

Original Research Article

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Efficacy of Plant Extract, Bio Agents against *A. solani* causing Early Leaf Spot of Tomato *in vitro* Conditions

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Tomato (*Solanum lycopersicum* L.) is the third most important crop in India that suffers from various biotic and abiotic stresses. Among the biotic stresses early blight caused by *Alternaria solani* [(Ell. and Mart.) Jones and Grout] is one of the most destructive diseases of tomato which leads losses up to 78 per cent. In the present study efficacy of plant extract, bioagents, cow by product and fungicides were evaluated against the pathogen .Among plant extracts , Datura showed maximum growth inhibition of 61.11 per cent followed by Neem Seed Kernel Extract (15 % concentration) which showed growth inhibition of 55.55 per cent *in vitro*. In case of bioagents *T. harzianum* showed maximum growth inhibition of 76.53 per cent *in vitro*.

Introduction

Tomato (*Solanum lycopersicum* L.) known as poor man's orange having high nutritive value and its fruit may be consumed either in fresh or in processed forms. It is rich in vitamin A, B and C and minerals (Khoso, 1994). It is the third most important crop in India after potato and onion in terms of area and production having 8.14 lakh ha and 20.51 mt respectively with the total productivity of 25.20 t ha⁻¹ (Anonymous 2018-19) out of this Rajasthan

contributes significant proportion having production of 9.02 mt with the productivity of 4.05 t ha⁻¹ over an area of 20366 hectare (2016-17) (Anonymous).

Despite of this abundance in quantity, its quality deteriorates and lags behind to meet up its domestic consumption due to various abiotic and biotic factors as it is affected by many fungal, bacterial, viral and nematodes diseases that affect tomato production and decrease its economical value. Among the

various diseases, early blight that is caused by *Alternaria solani* (Ellis and Martin) Jones and Grout is one of the most destructive diseases of tomato that mainly occurs in the tropical as well as in subtropical regions. It causes loss both at pre and post-harvest stages that leads to reduction of 35 to 78 percent in yield (Jones *et al.*, 1993). Early blight of tomato caused by *A. solani* was first recorded in 1882 in New Jersey, USA (Bose and Som, 1986). In India, this disease was first noticed by Butler in Faizabad, U.P in 1905. Datar and Mayee, (1981) reported the losses due to this disease from different part of India to the extent of 48-80 percent.

Abhinandan *et al.*, (2004) conducted a survey in Punjab during 2001 and reported that at Tapa District, maximum disease intensity of 49.5 percent was observed and minimum disease intensity of 8.2 percent was observed at Babakala District. The disease is air borne and its pathogen is soil inhabiting. Andrus *et al.*, (1945) proved the pathogenicity of *Alternaria* on tomato by using mycelial fragments as a source of inoculum of *A. solani*. *Alternaria* is saprophytic, endophytic and pathogenic in nature. The fungus survives in the crop debris in or on the soil; disease is favoured by high humidity, dew and rainfall. The spores are disseminated by water, wind, insects and other means include man and machinery. Under favourable conditions bull eyed pattern in which concentric rings with brown to black spot appear on lower side of infected leaves. Jambhulkar *et al.*, (2016) stated that the conidia survive on the soil surface as well as on old dry lower leaves of the plant and spread when suitable climatic condition prevails. The mycelium of fungus is haploid and septate. Morphologically it is branched, at initial growth stage it is light brown in colour while fully grown mycelium shown dark black in colour. The conidiophores are short, 50-90 μ m in length and are of dark black in colour. Conidia are

characterized by their size (120-296 x 12-20 μ m), beak, muriform, dark colour and are borne singly (Bose and Som, 1986). The conidia contain 5-10 transverse septa and 1-5 longitudinal septa (Singh, 1987).

In this study it is thought essential to test the efficacy of the most promising chemicals against early blight of tomato. However, in the recent years, the increase use of potentially hazardous fungicides in agriculture has been the subject of growing concern for both environmentalist and public health authorities. Therefore, novel approaches are required that uses low amount of chemicals which results in reduction of pollution hazards as well as the cost of production. So it is prerequisite to control plant disease by the integration of several methods. An integrated management approach that include use of bio agents, botanicals, cow by products as well as chemicals for the management of Early blight of tomato is evaluated in this report. Keeping in view of occurrence and losses incurred due to this disease and also lack of information in efficacy of some fungicides and botanicals against it was evaluated in this study.

Materials and Methods

This present study was conducted in the Laboratory of bio agent in college of agriculture, SKRAU Bikaner in order to develop integrated approach for management of early leaf spot of tomato via plant extracts, bioagents and by application of novel fungicides and cow by products. The pathogen was isolated by following Koch postulates from infected leaves of tomato and then these leaves were cut into small pieces of about 1.5 cm- 2 cm. surface sterilization was done with 0.1% mercuric chloride, then washed three times with distilled water and placed on Petri plates having potato dextrose agar (PDA). These Petri plates were incubated

at $26 \pm 1^\circ\text{C}$ for one week for sporulation. Pure culture was obtained by single spore technique by incubating at 28°C for a week. On the basis of symptomology and conidial characteristics, fungus was identified as *A. solani*, causal organism of early leaf spot of tomato.

Evaluation of plant extracts

Ten Plant extracts (*Datura stramonium* (Datura), *Allium sativum* (Garlic), *Allium cepa* (onion), *Azadirachta indica* (Neem), NSKE, *Calotropis procera* (Aak), *Eucalyptus globules* (Eucalyptus), *Citrullus colocynthis* (Tumba), *Tinospora cordifolia* (Giloy), *Curcuma longa* (Turmeric) known for antifungal properties were evaluated for their efficacy against *Alternaria solani* at four different concentrations viz. 5%, 7.5%, 10%, 15% under *in-vitro* condition through food poisoning technique against *A. solani*.

Plant extract preparation

Selected plants were collected from the surrounding areas of Bikaner, and washed thoroughly with tap water and air dried. One hundred gram of plant part was grind using pestle and mortar by adding equal amount (100 ml) of sterilized distilled water (1: 1, w/v). The pulverized mass was squeezed through the cheese cloth and the extracts were centrifuged at 10000 rpm for 5 - 10 minutes and used as stock solution. The stock solutions of different botanicals (5, 7.5, 10 and 15%) were mixed with 95, 92.5, 90, and 85 ml of PDA media, respectively so as to get 5, 7.5, 10 and 15 per cent concentrations and sterilized. Twenty ml of such medium was poured under aseptic condition into sterile Petri plates and allowed to solidify. Mycelial discs (5 mm) were cut out by using sterile cork borer and one such disc was placed on the centre of each Petri plate. The treatments were replicated thrice. Control was

maintained by growing the pathogen on PDA plates without plant extracts. Plates were incubated at room temperature ($28 \pm 1^\circ\text{C}$) for 7 days and radial growth was taken at the time when maximum growth occurred in the control plates.

Evaluation of antagonistic potential of bioagents

Efficacy of two fungal (*Trichoderma harzianum* and *T. viride*) and two bacterial (*Pseudomonas fluorescens* and *Bacillus subtilis*) bioagents was tested *in vitro* against *A. solani* through dual culture and paper disc method respectively.

Isolation and evaluation of fungal antagonists

In order to isolate fungal antagonists, ten gram soil was added in 90 ml sterilized water in Erlenmeyer flask and shaken gently for 4-5 minutes. Serial dilutions were made up to 10^{-7} and 0.2 ml soil suspension of suitable dilution (depending on stage of soil sampling) was added to the surface of *Trichoderma* selective medium (Elad and Chet, 1983) and Martin's Rose Bengal Agar media in Petri dishes for isolation of *Trichoderma spp.* Soil suspension was spread uniformly. The inoculated Petri dishes were incubated at $28 \pm 1^\circ\text{C}$ in BOD incubator for 7 days and the fungal colonies developed were sub cultured on potato dextrose agar media.

Dual culture technique was followed in order to ascertain the antagonistic capacity of *Trichoderma spp.* and other fungal antagonists. One mycelial disc (5 mm diameter) of each of the pathogen and antagonist was kept on the surface of potato dextrose agar medium in Petri dishes at 5 cm apart. The inoculated Petri dishes were incubated at $28 \pm 1^\circ\text{C}$ for 7 days. Three replications were kept for each fungal

antagonist. In case of control, the Petri dishes were inoculated with mycelial disc of the test pathogen only. The mycelial growth of test pathogen was measured after 4 days of inoculation.

Isolation and evaluation of antagonistic potential of bacterial bioagents

Stock soil solution was prepared by taking 10 g soil in 90 ml sterile distilled water in Erlenmeyer flask and shaken gently for 2 to 4 minutes. Serial dilutions were prepared up to 10^{-7} . A 0.2 ml soil suspension of suitable dilution was added on surface of the media in Petri dishes and spread uniformly. The inoculated Petri dishes were incubated at $28\pm1^{\circ}\text{C}$ for 48 hours and the colonies appeared were sub cultured on PAF (*Pseudomonas* agar fluorescens) media and NA (Nutrient agar) for *P. fluorescens* and *B. subtilis*.

Paper disc inoculation method was followed in order to test the antagonistic capacity of bacterial antagonists. Ten ml sterilized distilled water was added to each slant containing the fresh colony of respective bacterial antagonists and suspension was prepared by scrapping the bacterial growth with the help of sterilized inoculating needle. Sterilized filter paper discs (5 mm diameter) were dipped in respective bacterial suspension.

Four such inoculated discs were placed in opposite directions on the surface of potato-dextrose-agar media in Petri dishes. Mycelial discs (5 mm diameter) of *A. solani* were taken and placed at the center of Petri dishes containing the inoculated paper discs. In case of control, the Petri dishes were inoculated with mycelial discs of pathogen only. Three replications were kept for each bacterial antagonist. The inoculated Petri dishes were incubated at $28\pm1^{\circ}\text{C}$ in BOD incubator for 7 days.

Statistical analysis

Percent growth inhibition was calculated to evaluate the efficacy of plant extracts, bioagents, fungicides and cow by products against *A. solani* *in vitro* by formula given by Bliss (1934).

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

C = Mycelial growth of *A. solani* in control (mm)

T= Mycelial growth of *A. solani* in treatment (mm)

Results and Discussion

Efficacy of plant extracts tested against *A. solani* *in vitro*

In the present study ten different plant extracts were evaluated for their efficacy against *A. solani* at four different concentrations viz. 5%, 7.5%, 10%, 15% under *in-vitro* and the data are summarized in table 1 and plate 1. At 5% concentration, maximum per cent of inhibition was shown by Datura (40%), followed by NSKE (36.67%) and Neem leaves (33.33%) whereas minimum per cent inhibition was shown by Turmeric (11.11%).

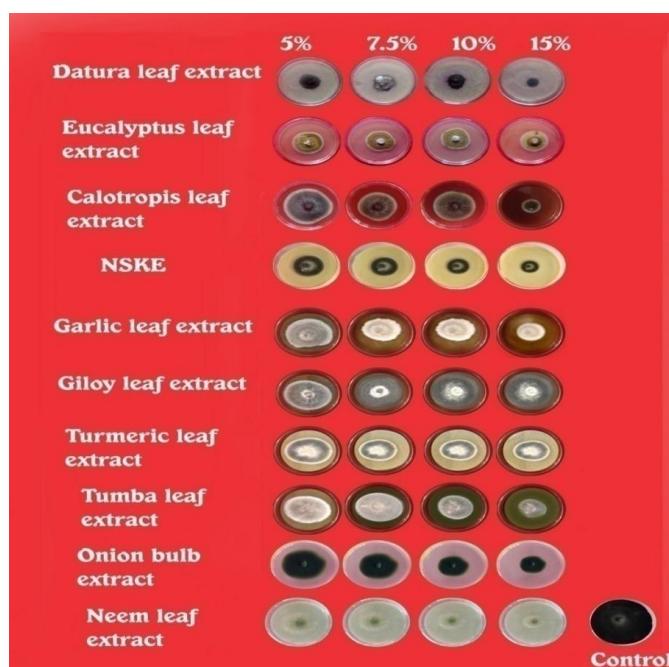
Percent inhibition increases gradually with the increase of concentration. *Allium sativum* and *Allium cepa* were equally effective (31.11%) against *Alternaria solani* at 7.5 concentration. Similarly Aak and Giloy were also equally effective (22.22%). At 10% concentration, growth inhibition by *Allium sativum* and *Citrullus colocynthis* was similar 38.89%.

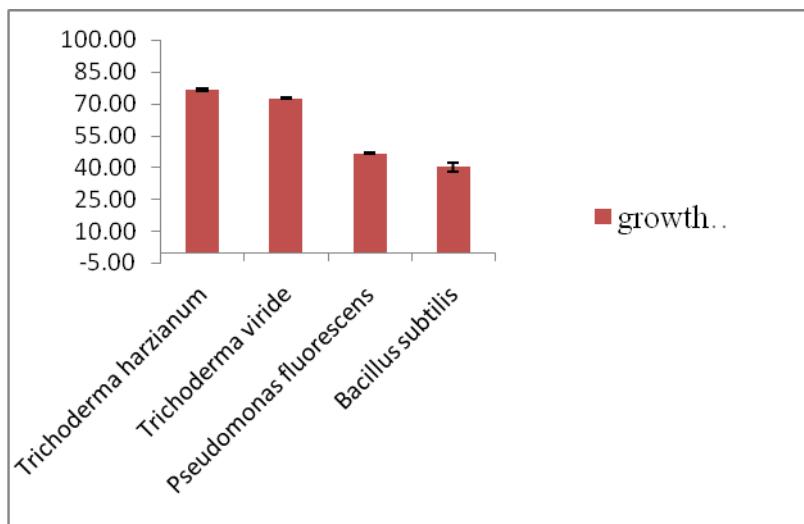
Maximum growth inhibition was observed by Datura at 15% concentration (61.11%), followed by NSKE (55.55%), Minimum growth inhibition was shown by Turmeric that was only (33.33%) at 15% concentration.

Table.1 Efficacy of plant extracts against *Alternaria solani* under *in vitro*

Treatment	5% Conc.	7.5% Conc.	10 % Conc.	15 % Conc.
	Growth Inhibition (%)	Growth Inhibition (%)	Growth Inhibition(%)	Growth Inhibition(%)
<i>Datura stramonium</i> (Datura)	40.00 (39.21)*	47.77 (43.41)	53.33 (46.76)	61.11 (51.37)
<i>Allium sativum</i> (Garlic)	24.44 (29.41)	31.11 (33.84)	38.89 (38.20)	48.89 (44.00)
<i>Allium cepa</i> (Onion)	27.78 (31.66)	31.11 (33.84)	36.67 (36.98)	46.67 (42.81)
<i>Calotropis procera</i> (Aak)	16.67 (23.68)	22.22 (27.92)	28.89 (32.10)	37.78 (37.60)
<i>Azadirachta indica</i> (Neem leaf)	33.33 (34.90)	35.55 (36.35)	44.44 (41.62)	53.33 (46.77)
NSKE	36.67 (37.32)	44.44 (41.62)	48.89 (44.01)	55.55 (47.96)
<i>Eucalyptus globule</i> (Eucalyptus)	22.22 (27.73)	24.44 (29.42)	42.22 (40.42)	50.00 (44.98)
<i>Citrullus colocynthis</i> (Tumba)	20.00 (26.54)	26.67 (30.76)	38.89 (38.21)	47.77 (43.40)
<i>Tinospora cordifolia</i> (Giloy)	13.33 (20.87)	22.22 (27.98)	31.11 (33.81)	44.44 (41.62)
<i>Curcuma longa</i> (Turmeric)	11.11 (19.98)	20.00 (26.55)	24.44 (29.36)	33.33 (35.10)
Control	-	-	-	-
S.Em±	0.972	1.016	1.127	1.312
CD (P=0.05)	2.888	3.019	3.347	3.897
CV%	5.781	5.307	5.115	5.215

*Figure in parentheses are angular transformed values

**Plate.1** Efficacy of plant extract against *A. solani* under *in vitro*

**Graph.1**

Efficacy of bio-agents against *A. solani* in vitro

The results presented in figure1 revealed that maximum growth inhibition was recorded in *T. harzianum* (76.53%) which was significantly superior over all the other bio-control agents followed by *T. viride* (72.44%), *Pseudomonas fluorescens* (46.66 %) and *Bacillus subtilis* (40.14%).

Efficacy of bioagents against *A.solani* in vitro

Early leaf spot of tomato is a foliar disease. The fungus survives in the crop debris in or on the soil; disease is favoured by high humidity, dew and rainfall. The spores are disseminated by water, wind, insects and other means includes man and machinery. The disease is air borne and soil inhabiting which is responsible for leaf blight, seedling collar rot and fruit rot of tomato (Datar and Mayee, 1981).The symptoms of the early blight disease appear as brown to dark leathery necrotic spots that appear first on the leaflets (Locke, 1949). Walker (1952) reported oval or angular shaped spots of 0.3 to 0.4cm diameter with usually narrow chlorotic zone around the spot. As the spots mature,

concentric rings of raised and depressed brown tissue are evident.

Management of *A. solani* should be done at initial stage and it should be done by organic and by natural ways as tomato is consumed directly or in processed form. So present study conferred the efficacy of plant extracts, bioagents against *A. solani* In the present study maximum percent inhibition of mycelial growth of *Alternaria solani* was recorded on 15 per cent concentration of Datura extract (61.11%) followed by NSKE(55.55%) and Neem leaf extract (53.33). Dalpati *et al.*, (2010) evaluated ten botanicals viz., Marigold, Neem, Custard apple, Lantana, Eucalyptus, Tamarind, Kanher, Garlic, Datura and Congress grass and stated that the per cent inhibition of fungus by botanicals ranged from 44.59 to 8.25 per cent. Lantana and Datura were found effective as it restricted 44.59 and 30.88 per cent respectively. Sadana and Didwania (2015) reported that the best plant extract found to be effective in inhibiting the growth of pathogen was *Eucalyptus obliqua* (15%) and it ranged from 64.9 to 88 per cent. This was followed by *Datura stramonium*, *Calotropis procera*, *Polyalthia longifolia* and *Azadirachta indica*.

Similarly bioagents were also reported to be effective against *A. solani* and the inhibition effect ranged from 76.53 % (*T. harzianum*) to 40.14 % (*B. subtilis*). Sanjeet kumar *et al.*, (2005) observed that all the three antagonist viz. *Trichoderma virens*, *T. harzianum* and *T. viride* grown rapidly than the colony of *Alternaria alternata* but *T. viride* parasitized the test fungus earliest in dual culture. Studies on hyphal interaction between antagonist and test fungus are based on disorganization of protoplasmic content and lysis of host hyphae. Dalpati *et al.*, (2010) evaluated different bio-agents (*Trichoderma harzianum*, *T. viride*, *Pseudomonas fluorescens* and *Bacillus subtilis*). Among the four bio-agents, *T. harzianum* was found superior as compared to other in inhibition (76.66 per cent) followed by *Bacillus subtilis* (73.66 per cent).

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