JOURNAL OF PURE AND APPLIED MICROBIOLOGY, September 2013. Vol. **7**(3), p. 1925-1932

Bioactivity of Garlic Bulb Extract Compared with Fungicidal Treatment against Tomato Phytopathogenic Fungi

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(Received: 21 March 2013; accepted: 25 May 2013)

Antifungal activity of methanolic garlic extract (*Allium sativum*) was investigated to find a natural alternative to synthetic fungicides currently used in the control of tomato phytopathogenic fungi (*Fusarium oxysporum*, *Pythium aphanidermatum* and *Rhizoctonia solani*) the causative agents of damping-off diseases. *A. sativum* extract was strongly active and showed fungicidal and fungistatic activities against *P. aphnidermatum* and *R. solani* with MIC of 4 mg/ml and MFC of 8 mg/ml while *F. oxysporum* was less sensitive and its MFC reached to 16 mg/ml. Carbendazim fungicide was more effective than methanolic garlic extract inhibiting mycelial growth of all phytopathogenic fungi at 8 ppm and a huge concentration reached to 8 mg/ml was required to attain the same effect. Chemical analysis of *A. sativum* extract by GC/MS revealed that garlic extract was mainly composed of organosulfur compounds especially diallyl disulfide (allicin), diallyl trisulfide and ajoene which inhibit different microbial enzymes essential for fungal growth. The present study has demonstrated that methanolic garlic extract was found to be effective in controlling tomato damping-off diseases and may be an attractive natural alternative to chemical fungicides used to control tomato damping-off diseases.

Key words: *Alllium sativum*, antifungal activity, Carbendazim, GC-MS analysis.

Tomatoes are considered an important tropical crop in the basis of its high consumption, nutritional and economic value to farmers (Clinton, 1998 and Ejechi *et al*., 1999). The main diseases in tomatoes responsible for reduction of yield and quality are seedling damping-off caused by *Pythium aphanidermatum* and *Rhizoctonia solani* and Fusarium wilt caused by *Fusarium oxysporum* (Schwarz & Grosch, 2003 and Song *et al*., 2004). These diseases can be controlled by application of chemical fungicides, crop rotation and the use of pathogen-resistant varieties (Manoranjitham *et al*., 2001 and Soylu *et al*., 2010). Despite the proven efficiency of chemical fungicides in the prevention and spread of fungal diseases, their repeated application has resulted in the accumulation of residual toxicity in tomatoes, environmental pollution, altered the biological balance in the soil by decimating beneficial microorganisms and widespread development of resistance to various types of fungicides (Georgopoulos, 1987; Staub, 1991; Pandy, 2003 and Bharathi *et al*., 2004). Recently, efforts have been focused on developing potentially effective, environmentally safer biocontrol agents. Within this context, is the utilization of plant extracts as biological control of tomato bacterial and fungal pathogens. These plant extracts considered as natural sources of antimicrobial substances, regarded as

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environmentally safe, degraded by soil microbes and they don't pose any health residual or environmental problems at any used concentration (Isman, 2000, Hsieh *et al*., 2001, Satya *et al*., 2005, Balestra *et al*., 2009 and Al-Rahmah *et al*., 2011). Garlic (*Allium sativum*) extract has been proposed to control plant diseases caused by bacterial and fungal pathogens and there are many examples of pathogens effectively controlled by the application of such garlic extracts: *Aspergillus niger* and *Fusarium pallidorosum* (Arya *et al*., 1995), three *Aspergillus* species (Yin and Tsao, 1997), *Pythium ultimum*, *Rhizoctonia solani* and *Fusarium solani* (Biachi *et al*., 1997), *Sclerotium capivorum* (Ceron *et al*., 1999), *Phytophthora infestans* (Cao and Van Bruggn, 2001), potato bacterial late blight (Cao and Forrer, 2001), citrus green and blue molds (Obagwu and Korsten, 2003), *A. niger*, *Alternaria alternata* and *Botryosphaera obtuse* (Bisht and Kamal, 2004), *A. niger*, *Penicillium cyclopium* and *F. oxysporum* (Benkeblia, 2004), tomato bacterial speck (Curtis *et al*., 2004 and Balestra *et al*., 2009), *Pythium aphanidermatum* (Manoranjitham *et al*., 2001, Narayana Bhat and Shukla, 2001, Ramanathan *et al*., 2004 and Muthukumar *et al*., 2010) and *Botryrtis cinera* the causal agent of tomato grey mould disease (Soylu *et al*., 2010). Bioactivity of garlic extract against phytopathogenic fungi reduced plant diseases and effectively inhibited spore germination of the pathogenic fungi (Abou-Jawdah, 2002). The antifungal activity of garlic extract suppress plant diseases could be attributed to fungitoxic sulfur compounds especially allicin (Rabinkov *et al*., 1998) and ajoene resulting from conjugation of three allicin molecules (Ankri and Mirelman, 1999). Allicin and ajoene inhibit different microbial enzymes essential for fungal growth (Fujisawa *et al*., 2008). However, far less attention has been paid to the application of garlic extracts as biocontrol agents against tomato phytopathogenic fungi (Latha *et al*., 2009). The objectives of the present study are planned to evaluate antimicrobial activity of garlic extract against the three phytopathogenic fungi (*Pythium aphanidermatum*, *Rhizoctonia solani* and *Fusarium oxysporum*) in vitro and to compare efficacy of garlic extract with that of reference fungicide (Carbendazim) in controlling phytopathogenic fungi.

MATERIALS AND METHODS

Preparation of garlic extract

Methanolic garlic (*A. sativum*) extract was prepared by soaking 50 gm of freshly crushed garlic bulb in methanol (10 ml of methanol /gm of garlic) with stirring for 48 hrs then filtered through double layers of muslin, centrifuged at 9000 rpm/min for 10 minutes and finally filtered again through Whatman filter paper No. (41) to remove garlic debris and obtain a clear filtrate. The filtrate was transferred into sterile 50 ml flask, evaporated and concentrated under reduced pressure and temperature below 40°C. The average yield was approximately 14.6 gm of sticky brownish extract from 50 gm of garlic fresh weight. The sticky garlic extract was sealed and preserved in refrigerator at 4°C till used.

Fungal cultures

Fungal cultures of *Pythium aphanidermatum*, *Fusarium oxysporum* and *Rhizoctonia solani* were isolated from tomato crop by hyphae point and monsporic technique (Anguiz, 1989) and preserved in slants containing potato dextrose agar (PDA) till used. The tomato phytopathogenic fungi were provided from the culture collection of Botany and Microbiology Department, King Saudi University, Riyadh, K.S.A

Antifungal activity of garlic extract

Antifungal activity was evaluated on tomato phytopathogenic fungi using the food poisoning technique (Kumar *et al*., 2008). Sticky garlic extract was re-dissolved in (5ml) of methanol, sterilized in disposable Millipore filter (0.22 µm pores) and mixed with sterile potato dextrose agar medium (PDA) to obtain the final concentration of 5 mg ml^{-1} of garlic extract then poured in sterile Petri dishes (90 mm diameter). For control, five ml of Millipore-sterilized methanol was added to (PDA) medium and discs of 7 mm diameter of phytopathogenic fungi were cut from the periphery of 6 days old cultures and inoculated aseptically to the center of poured Petri dishes of treatment and control sets and incubated at 25°C ± 2°C for 7 days.

Fungal colony diameter of treatments and control sets were measured and percentage of mycelial inhibition was calculated using the following formula.

Percentage of mycelial inhibition = [C – T / C] x 100 (where, C and T are the growth diameter (mm) in control and treatment respectively).

Fungicidal analysis of garlic extract compared with reference fungicide (Carbendazim)

Garlic (*A. sativum*) extract was manipulated to determine its minimal inhibitory concentration (MIC) and minimal fungicidal concentrations (MFC) to evaluate its efficiency in controlling tomato phytopathogenic fungi. Different concentrations of garlic extract (0.0, 2.0, 4.0, 8.0 and 16.0 mg/ml) were prepared separately by dissolving their requisite amount in 5 ml of methanol, sterilized through Millipore filter and mixed with (PDA) medium to obtain the final concentrations. To compare efficacy of garlic extract with that of fungicide (Carbendazim) in controlling the tomato phytopathogenic fungi, different concentrations (0.0, 2.0, 4.0, 8.0 and 16.0 ppm) of Carbendazim of 98% active ingredients were prepared by mixing weighted powder of fungicide with a known volume of sterile (PDA). Fungal plugs (7 mm in diameter) were obtained and placed at the center of petri dish in potato dextrose agar medium (PDA) with garlic extract of various concentrations and fungicide. The cultures were incubated at 25°C ± 2°C and radial growth of mycelia was measured after 6 days. Fungicidal effect of garlic extract was measured and MIC and MFC were determined then compared with fungicidal effect of the reference fungicide under a totally random design with four replications.

GC/GC-MS analysis of the effective plant extracts

Garlic (*A. sativum*) extract was analyzed through Gas Chromatography and Mass Spectroscopy (GC-MS) Varian model, 450 equipped with a flame ionization detector and quantization was carried out by the area normalization method neglecting response factors. The analysis was carried out using a VF-5MS capillary column (30 m x 0.25 mm; 0.25 µm film thickness). The operating conditions were as follow: injection and detector temperature, 250 and 300 °C respectively; split ratio, 1 : 50; carrier gas, Helium with flow rate (1.0 ml/min). Oven temperature program was 50 – 300 ºC at the rate of 7 ºC/min. Mass spectrometer conditions were: ionization potential, 70 eV; mass range from m/z, 40 – 400 amu; electron multiplier energy, 2000 V. The components of garlic extract were identified by comparison of their relative retention times and the mass spectra with those authentic reference compound shown in the literature (Adams, 2007) and by computer matching of their MS spectra with Wiley and Nist 8 mass spectral library.

Statistical analysis

All experiments were conducted in four replicates for each treatment and the data were reported as mean ± SE (standard error). The data were also analyzed statistically using One-way analysis of variance (ANOVA) and differences among the means were determined for significance at $P \leq 0.05$ (by SPSS, 16.1 Chicago, USA).

RESULTS

Antifungal activity of garlic methanolic extract was studied and evaluated against tomato phytopathogenic fungi (*F. oxysporum*, *P. aphanideratum* and *R. solani*). Garlic extract at 5 mg/ml was effective in suppressing the mycelial growth of all concerned tomato phytopathogenic fungi compared to non-treated control. Garlic extract was highly effective in suppressing the mycelial growth of *P. aphanidermatum* and its mycelial growth was inhibited to 77.43% while *R. solani* and *F. oxysporum* were less sensitive and

Table 1. Antifungal screening test of methanolic garlic (*A. sativum*) extract (5mg/ml) against tomato phytopathogenic fungi

Garlic extract	Mean diameter of mycelial growth (mm)			Percentage of mycelial growth inhibition		
conc. (mg/ml)	*F. o*	*P. a*	*R. s*	*F. o*	*P. a*	*R. s*
5.00	29.3* ± 0.12	18.8* ± 0.10	24.0* ± 0.08	55.94	77.43	69.35
0.00	66.5* ± 0.16	83.3* ± 0.27	78.3* ± 0.13	0.00	0.00	0.00

F. o: Fusarium oxysporum; P. a: Pythium aphanidermatum; R. s: Rhizoctonia solani
Values in the same column followed by asterisk (*) are significantly different at (P = 0.05)
Data are means (n = 4) ± standard error of four replicates

their mycelial growth were inhibited to 69.35 and 55.94% at 5 mg/ml respectively (Table 1).

The MIC and MFC of the effective garlic extract (*Allium sativum*) in comparison with carbendazim as a reference fungicide were employed by poisoned food technique to assess garlic fungicidal and fungistatic properties. As illustrated in Table, 2 Carabendazim shows a various capabilities to suppress tomato phytopathogenic fungi on solid medium. *P. aphanidermatum* was more sensitive to carbendazim and its mycelial growth was completely inhibited at 4 ppm while *R. solani* and *F. oxysporum* were less sensitive and their mycelial

Table 2. Effect of different concentrations of reference fungicide (Carbendazim) on mycelial growth of tomato phytopathogenic fungi

Carbendazim	Mean diameter of mycelial growth (mm)			Percentage of mycelial growth inhibition		
conc. (ppm)	*F. o*	*P. a*	*R. s*	*F. o*	*P. a*	*R. s*
0.00	86.8* ± 0.08	89.5* ± 0.05	84.8* ± 0.09	0.00	0.00	0.00
2.00	68.5* ± 0.13	52.8* ± 0.17	63.3* ± 0.10	21.08	41.01	25.35
4.00	37.3* ± 0.13	0.00 ± 0.00	28.5* ± 0.13	57.03	100.0	66.39
8.00	0.00 ±0.00	0.00 ± 0.00	0.00 ± 0.00	100.0	100.0	100.0
16.0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	100.0	100.0	100.0

F. o: Fusarium oxysporum; P. a: Pythium aphanidermatum; R. s: Rhizoctonia solani
Values in the same column followed by asterisk (*) are significantly different at (P = 0.05)
Data are means (n = 4) ± standard error of four replicates

Table 3. Effect of different concentrations of garlic (*A. sativum*) extract on mycelial growth of tomato phytopathogenic fungi.

Garlic extract	Mean diameter of mycelial growth (mm)			Percentage of mycelial growth inhibition		
conc. (mg/ml)	*F. o*	*P. a*	*R. s*	*F. o*	*P. a*	*R. s*
0.00	68.5* ± 0.08	84.3* ± 0.05	76.8* ± 0.09	0.00	0.00	0.00
2.00	62.8* ± 0.05	53.8* ± 0.12	46.3* ± 0.06	8.32	36.18	39.71
4.00	34.3* ± 0.07	22.3* ± 0.00	27.3* ± 0.11	49.93	73.55	64.45
8.00	4.8* ±0.00	0.00 ± 0.00	0.00 ± 0.00	92.99	100.0	100.0
16.0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	100.0	100.0	100.0

F. o: Fusarium oxysporum; P. a: Pythium aphanidermatum; R. s: Rhizoctonia solani
Values in the same column followed by asterisk (*) are significantly different at (P = 0.05)
Data are means (n = 4) ± standard error of four replicates

Table 4. Phytochemical composition and relative contents of garlic (*A. sativum*) extract

Retention time	Compound name	Molecular formula	Molecular weight	Peak area
5.42	S-[2-Aminoethyl]dl-cysteine	$C_5H_{12}N_2O_2S$	164	2.432
8.48	1,3 Dithiane	$C_4H_8S_2$	120	7.463
10.55	Dimethyl trisulfide	$C_2H_6S_3$	126	4.829
13.56	Diallyl disulfide	$C_6H_{10}S_2$	146	22.186
13.97	±-D- Glucopyranoside	$C_{13}H_{26}BNO_6Si$	331	1.039
14.15	Diallyl thiosulfinate	$C_6H_{10}OS_2$	162	5.541
14.94	Ajoene	$C_9H_{14}OS_3$	234	9.320
15.22	Allyl methyl trisulfide	$C_4H_8S_3$	152	13.456
19.33	Diallyl trisulfide	$C_6H_{10}S_3$	178	22.262
22.24	Diallyl sulfide	$C_6H_{10}S$	114	11.472

growth were inhibited to 66.39 and 57.03% respectively at the same concentration.

The concentration effect of carbendazim is presented in Fig.1, where the inhibitory effect started at 2 ppm and increased in proportion to carbendazim concentrations reached to maximum in final concentration of 8 ppm.

Carbendazim was strongly effective against *P. aphanidermatum* with MIC of 2 ppm and MFC of 4 ppm while it showed fungistatic activity against *F. oxysporum* and *R. solani* with MIC 4 ppm and MFC of 8 ppm. On the other hand, *A. sativum* extract showed antifungal activities against the tomato phytopathogenic fungi and the inhibitory effect was increased in proportion to garlic concentrations, reached to maximum at 8 mg/ml except *F. oxysporum* which was inhibited to 92.99% at the same concentration and completely inhibited at 16 mg/ml. These inhibitions were reported to be significant for the effective garlic extract at the level of 0.05 (ANOVA)

A. sativum extract was also strongly active and showed fungicidal and fungistatic activities against the tested *P. aphanidermatum* and *R. solani* with MIC of 4 mg/ml and MFC of 8 mg/ml while *F. oxysporum* was less sensitive and its minimal fungicidal concentration reached to 16 mg/ml (Table, 3). The concentration effect of the effective garlic extract (*A. sativum*) on mycelial growth is shown by plotting their logarithm concentration against mycelial growth of the phytopathogenic fungi (Fig.2). Growth inhibitions of phytopathogenic fungi were observed and increased with concentration reached to maximum at 8 mg/ml except for *F. oxysporum* which was completely inhibited at 16 mg/ml of the extract.

The methanolic garlic extract was chemically analyzed by GC-MS to determine and

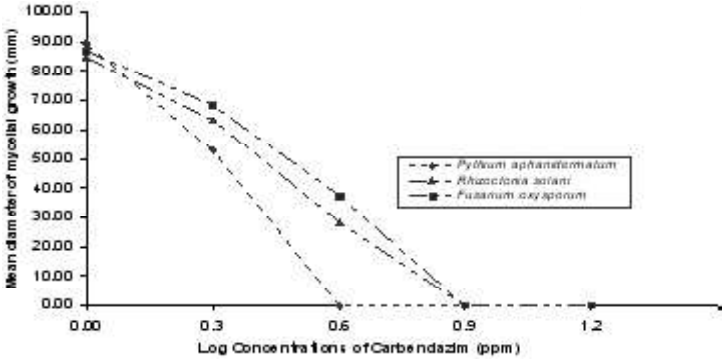

Fig. 1. Effect of different concentrations of Carbendazim on colony radial growth of the tomato phytopahtogenic fungi

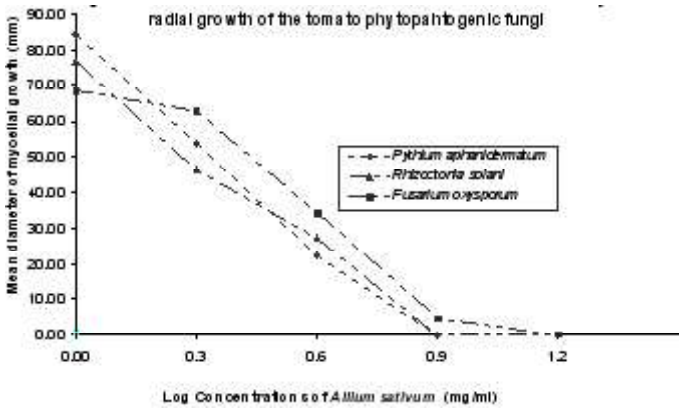

Fig. 2. Effect of different concentrations of *Allium sativum* on colony radial growth of the tomato phytopahtogenic fungi

identify the chemical constituents of the extract. The results revealed that 10 compounds were present in garlic extract. The compound name, chemical formula, molecular weight, retention time and peak area percentage of garlic constituents were given in table (4). The main components of garlic extract are sulfur compounds especially diallyl disulfide (DADS), diallyl trisulfide (DATS), allyl methyl trisulfide (AMTS), diallyl sulfide (DAS) and Ajoene which were transformed from allicin (DADS) responsible for growth inhibition of tomato phytopathogenic fungi.

DISCUSSION

The management of tomato seedlings damping-off diseases relies on the use of chemical fungicides. As there is a strong debate about the safety aspects of fungicides in use since they are considered responsible for many carcinogenic and tertogenic attributes as well as residual toxicity. Popular trend towards environment friendly organic production methods in agriculture is increased and the necessity of finding acceptable "natural" effective alternatives to "artificial" chemical fungicides against fungal plant pathogens is of great interest. Within this context, plant extracts as *A. sativum* extract can be used to control tomato damping-off diseases. Methanolic garlic extract was screened *in vitro* at 5 mg/ml to evaluate its efficiency in controlling tomato phytopathogenic fungi (*Pythium aphanidermatum*, *Rhizoctonia solani* and *Fusarium oxysporium*). Assays showed that, garlic extract provided a significant inhibition of mycelial growth of all concerned phytopathogenic fungi and their sensitivity was varied greatly. *Allium sativum* extract was highly effective in suppressing the mycelial growth of *P. aphanidermatum* and its mycelial growth was inhibited to 77.43% while *R. solani* and *F. oxysporum* were less sensitive and their mycelial growth were inhibited to 69.35 and 55.94% at 5 mg/ml respectively

These results are in accordance with that of Ramanathan *et al*., 2004 and Jung *et al*., 2003. A variation on fungitoxicity of the *A. sativum* extract against tomato phytopathogenic fungi may be due to variation in fungal species itself (Manoranjithan *et al*., 2001 and Narayana Bhat and Shukla, 2001).

The study of MIC and MFC of the fungitoxicants compared with reference fungicide are necessary to evaluate their efficacy in suppressing mycelial growth of the phytopathogenic fungi. *A. sativum* extract was strongly active against the tomato phytopathogenic fungi but its MIC with MFC were comparatively higher than that of reference fungicide. However, carbendazim 98% fungicide was the most effective fungitoxicant suppressing growth of phytopathogenic fungi than extracts of *A. sativum* as mycelial growth of the three phytopathgenic fungi were completely inhibited at 8 ppm while a huge concentration reached to 8 mg/ml was required for *A. sativum* extract to attain the same effect.

Antifungal compounds present in *A. sativum* extract were analyzed by GC-MS and 10 compounds were identified. Rabinkov *et al*., (1998) reported that allicin and ajoene identified form *A. sativum* extract through GC-MS analysis were found to possess antifungal activity and the suppressive effect of *A. sativum* extract against the tomato phytopathogenic fungi could be attributed to fungitoxic sulfur compounds especially allicin and ajoene resulting from conjugation of three allicin molecules (Ankri and Mirelman, 1999). Allicin and ajoene inhibit different microbial enzymes essential for fungal growth (fujisawa *et al*., 2008).

Some researchers have suggested that antimicrobial sulfur components of the garlic extract cross the cell membrane interacting with the fungal enzymes suppressing their action (Pane *et al*., 2011 and Omidbeygi *et al.*, 2007). Other researcher attributed the inhibitory effect to hydrophobicity characters of garlic extract and their components. This enables lipid-soluble sulfur compounds of garlic extract to partition in the lipids of the fungal cell wall membrane and mitochondria disturbing their structure and rendering them more permeable. Leaking of ions and other cell contents can then occur causing cell death (Burt, 2004).

Efforts should be made to evaluate penetration property of *A. sativum* extract into the plants and to identify an acceptable fragrance for *A. sativum* extract capable of disguising its unpleasant odor without affecting its antifungal activity (Obagwu and Korsten, 2003)

Antifungal activity of *A. sativum* extract

gives new opportunity to improve control against different tomato damping-off diseases that cause losses at seedling stage especially in organic agriculture. Further studies should be done in greenhouse and in open fields to evaluate any effect of these natural substances on tomato plants development and on qualitative and quantitative tomato production

The present study indicated that application of *A. sativum* extract as biocontrol agents was found to be effective in controlling tomato damping-off diseases and that garlic extract may be an attractive alternative for the use of natural product to control tomato phytopathogenic fungi avoiding chemical fungicide application.

ACKNOWLEDGMENTS

The Authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project (Project No. RGP-VPP-184).

REFERENCES

1. Abou-Jawdah, Y.; Sobh, H. and Salameh, A., Antimycotic activities of selected plant flora growing wild in Lebanon against phytopathogenic fungi. *J. Agri. &Food Chem.,* 2002; **50**: 3208 - 3213
2. Adams, R. P., Identification of Essential Oils Components by Gas Chromatography and Mass Spectroscopy. Allured Publishing Corp., Carol Stream, IL. USA, 2007.
3. Al-Rahmah, A. N.; Mostafa, A. A. and Abel-Megeed, A., Antifungal and antiaflatoxigenic activities of some plant extracts. *Afr. J. Microbiol. Res*.; 2011; **5**(11): 1342 - 1348
4. Anguiz, R., Anatomosis groups pathogenicity and other characteristics of *Rhizoctonia solani* isolated from potatos in Peru. *Plant Dis.,* 1989; **73**: 199-201
5. Ankri, S. and Mirelman, D., Antimicrobial properties of allicin from garlic. *Microbes & Infection*, 1999; **2**: 125 - 129
6. Appleton, J.A. and Tansey, M. R., Inhibition of growth of zoopathogenic fungi by garlic extract. *Mycologia,* 1975; **67**: 882-885
7. Arya, A.; Chauhan, R. and Arya, C., Effect of allicin and extracts of garlic and bignonia on two fungi. *Ind. J. Mycol. Plant Pathol*., 1995; **23**: 316-318
8. Balestra, G. M.; Heydari, A.; Ceccarelli, D.; Ovidi, E. and Quattrucci, A., Antibacterial effect of *Allium sativum* and *Ficus carica* extracts on tomato bacterial pathogens. *Crop Prot*., 2009; **28**: 807 - 811
9. Benkeblia, N., Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum* L.*). Lebensm-Wiss-U-Technol*., 2004; **37**: 263 – 268
10. Bharathi, R.; Vivekananthan, R.; Harish, S.; Ramanathan, A. and Samiyappan, R., Rhizobacteria based bio-formulations for the management of fruit rot infection in chilies. *Crop Prot*., 2004; **23**: 835 - 843
11. Bianchi, A.; Zambonelli, A.; D'Auleria, A. Z. and Bellesia, F., Ultrastructural studies of the effects of *Allium sativum* on phytopathogenic fungi in vitro. *Plant Dis*., 1997; **81**: 1241- 1246
12. Bisht, S. S. and Kamal., Garlic extract – an effective antifungal treatment for the control of storage rot of apple. *Proc. Nat. Acd. Sci. India,* 1994; **64**(B): 233 – 234
13. Burt, S., Essential oils; their antimicrobial properties and potential applications in foods – a review. *Int. J. Food Microbiol*., 2004; **94**: 223 – 253
14. Cao, K. Q. and Forrer, H. R., Current status and prosperity on biological control of potato late blight. J. Agric. Univ. Hebei. J. Agric. Univ. Hebei. 2001: 24 http://www.cipotato.org/gilb/Pubs/proceedings_easa/caokeqiang%2821%29.pdf, 2001.
15. Cao, K. Q. and Van Bruggen, A. H.C., Inhibitory efficacy of several plant extracts on *Phytophthora infestans*. *J. Agric. Univ. Hebei*. http://www.cipotato.org/gilb/Pubs/ proceedings_easa/caokeqiang%2821%29.pdf, 2001.
16. Ceron, S. M.; Pina, R. E.; Avila de Moreno, C.; Gil, A. L.; Numpaque, P. V.; Villate, C. R. and Lerena, H. G., In vitro application of extracts of eucalyptus, garlic, calendula, black berry herb, chamomile and nettle for the control of fungus *Sclerotium capivorum*. Fitopathologia. Colombiana, 1999; **23** (1-2): 68 – 71
17. Clinton, S. K., Lycopene; chemistry, biology and implications for human health and diseases. *Nutr. Rev.,* 1998; **56**(2): 35 - 51
18. Curtis, H.; Noll, U.; Stormann, J. and Slusarenko, A. J., Broad-spectrum activity of the volatile phyoanticipin allicin in extracts of garlic (*Allium sativum* L.) against plant pathogenic bacteria, fungi and Oomycetes. *Physiol. & Mole. Plant Pathol*., 2004; **65**: 79 - 89
19. Ejechi, B. O.; Nwafor, O. E. and Okoko, F. J., Growth inhibition of tomato-rot fungi by

phenolic acid and essential oil extracts of pepper fruit (*Dennetia tripetala*). *Food Res. Int.,* 1999; **32**: 395 – 399

20. Fujisawa, H.; Suma, K.; Origuchi, K.; Kumagal, H.; Seki, T. and Ariga, T., Biological and chemical stability of garlic-derived allicin. *J. Agri & Food Chem*., 2008; **56**: 4229 – 4235
21. Georgopoulos, S. G., The development of fungicide resistance. In: Wolfe, M., Caten, CE (Eds.). Populations of plant pathogens- their dynamics and genetics. Blackwell Scientific Publications, Oxford. 1987; 239 - 251
22. Hsieh, P. C.; Mau, J. L. and Huang, S. H., Antimicrobial effect of various combination of plant extracts. *Food Microbiol.,* 2001; **18**: 35-43
23. Isman, B. M., Plant essential oils for pest and disease management. *Crop Prot.,* 2000; **19**: 603 - 608
24. Kumar, A.; Shukla, R.; Singh, P.; Prasad, C. S. and Dubey, N. K., Assessment of *Thymus vulgaris L*. essential oil as a safe botanical preservative against post harvest fungi infestation of food commodities. Innovat. *Food Sci. Emerg. Technol.,* 2008; **9**: 575 – 580
25. Latha, P.; Anand, T.; Ragupathi, V.; Prakasam, R. and Samiyappan, R., Antimicrobial activity of plant extracts and induction of systemic resistance in tomato plants by mixtures of PGPR strains and Zimmu leaf extract against *Alternaria solani*. *Biol. Control,* 2009; **50**: 85 - 93
26. Manoranjitham, S. K.; Prakasam, V.; Rajappan, K., Biocontrol of damping-off of tomato caused by *Pythium aphanidermatum*. *Ind. Phytopatho*l., 2001; **54**: 59-61
27. Mostafa, A. A.; Al-Rahmah, A. N. and Adel-Megeed, A., Evaluation of some plant extracts for their antifungal and antiaflatoxigenic activities. *J. Med. Plant. Res*., 2011; **5**(17): 4231-4238
28. Muthukumar, A.; Eswaran, A.; Nakkeeran, S. and Sangeetha, G., Efficacy of plant extracts and biocontrol agents against *Pythium aphanidermatum* inciting chilli damping-off. *Crop Prot.,* 2010; **29**: 1483-1488
29. Narayana Bhat, M. and Shukla, B. K., Evaluation of some leaf extract against *Pythium aphanidermatum* in vitro and pot culture. *Ind. Phytopathol*., 2001; **54**: 395-397
30. Obagwu, J. and Korsten, L., Control of citrus green and blue molds with garlic extracts. Eur. *J. Plant Pathol*., 2003; **109**: 221-225
31. Omidbeygi, M.; Barzegar, M.; Hamidi, Z. and Naghdibadi, H., Antifungal activity of thyme, summer savory and clove essential oils against Aspergillus flavus in liquid medium and tomato paste. *Food Control*, 2007; **18**: 1518 – 1523
32. Pandy, R., Pesticides and sterility. Everyman's. Sci., 2003; **38**: 84 -86
33. Pane, C.; Spaccini, R.; Piccolo, A.; Scala, F. and Bonanomi, G., Compost amendments enhance peat suppressiveness to *Pythium ultimum*, *Rhizoctonia solani* and *Sclerotinia minor*. *Biological Control*, 2011; **56**: 115 - 124
34. Qi, R. and Wang, Z., Pharmacological effects of garlic extract. *Trends Pharmacol. Sci*., 2003; **24**: 62 – 63
35. Rabinkov, A.; Miron, T.; Konstantinovski, L.; Wilchek, M.; Mirelman, D. and Weiner, L., The mode of action of allicin : trapping of radicals and interaction with thiol containing protein. *Biochem. Biophys. Acta*., 1998; **1379**: 233 - 244
36. Ramanathan, A.; Marimuthu, T. and Raguchander, T., Effect of plant extracts on growth in *Pythium aphanideramtum*. *J. Mycol. Plant Pathol.*, 2004; **34**: 315 – 317
37. Satya, V. K.; Radhajeyalakshmi, R.; Kavitha, K.; Paranidharan, V.; Bhaskaran, R. and Velazhahan, R., In *vitro*, Antimicrobial activity of Zimmu (*Allium sativum* L. x *Allium cepa* L.) leaf extract. *Arch. Phytopathol. Plant Prot*., 2005; **38**: 185 - 192
38. Schwarz, D and Grosch, R., Influence of nutrient solution concentration and a root pathogen (*Pythium aphanidermatum*) on tomato root growth and morphology. *Sci. Hortic*, 2003; **97**: 109 – 120
39. Singh, U. P.; Pandey, V. N.; Wagner, K. G. and Singh, K. P., Antifungal activity of ajoene, a constituent of garlic (*Allium sativum*). *Can. J. Bot*.,1990; **68**: 1354 - 1360
40. Song, W.; Zhou, L.; Yang, C.; Cao, X.; Zhang, L. and Liu, X., Tomato fusarium wilt and its chemical control strategies in hydroponic system. *Crop prot*., 2004; **23**: 243-247
41. Soylu, E. M.; Kurt, S. and Soylu, S., *In vitro* and *in vivo* antifungal activities of the essential oils of various plants against tomato grey mould disease agent *Botrytis cinerea*. *Int. J. Food Microbiol*., 2010; **143**: 183 - 189
42. Staub, T., Fungicide resistance; practical experience with anti-resistance strategies and the role of integrated use. *Annu. Rev. Phytopathol.,* 1991; **29**: 421 - 442
43. Yin, M. C. and Tsao, S. M., Inhibitory effect of seven *Allium* plants upon three *Aspergillus* species. *Inter. J. Food Microbiol.,* 1997; **49**: 49-56.