Journal of Agricultural Technology 2010 Vol.6(3): 409-419
Available online http://www.ijat-rmutto.com
ISSN 1686-9141

Antifungal activity and biocontrol potential of metabolite produced by an endophytic *Fusarium* (MTCC-9622) against some postharvest pathogens

Tayung, K.[1*], Barik, B.P.[2] and Jha, D.K.[3]

[1]Department of Botany, North Orissa University, Takatpur- 757003, Orissa, India.
[2]Department of Bioinformatics, North Orissa University, Takatpur- 757003, Orissa, India.
[3]Microbial Ecology Laboratory, Gauhati University, Guwahati-780014, Assam, India.

Tayung, K., Barik, B.P. and Jha, D.K. (2010). Antifungal activity and biocontrol potential of metabolite produced by an endophytic *Fusarium* (MTCC-9622) against some postharvest pathogens. Journal of Agricultural Technology 6(3): 409-419.

Biocontrol potential of metabolite produced by an endophytic *Fusarium*, isolated from *Taxus baccata* bark was investigated against some postharvest pathogens of vegetables. *In-vitro* antifungal activity and effective control analysis of the metabolite against the pathogens indicated that the metabolite effectively inhibit postharvest pathogens and reduced the disease incidence by 50%. The metabolite showed significant antifungal fungal activity against three postharvest pathogens (*Fusarium oxysporum*, *Aspergillus niger* and *Rhizopus stolonifer*). Based on morphological and molecular characterization, the fungus was identified as *Fusarium solani*. The crude metabolite was separated into two fractions by column chromatography over silica gel (60-120mesh) using a mixture of petroleum ether (40-60^{0}C) and ethyl acetate (20:1) as the eluent. The active compound was characterized on the basis of its spectral data obtained from GC/MS analysis. On the basis of spectral data, all the major compounds were identified to be hydrocarbons. The present investigation is a first report of an endophytic *Fusarium* producing hydrocarbons metabolite active against postharvest pathogens. The study revealed that endophytic fungi could be potentially applied as biological control agents and in biocontrol postharvest technology.

Key words: Antifungal activity, biocontrol potential, postharvest pathogens, endophytic *Fusarium*, hydrocarbons metabolite

Introduction

Fruits and vegetables that are grown and consumed worldwide often get spoilage due to infection caused by postharvest pathogens. Losses from these infections are much greater than generally realized because the value of fresh fruits and vegetables increases several fold while passing from the field to the consumer (Sams, 1994). It is estimated that postharvest losses range from 10 to

[*]Corresponding author: K. Tayung; e-mail: kumanandbotnou@rediffmail.com

30% per year despite the use of modern storage facilities and techniques (Harvey, 1978). Such losses are even greater in developing countries due lack of modern storage facilities. Postharvest diseases are mainly due to infections cause by bacteria and fungi that occur during the growing season, at harvest time, during handling, storage, transport and marketing, or even after purchase by the consumer (Dennis, 1983). The reduction of losses in perishable food crops because of postharvest diseases has become a major objective of international organizations (Kelman, 1989).

Adequate storage facilities and more importantly slowing down the development of pathogens can prevent or reduce the losses to a great extend. To overcome such problems, normally chemicals and antibiotics are used to prevent from decay and ensure product protection. However, increasing use of chemical inputs have causes several negative effects to living organisms and degrading the environment. The excessive use and misuse of these agrochemicals have also resulted into development of fungicide resistance pathogens (Gerhardson, 2002). Moreover, fear-mongering by some opponents to chemicals and consumer demand for chemicals free food has led to a search for substitute of these products. In such cases, biological control method has been highly appreciated around the world as an eco-friendly alternative to agrochemicals.

Postharvest biological control is a relatively new approach with several advantages over conventional biological control method (Pusey, 1989). Several workers have demonstrated that many antagonistic strains of bacteria and fungi are used as biological control of postharvest diseases of fruits and vegetables (Wisniewski and Wilson, 1992). Research to elucidate whether these organisms could potentially be used as biological control agents to combat diseases has intensified over the past 20 years, and this has led to the commercial development of several registered microbial agents for vegetable crop disease management, as well as for diseases on other crops (Punja and Utkhede, 2003). More recently, endophytic fungi that reside in healthy inner tissues of plants have been identified and used as potentials biological control agents against wide variety of pathogens and insect pests (Azevedo *et al*., 2000; Backman and Sikora, 2008; Herre *et al*., 2007; Rubini *et al*., 2005). Various endophytic and non-endophytic *Fusarium* isolates have been shown to produce secondary and toxic metabolites with biocontrol ability against nematodes (Fuchs *et al*., 1999; Hallmann and Sikora, 1994b; Vu *et al*., 2004). Yet little is known about metabolites produced by endophytic *Fusarium* against postharvest pathogens.

The aim of this study was to evaluate antifungal activity and biocontrol potential of metabolite produced by an endophytic *Fusarium* (MTCC-9622), against some postharvest pathogens of vegetables, molecular identification

based on rDNA-ITS sequence analysis and characterization of the active metabolites of the fungus.

Materials and methods

The fungus used in this study was obtained as endophyte from *Taxus baccata* bark located in Dibang valley of Arunachal Pradesh, a part of Eastern Himalaya, India. The procedure used to isolate this organism has been described previously and include surface sterilization technique by immersing the bark sequentially in 70% ethanol for 3 min and 0.5% sodium hypochloride (NaOCl) for 1min and rinsed thoroughly with sterile distilled water, carefully excising the inner tissues and placed on petri-plates containing potato dextrose agar (PDA) and water agar (WA) media (Tayung and Jha, 2006). The fungus was induced to sporulate for identification. It was among the several endophytic isolates that displayed antifungal activity against postharvest pathogens during preliminary screening and was selected for further study. The fungus was cultivated on Potato dextrose broth (Hi-media) by placing agar blocks of pure culture (3 mm in diameter) of actively growing culture in 500ml Erlenmeyer flasks containing 200 ml of the medium. The flasks were incubated in BOD shaking incubator for 3 weeks at 24 ± 2^0C with periodic shaking at 150 rpm. The fermentation broth was then filtered through sterile cheesecloth to remove the mycelia mats. The filtrate was extracted thrice with ethyl acetate at room temperature. The organic solvent was evaporated in vacuum evaporator and the resultant compound was dried with $MgSO_4$ and concentrated to yield the crude metabolite. The metabolite was dissolved in Dimethly Sulphoxide (DMSO) for antifungal assay against post harvest pathogens.

The test pathogens were isolated from naturally infected disease vegetables i.e. *Rhizopus stolonifer* from bean, *Fusarium oxysporum* from ginger, *Aspergillus niger* from yam and *Geotrichum candidum* from tomato. The aggressiveness of the pathogens was verified based on their infection, producing largest lesions and ability to cause rapid spoilage in the local storage market. The fungi were purified and maintained on potato dextrose agar (PDA) and stored at 4°C, with periodic transfers into the vegetables to maintain its aggressiveness. The crude metabolite of the endophytic fungus was evaluated for antifungal activity against the postharvest pathogens by agar cup diffusion assay. The test pathogens were culture on potato dextrose broth and spore suspension of 7 days old culture were inoculated into petri-plates containing potato dextrose agar medium. The spore suspensions were evenly spread out in the plates with a help of a sterile cotton swab. Then agar cups were prepared in the middle of the plates with a help of a cork borer (6 mm diameter). Each cup was then loaded with 100µl of the crude metabolite. The plates were incubated at 27°C for 3 days and

the zone of inhibition was measured and compared with the control (i.e. cups filled DMSO only). Three replicates were maintained in each case.

Evaluation on effectiveness of the metabolite against postharvest pathogens was done following method describe by Taqarort *et al*. (2008). Healthy vegetables were selected and washed with sterile phosphate buffer (0.05 M, pH 6.5) for 10 min with shaking and rubbing with a sterilized paint brush. Washed vegetables were disinfected with 0.1% (v/v) sodium hypochlorite, rinsed three times in sterile distilled water and then air dried prior to wounding. Four wounds (3 mm deep and 3 mm wide) were made using a sterile needle at the equatorial side. A 30 µl of the crude metabolites was pipette into each wound site. Controls were treated with 50 µl of sterile distilled water under the same conditions. After 24 h of incubation at 27°C each wound were inoculated with 20 µl of spore suspension of the respective pathogens. The vegetables were stored at 27°C and at ~ 90% RH for a week. Three replicates were maintained in each case. After incubation period, wounds were examined and the percentage of disease incidence (I) was determined as follow:

$$I\ (\%) = \frac{\text{(Number of decayed wounds)}}{\text{(Number of total wounds)}} \times 100$$

Total genomic DNA was extracted from mycelia of the fungus grown on potato dextrose agar medium by using CTAB method (Cai *et al*., 2006). DNA amplification was performed by PCR. For ITS-rDNA amplification ITS4 and ITS5 primers were used according to the method described by White *et al*. (1990). The amplification reaction was performed in a 50l of reaction volume. The PCR product, spanning approx. 500-600 bp was checked on 1% agarose electrophoresis gels. It was then purified using quick spin column and buffers (washing buffer and elution buffer) according to the manufacturer's protocol (QIA quick gel extraction kit Cat No. 28706). DNA sequencing was performed using the above mentioned primers in an Applied Biosystem 3130xl analyzer. The crude metabolites was separated into two fractions by column chromatography over silica gel (60 - 120 mesh) using a mixture of petroleum ether (40-60 ^{o}C) and ethyl acetate (20:1) as the eluent. The separated active compounds were characterized on the basis of its spectral data obtained from GC/MS analysis. GC/MS analysis were performed on an HP 5890 Gas Chromatograph equipped with a fused silica 30 m × 0.25 mm, SE-30 capillary column, and a HP 5971 series Mass Selective Detector. The column was temperature programmed from 100-290°C at 3°C/min with a carrier gas (He) at a flow rate 1 ml/min. The identification of the compounds was based on comparing their relative retention times and mass spectra with those of Wiley

Registry and NBS Libraries (Massada, 1976) and through library comparison using the NIST database on the mass spectrometer.

Results

The morphological traits of the isolate were white, then pink, cottony, nearly round margins. Microscopic morphologies of the fungus indicated erect septate hyphae, simple or branched and sickle-shaped conidia differentiated from mycelia tips. Morphological characters of the fungus revealed *Fusarium* sp., in taxonomy. To known its phylogenetic affiliations the sequence of the fungus was compared with the GenBank database available in NCBI using the BLAST. Based on NCBI-BLAST search analysis of rDNA-ITS sequence, the fungus was identified as *Fusarium solani* (Fig. 1). A living culture of the strain has been deposited in microbial type culture collection and gene bank (MTCC), Chandigarh, India with accession no. 9622. The agar cup diffusion assay showed that all the tested postharvest pathogens were susceptible to the crude metabolite (Table 1). The metabolite effectively controls *Fusarium oxysporum* which was depicted by highest zone of inhibition. Similarly, significant antifungal activity of the crude metabolites was observed against *Aspergillus niger* and *Rhizospus stolonifer*. However, the metabolite showed less activity against *Geotrichum candidum* which was indicated by low inhibition zone.

The metabolite effectively reduced the disease incidence by 50% against the pathogens at spore suspension of 10^5 ml^{-1}, the percentage of rot incidence varying between10% to 40% while it was 75% for the control (Fig. 2). Among the pathogens, the disease incidence of *Fusarium oxysporum* and *Aspergillus niger* were significantly reduced with percentage rot incidence of 8% and 16% respectively. Effective reduction of disease incidence was also observed in *Rhizospus stolonifer* with rot percentage incidence of 19%. However, the metabolite showed less reduction disease incidence potential towards *Geotrichum candidum* with rot percentage incidence of almost 50%. The crude metabolite (0.543 g) was separated and purified into two fractions by column chromatography over silica gel (60-120 mesh) using a mixture of petroleum ether (40-60^0C) and ethyl acetate (20:1). The first fraction was colorless liquid which was subjected to GCMS analysis that showed a mixture of several compounds (Fig. 3a). From mass spectral analysis six compounds were identified and they together accounted for at least 86% of the total mass. The second fraction which was red and semi-solid mass was also subjected to GCMS analysis (Fig. 3b). From GCMS analysis it was evident that second compound consisted of only five compounds and out of these five there were only two major components, which together accounted for 70% of the total mass. All the major compounds were identified and presented in Table 2.

Table 1. *In vitro* antifungal activity of the crude metabolites against the post harvest pathogens.

Pathogen	Crude metabolites (100 µl)
	Zone of inhibition (mm)
Rhizopus stolonifer	26 ± 0.58
Fusarium oxysporum	32 ± 1.55
Aspergillus niger	28 ± 0.58
Geotrichum candidum	16 ± 1.54

Values are mean of three replicates
± Standard Deviation

Table 2. Major identified compounds produced by *Fusarium* (MTCC-9622).

Compound*	RT (min)	Relative amount (%)
Dodecene	04.19	06.5
Dodecane	04.32	04.1
Hexylcyclohexane	05.28	05.4
1-Tetradecene	10.77	43.8
Tetradecane	11.12	09.8
Octylcyclohexane	14.20	16.5
2-Undecanone	1.69	6.13
2,4-Di-tert-butylphenol	2.21	9.88
8-Pentadecanone	2.96	29.68
8-Octadecanone	4.84	41.36
10-nonadecanone	8.76	12.95

*Several minor peaks were omitted from the total analysis since they represent very small amount.

Discussion

Recent finding have shown that endophytic fungi, successfully control wide variety of plant pathogenic fungi, viruses and nematodes (Dingle and Mcgee, 2003; Hallmann and Sikora, 1996c; Kim *et al*., 2007; Lehtonen *et al*., 2006). The importance of endophytic fungi in this regards eventually led to development of a symposium by Biological Control Committee of the American Phytopathological Society on endophytes as emerging tools for biological control agents and key to plant protections (Backman and Sikora, 2008).

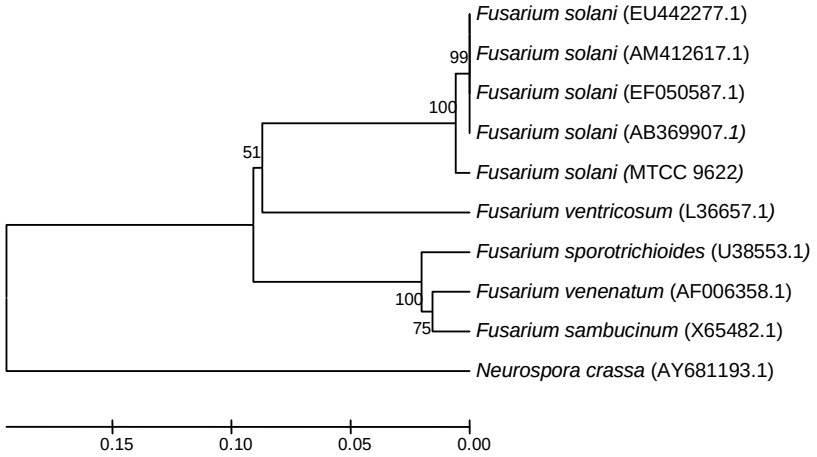

Fig. 1. Phylogenetic tree of *Fusarium* (MTCC-9622) using UPGMA method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The values in parentheses are accession no. of ITS sequences obtained from NCBI database. *Neurospora crassa* is used as an out group.

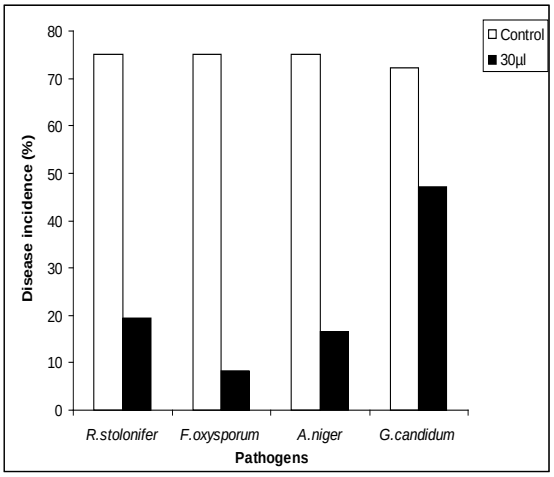

Fig. 2. Reduction of postharvest pathogens by the metabolite produced by *Fusarium* (MTCC-9622). Values are mean of three replicates; 30µl- crude metabolite; Control- DMSO solution.

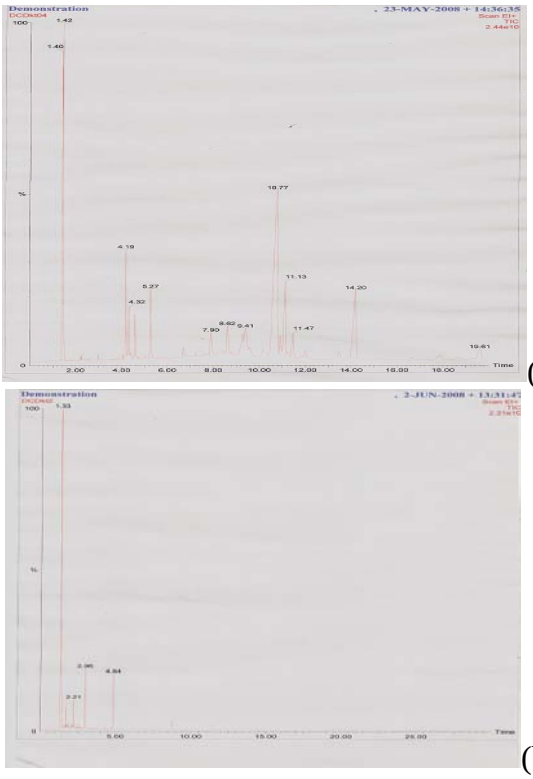

Fig. 3. GCMS analysis of the metabolite produced by *Fusarium* (MTCC-9622) showing mass spectrum of the first (a) and second fraction (b).

Control of postharvest fungal pathogens by bacteria and yeast antagonists such as *Bacillus subtilis, Pseudomonas cepacia*, *Pseudomonas syringae* and *Debaryomyces hansenii* has been described for a variety of stored vegetables and fruit including apple, apricot, cherry, citrus, grape, nectarine, peach, pear, pepper, persimmon, plum, potato and tomato (Wisniewski and Wilson, 1992). Yet little is known about the use of endophytic fungi against postharvest pathogens. In this study we have demonstrated metabolite derived from endophytic *Fusarium*, exhibited significant antifungal activity and biocontrol potential against postharvest pathogens of vegetables. The genus *Fusarium* is diverse and widespread, commonly causing diseases in plants and animals including humans (Smith, 2007). Therefore, many pathologists believed to be a pathogen and never to be an endophyte. But, recently many *Fusarium* species have been isolated and reported as endophytes from several plant species suggesting their non-pathogenic forms. Various non-pathogenic *Fusarium* isolates have shown their ability to suppress plant pathogens (Fuchs *et al*., 1999; Hallmann and Sikora, 1994). Similarly, endophytic *Fusarium* has also been reported as biocontrol agents against nematodes and soil borne

pathogenic fungi (Hallmann and Sikora, 1996). However, use of endophytic fungi as biocontrol agents against postharvest pathogens are less investigated. Our result, on *in-vitro* antifungal activity and effective control analysis of the metabolite produced by endophytic *Fusarium* strongly suggests the use of the metabolite to control postharvest pathogens. Among several fungal pathogens, *Rhizopus stolonifer, Aspergillus niger* and *Geotrichum candidum* are described as serious postharvest pathogens causing rapid spoilage on vegetables and fruits and in most cases for effective control of these pathogens, fungicides are used (Northover and Zhou, 2002). However, increasing use of chemicals input have caused several negative effects and generated fungicide resistance pathogens. Thus, as an alternatives to chemicals and growing global chemophobia among people, biological control measure has been highly accepted an eco-friendly approach to solve such serious problems. Although, several biological control agents are reported few have been successfully applied against postharvest pathogens. *In-vitro* antifungal assay of the metabolites showed effective inhibition against *Rhizopus stolonifer, Aspergillus niger* and *Fusarium oxysporum* without any controlled environmental conditions. Such result suggests the use of the metabolites as alternatives to fungicides in managing postharvest pathogens. Several mode of actions have been described for biological control, such as mycoparasitism, induced resistance, competition, production of lytic enzymes, e.g. β-1,3-glucanase, chitinase and antibiotics, e.g. 2,4-diacetylphloroglucinol, Phenazines, Cyclic lipopeptides (Pal and Gardener, 2006). In the present study, the antagonistic activity of endophytic *Fusarium* toward postharvest pathogens was through production of secondary metabolite, suggests the mode of action to be through antibiotic production. Biocontrol through antibiotics are reported from few microorganisms and most antibiotics used as biological control agents are phenolic compounds derived from strains of *Pseudomonas* isolated from plant rhizosphere (Dwivedi and Johri, 2003). The active metabolite produced by the endophyte showing biocontrol potentials were found to be hydrocarbon in nature. Although hydrocarbon compounds are not well documented in endophytes but recently it has been reported from an endophytic fungus, *Gliocladium roseum* (NRRL 50072) isolated from *Eucryphia cordifolia* (Strobel *et al*., 2008). Similarly, Ramin *et al*. (2007) have reported that volatiles metabolite produced by endophytic fungus, *Muscodor albus* effectively control postharvest disease of apple. Such results strongly agree with the facts that fungal endophytes produce diverse and interesting metabolites and are recognized as a repository of novel bioactive substances for potential applications in agriculture and industry (Strobel and Daisy, 2003; Tan and Zou, 2001).

Acknowledgements

The authors are thankful to Prof. D. K. Deka, Organic Chemist, University of Gauhati, India for GCMS analysis of the metabolite and to Dr. B.D. Shenoy, Scientist IMTECH, Chandigarh, India for DNA sequencing.

References

Azevedo, J.L., Jr Maccheroni, W., Pereira, J.O. and Araujo, W.L. (2000). Endophytic microorganisms: a review on insect control and recent advances on tropical plants. Electronic Journal of Biotechnology 3: 41-65.

Backman, P.A. and Sikora, R.S. (2008). Endophytes: An emerging tool for biological control. Biological control 46: 1-3.

Cai, L., Jeewon R. and Hyde, K.D. (2006). Phylogenetic investigation of sordariaceae based on multiple gene sequences and morphology. Mycological Research 110: 137-150.

Dennis, C. (1983). Postharvest Pathology of Fruits and Vegetables. Academic Press, London.

Dingle, J. and Mcgee, D.A. (2003). Some endophytic fungi reduce the density of pustules of *Puccinia recondita* f. sp. *tritici* in wheat. Mycological Research 107: 310-316.

Dwivedi, D. and Johri, B.N. (2003). Antifungal from *fluorescent Pseudomonas*: Biosynthesis and regulation. Current Science 85: 1693–1703.

Fuchs, J.G., Moenno-Loccoz, Y. and Defago, G. (1999). Ability of nonpathogenic *Fusarium oxysporum* Fo47 to protect tomato against *Fusarium* wilt. Biological Control 14: 105-110.

Gerhardson, B. (2002). Biological substitutes for pesticides. Trends Biotechnology 20: 338-343.

Hallmann, J. and Sikora, R.A. (1994). In-vitro and in-vivo control of *Meloidogyne incognita* with culture filtrate from non-pathogenic *Fusarium oxysporum* on tomato. Journal of Nematology 26: 102

Hallmann, J. and Sikora, R.A. (1996). Toxicity of fungal endophyte secondary metabolites to plant parasitic nematodes and soil-borne plant pathogenic fungi. European Journal of Plant Pathology 102: 155-162.

Harvey, J.M. (1978). Reduction of losses in fresh fruits and vegetables. Annual Review of Phytopathology 16: 321-341.

Herre, E.A., Mejia, L.C., Kyllo, D.A., Rojas, E., Maynard, Z., Butler, A. and Van Bael, S.A. (2007). Ecological implications of anti-pathogen effects of tropical fungal endophytes and mycorrhizae. Ecology 88: 550–558.

Kelman, A. (1989). Introduction: the importance of research on the control of postharvest diseases of perishable food crops. Phytopathology 79: 1374-1385.

Kim, H.Y., Choi, G.J., Lee, H.B., Lee, S.W., Lim, H.K., Jang, K.S., Son, S.W., Lee, S.O., Cho, K.Y., Sung, N.D. and Kim, J.C. (2007). Some fungal endophytes from vegetable crops and their anti-oomycete activities against tomato late blight. Letters in applied microbiology 44: 332-337.

Lehtonen, P.T., Helander, M., Siddiqui, S.A., Lehto, K. and Saikkonen, K. (2006). Endophytic fungus decreases plant virus infections in meadow ryegrass (*Lolium pratense*). Letters in Biology 2: 620-623.

Massada Y. (1976). Analysis of Essential Oil by Gas Chromatography and Spectrometry. John Wiley and Sons, New York.

Northover, J and Zhou, T. (2002). Control of rhizopus rot of peaches with postharvest treatments of tebuconazole, fludioxonil, and *Pseudomonas syringe*. Canadian Journal of Plant Pathology 24:144-153.

Pal, K.K and Gardener, B.M. (2006). Biological Control of Plant Pathogens. The Plant Health Instructor DOI: 10.1094/PHI-A-2006-1117-02.

Punja, Z.K and Utkhede, R.S. (2003). Using fungi and yeast to manage vegetable crop diseases. Trends in Biotechnology 21 (9): 400-407.

Pusey, P.L. (1989). Use of *Bacillus subtilis* and related organisms as biofungicides. Pesticide Science 27:133-140.

Ramin, A.A., Robert, K., Prange, P., Braun, G. and Delong, J.M. (2007). Biofomigation of Postharvest Fungal Apple Decay with *Muscodor albus* Volatiles. Journal of Applied Sciences Research 3(4): 307-310.

Rubini, M.R., Silva-Ribeiro, R.T., Pomella, A.W.V., Maki, C.S., Araujo, W.L., Santos, D.R. and Azevedo, J.L. (2005). Diversity of endophytic fungal community of cacao (*Theobroma cacao* L.) and biological control of *Crinipellis perniciosa*, causal agent of Witches' Broom Disease. International Journal Biological Science 1: 24–33.

Sams, C.E. (1994). Management of postharvest disease resistance in horticultural crops: introduction to the colloquium. HortScience 29:746.

Smith, S.N. (2007). An Overview of Ecological and Habitat Aspects in the Genus *Fusarium* with Special Emphasis on the Soil-Borne Pathogenic Forms. Plant Pathology Bulletin 16: 97-120.

Strobel, G.A. and Daisy, B. (2003). Bioprospecting for Microbial Endophytes and Their Natural Products. Microbiology and Molecular Biology Reviews 67: 491-502.

Strobel, G.A., Knighton, B., Kluck, K., Ren, Y., Livinghouse, T., Griffin, M., Spakowicz, D. and Sears, J. (2008). The production of myco-diesel hydrocarbons and their derivatives by the endophytic fungus *Gliocladium roseum* (NRRL 50072). Microbiology 154: 3319-3328.

Tan, R.X. and Zou, W.X. (2001). Endophytes: a rich source of functional metabolites. Natural Product Report 18: 448-459.

Taqarort, A., Echairi., A., Chaussod, R., Nouaim, R., Boubaker, H., Benaoumar, A.A. and Boudyach, E. (2008). Screening and identification of epiphytic yeasts with potential for biological control of green mold of citrus fruits. World Journal of Microbiology and Biotechnology 24: 3031–3038.

Tayung, K. and Jha, D.K. (2006). Antimicrobial evaluation of some fungal endophytes isolated from the bark of Himalayan yew. World Journal of Agricultural Sciences 2: 489- 494

Vu, T.T., Sikora, R.A. and Hauschild, R. (2004). Effect of endophytic *Fusarium oxysporum* toward *Radopholus similes* activity in the ebsence of banana. Communication in Agricultural and Applied Biological Sciences 69: 381-385.

White, T.J., Bruns, T.D., Lee, S. and Taylor, J.W. (1990). Amplification and direct sequencing of fungal rRNA genes for phylogenetics. In PCR protocols: a guide to methods and application, Eds., Innis, M.A., D.H. Gelfand., J. Sninsky and T.J. White. Academic Press, San Diego, pp:315-322.

Wisniewski, M.E and Wilson, C.L. (1992). Biological control of postharvest diseases of fruits and vegetables: Recent advances. HortScience 27:94-98.

(Received 6 August 2009; accepted 5 May 2010)