



Full Length Article

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. © 2015 Friends Science Publishers

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 ineffective. 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

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 Nmeko, 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 μ L) from 80% concentration of each extract was mixed with a drop (10 μ L) 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 $25 \pm 2^\circ\text{C}$ 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 $\times 100$ (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 40°C 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 83 and 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

Table 1: Morphological and cultural characteristics of *Fusarium oxysporum* f.sp *lycopersici* and *F. solani*

| | Colony propagule | Color | Shape | Septation |
|---|------------------|-----------------------------------|--------------------------------|-----------|
| <i>Fusarium oxysporum</i> f.sp <i>lycopersici</i> | 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 |
| | Chlamydo spores | Hyaline | Smooth and rough walled | |
| <i>Fusarium solani</i> | Colony | White to cream, cottony | cottony | |
| | Mycelium | Cream, brown | Sparse,Branched | Septate |
| | Microconidia | Cream | Oval,ellipsoid 1-2celled | Septate |
| | Macroconidia | Cream | Fusiform | Septate |
| | Chlamydo spores | | 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 (%) | | | | | | | | | | | |
|----------------------------|--|---------|----------|----------|---------------|-----------|------------------------|---------|---------|----------|---------------|-----------|
| | <i>Fusarium oxysporum</i> f. sp <i>lycopersici</i> | | | | | | <i>Fusarium solani</i> | | | | | |
| | 20% | 40% | 60% | 80% | Corr value(r) | Sig value | 20% | 40% | 60% | 80% | Corr value(r) | Sig value |
| <i>Allium sativum</i> | 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 |
| <i>Zingiber officinale</i> | 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 |
| <i>Curcuma longa</i> | 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

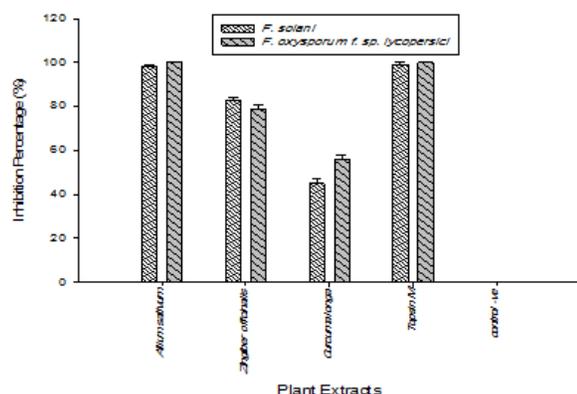


Fig. 1: *In vitro* screening for spore inhibition (mean ± SD) of *F. oxysporum* f. sp *lycopersici* and *F. solani* by crude extracts of plant rhizomes and a fungicide (Topsin-M)

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 Nmeke, 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 Alkhalil, 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 beetle extract as compared to Carbendazim alone and in mixture with piper beetle 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- allacin having strong antimicrobial activity against plant

Table 3: Phytochemical analysis of crude extracts of some plants

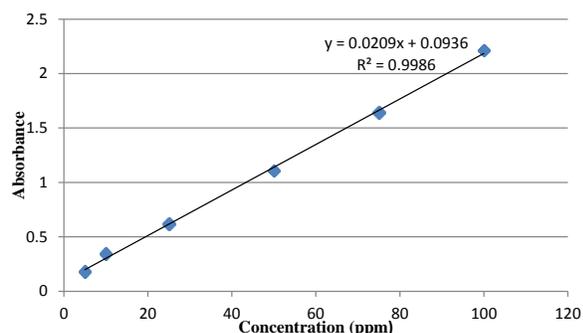
| Plant sample | Flavonoid | Terpenoid | Saponin | Steroids | Tannins | Phlobatanins | Cardiac glycoside | Caumarins |
|----------------------------------|-----------|-----------|---------|----------|---------|--------------|-------------------|-----------|
| <i>Allium sativum</i> L. | + | + | + | + | + | - | + | + |
| <i>Curcuma longa</i> L. | + | + | + | - | - | - | - | - |
| <i>Zingiber officinale</i> 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)* |
|----------------------------------|-----------------------------|
| <i>Allium sativum</i> L. | 54.25 ± 0.15 |
| <i>Zingiber officinale</i> Rosc. | 29.80 ± 0.45 |
| <i>Curcuma longa</i> 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, Intiaj *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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