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Phytochemical Analysis and Antifungal Efficacy of Rhizome Extracts of various plants against Fusarium Wilt and Root Rot of Tomato

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Abstract

Fungal pathogens are serious problems on tomato plants. Among them, *Fusarium oxysporum* f. sp. *lycopersici* and *F. solani*, causal agents of wilt and root rot, respectively, affect tomato production under greenhouse and field conditions. Aqueous extracts of three plant rhizomes viz., *Curcuma longa* Val., *Allium sativum* L. and *Zingiber officinale* Rosc. tested @ 20, 40, 60 and 80% concentrations. *In vitro* study revealed that mycelial growth and spore germination was inhibited significantly ($P< 0.05$) with all extracts. *A. sativum* completely reduced the mycelial growth of *F. oxysporum* f. sp. *lycopersici* and *F. solani* at highest concentration. *Z. officinale* showed moderate inhibition ranging from 37.77-48.47% against *F. solani* and 30.33–44.49% against *F. oxysporum* f. sp. *lycopersici. C. longa* exhibited moderate inhibition of *F. oxysporum* f. sp. *lycopersici*, whereas, least inhibition was observed against *F. solani.* Correspondingly, conidial germination of test fungi was almost completely reduced by *A. sativum* extract. Phytochemical screening of crude extract revealed the presence of flavonoids, terpenes, saponins, whereas, *A. sativum* was also found rich in steroids, tannins, glycosides and coumarins. Tannins were not detected in *C. longa.* Total phenol contents (TPC) were present in highest quantity ((54.24GAEmg/g) in *A. sativum* followed by *Z. officinale* (29.80GAEmg/g), whereas, rhizomes of *C. longa* contained lowest TPC (25.45GAEmg/g). It was concluded that rhizome extracts of *A. sativum* possesses sufficient antifungal activity under controlled conditions to warrant a further investigation under field conditions.

Keywords: Antimicrobial activity; *Allium sativum*; *Curcuma longa*; *Zingiber officinale*; *Fusarium oxysporum* f. sp. *Lycopersici*; *F. solani*; Secondary metabolites

Introduction

Plant fungal diseases are traditionally controlled by using synthetic fungicides which although are cost effective but have created different types of toxicological and environmental problems (Malkhan *et al*., 2012). Moreover, fungicides and other synthetic chemicals are sources of carcinogenicity, adverse effects on human health and food borne hormonal imbalance (Kumar *et al*., 2007) and resistance by pathogen to fungicides has made certain fungicides infective. Furthermore, there is increasing public concern over the level of pesticide residues on vegetables, fruits and other cereal crops. In an attempt to minimize the use of synthetic chemicals attention has been paid during the past few years towards exploitation of ecofriendly novel plant products having antimicrobial activities (Jamil *et al*., 2007). This situation has led the researchers to search alternative solutions to synthetic chemicals.

Tomato belonging to nightshade family is most widely consumed vegetable crop due to its savory fruit; flavor and nutritive values, thus ranks first in the relative contribution to human nutrition (Saltueit, 2003). But production of tomato crop is greatly reduced due to foliar and fruit diseases incited by fungi and Oomycetes under favorable climatic conditions (Agrios, 2005). These diseases are serious threat to tomato growers in open field and under controlled cultivation. Fusarium wilts caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder and Hansen) (Carrillo-Fasio *et al*., 2003) and root rots caused by *F. solani* are among major diseases of tomato.

The hidden potential of indigenous medicinal plants of Pakistan has been continuously explored by researchers. These plants are rich in extensive variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, etc, having antimicrobial properties (Dahanukar *et al*., 2000; Aidah *et al*., 2014). Zia-ul-Haq *et al*. (2011, 2012) tested methanolic and ethanolic extracts of seeds of medicinal plants against various species of bacteria, fungi and nematode. Most of the botanical compounds of higher plants degrade more rapidly within few days or few hours as compared to synthetic pesticides, safe and ecofriendly and

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have been successful in management of plant diseases (Riaz *et al*., 2010). A large number of plant species have been reported to possess natural substances that are lethal to many diseases caused by fungi in plants (Sateesh *et al*., 2004). Several workers studied the antifungal activity of plant extracts like *Allium sativum*, *Azadirachta indica*, on various fungi *Botrytis cinerea*, *F. oxysporum f. sp. Lycopersici* and *Rhizoctonia solani* (Aba Alkhail, 2005; Agbenin and Marley, 2006). Nashwa and Abo-Elyousr (2012) evaluated the antifungal activity of *Ocimum basilicum*, *Azadirachta indica*, *A. sativum*, *Eucalyptus chamaldulensis*, *Nerium oleander* and *Datura stramonium* against *Alternaria solani in vitro* and *in vivo* and reported that the extracts of *A. sativum* significantly reduced the mycelial growth and disease severity of *A. solani* up to 42.2% (*in vitro*) 46.1% (*in vivo*), respectively.

Allium sativum L. belonging to Alliaceae family is a widely cultivated aromatic perennial herb throughout the world. The active component-(allicin) having strong antifungal activity effectively controlled various plant diseases including Late blight of tomato and potato (Slusarenko *et al*., 2008). Shrestha and Tiwari (2009) recorded complete inhibition of mycelia growth of *F. solani* at 40% concentration of garlic. *Z. officinale* Rosc. a perennial herb belongs to the family Zingiberaceae consists of underground rhizomes and erect shoots (Chandarana *et al*., 2005). Presence of chemical compounds like caprylic acid makes *Z. officinale* a plant with potent antifungal properties (Okigbo and Nmeka, 2005). Abdel-Kader *et al*. (2012) and Fawzi *et al*. (2009) evaluated antifungal potential of various plant extracts against *Alternaria alternata*, *F. solani*, *F. oxysporum*, *Rhizoctonia solani*, *Macrophomina phaseolina*, *Alternaria solani* and *pythium sp*. The results revealed that ginger extract had maximum inhibition on the growth of pathogenic fungi. *Curcuma longa* Val. commonly known as turmeric, (family Zingiberaceae), is also a medicinal plant consisting of natural compounds that are analgesic (Chattopadhyay *et al*., 2004). Lee *et al*. (2003) evaluated the fungicidal activity of *C. longa* against six fungal species of rice in a glasshouse experiment. It was observed that *C. longa* extract reduced 80% disease severity of rice sheath blight at highest concentration (2000 ppm). Balbi-Peña *et al*. (2006) found inhibitory effect of curcuma on mycelial growth of *Alternaria solani* whereas; Imtiaj *et al*. (2005) observed its efficacy against conidial germination of *Colletotrichum gleosporioides*.

In Pakistan, few reports are available on exploitation of extracts obtained from medicinal plants as antifungal agents and there is lack of work in this field. To cope with aforementioned risks and increasing public concern there is a need to develop alternative approaches or disease management systems to reduce the dependence on synthetic chemicals. Therefore, present study was planned to explore the potential of medicinal plants such as *A. sativum*, *C. longa* and *Z. officinale* against two economically important fungal pathogens of tomato viz. *F. oxysporum* f. sp. *lycopersici* and *F. solani* under *in vitro* conditions and to ascertain the chemical composition of these plants.

Materials and Methods

Plant Materials

Plant samples (*A. sativum*, *Z. officinale* and *C. longa*) were collected from local market. Fresh bulbs of plants were washed under sterile distilled water and blot dried. Samples (100g) were sliced into small pieces, macerated with 100 mL of sterile distilled water (1:1 w/v) using a blender grinder (Anex, Hong Kong) for 10 min. The extracts thus obtained was passed through double layered sterilized cheese cloth and then centrifuged at 6000 rpm for 15 min at 4°C. The supernatants were collected and filtered using Whatman No.42 filter paper (Sigma-Aldrich). The extracts were then kept at 4°C until use within 24 h. The final concentrations of 20, 40, 60 and 80% were utilized.

Plant Pathogens

F. oxysporum f.sp. *lycopersici* (Sacc.) Snyder and Hansen, and *F. solani* (Mart.) Sacc. was isolated from diseased tomato plants from open fields. All the fungal strains were cultured on potato dextrose agar (PDA) (Difco™) medium and purified using single spore or hyphal tip technique (Brown, 1924). The fungal isolates were identified on the basis of cultural, morphological and microscopic characteristics as described by Barnett and Hunter (1972) and Ellis (1971). Pure cultures were maintained on PDA medium at 4°C until further used.

In vitro Antifungal Activity of Plant Extracts on the Mycelial Growth of Test Fungi

The antifungal *in vitro* assays were carried out following the modified method of Okigbo *et al*. (2009). One mL extract of each concentration and recommended fungicide Topsin-M (Thiophenate-methyl) (Arista Life Sciences Company) and nine mL of molten PDA medium was poured into a sterilized Petri dish (90 mm) in four replications to prepare a PDA-extract mixture with corresponding 2, 4, 6 and 8% extract and 2500 ppm fungicide concentrations. Agar plates with sterile distilled water (SDW) served as negative controls while positive control was set up using a fungicide (Topsin-M). A 6 mm fungal culture disk taken from actively growing PDA cultures of test fungi was seeded onto the centre of each Petri plate. The plates were incubated for 7 days at 25±2°C. The colony diameter was measured and the percent inhibition of the growth was calculated using the formula suggested by Vincent (1947): $I = (C - T)/C \times 100$, where 'I' is percentage growth inhibition (mm), C is diameter of fungal colony (mean) in control and T is diameter of fungal colony (mean) with plant extract.

Inhibition of Spore Germination by Plant Extracts

The antifungal activity of plant extracts on spore germination of test fungi was assayed using the cavity slide method suggested by Nair and Ellingboe (1962). One drop (10 uL) from 80% concentration of each extract was mixed with a drop (10 uL) of spore suspension of phytopathogenic fungi (10^5 spores mL^{-1}) to a sterile cavity slide. Positive and negative control treatments were prepared as a film of recommended fungicide and sterile distilled water respectively. The slides were kept in moist chamber lined with filter paper in triplicate and incubated at 25±2ºC for 48 h. A drop of lactophenol-cotton blue (Sigma-Aldrich) was added to wells immediately after incubation to stop further germination of spores. Percent inhibition of spore germination was calculated by using the formula, Percentage inhibition = no. of conidia germinated in control-number of conidia germinated in Treatment / no. of conidia germinated in control × 100 (Kishore *et al*., 2001).

Phytochemical Analysis

Dried plant materials of each sample viz., *A. sativum*, *C. longa* and *Z. officinale* was refluxed with sterile distilled water, filtered and evaporated under reduced pressure at 40ºC and stored in crude form to be used for phytochemical analysis. Preliminary qualitative tests were carried out for identification and presence of biochemical constituents like flavonoids, tannins, terpenoids, saponins, tannins, phlobatannins and glycosides by using the procedure of Harborne (1998) and Trease and Evans (2009).

Estimation of Total Phenol Contents

Total phenol contents were estimated using protocol of Singleton and Rossi (1965) with slight modification. For each replicate, 1 mL of plant extracts (0.5g/20 mL), prepared in sterile distilled water was added to 4 ml of Folin-Ciocalteu's reagent (Sigma, USA). After 7 min, 5 mL of 20% sodium carbonate was added to each solution. The resultant solutions were incubated in darkness for 2 h at room temperature. The absorbance was measured at 740 nm with a spectrophotometer (UV 3000, ORI. Germany). Gallic acid (5, 10, 25, 50, 75 and 100 mg L^{-1}) was used as a standard chemical for calibration curve. Quantification of TPC was expressed in terms of Gallic acid equivalent (GAE) mg g^{-1} of dried fraction. All samples were analyzed in triplicate.

Statistical Analysis

Analysis of variance (ANOVA) was performed on the data and means were separated by using Tukey's HSD test-using SAS (SAS/IML software; Version 6; SAS, Institute) program (Steel *et al*., 1997).

Results

Fungi Isolated from Tomato

Isolation of two *Fusarium* species was carried out from naturally infected tomato plants and identified on basis of cultural and morphological characteristics as *F. oxysporum* f. sp. *lycopersici* and *F. solani* (Table 1).

Antifungal Activity of Plant Extracts on Mycelial Growth

The correlation between plant extracts and different concentrations was found statistically significant (Table 2). *A. sativum* significantly suppressed the mycelial growth of tested fungi as compared to control (SDW) at all concentrations. Complete (100%) inhibition was observed at higher (80%) concentration against *F. oxysporum* f. sp. *lycopersici* and *F. solani*, similar to fungicide control (Topsin-M). Antifungal activity of *Z. officinale* was observed against *F. solani* ranging from 37.77–48.47% and a range (30.33–44.49%) of mycelial growth reduction was noticed against *F. oxysporum* f. sp. *lycopersici* at various concentrations. Subsequently, *C. longa* showed 32.93–47.67 percent inhibition against *F. oxysporum* f. sp. *lycopersici* at different concentrations. While *C. longa* showed the least inhibition of *F. solani* and there was no significant difference between inhibitory effect at 60% and 80% concentrations (Table 2). In general, the rate of mycelial growth reduction was corroborated with the concentrations.

Effect of Plant Extracts on Spore Germination

There was a significant difference observed between the inhibitory effects of plant samples at highest concentration (80%) on spore germination of tested fungi as compared to control (Fig. 1). Aqueous extract of *A. sativum* bulbs completely (100%) inhibited the conidial germination of *F. oxysporum* f. sp *lycopersici*. However, almost complete (98%) inhibition of spore germination was observed against *F. solani* sporulation that was comparable to fungicide treatment. Rhizome extract of *Z. officinale* ranked next with 83and 79 percent spore inhibition of *F. solani* and *F. oxysporum* f. sp *lycopersici*, respectively. Among three plants extracts *C. longa* showed lowest inhibition of conidial germination of both fungi as compared to control (Fig. 1).

Qualitative and Quantitative Phytochemical Analysis of Crude Extracts

All crude extracts of plants were rich in flavonoids, terpenes, saponins, whereas, *A. sativum* was also found rich in steroids, tannins, glycosides and coumarins. Tannins were also present in *Z. officinale* but absent in *C. longa*. In contrast, all the extracts were poor in phlobatannins (Table 3). Total phenol contents were expressed as mg/g gallic acid

Table1: Morphological and cultural characteristics of *Fusarium oxysporum* f.sp *lycopersici* and *F. solani*

	Colony propagule	Color	Shape	Septation
Fusarium oxysporum f.sp lycopersici	Colony	Initially white, salmon to purple	Floccose,fluffy	
	Mycelium	White to purple	Discrete sporodochia	septate
	Microconidia	Hyaline	Oval, elliptical	Aseptate
	Macroconidia	Hyaline	Fusiform,3-5celled	Septate
	Chlamydospores	Hyaline	Smooth and rough walled	
Fusarium solani	Colony	White to cream, cottony	cottony	
	Mycelium	Cream, brown	Sparse,Branched	Septate
	Microconidia	Cream	Oval,ellipsoid 1-2celled	Septate
	Macroconidia	Cream	Fusiform	Septate
	Chlamydospores		Globose to oval, smooth walled	

Table 2: Efficacy of plant extracts (*in vitro*) at different concentrations on mycelial growth inhibition of pathogenic fungi of tomato

Plant extracts	Inhibition (%)											
	Fusarium oxysporum f. sp *lycopersici*						*Fusarium solani*					
	20%	40%	60%	80%	Corr value(r)	Sig value	20%	40%	60%	80%	Corr value(r)	Sig value
Allium sativum	90.31 d	92.91 c	96.38 b	100.00 a	0.9973*	0.0027	91.11 d	93.05 c	95.41 b	100.00 a	0.9781*	0.0219
Zingiber officinale	30.33 j	33.94 i	39.87 gh	44.49 f	0.9961*	0.0039	37.77 h	43.05 g	45.55 f	48.47 e	0.9845*	0.0155
Curcuma longa	32.93 i	38.57 h	41.02 g	47.67 e	0.9866*	0.0134	2.91 k	5.27 j	7.77 i	8.61 i	0.9811*	0.0189
Topsin-M	100.00 a						98.75 a					
Control negative	0.00 k						0.00 l					
$LSD_{0.05}$	1.7204						1.9296					

Means sharing different letters are statistically significant at p=0.05

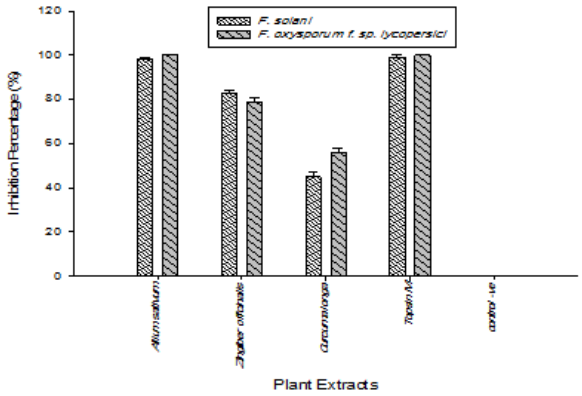

Fig. 1: *In vitro* screening for spore inhibition (mean ± SD) of *F. oxysporum* f. sp *lycopersici* and *F. solani* by crude extracts of plant rhizomes and a fungicide (Topsin-M)

equivalent using the standard curve (Fig. 2). Quantitative analysis of crude extracts of plants revealed that bulbs of *A. sativum* had the highest contents of phenols (54.24GAE mg/g) followed by *Z. officinale* (29.80 GAE mg/g), whereas, rhizomes of *C. longa* contained 25.45 GAE mg/g phenol contents (Table 4).

Discussion

The possibility of controlling tomato fungal diseases with plant products has an unusual significance in the context of environmental pollution, toxicity of the produce and development of resistance by plant pathogens. Preliminary assessment of the antifungal potential of different plant extracts, under controlled conditions established significant antimicrobial activity on fungal diseases of tomato plants. Consequently, this is in line with the earlier reports on the efficacy of these plant extracts on the phytopathogens of other crops (Ijato *et al*., 2010; Okigbo and Nmeka, 2005). The present study revealed that *A. sativum* extract was more effective than *Z. officinale* and *C. longa* in suppressing the growth and sporulation of *F. oxysporum* f. sp *lycopersici* and *F. solani.* These findings also corresponded with previous reports (Aba Alkhail, 2005; Shrestha and Tiwari, 2009; Taskeen-Un-Nisa *et al*., 2011) on the *in vitro* antifungal effect of *A. sativum*. The authors found *A. sativum* to be most effective at highest concentration in reducing the spore germination of *F. oxysporum* the cause of tomato wilt and *F. solani*. Similarly, Muhsin *et al*. (2000) observed reduction in mycelial growth of Fusarium spp. and Rhizoctonia spp. whereas Agbenin and Marley (2006) reported reduction in *F. oxysporum* f sp. *lycopersici* by garlic extract. Conversely, Singha *et al*. (2011) observed that *F. oxysporum* f. sp *lycopersici* population was reduced more efficiently by amending the tomato soil with piper betle extract as compared to Carbendazim alone and in mixture with piper betle extract.

The inhibitory magnitude of plant extracts is dose/concentration dependent. Use of high concentration of plant extracts for the control of pathogenic fungi has been documented by Bianchi *et al.* (1997), Udo *et al.* (2001) and Chiejina and Ukeh (2012). This is in an agreement with our findings that *A. sativum* exhibited significantly higher results as the concentration increased. This inhibitive action of *A. sativum* bulb extract has been attributed to a component-allicin having strong atimicrobial activity against plant

Table 3: Phytochemical analysis of crude extracts of some plants

Plant sample	Flavonoid	Terpenoid	Saponin	Steroids	Tannins	Phlobatanins	Cardiac glycoside	Caumarins
Allium sativum L.	+	+	+	+	+	-	+	+
Curcuma longa L.	+	+	+	-	-	-	-	-
Zingiber officinale Rosc.	+	+	+	-	+	-	-	-

Presence = (+) absence = (-). Each datum is the average of two independent determinations

Table 4: Total polyphenol contents of plant extracts

Sample	Phenol Contents (GAE mg/g)*
Allium sativum L.	54.25 ± 0.15
Zingiber officinale Rosc	29.80 ± 0.45
Curcuma longa L.	25.45 ± 0.48

*values are expressed as means ± standard deviation (n=3)

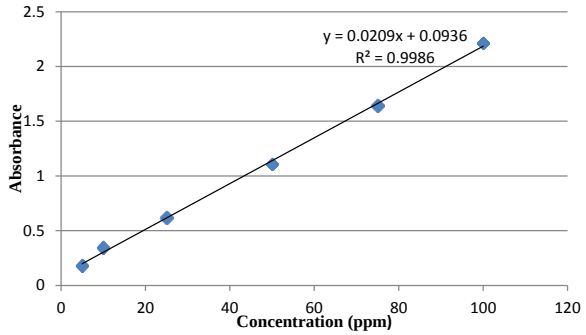

Fig. 2: Standard Curve of Gallic Acid

diseases (Slusarenko *et al*., 2008; Ameh *et al.*, 2013).

Zingiber officinale rhizome extract comes next to garlic extract though *C. longa* extract showed least efficacy against the test fungi in our findings. In contrast, several workers found *Z. officinale* extract with highest inhibition effect on *F. solani* and *F. oxysporum* f sp. *lycopersici* growth in diseased potato tubers (Anukwuorji *et al*., 2013) and in tomato (Abdel-Kader *et al*., 2012). Furthermore, according to Lakshmi (2012) *C. longa* depicted the least inhibition against *F. oxysporum.* Similar effects of plant extracts were observed in present findings. Besides inhibiting the mycelial growth plant extracts also inhibited spore germination of *F. oxysporum* f. sp. *lycopersici* and *F. solani*. This is in agreement with the work of Agbenin and Marley (2006). The inhibition rate to spore germination and mycelial growth of *Fulvia fulva* in tomato reached almost 100% when garlic extract was used at 40 mg ml^{-1} and 80 mg ml^{-1} concentrations (Ting-Ting *et al*., 2011). On the other hand, Imtiaj *et al.* (2005) recorded highest conidial germination inhibition of *Colletotrichum gleosporioides* on mango with *C. longa* and *Z. officinale* while lowest inhibition was recorded with *A. sativum.*

For instance, higher plants are large reservoirs of antifungal compounds, being biodegradable, are considered valuable in disease resistance (Okigbo and Ajalie, 2005; Siva *et al*., 2008). Therefore, they have been implicated in pathogenesis of many plant diseases. Flavonoids, isoflavonoids, glycosides, tanins, coumarins, terpenes, alkaloids and phenolic compounds are secondary metabolites synthesized by plants (Simões *et al*., 1999).

Results indicated the presence of flavonoids, terpenes, saponins, steroids, tannins, glycosides and coumarins in the ranking order of *A. sativum* > *Z. officinale* > *C. longa*. Our results are in an analogy, at least in part, with some of the previous reports. Qusti *et al*. (2010) observed highest antioxidant activity of *Z. officinale* but garlic showed moderate activity. According to Ameh *et al*. (2013) methanolic extract of garlic bulbs revealed higher concentrations of chemical compounds such as glycosides, carbohydrates and proteins while steroids, oils, reducing sugars and flavonoids in medium to lower quantities. However, tannins, terpenoids and resins are absent. Steroids, glycosides, terpenoids and flavonoids were found in *C. longa* while tannins were absent (Chhetri *et al*., 2008). Anukwuorji *et al*. (2013) revealed that in *Z. officinale* saponins were abundantly present while tannins, glycosides and flavonoids were moderately present and steroids were not detected. This also supports our findings.

Difference in antioxidant activity may be due to factors such as sample preparation and analytical procedures (Al-Farsi *et al*., 2007). It is well known that polyphenols are present in plant kingdom in plenty of amount, even in surprisingly high quantity (Harborne, 1993) affecting the germination and growth of many fungal pathogens (Amadi *et al*., 2010).

Previous findings indicated that polyphenols are good source of antioxidants that are naturally present in plants (Amiot *et al*., 1997) and this antioxidant activity of phenolics is mainly due to their redox properties (Atoui *et al*., 2005). Consequently, higher quantity of phenolic compounds was obtained from *A. Sativum* in our study. This is consistent with reports of several workers (Benkeblia, 2005; Tepe *et al*., 2005; Leelarungrayub *et al*., 2006). Conversely, Temitope *et al.* (2010) reported that turmeric contained high contents of total phenols as compared to ginger and garlic. Also Wangcharoen and Wallaya (2007) noticed low TPC's in garlic. Similarly, Qusti *et al.* (2010) determined high contents (812.67 mg GAE/g) of phenols in ginger but moderate (367.60 mg GAE/g) in garlic.

Conclusion

Crude extracts of plant rhizomes consistently showed significant *in vitro* antifungal activity against plant pathogens. As the chemical fungicides are cost effective and out of reach of the small land holders. Also the consumers are quite doubtful on the production of synthetic chemicals and there has always been a good acceptance when it comes to natural antioxidants. This made a rationale for exploitation of plant products in disease management. It is envisaged that application of plant extracts as a natural fungicides to treat different pathogens will be useful for small-scale farmers that are not able to afford costly fungicides.

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References

Aba Alkhail, A.A., 2005. Antifungal activity of some extracts against some plant pathogenic fungi. *Pak. J. Biol. Sci*., 8: 413–417

Abdel-Kader, M.M., N.S. El-Mougy, N.G. El-Gammal, F. Abd-El-Kareem and M.A. Abd-Alla, 2012. laboratory evaluation of some chemicals affecting pathogenic fungal growth. *J. App. Sci. Res*., 8: 523–530

Aidah, N., N. Abdullah, E. Oskoueian, C.C. Sieo and W.Z. Saad, 2014. Membrane-active antibacterial compounds in methanolic extracts of *Jatropha curcas* and their mode of action against *Staphylococcus aureus* S1434 and *Escherichia coli* E216. *Int. J. Agric. Biol.*, 16: 723–730

Agrios, G.N., 2005. *Plant Pathology*, 5th edition. Acad. Press, USA

Agbenin, O.N. and P.S. Marley, 2006. *In-Vitro* assay of some plant extracts against *Fusarium oxysporum f. sp lycopersici*, causal agent of Tomato Wilt. *J. Plant. Prot. Res*., 46: 215–220

Al-Farsi, M., C. Alasalvar, M. Al-Abid, K. Al-Shoaily, M. Al-Amry and F. Al-Rawahy, 2007. Compositional and functional characteristics of dates, syrups, and their by-products. *Food Chem*., 104: 943–947

Amadi, J.E., S.O. Salami and C.S. Eze, 2010. Antifungal properties and phytochemical screening of extracts of African Basil (*Ocimum gratissimum* L.). *Agric. Biol. J. North Amer*., 1: *ISSN Print: 2151-7517, Online 2151–7525*

Ameh, G.I., S.C. Eze and F.U. Omeje, 2013. Phytochemical screening and antimicrobial studies on the methanolic bulb extract of *Allium sativum* L. *Afr. J. Biotechnol*., 12: 1665–1668

Amiot, M.J., A. Fleuriet, V. Cheynier and J. Nicolas, 1997. *Phytochemistry of Fruits and Vegetables*. F. A. Tomas-Barberan and R. J. Robins (Eds.), Ch. 4. Clarendon Press, Oxford

Anukwuorji, C.A., C.L. Anuagasi and R.N. Okigbo, 2013. Occurrence and control of fungal pathogens of potato (*Ipomoea batatas* L. Lam) with plant extracts. *Ph Tech Med*., 2: 278–289

Atoui, A.K., A. Mansouri, G. Boskou and P. Kefalas, 2005. Tea and herbal infusions: Their antioxidant activity and phenolic profile. *Food Chem*., 89: 27–36

Bianchi, A., A. Zambonelli, A.Z. D'Aulerio and F. Bellesia, 1997. Ultrastructural studies of the effects of *Allium sativum* on phytopathogenic fungi *in vitro*. *Plant Dis.*, 81: 1241–1246

Balbi-Peña, M.I.B., A. Becker, J.R. Stangarlin, G. Franzener, M.C. Lopes and K.R.F. Schwan-Estrada, 2006. Control of *Alternaria solani* in tomato by *Curcuma longa* and curcumin- I. *In vitro* evaluation. *Fitopat. Brasil*., 31: 310–314

Barnett, H.L. and B.B. Hunter, 1972. I*llustrated Genera of Imperfect Fungi*, 4th ediyion. Burgess Publication Ltd. St.Paul, Minnesota, USA, 1–216

Benkeblia, N., 2005. Free-radical scavenging capacity and antioxidant properties of some selected onions (*Allium cepa* L.) and garlic (*Allium sativum* L.) extracts. *Braz. Arch. Biol. Technol*., 48: 753–759

Brown, W., 1924. Two mycelial methods. II. A method of isolation single strains on fungi by cutting out a hyphal tip. *Ann. Bot*., 38: 402–404

Carrillo-Fasio, J.A., T.J. Montoya-Rodriguez, R.S. Garciaestrada, J.E. Cruz-Ortega, I. Marquezzequera and A.J. Sanudo-Barajas, 2003. Razas de *Fusarium oxysporum* f. sp. *lycopersici* Snyder y Hansen, en tomate (*Lycopersicum esculentum* Mill.) en el Valle de Culiacán, Sinaloa, México. *Revista Mexicana de Fitopatología*., 21: 123–127

Chattopadhyay, I., K. Biswas, U. Bandyo Padhyay and R.K. Banerjee, 2004. Turmeric and curcumin: Biological actions and medicinal applications. *Curr. Sci*., 87: 44–53

Chandarana, H., S. Baluja and S.V. Chanda, 2005. Comparison of antibacterial activities of selected species of Zingiberaceae family and some synthetic compounds. *Turk. J. Biol*., 29: 83–97

Chhetri, H.P., N.S. Yogol, J. Sherchan, K.C. Anupa, S. Mansoor and P. Thapa, 2008. Phytochemical and antimicrobial evaluation of some medicinal plants of Nepal. *Kathmandu Uni. J. Sci. Eng. Technol*., 1: 49–54

Chiejina, N.V. and J.A. Ukeh, 2012. Antimicrobial properties and phytochemical analysis of methanolic extracts of *Aframomum melegueta* and *Zingiber officinale* on fungal diseases of tomato fruit. *J. Nat. Sci. Res*., 2: 10–15

Dahanukar, S.A., R.A. Kulkarni and N.N. Rege, 2000. Pharmacology of medicinal plants and natural products. *Ind. J. Pharmacol*., 2: S81–S118

Ellis, M.B., 1971. *Dematiaceous Hyphomycetes*. Commonwealth Mycological Institute, Kew, Surrey, England

Fawzi, E.M., A.A. Khalil and A.F. Afifi, 2009. Antifungal effect of some plant extracts on *Alternaria alternata* and *Fusarium oxysporum*. *Afr. J. Biotechnol*., 8: 2590–2597

Harborne, J.B., 1993. *New Naturally Occurring Plant Polyphenols*. In Polyphenolic phenomena. Paris: Scalbert, INRA

Harborne, J.B., 1998. *Phytochemical Methods*. A guide to modern techniques of plant analysis, 3rd edition. Chapman and hall, London. UK

Ijato, J.Y., S.D. Oyeyemi, J.A. Ijadunola and J.A. Ademuyiwa, 2010. Allelopathic effect of leaf extract of *Azardirachta indica* and *Chromolaena odorata* against post harvest and transit rot of tomato (*Lycopersicum esculentum* L). *J. Amer. Sci*., 6: 1595–1599

Imtiaj, A., S.A. Rahman, Shahidul Alam, R. Parvin, K.M. Farhana, S. Kim and T. Lee, 2005. Effect of Fungicides and Plant Extracts on the Conidial Germination of *Colletotrichum gloeosporioides* Causing Mango Anthracnose. *Mycobiology*., 33: 200–205

Jamil, A.M.S., M.M. Khan and M. Ashraf, 2007. Screening of some medicinal plants for isolation of antifungal proteins and peptides. *Pak. J. Bot*., 39: 211–21

Kishore, G.K., S. Pande and J.N. Rao, 2001. Control of Late Leaf Spot of Groundnut (*Arachis hypogaea*) by Extracts fromNon-Host Plant Species. *Plant. Pathol. J*., 17: 264–270

Kumar, A.S., N.P.E. Reddy, K.H. Reddy and M.C. Devi, 2007. Evaluation of Fungicidal Resistance Among *Colletotrichum gloeosporioides* isolates Causing Mango Anthracnose in Agric. Export Zone of Andhra Pradesh, India. *Plant Path. Bull*., 16: 157–160

Lakshmi, B.S., 2012. Bio Control of Cereal Crop Pathogens by *Curcuma longa* L. Rhizomes. *J. Biotechnol. Biotherapeutics*., 2: 8–12

Lee, H.S., K.J. Choi, K.Y. Cho and Y.J. Ahn, 2003. Fungicidal activity of ar-turmerone identified in curcuma longa rhizome against six phytopathogenic fungi. *Agric. Chem. Biotechnol*., 46: 25–28

Leelarungrayub, N., V. Rattanapanone, N. Chanarat and J.M. Gebicki, 2006. Quantitative evaluation of the antioxidant properties of garlic and shallot preparations. *Nutrition*, 22: 266–274

Malkhan, S.G., A. Shahid, A. Masood and S.S. Kangabam, 2012. Efficacy of plant extracts in plant disease management. *J. Earth. Envir. Sci*., 3: 425–433

Muhsin, T.M., S.R. Al-Zubaidy an T.A. Eman, 2000. Effect of garlic bulb extract on the growth and enzymatic activities of rhizosphere and rhizoplane fungi. *Mycopathologia*, 152: 143–146

Nair, K.R.S. and A.H. Ellingboe, 1962. Method of controlled inoculations with conidiospores of *Erysiphe graminis* var. *tritici*. *Phytopathology*, 52: 714

Nashwa, S.M.A. and K.A.M. Abo-Elyousr, 2012. Evaluation of various plant extracts against the early blight disease of tomato plants under greenhouse and field conditions. *Plant Prot. Sci*., 48: 74–79

Okigbo, R.N. and A.N. Ajalie, 2005. Inhibition of some human pathogens with tropical plants extracts of *Chromoleana odorata* and *Citrus aurantifolia* and some antibiotics. *Int. J. Mol .Adv. Sci. Pak*., 1: 34–40

Okigbo, R.N. and I.A. Nmeka, 2005. Control of yam tuber rot with leaf extracts of *Xylopia aethiopia* and *Zingiber officinale*. *Afr. J. Biotechnol*., 4: 804–807

Okigbo, R.N., R.E. Okorie and R.R. Putheti, 2009. *In vitro* effects of garlic (*Allium sativum* L.) and african basil (*Ocimum gratissimum* L.) on pathogens isolated from rotted cassava roots. *Interciencia*, 34: 742–747

Qusti, S.Y., A.N. Abo-khatwa and M.A. Bin Lahwa, 2010. Screening of antioxidant activity and phenolic content of selected food items cited in the holly quran. *Eur. J. Biol. Sci*., 2: 40–51

Riaz, T., S.N. Khan and A. Javaid, 2010. Management of corm-rot disease of gladiolus by plant extracts. *Nat. Prod. Res*., 24: 1131–1138

Saltveit, M.E., 2003. Mature fruit vegetables. *In*: *Postharvest Physiology and Pathology of Vegetables*, pp: 707–732. J.A. Bartc and J.K. Brecht (Eds). Marcel Dekker, New York, USA

Sateesh, K., T. Marimuthu, B. Thayumanavan, R. Nandakumar and R. Samiyappan, 2004. Antimicrobial activity and induction of systemic resistance in rice by leaf extract of Datura metel against *Rhizoctonia solani* and *Xanthomonas oryzae* pv. *oryzae*. *Physiol. Mol. Plant Pathol*., 65: 91–100

Simões, C.M.O., E.P. Schenckel, G. Gosman, J.C.P. Mello, L.A. Mentz and P.R. Perovick, 1999. Farmacognosia: da planta ao medicamento, pp: 821. Santa Catarina, Porto Alegre, Florianópolis: ed. da UFRGS; ed. da UFSC

Singha, I.M., Y. Kakoty, B.G. Unni, M.C. Kalita, J. Das, A. Naglot, S.B. Wann and L. Singh, 2011. Control of Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* using leaf extract of *Piper betle* L.: A preliminary study. *World J. Microbiol. Biotechnol*., 27: 2583–2589

Singleton, V.L. and J.A. Rossi, 1965. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *Amer. J. Enol. Vitic*., 16: 144–158

Siva, N., S. Ganesan, N. Banumathy and Muthuchelian, 2008. Antifungal effect of leaf extracts of some medicinal plants against *Fusarium oxysporum* causing wilt disease of *Solanum melogena* L. *Ethnobot. Leaflets*., 12: 156–163

Shrestha, A.K. and R.D. Tiwari, 2009. Antifungal activity of crude extracts of some medicinal plants against *Fusarium solani* (Mart.) Sacc. *Ecoprint. An Int. J. Ecology*., 16: 75–78

Slusarenko, A.J., A. Patel and D. Portz, 2008. Control of plant diseases by natural products: Allicin from garlic as a case study. *Eur. J. Plant Pathol*., 121: 313–322

Steel, R.G.D., J.H. Torrie and D.A. Dickey, 1997. *Principles and Procedures of Statistics*. A Biometrical approach. 3rd edition., McGraw Hill Book Co., New York, USA

Taskeen-Un-Nisa, A.H. Wani and R.A. Mir, 2011. Antimycotic activity of plant extracts on the spore germination of somepathogenic fungi. *Mycopath*., 8: 65–69

Trease, G.E. and W.C. Evans, 2009. *Pharmacognosy*. 16th edition, Bailliere Tindall Ltd. London. Elsevier Publishers. 1–571

Temitope, A.O., A.G. Olufemi and F.T. Alaba, 2010. Effect of heat treatment on antioxidant activity of some spices. *Continental J. Food Sci. Technol*., 4: 53–59

Tepe, B., M. Sokmen, H.A. Akpulat and A. Sokmen, 2005. *In vitro* antioxidant activities of the methanol extracts of five Allium species from Turkey. *Food Chem*., 92: 89–92

Ting-Ting, W., C. Zhi-Hui, M.A. Khan, N. Qing and H. Ling, 2011. The inhibitive effects of garlic bulb crude extract on *fulvia fulva* of tomato. *Pak. J. Bot*., 43: 2575–2580

Udo, S.E., B.E. Madunagu and C.D. Isemin, 2001. Inhibition of growth and sporulation of fungal pathogens on sweet potato and yam by garlic extract. *Nigerian J. Bot*., 14: 35–39

Vincent, J.M., 1947. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*, 150: 850

Wangcharoen, W. and W. Morasuk, 2007. Antioxidant capacity and phenolic content of some Thai culinary plants. *Maejo. Int. J. Sci. Tech*., 1: 100–106

Zia-Ul-Haq, M., A. Mansoor, J.M. Noor, A. Shakeel, Q. Mughal and M.K. Inamullah, 2011. Antimicrobial screening of selected flora of Pakistan. *Arch. Biol. Sci*., 63: 691–695

Zia-Ul-Haq, M., M.R. Shah, M. Qayum and S. Ercisli, 2012. Biological screening of selected flora of Pakistan. *Biol. Res*., 45: 375–379

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