GLOBAL JOURNAL OF BIO-SCIENCE AND BIOTECHNOLOGY

www.scienceandnature.org

IN VITRO EVALUATION OF ETHANO-BOTANICALLY IMPORTANT PLANT EXTRACTS AGAINST EARLY BLIGHT DISEASE (*ALTERNARIA SOLANI*) OF TOMATO

C. Maya[1] & M. Thippanna[2]*
Department of Botany, Jnanabharathi campus, Bangalore University, Bangalore-560056 (Karnataka) INDIA
*Corresponding author's, Email:tgowthamsandesh@gmail.com

ABSTRACT
The Tomato *(Lycoperiscum esculantum)* is a diploid species with 2n=24 chromosomes and belongs to the family *Solanaceae*. It is the world's largest vegetable crop after potato. The area under tomato in India is about 4.97 lakh hectares with a production of about 86 lakh tons. Early blight is caused by the fungus *Alternaria solani* Sorauer. This disease in severe cases can lead to complete defoliation and is most damaging on tomato in regions with heavy rainfall, high humidity and fairly high temperatures (24^{o}C-29^{o}C). *A. solani* is characterized by septate beaked muriform conidia borne singly on simple conidiophore, In the present study Ten locally available plants which are ethano-botanically important are selected *viz., Amaranthus caudatus, Anacardium occidentale, Azadirachta indica, Bambusa arundinacea , Capsicum annuum, Ecballium elaterium, Eucalyptus gobules, Ficus religiosa , Lantana camara and Morus alba* aquoes and thier extracts were evaluated against *A. solani* by poison food technique. The results revealed that leaf and seed extracts of *A.indica* recorded maximum mycelial inhibition with 78.83% followed by *L. camara* with 59.9% and *E. globules* with 59.7% inhibition in mycelial growth and *B. arundinacea* exhibited least mycelial inhibition with 3.7%.

KEYWORDS: Tomato, early blight, *Alternaria solani*, plant extracts, antifungal, mycelial inhibition.

INTRODUCTION

The Tomato *(Lycoperiscum esculantum)* is a diploid species with 2n=24 chromosomes. Tomato is an herbaceous plant, belongs to the family *Solanaceae*. It is one of the most important "Protective food" both because of its special nutritive value and its wide production. Tomato occupies a significant position in vegetable production. Total world production 152.9 million ton with value of $74.1 billion. (FAOSTAT database, 2009). The area under tomato in India is about 4.97 lakh hectares and is about 7.3% of the total cropped land under vegetables with a production of about 86 lakh tons (NHB database 2010). Tomato production has increased by almost 15 times, from a mere 0.54 million tons in 1961 to about 8.6 million tons in 2005. (FAO, 2007). Early blight is a three-phase disease, which produce leaf spots, stem canker and fruit rot, but the foliar phase is the most common and destructive part of the disease (Maiero and Barksdale, 1989), responsible for significant economic losses sustained by Tomato producer each year. *A. solani* can cause extensive defoliation leading to a reduction of economic fruit yield (Spletzer and enyedi, 1999). The conidia of *A. solani* are muriform and beaked (Neergaard, 1945; Ellis and Gibson, 1975). *A. solani* has transverse and longitudinal septate conidia, multinucleate cells, and dark-colored cells (Rotem, 1994). The melanins protect against adverse environmental conditions including resistance to microbes and hydrolytic enzymes (Rotem, 1994). Control of early blight disease has been accomplished primarily by the application of chemical fungicides (Jones *et al.,* 19991). Several effective pesticides have been recommended for use against *A. solani*, but are not considered to be long-term solutions, due to concerns of expense, exposure risks, fungicide residues and other health and environmental hazards. In an attempt to modify this condition, some alternative methods of control have been adopted. Natural products isolated from plant appear to be minimal environmental impact and danger to consumers in contrast to synthetic pesticides (Varma and Dubey, 1999). Control of microorganism linked plant disease with plant extracts as components in integrated pest management strategy has been tested by many researchers.

The use of plant extracts has been shown to be eco-friendly and effective against many plant pathogens (Saadabi 2006; Gachomo and Kotchoni, 2008; Thobhunluepop, 2009; Duru and Onyedineke, 2010). Most of these substances were evaluated in order to find safe alternative control methods to the human and the environment. The objective of the present study is to evaluate the antifungal activity of aqueous extracts of 10 plant species which were ethano-botanically important were screened against *A. solani* under *in vitro* conditions hence Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds etc. (Gordon and David, 2001). The beneficial effects of the plant materials typically results from the combination of secondary products present in the plant. Plant products and their active constituents played an important role in plant disease control by combating growth and development of pathogens and including resistance in plants.

MATERIALS AND METHODS

The pathogenic isolates of *A. solani* was isolated from the tomato leaves showing typical symptoms of early blight

by using potato dextrose agar (PDA), medium and identified as *A. solani* according to Simmsons (2007).

Collection of botanicals/plant materials

Fresh healthy ten disease free botanical plant parts viz., leaves, inflorescence, bulbs and seeds were collected from the Bangalore University Campus, Bangalore, Karnataka, India and in regions of Kolar and Chickballapura district local forests (Table-1) which are ethano-botanically important for mankind in treating various disorders. An authenticated voucher specimen of the plant has been deposited in the herbarium, Department of Botany, Bangalore University, Bangalore, Karnataka, India.

TABLE 1. Summarizes the ethnobotanical data of the plant species selected for study.

Botanical name	Family	Part used [a]	Collection site [b]	Popular uses [c]
Amaranthus caudatus	*Amaranthaceae*	L	K	Treatment of diarrhoea, healing of mouth ulcers
Anacardium occidentale,	*Anacardiaceae*	L & I	K & C	Treatment of ringworm, leprosy, antihelmintic
Azadirachta indica Juss.	*Meliaceae*	L & S	K & C	Treatment of eczema, leucoderm and malarial fever
Bambusa arundinacea	*Poaceae*	L	B	Treatment of skin diseases, intestinal worms
Capsicum annuum,	*Solanaceae*	F	K	Treatment of headaches, indigestion, colds, fever
Ecballium elaterium,	*Cucurbitaceae*	L	C	Treatment of kidney problems, paralysis
Eucalyptus gobules Labill.	*Myritaceae*	L	K	Treatment of chronic cough, asthma and bronchitis
Ficus religiosa	*Moraceae*	L	B	Treatment of asthma , haemorrhages, wounds
Lantana camara	*Verbenaceae*	L & S	K	Treatment of tetanus, dysentery, ulcers, eczema.
Morus alba	*Moraceae*	L	K	Treatment of cough , facial dropsy , injury

[a]Plant part : L-Leaves, I-Inflorescence , B-Bulbs ,S-Seeds and F-Fruits

[b]Collection site: B-Bangalore University Campus , K-Kolar local forests , C-Chickbalapur local forests.

[c]Based on ethnobotanical surveys. (Prajapati, N,D.,Purohit, S.S., Sharma,A.K., Tarun kumar , 2004).

All the ten botanical extracts tested showed varied degree of inhibition over control in the mycelial growth (Table-2 and Fig-1) of the pathogen *A. solani* at different concentrations.

Preparation of aqeous plant extracts

Ten locally available plant species namely *Amaranthus caudatus , Anacardium occidentale, Azadirachta indica, Bambusa arundinacea , Capsicum annuum, Ecballium elaterium, Eucalyptus gobules, Ficus religiosa, Lantana camara and Morus alba* were selected to prepare the aqueous extracts by modified weight/ volume(w/v) method (Parveez et al., 2009) .Healthy plant parts viz., leaves, inflorescence ,bulbs and seeds collected from fields were surface sterilized with 0.1% mercuric chloride ($HgCl_2$) .2-3 subsequent washings was done with sterile distilled water and kept for few minutes till they become semi dried. Plant materials were chopped aseptically and homogenized in mixer grinder using sterile distilled water at the rate of 1:1 ratio (i.e.100g of plant material in 100 ml of sterile distilled water). The homogenized extracts were filtered through double layered muslin cloth. The filtrates were collected by sterile Whatman No.1 filter paper. The extracts thus obtained were considered as standard (100%) stock solution and used to prepare desired test concentrations of 10%, 20% and 30% for further studies.

***In vitro* screening of aqueous plant extracts by poison food technique**

All the selected plants (Table-1) were subjected to poisoned food technique (Manmohan and Govindaiah, 2012) to evaluate the efficacy of botanicals in laboratory against *A. solani* at concentrations of 10%, 20% and 30% with 3 replications each of different botanicals. Potato dextrose agar (PDA) was used as nutrient medium and required quantity of each botanical extract was added separately so as to get a requisite concentration of the botanical extract. The botanical extract were thoroughly mixed by stirring and sterilized. About 15 ml poisoned medium was poured to each of the 90 mm Petridishes and allowed for solidification. The actively growing periphery of the nine day old culture of *A. solani* was carefully cut using a gel cutter and transferred aseptically to the centre of each Petridish containing the poisoned solid medium. Suitable control was maintained by growing the cultures on PDA without the botanical extract. Triplicates have been maintained, all the plates were incubated at 27±2ºC for nine days. After incubation period, the effects of extracts were determined by measuring the radial mycelial growth of the pathogen in the test plates. This was compared with control to calculate the percentage inhibition of mycelia of the pathogen (Manmohan and Govindaiah, 2012).

$$P\,I\,(\%) = \frac{Mc - Mt}{Mc} \times 100$$

Where, Mc=Mycelial growth in control

Mt=Mycelial growth in treatment

RESULTS

All the ten botanical extracts tested showed varied degree of inhibition over control in the mycelial growth (Table-2 and Fig-1) of the pathogen *A. solani* at different concentrations. Results were interpreted in the terms of their mean value. The maximum inhibitions of mycelial growth was recorded in A. indica with 78.83%, followed by L. camara with 59.9% and E. globules with 56.20% inhibition in mycelial growth and were highly significant. Significant results were also observed in *C. annum* with 54.8%, Minimum results were observed in *A. occidentale* 35.8%, inhibition in mycelial growth was observed are less significant, least inhibition was recorded in *B. Arundinacea with 3.7*% inhibition in mycelial growth of *A. solani* (Table-2, Fig-1). It was observed that in most of the treatments there was significant interaction with respect to the concentrations. With the increase in the concentration of the extract, there was corresponding increase in the inhibition of the pathogen.

TABLE 2: *In vitro* screening of aqueous plant extracts against *Alternaria solani* by poison food technique

		Percentage inhibition over control			
		Concentrations			Mean
SI No	Treatments	10%	20%	30%	
1	Control	0.0	0.0	0.0	0.0
2	*Amaranthus caudatus*	5.2	10.4	16.2	10.6
3	*Anacardium occidentale*	27.6	35.6	44.2	35.8
4	*Azadirachta indica Juss*	76.3	78.2	82.0	78.8
5	*Bambusa arundinacea*	2.7	4.6	4.6	3.7
6	*Capsicum annuum*	44.0	57.2	63.4	54.8
7	*Ecballium elaterium*	4.2	8.0	12.3	8.1
8	*Eucalyptus gobules Labill*	53.5	58.5	66.5	59.5
9	*Ficus religiosa*	5.6	10.9	16.3	10.9
10	*Lantana camara*	51.2	61.3	67.2	59.9
11	*Morus alba*	8.1	13.8	18.0	13.3
	CD@5%	0.787	0.849	0.923	

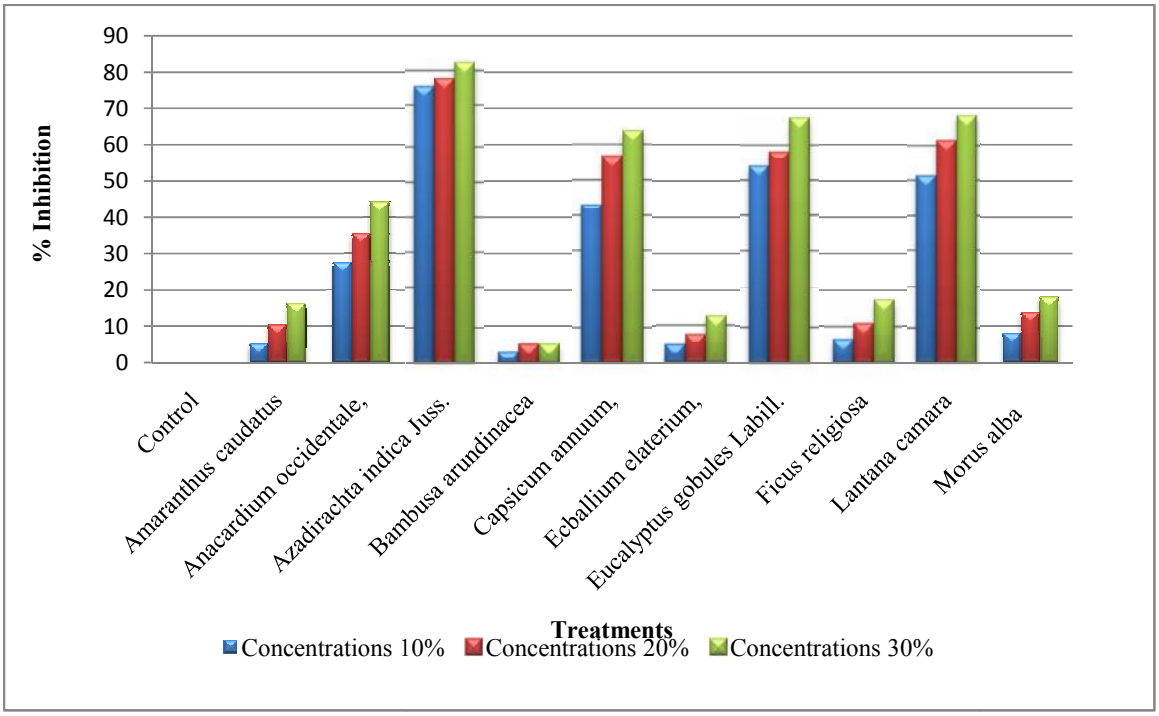

FIGURE 1: Graphical representation of *In vitro* screening of aqueous plant extracts against *A. solani*

DISCUSSION

In the present study, the antifungal activity of the extracts of 10 plant species aganist early blight pathogen (*A. solani*) was evaluated. Leaf and seed extract of *A.indica* (30%) was highly effective in reducing the radial mycelial growth of *A. solani*. At some concentrations, extracts from *Lantana camara* (leaf and inflorescence), *Eucalyptus gobules* (leaf) and *Capsicum annuum* (Fruits) also inhibited the mycelial growth of the *A. solani* over 50%. Similar effect of other various plants extracts effective aganist *Alternaria spp* have been reported by several workers (Hassanein *et al.,* 2008; Patil *et al.,* 2001; Srivastava *et al.,* 1997*)*. The results are confirmatory with those reported by Mohana and Raveesha (2007) stating that the aqueous extract from *Decalepis hamiltonii* at 30% concentration caused 84.83% mycelial growth inhibition on *A. alternata* and increase in extract concentration up to 50% resulted in 100% inhibition further, the aqueous neem extracts inhibited the mycelial growth of *A. solani*. The present result corroborates with Hassanein, *et al.,* 2008.

CONCLUSION

The study undertaken is inferred that early blight caused by *Alternaria solani* is susceptible to some selected plant aqueous extracts of *Azadirachta indica, Lantana camara, Eucalyptus gobules* and *Capsicum annuum* at higher concentrations and exhibited promising inhibition in the

radial growth of the mycelia of the pathogen. So these extracts could be useful in the treatment of fungal infections caused by *A. solani*. These botanicals will be further evaluated in field conditions. Usage of botanicals in the disease management is eco-friendly and economically feasible. Plant is biosynthetic laboratory, not only for chemicals compounds such as carbohydrates, proteins, lipids that are utilized as food by man, but also for a multitude of compounds like glycosides, alkaloids, coumarins, flavonoids, terpenoids, phenolic compounds etc. are important constituents of plants. Since antiquity, plants and plants products have been known to display not only their pharmacological benefits but others biological properties including pesticide activities. As plants and their products are known to posses various secondary metabolites, which showed inhibitory effect against the growth of pathogens, therefore, the plant and their products should be utilized to combat the disease causing pathogens.

ACKNOWLEDGMENT

Authors are thankful to University Grants Commission (UGC), New Delhi for funding the fellowship under **CSIR-UGC-JRF scheme** to conduct the work. I am also thankful to the Department of Botany, Bangalore University, Bangalore for providing laboratory facilities.

REFERENCES

Duru, C. M. and Onyedineke, N. E. (2010) In vitro study on the antimicrobial activity and phytochemical analysis of ethonalic extracts of the mesocarp of Voacanga africana. Am. J. Plant Physiol. **5**:163-169.

Ellis, M.B. and Gibson, I.A.S. (1975) *Alternaria solani* no. 45 set **48**. *Commonwealth Mycological Institute*, Kew, Surrey, UK.

Falck, R. (1907) *Wachstumgesetze, Wachstufakoren und Temperaturwerte der holzer storenden.* Mycelien. **1**: 153-154.

FAO. (2007) *Food and Agriculture Organization. FAOSTAT citation database results. FAO.* http://faostat. fao.org/faostat.

FAO. (2009) *Food and Agriculture Organization. FAOSTAT citation database results. FAO.* http://faostat. fao.org/faostat.

Gachomo, E. W. and Simeon Kotchoni . O. (2008) *Extract from drought-stress leaves enhances disease resistance through induction of pathogenesis related protiens and accumulation of reactive molecules.* Biotechnology. **7:** 273-279.

Gordon, M. C. and David, J. N. (2001) *Natural product drug discovery in the next millennium.* Pharm. Biol., 39, **8-17**. 2001.

Hassanein, N. M. Abou zeid, M. A, .Youssef, K. A. and Mahmoud., D. A. (2008) *Efficacy of Leaf Extracts of Neem (Azadirachta indica) and Chinaberry (Melia azedrach) Against Early Blight and Wilt Diseasesof Tomato*. Aust. J. Basic Appl. Sci. **2(3)**: 763-772.

Jones, J. B., Jones, J. P., Stall, R. E. and Zitter, T. A. (1991) *Infectious diseases: Diseases caused by fungi. In: Compendium of Tomato diseases.* Am. Phytopathol. Soc., St. Paul, MN. pp. 9-25.

Locke, S.B. (1949) *Resistance to early blight and septoria leaf spot in the genus Lycopersicon.* Phytopathol. **39**: 829-836.

Maiero, M.T. and Barksdale, T. H. (1989) *Combining ability estimates for early blight resistance in tomato*. J. Am. Soc. Hort. Sci. **114**: 118-121.

Manmohan, M. S and Govindaiah. (2012) *In vitro screening of aqueous plant extracts against Fusarium solani (Mart.) Sacc. causing root rot in mulberry.* Green Farming – Int. J. App. Agri. Hort. Sci. **3(2):** 223-225.

Mohana, D. C. and Raveesha, K. A. (2007) *Anti-fungal evaluation of some plant extracts against some plant pathogenic field and storage fungi.* J. Agric. Technol. **4(1)**: 119-137.

Neergaard, P. (1945) *Danish species of Alternaria and Stemphylium: taxonomy, parasitism, economic significance.* Oxford University Press, London. pp 260–287.

NHB (2009-10) National *Horticulture Board.NHB database.* Ministry of Agriculture. Gurgon, India. www. nhb. gov.in.

Parveez Gulzar, Tasneem, N., Tanki, Sahaf, K. A., Shahazad ahmad, N. A., Zagar, M. A. and Raja, T. A. (2009) *In vitro evaluation of various botanical extracts aganist Fusarium pallidoroseum (Cooke) Sacc.-The causal pathogen of twig blight of mullberry* . Indian J. Seric. **48(s)**: 133-137.

Patil, M. J., Ukey, S. P. and Raut, B. T. (2001) Evaluation *of fungicides and botanicals for the management of early blight (Alternaria solani) of tomato*. PKV-Res. J. **25(1)**: 49-51.

Prajapati, N. D. Purohit, S. S. Sharma, A. K and Tarun kumar. (2004) *A handbook of medicinal plants, a complete source book. 1st edn reprinted.* Agrobios, India.

Rotem J. and Reichert**,** I. **(**1964) *Dew – a principal moisture factor enabling early blight epidemics in a semiarid region of Israel.* Plant. Dis. Rep. **48**: 211–215.

Saadabi, A.M.A. (2006) *Antifungal activity of some saudi plants used in traditional medicine.* Asian J. Plant. Sci. **5:** 907-909.

Simmsons, E. G. (2007) *Alternaria an identification manual,* CBS Fungal biodiversity center, Utrecht, the Netherlands. pp. 379.

Spletzer, M. E. and Enyedi, A. J. (1999) *Salicylic acid induces resistance to Alternaria solani in hydroponically grown tomato.* Phytopathol. **89**: 722-727.

Srivastava, A. K. Bihari, B. Lal, B. (1997) *Studies on biofungicidal properties of leaf extract of some plants.* Indian Phytopathol. **50(3)**: 408-411.

Thobhunluepop, P. (2009) *Implementationof bio-fungicides and seed treatment in organic rice cv.KDML 105 farming.* Pak.J.Biol.Sci. **12**: 1119-1126.

Varma J. and Dubey, N. K. (1999) *Prospectives of botanical and microbial products as pesticides of Tomorrow.* Curr. Sci. **76:**