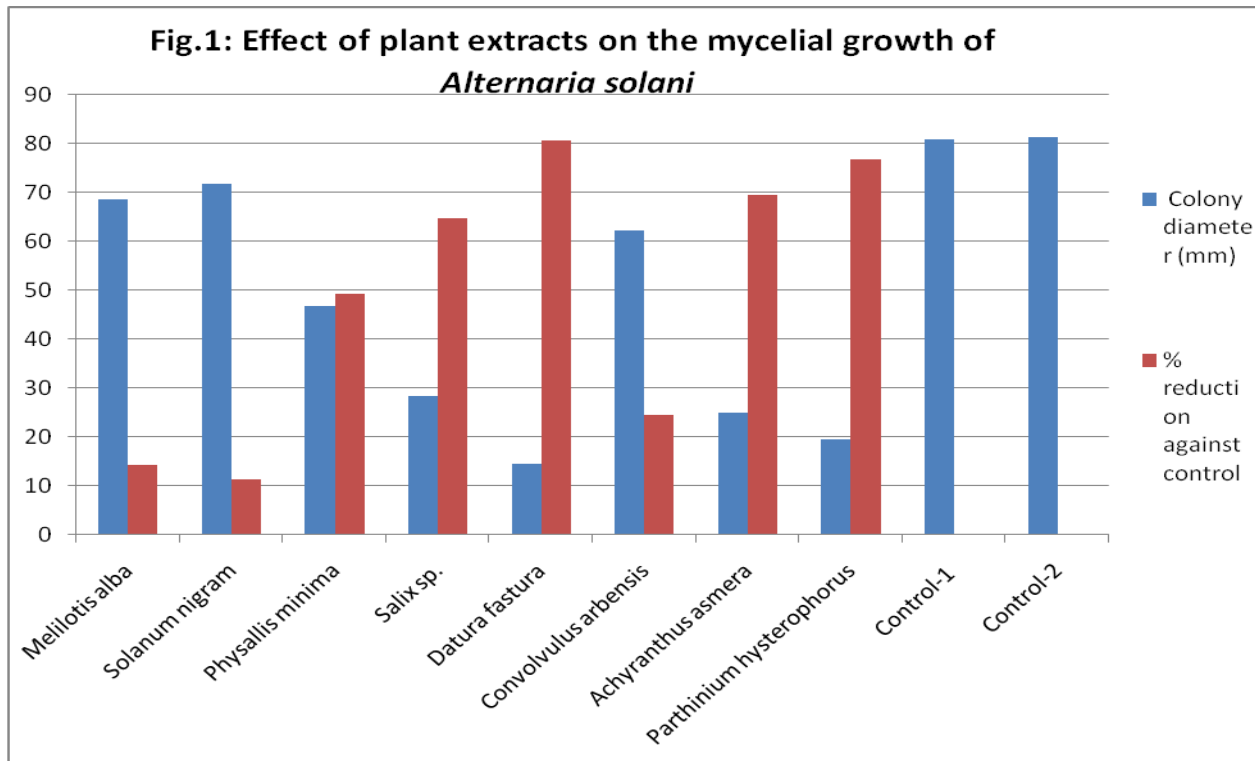
The results of in vitro antifungal activity of 8 plant extracts are summarized in table 1. The extracts produced different levels of antifungal activity against *A. solani*. Results indicated that all the extracts significantly reduced the radial growth of *A. solani*, in comparison with the control. The antifungal activity of different plant extract was determined *in vitro* following Poison Food Technique. The results are presented in table 1 shows that maximum inhibition was obtained by *Datura* extract (80.78 reduction) followed by *Parthenium* (76.48), *Achyranthus* (69.66), *Salix sp.* (65.14) and *Physallis minima* (49.53) (Fig. 1). The other treatments were also significantly controlled the mycelium growth of *Alternaria solani*. Thus,

the extract of, *Datura* and *Parthenium* could be a possible source to obtain new and effective biofungicides to control *A. solani* in various crops. Biofungicides are easily biodegradable, selective and locally produced, especially for the farmers who cannot afford

expensive synthetics fungicides. By using weed plant species as raw materials for plant derived fungicides, can manage the disease, and at the same time might create economic uses for these unwanted species (Shivastava and Singh, 2011).

Table.1 Effect of plant extracts on the mycelial growth of *Alternaria solani*

Treatment	Colony diameter (mm)	Reduction against control
<i>Melilotis alba</i>	68.56	14.34
<i>Solanum nigrum</i>	71.87	11.45
<i>Physallis minima</i>	46.86	49.37
<i>Salix sp.</i>	28.32	64.71
<i>Datura fastusa</i>	14.59	80.68
<i>Convolvulus arvensis</i>	62.18	24.57
<i>Achyranthus aspera</i>	25.00	69.47
<i>Parthiniun hysterophorus</i>	19.56	76.75
Control-1	81.00	-
Control-2	81.46	-
S.E.	2.456	-
C.D. (P=0.05)	1.177	-



Present findings also supported by Jalender and Gachande (2012) the leaf extract of *D. stramonium* and *D. innoxia* at 20% concentration was found more inhibitory activity against *F. oxysporum udum*, while the extract of *D. stramonium* at 20% concentration was inhibitory against *A. solani*. Shrestha and Tiwari (2009) reported complete inhibition of mycelial growth of *Fusarium solani* (Mart.) Sacc. Causing dry potato tuber rot when treated with *Allium sativum* extract. The inhibitory effect of *Salix sp.* (willow) aqueous extract against *Alternaria solani* could be explained by the presence of salicin i.e. glucoside of salicyl alcohol. Salicin may bind with receptors on the surface of pathogen cells and penetrates to the fungal cells which could be killed through denaturation of some enzymes and proteins. Salicin may be the major compound that shows the antifungal effect but other metabolites may increase the potency of the *Salix sp.* (willow) extracts compared with pure salicin (El-Shemy *et al.*, 2007). Pal and Kumar (2013) also reported in-vitro mycelia inhibition of *Fusarium* with *Achyranthes aspera* and *Parthenium hysterophorus* extracts.

In order to maintain the productivity, more and more chemicals are being added in the natural environment, which enter the food chain through water, soil, and air resulting serious harmful effects on human health (Ramachandra *et al.*, 2003). According to the survey made by the WHO, more than 50,000 people in developing countries are annually poisoned and 5,000 die as a result of the effects of toxic agents, used in agriculture. In India 35,000 – 40,000 tons of hazardous chemicals are sprayed on the crops every year, instead of helping the poor, these chemicals are causing cancer, sterility and death (Das, 1983).

To avoid the use of these horrible diseases causing synthetic chemicals, the plants and

their products should be utilized to combat phytopathogens. As plants are known to possess various secondary metabolites, which showed inhibitory effect against the growth of pathogens. Keeping these problems in view, efforts are underway to search economic safe phytochemicals, which could be utilized for disease control.

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