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IN VITRO ANTIFUNGAL EVALUATION OF VARIOUS BOTANICAL EXTRACTS AGAINST EARLY BLIGHT DISEASE *(ALTERNARIA SOLANI)* OF TOMATO

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ABSTRACT
Tomato (*Lycopersicum esculantum*) belongs to the important fruit vegetable for human nutrition and is cultivated across all continents in fields or in protected culture. 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 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. 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 (24ºC-29ºC). *A. solani* is characterized by septate, beaked, muriform conidia, borne singly on simple conidiophores. In the present study, ten locally available aqueous botanical extracts *viz*. *Accacia catechu, Cassia fistula, Cassia tora, Eupatorium odoratum, Melia azardichita, Pongamia pinnata, Psidium guajava, Tamarindus indica, Vitex nigundo and Zinger officinalea* were evaluated against *A. solani* by poison food technique. The results revealed that Rhizome extracts of *Zinger officinalea* recorded maximum mycelial inhibition with 80.70% followed by *M. azardichita* 73.64%, *P. guajava* 71.75 %, *P. pinnata* 68.92% and *E. odoratum* 63.71% and *Cassia tora* with 13.74% least mycelial inhibition.

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

INTRODUCTION

Tomato *(Lycoperiscum esculantum)* is an herbaceous plant. 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 86lakh tons (NHB database 2010). 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 & 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). Controls of early blight has been accomplished primarily by the application of chemical fungicides (Jones *et al*., 1991), 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). 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 a safe alternative control methods to the human and the environment. The present work was designed to investigate the antifungal activity of aqueous extracts of 10 botanicals against *A. solani* under *in vitro* conditions.

MATERIALS & 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, rhizomes and seeds were collected from in and around Bangalore University Campus, Bengaluru, Karnataka, India and also 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, Bengaluru, Karnataka, India. Preparation of aqueous plant extracts: Ten locally available plant species namely *Accacia catechu,Cassia fistula,Cassiatora, Eupatorium odoratum, Melia azardichita, Pongamia pinnata,Psidium guajava, Tamarindus indica, Vitex nigundo and Zinger officinalea* were selected to prepare the aqueous extracts by modified weight/ volume(w/v) method (Parveez *et al*., 2009). Healthy plant parts *viz*., leaves, inflorescence, rhizomes and seeds collected from fields were surface sterilized with 0.1% sodium hypochlorate (NaHCl), 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 Whattman 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 botanical 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 Petri dishes and allowed for solidification.

TABLE 1: Summarizes the ethno-botanical data of the plant species selected for study

Botanical name[a]	Family	Part used	Collection site [b]	Popular uses [c]
Accacia catechu	Mimosaceae	L	K	used as stimulant, astringent
Cassia fistula	Caesalpiniaceae	L	K	Treatment of constipation, common cold, fever
Cassia tora	Fabaceae	L	K	Treatment of ringworm, leprosy, psoriasis
Eupatorium odoratum	Asteraceae	L	B	used as antiseptic, laxative, stimulant
Meliaazardichita	Meliaceae	L, F	B	Treatment of skin disorders, anti-ulcer, antimicrobial
Pongamiapinnata	Fabaceae	L, F	K	Treatment of diarrhea, cleaning teeth and gums
Psidium guajava.	Myritaceae	L	C	used as anti-diarrheal, anti-inflammatory
Tamarindusindica	Fabaceae	L	K	Treatment of gastric, reducing malaria
Vitexnigundo	Lamiaceae	L	C	Treatment of eczema, ringworm, rheumatic pain
Zinger officinalea	Zingiberaceae	R	K	Treatment of nausea, morning chemotherapy sicknes

[a]Plant part : L-Leaves, F-Fruits , R-Rhizome .
[b]Collection site: B-Bangalore University Campus , K-Kolar local forests , C-Chickbalapur local forests.
[c]Based on ethno-botanical surveys. (Prajapati,N,D., et al. 2004).

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 Petri dish 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).

$$PI = \frac{Mc - Mt}{Mc} X\ 100\%$$

Where, Mc = Mycelial growth in control
Mc = 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.

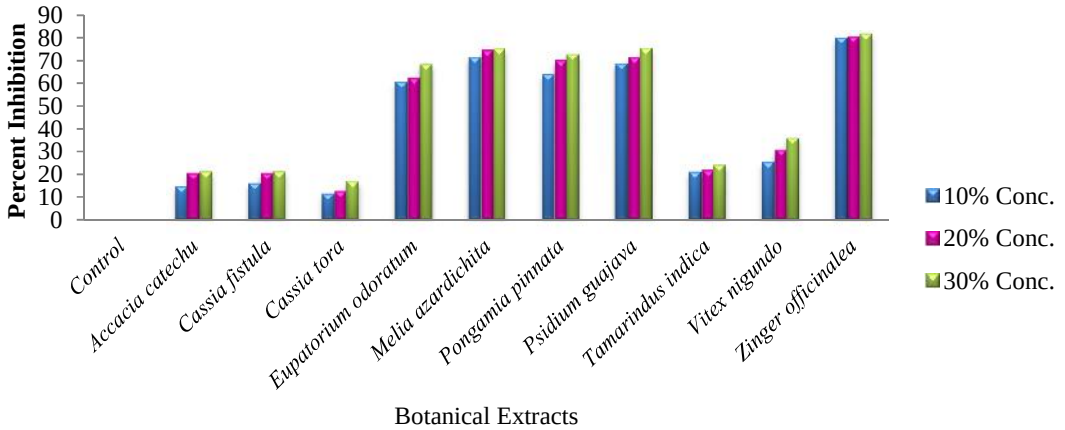

FIGURE 1: Graphical representation of *in vitro* screening of aqueous botanical extracts against *Alternaria solani* by poison food technique

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

SI. No.	Botanicals	Concentrations of Plant Extracts 10% Inhibition (%)	20% Inhibition (%)	30% Inhibition (%)	Mean Inhibition %
1	Control	0.00	0.00	0.00	0.00
2	*Accacia catechu*	14.66 ± 1.89	20.44 ± 1.97	21.27 ± 1.79	18.79
3	*Cassia fistula*	15.99 ± 2.47	20.44 ± 1.97	21.27 ± 1.79	19.23
4	*Cassia tora*	11.48 ± .64	12.73 ± .55	17.03 ± 2.31	13.74
5	*Eupatorium odoratum*	60.66 ± 1.35	62.14 ± 1.11	68.33 ± .62	63.71
6	*Melia azardichita*	71.33 ± 1.73	74.36 ± .55	75.25 ± .55	73.64
7	*Pongamia pinnata*	64.03 ± .63	70.03 ± .17	72.70 ± .72	68.92
8	*Psidium guajava*	68.59 ± 1.44	71.33 ± .47	75.33 ± 1.54	71.75
9	*Tamarindus indica*	21.10 ± 1.38	21.85 ± .64	24.07 ± .77	22.34
10	*Vitex nigundo*	25.38 ± 1.24	30.77 ± 2.29	35.84 ± 1.72	30.66
11	*Zinger officinalea*	79.96 ± .17	80.44 ± .44	81.70 ± .46	80.70

The results obtained from three different concentrations were interpreted in the terms of their mean value of three replications. The maximum inhibitions of mycelial growth was recorded in *Z. officinalea* with 80.79%, followed by *M. azardichita* with 73.64% and *P. guajava* 71.75% inhibition in mycelial growth and were highly significant. Significant results were also observed in *P. pinnata* 68.92%, *E. odurantum* 63.71% in inhibiting the mycelial growth. Minimum results were observed in *V. nigundo* 30.66% and *T. indica* 22.34% inhibition in mycelial growth was observed are less significant, least inhibition was recorded in *C. tora* 13.74% inhibition in mycelial growth of *A. solani* (Table-2 and 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.

DISCUSSION

In the present study, the tested botanical extracts showed antifungal activity against early blight pathogen (*A. solani*) were evaluated. Rhizome extract of *Z. officinalea* (30%) was highly effective in reducing the radial mycelial growth of *A. solani*. At some concentrations, extracts from *M. azardichita* (leaves+Fruits), *P. guajava* (leaves), *P. pinnata* (leaves) and *E. odoratum* (leaves) also inhibited the mycelial growth of the *A. solani* over 50%. Similar effects of other various botanical extracts effective against *Alternariaspp* 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. alternate* and increase in extract concentration up to 50% resulted in cent percent inhibition further, the aqueous neem extracts inhibited the mycelial growth of *A. solani.* The present result corroborates with Hassanein *et al*., 2008.

CONCLUSION

The fungicidal activity of the extracts against *A.solani* indicates the potential of some plant species as a natural source of fungicidal material. Antifungal activity was confirmed in all the tested plant species, although the results showed that different botanical extracts varied in their effectiveness in inhibiting the mycelia growth of different pathogens tested. A*lternaria solani* is susceptible to some selected plant aqueous extracts of *Zinger officinalea, Melia azardichita, Psidium guajava, Pongamia pinnata and Eupatorium odoratum* 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. This approach can contribute in reducing the amount applied of fungicides and subsequently minimize its hazards to the environment and human health. Usage of botanicals in the disease management is economically feasible and eco-friendly.

RECOMMENDATIONS

A*lternaria solani* is susceptible to some selected aqueous plant extracts of *Zinger officinalea, Melia azardichita, Psidium guajava, Pongamia pinnata and Eupatorium odoratum* at higher concentrations and exhibited promising inhibition in the radial growth of the mycelia of the pathogen. Hence, I recommend the extract of *Zinger officinalea* could be useful in the treatment of fungal infections caused by *A. solani* in field conditions.

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REFERENCES

Duru, C.M. & Onyedineke, N.E. (2010) In vitro study on the antimicrobial activity and phyto-chemical analysis of ethonalic extracts of the mesocarp of *Voacanga africana*. Am.J. plant Physiol., 5.163-169.

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

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. & Simeon Kotchoni, O. (2008) Extract from drought-stress leaves enhances disease resistance through induction of pathogenesis related proteins and accumulation of reactive molecules. Biotechnology, 7. 273-279.

Hassanein, N.M. Abouzeid, M.A., Youssef, K.A. & Mahmoud, D.A. (2008) Efficacy of Leaf Extracts of Neem (*Azadirachtaindica*) and Chinaberry (*Melia azedrach*) Against Early Blight and Wilt Diseases of Tomato. Aust. J. Basic Appl. Sci. 2(3).763-772.

Jones, J.B., Jones, J.P., Stall, R.E. & 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*. Phytopathology, 39. 829-836.

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

Manmohan, M.S. & 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 against *Fusarium pallidoroseum* (Cooke) Sacc.-The causal pathogen of twig blight of mulberry. Indian J. Seric., 48(s):133-137.

Patil, M.J. Ukey, S.P. 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 Tarunkumar (2004) A handbook of medicinal plants , a complete source book. 1st edn reprinted. Agrobios, India.

Rotem, J., Reichert, I. (1964) Dew – a principal moisture factor enabling early blight epidemics in a semiarid region of Israel. Plant D is Rep48. 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 Manuel, CBS Fungal biodiversity center, Utrecht, the Netherlands, p. 379.

Spletzer, M.E. & 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 bio-fungicidal properties of leaf extract of some plants. Indian Phytopathol .50(3). 408-411.

Thobhunluepop, P. (2009) Implementation of bio-fungicides and seed treatment in organic rice cv. KDML 105 farming. Pak .J. Biol. Sci., 12.1119-1126.

Varma, J. & Dubey, N.K. (1999) Prospectives of botanical and microbial products as pesticides of Tomorrow. Curr. Sci. 76. 172-179.