Research Paper :

Secondary metabolites of *Chaetomium globosum* used as antifungal against post harvest pathogens

NEETA SHARMA AND MADHU PRAKASH SRIVASTAVA

International Journal of Plant Protection, **Vol. 4 No. 2 (October, 2011) : 353-358**

See end of the article for authors' affiliations

Correspondence to :
MADHU PRAKASH SRIVASTAVA
Department of Botany, Mycology and Plant Pathology Division University of Lucknow, LUCKNOW (U.P.) INDIA
Email madhusrivastava 2010@gmail.com

SUMMARY

***Chaetomium globosum* strain F0140, which was isolated from *Butea monosperma*, has been identified as a potential antagonist of post–harvest pathogen. Production of antifungal compound by *Chaetomium globosum* and its role in suppression of test fungus *in vitro* has been evaluated. Bi-cultural test in laboratory showed that *C. globosum* gave the highest inhibition activity against test fungal pathogen. Inhibition of radial growth and clear zone of inhibition were 95.24 % and 0.35 cm observed, respectably. Crude extract also showed 95% inhibition at the 100 % concentration. The 10 h exposure of extract showed 100% inhibition of spore germination. Culture study showed the best medium for *C. globousm* was MYEA at 20°C in pH-6. This result showed high antifungal metabolite produce by isolate which gave maximum bioefficacy under laboratory conditions against post -harvested pathogen. Significance in antagonism between isolates and test pathogen was observed.**

Sharma, Neeta and Srivastava, Madhu Prakash(2011). Secondary metabolites of *Chaetomium globosum* used as antifungal against post harvest pathogens. *Internat. J. Plant Protec.,* **4**(2): 353-358.

Key words : *Chaetomium globosum*, Antagonism, Antifungal compound, Pathogen inhibition, Plant pathogen

Received : *June, 2011*
Revised : *August, 2011*
Accepted : *September, 2011*

Pathogenic fungi cause plant diseases that result in considerable losses to crop yields. Crop growers generally apply synthetic fungicides as preventive and therapeutic measures to control plant diseases. The indiscriminate and excessive use of a wide range of fungicides has led to environmental pollution and the production of resistant pathogen populations. Therefore, the demand for organic agricultural products cultivated without using any agricultural chemicals or chemical fertilizers is increasing. These events have caused many scientists to conduct research in to the integrated control of fungal diseases, including biological controls using antagonistic microorganisms and safer chemicals such as food preservatives and plant-derived products (Copping *et al.,*. 2000, Istvan, 2002). Bio-control agents possess a number of important advantage over traditional and chemical pesticide which makes their commercial outlook particularly promising, as in general they were considered non-hazardous to humans and animals : biodegradable and environmental friendly, attack specific target organism leaving other beneficial organism unaffected (Adaskaveg *et al.*, 2002).

An antagonist of several soil borne and airborne plant pathogenic fungi, as liquid cultures of *C. globosum* , which was isolated from barnyard grass, showed potent *in vivo* antifungal activity against rice blast (*Magnaporthe grisea*) and wheat leaf rust (*Puccinia recondita*), and moderate *in vivo* antifungal activity against tomato late blight (*Phytophthora infestans*). The production of antifungal substances by this organism is thought to play an important role in its antifungal activity.

Di Poetro *et al.* (1992) reported that the ability of *C. globosum* strains to produce chaetomin in liquid culture is correlated with their activity against damping off of sugar beet caused by *Pythium ultimum*. High antifungal metabolite production by *C. globosum* results in potent *in vivo* antifungal activity against spot blotch (*Cochliobolus sativus*) of wheat under laboratory and glasshouse conditions (Aggarwal *et al.,* 2004). Several antagonistic mechanisms may play a vital role in disease

suppression by *C. globosum*. *In vitro* hyphal coiling was observed in dual culture with *Aspergillus niger, Rhizoctonia solani* and *Alternaria alternata* indicating potential antibiosis of *C. globosum* against these pathogens (Mandal, *et al.,* 2004 and Vannacci and Harman, 1988).

Therefore, the present study was undertaken to determine the role of antibiosis in biological control of pathogen by isolates of *C. globosum*. During a search for an antagonist of several soil borne and airborne plant pathogenic fungi, it was found that liquid cultures of *C. globosum*, showed potent *in vitro* antifungal activity against pathogenic fungi. The production of antifungal substances by this organism is thought to play an important role in its antifungal activity. The objective of this work was to purify and identify the antifungal substances produced by this fungus that are responsible for the suppression of several plant diseases.

MATERIALS AND METHODS

Fungal isolation and identification:

The endophytic isolate used in the experiment was *C. globosum* isolated from *Butea monosperma* leaves during summer season (2007) in Lucknow University campus, Lucknow. The isolate was incubated on Potato dextrose agar (PDA) at 25±1ºC for 15 days with 12 h of illumination daily. Mycelial fragments, ascomata, and ascospores were collected, placed on glass slides, and observed. The isolate was identified by Agharker Research Institute, Pune, to the species level based on the criteria described by Ellis (1971), including the presence or absence of aleuriconidia and the shape and size of the ascomata and ascospores.

Culture studies of fungal antagonist *Chaetomium globosum*:

The antagonist *C. globosum* was grown on seven different media, at different temperatures and different pH.

Six different culture media as Potato dextrose agar (PDA), Czapek (dox) agar, Malt extract, Malt yeast extract agar, Nutrient yeast agar medium and Richard's medium were studied, the pH of the medium was adjusted and sterilization was done at 15 lbs pressure for 20 minutes.

For effect of different pH, PDA medium was used for these experiments. The pH of the medium was adjusted from 4, 5, 6, 7 and 8 and sterilization was done at 15 lbs pressure for 20 min. 20ml of each medium was poured in Petri dishes and allowed to solidify. Fungal discs of 5 mm diameter of *C. globosum* were cut with the help of the sterile cork borer from periphery of 3 days old culture .The plates were incubated with *C. globosum* at 25±1^{0}C. Mycelial growth and colony characters were evaluated.

For effect of different temperatures, PDA was used and pH was adjust 6.0, Petri dishes of 80 mm diameter containing 20 ml medium were centrally inculcated with 5 mm myeclial size from the margin of three days old culture of *C. globosum* and incubated at 10°C, 15°C, 20°C, 25°C and 30°C.

Day by day the radial growth of *C. globosum* was examined. Three replicates of each setup were maintained and the experiments were repeated thrice.

Bio-efficacy of *C. globosum in vitro* by dual cultures:

Bio-efficacy of *C. globosum* isolates against the microorganisms, *Alternaria alternata, Fusarium oxysporum, Aspergillus niger, Penicillum digitatum,* and *Helminthosporium solani* was tested under *in vitro* conditions by dual culture technique. All cultures were grown on PDA in Petri plates and incubated at 26±1^0 C for 7 days in three replications. The colony diameter of test fungus in dual culture with each isolate of *C. globosum* was measured and growth inhibition was expressed as a percentage of the control. In the corresponding control an equal amount of PDA was added. Day by day the radial growth of test fungi was observed.

Antimicrobial activity was expressed in term of percentage of mycelial growth inhibition and calculated as per formula.

In Petri plate,

Percentage of mycelia growth inhibition=dc-dt×100/dc

where,

dc= Average diameter of fungal colony in control

dt= Average diameter of fungal colony in treatment

Effect of culture filtrate on growth of *Aspergillus niger* in liquid medium:

The inhibitory effect of extracts of *C. globosum* isolates on mycelial growth of *Aspergillus niger* was studied in *in vitro* bioassays.50 ml of Potato dextrose broth (Hi Media) was autoclaved in 150 ml Erhlenmeyer flasks and three agar plugs of isolate of *C. globosum* were transferred to each flask. Uninoculated flasks were used as negative controls. The flasks were incubated for 21 days at 26 ± 2ºC before being clarified by centrifugation at 15,000 rpm for 10 min. The supernatant

of each isolate was sterilized through a syringe filter (X-60, 0.45 µm) and added at a ratio of 1:4 (v/v) to flasks containing Potato dextrose broth medium that had been autoclaved and cooled at 45ºC The flasks were inoculated from a 4-day-old culture of *A. niger*. Broth from the original uninoculated flasks was used for negative controls. The flasks were incubated at 26 ± 2ºC for 15 days. The fresh mycelial weight of *A. niger* in each flask was recorded and compared with the control so as to calculate per cent inhibition. Three replications were kept for isolate.

Assay for the production of inhibitory metabolites by *C. globosum:*

Two hundred milliliters of Potato dextrose broth (Hi Media) was autoclaved in 1000 ml Erlenmeyer flasks and agar plugs from isolate of *C. globosum* were transferred to each flask separately and incubated at 26± 2ºC for 3 weeks as static cultures. The cultures were clarified by centrifugation at 15,000 rpm for 10 min and filtered through filter paper (Whatmen filter No. 1). The antifungal metabolite present in the culture filtrate was extracted by solvent extraction.

Extraction of antifungal metabolites:

Antifungal metabolites were extracted by adding equal volume of ethyl acetate to culture filtrate in a separating funnel. In the ethyl acetate extract, sodium sulphate was added to remove moisture. The solvent was evaporated on a rotary evaporator and the crud extract (oily residues) obtained from each isolate was dissolved individually in methanol and stored in plastic vials.

Effect of duration of exposure of crude extract to the spore of fungus:

Conidial suspensions of *Aspergillus niger* (10^4 conidia/ml) were prepared in sterilized water. Spores were mixed with the crude extracts and kept for 1, 5, 10, 15, and 24 h at 25 ±2ºC .After the specified periods of time, the cotton blue stained spore suspension was filtered through Whatman filter paper No 1. The spores retained on the filter paper were washed, 4-5 times with distilled water to remove traces of the leaf extract. Such spores were mixed in a drop of sterile distilled water on glass slides and incubated for 24 h at 25 ±2ºC. Control sets were prepared in the same way in sterile distilled water only. Conidial germination was monitored at 10X and per cent inhibition over control was calculated.

RESULTS AND DISCUSSION

The endophytic fungi isolated from *Butea monosperma* was identified as *C. globosum* based on the criteria of Ellis (1971). Colonies on PDA were pale olivaceous, perithecia scattered or gregarious broadly ovate or ellipsoid often pointed at the base, 250-300 x 200-250µm, in fresh condition olivaceous but in dry specimen dark brown. Apical hairs somewhat coarser than the other. Simple sparingly septate, minutely scabrous, 3-4µm thick and long. Asci oblong clavate, slightly apiculate at both ends, ascospores pointed at both ends.

C. globosum was grown on six different solid media in order to find the best medium studied for its growth. The perusal of Fig. 1 revealed that Malt yeast agar medium supported the best growth of antagonist significantly superior to other media tested. Radial growth of *C. globosum*, 64.96 mm on MYEA, 30.23 mm on NA YD, 27.53 mm on PDA and 21.62 mm on MEA, while poor growth was recorded on Czepek medium (11.56 mm) and Richards medium (7.80 mm).

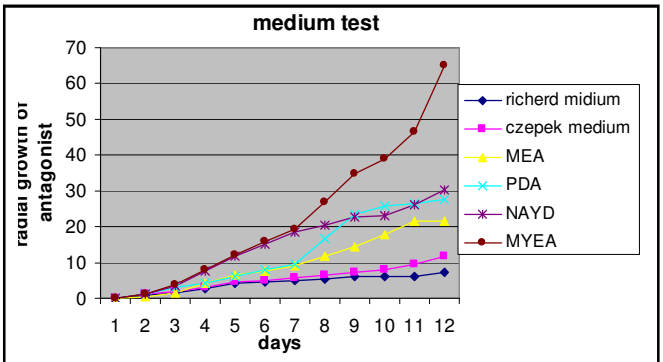

Fig. 1 : Effect of different media on the growth of *C.globosum*

C. globosum was grown on different pH as 4 to 8. PDA medium was used to select the best pH for the growth on the fungus. The data on average radial growth are presented in Fig. 2. Excellent growth on solid media

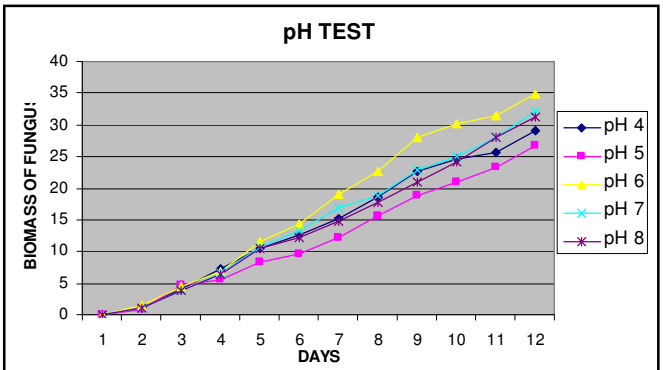

Fig. 2 : Effect of different pH on the growth of *C.globosum*

was recorded at pH 6. The growth on different pH was on pH 6 (34.82 mm) then on pH 7 (31.98 mm), pH 8 (31.33 mm), pH 4 (29.17 mm) and poor growth observed on pH 5 (26.79 mm).

The antagonist was grown at five different temperatures in order to find out the best temperature suited for its growth. The data on average radial growth are presented in Fig. 3. Optimum temperature for radial growth of *C. globosum* appeared to be in between 25-30°C. Excellent growth on solid media was recorded at 30°C while good growth on solid media was recorded at 25°C. The growth on different temperature were 30°C (38.72 mm), 25°C (25.80 mm), 20°C (15.04 mm),while almost nil growth on 15°C and 10°C (Fig. 3).

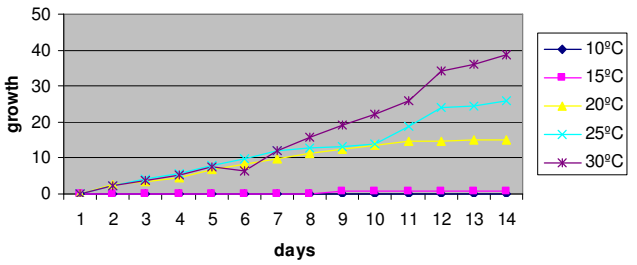

Fig. 3 : Effect of different temperatures on the growth of *C.globosum*

Isolate of *C. globosum* suppressed the radial growth of post harvest pathogen *C.globosum* gave the maximum reduction in diameter .The least reduction (33%) was in dual culture with the *C.globosum* and *A.niger*. In dual culture a clear zone of inhibition was observed exhibiting antibiosis between the pathogen and antagonists. It was observed that *C. globosum* is most effective to reduce the growth the test pathogen *Helminthosporium solani* (55%), *Alternaria alternate*(51%), *Penicillium digitatum* (49%), *Fusarium oxysporum*(43%)and *Aspergillus niger* (33%) (Table 1)

A liquid culture of *C.globosum* showed potential antifungal activity against *A. niger* on PDB medium. Culture filtrate of *C.globosum* completely inhibited mycelial growth of test pathogen. This result indicated that crude extract of *C.globosum* reduces the biomass of *A. niger* by 94.24% at the 100% concentration of antagonist where at 70% concentration 74.51. at 50% concentration 65.51% and at 30% concentration 56.32% and at 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1% concentration 42.52%,37.93% 32.18% 31.03% 29.88% 28.73% 26.43% 20.68% 18.39% 16.06%, radial growth observed, respectively. The maximum inhibition of mycelial growth (94.25%) was observed in 100% concentration of *C.globosum*. (Table 2)

Table 2 : To demonstrate the growth of test pathogen (*Aspergillus niger*) in culture filtrate of *C. globosum*

Concentration of culture filtrate	% inhibition in the growth of pathogen (in %)
Control	0
1%	16.09
2%	18.39
3%	20.68
4%	26.43
5%	28.73
6%	29.99
7%	31.03
8%	32.18
9%	37.93
10%	42.52
30%	56.32
50%	65.51.
70%	74.71
100%	94.24

The exposure of the spore to the extract for different periods of time was studied. An exposure of 1 h inhibited spore germination by 40%, where as 100% inhibition was observed after 10 h exposure (Table 3).

Table 1 : *In vitro* screening of biocontroal agent *C. globosum* against various post harvested pathogens far a myecial growth inhibition

Post harvest pathogen	Radial myceial growth in dual culture	Inhibition %	Dual culture	Zone of inhibition	Degree of antagonism
Helminthosporium solani	13.2(68.8)c	13.16(34.18)	55%	+	HA
Alternaria alternata	15.5(67.24)	13.15(34.18)	51%	+	HA
Penicillium digitatum	15.4(51.84)	22.6(34.18)	49%	+	HA
Fusrium oxysporum	17.4(59.54)	17.4(34.18)	43%	+	MA
Aspergillus niger	33.6(67.24)	12.72(34.18)	33%	+	MA

HA - Highly antagonist MA - Modrate antagonist + - Incateres zone of inhibition C - Radial growth in control

Table 3 : Effect of the duration of exposure of the crude extract of *C. globosum* on spore germination of *Aspergillus niger*

Treatments	Inhibition of spore germination (%)
1 h	40
5 h	65
10 h	100
15 h	100
24 h	100
Control	0.0

In present investigation, eleven endophytic fungi were screened for their effectiveness in controlling the growth of post-harvest pathogens *Alternaria alternata Aspergillus niger, Fusarium oxysporum Helminthosporium solani* and *Penicillium digitatum,* under *in vitro* conditions. It has been observed that the most effective endophytic fungus was *C. globosum* isolated from *Butea monosperma*, which showed great antibiosis to these post harvest pathogens. The soil borne saprobic fungus, *C. globosum* is known as an antagonist of several soil and seed-borne plant pathogens and has the potential to control certain diseases Kohl *et al.* (1995) found that *C. gobosum* has high competitive ability which is prerequisite for successful saprobic antagonists. They concluded that *Chaetomium s*pp. are strongly competitive and have the ability to colonize organic compost and suppress pathogens in the soil. It also exhibits antagonism against many soil and seed borne pathogens, providing successful control to many diseases (Habbard *et al.,* 1982 and Walther and Gindrat, 1988).

Kommendah1 and Chand (1975) stated that antagonistic activity from the hyphae of *Chaetomium* sp. is effective only when the hypha of test pathogens were near, implying antibiosis, as the mechanism of the biocontrol. The application of *Chaetomium* spp. biological products reduced the pathogen inoculum and disease incidence of Phytophthora rot of sweet orange (Usuwan and Soytong, 1998).

Specific strains of antagonistic fungi e.g. *Chaetomium cupreum* and *C. globosum* were isolated and screened for biological control of many plant pathogens (Soytong, 1995). The biocontrol agent, *C. globosum* has been found to producing antifungal metabolites. Six different isolates of *C. globosum* were characterized for the production of antifungal metabolites. One of the purified compounds from isolate Cg2 when used for bioassay against *Bipolaris sorokiniana, Rhizoctonia solani, Fusarium udum* and *Macrophomina phaseolina* pathogens proved effective in inhibiting the growth up to 70 per cent (Aggarwal *et al.,* 2007). Screening for antagonism should not be limited to species, but should consider specific strains within species because these can differ in antagonistic activity (Soytong, 1992).

In dual culture test, a clear inhibition zone was also observed between the growth of the pathogen and antagonist, clearly indicating antibiosis that is, there was release of some chemical metabolites by the antagonistic fungi, which was inhibitory to the growth of the pathogen. Antibiosis occurs during interactions involving low-molecular weight diffusible compounds or antibiotics produced by *Chaetomium* strains that inhibit the growth of the other microorganisms. *C. globosum* strain FO142, which was isolated from barnyard grass, showed potent disease control efficacy against rice blast (*Magnaporthe grisea*) and wheat leaf rust (*Puccinia recondita*). Two antifungal substances were purified from broth from this organism and identified as Chaetoviridin A, which suppressed rice blast and wheat leaf rust over 80% more than Chaetoviridin B (Kim and Hwang, 2007).

In the present investigation, the activity of crude extract filtrate of *C. globosum* on *Aspergillus niger* was studied. It was found that the maximum inhibition in mycelial growth at 100% concentration of crude extract filtrate (CCF). Culture washings of the *Chaetomium* spp. isolates may have activated defense reactions of the plant, there by limiting pathogen spread and replication, other reports of biological control by *Chaetomium* spp. imply inhibition (Tveit and Moore, 1954), though these metabolites are often unstable when applied in the field (Misato *et al.,* 1977). Extracts of culture filtrate of fungal taxa belonging to *Chaetomium* sp. isolated as entophytes were tested for their *in vitro* acetyl cholinesterase and butyrl cholinesterase inhibitory activity, the result showed high rate of inhibitory activity (Rodrigues *et al.,* 2005). Biological control of plant pathogens has been shown to have potential control of many diseases in plantations. *Chaetomium, Trichoderma* species are biological control agents that have the potential to control plant diseases. Kommendahl and Chand, (1975) stated that antagonist from the hyphae of *Chaetomium* spp. is effective only when the hyphae of test pathogens were near, implying antibiosis, as the mechanism of the biological control. A new endophytic fungus *C. spirale* ND35 from *Populus tomentosa*, was reported and bio-control trials of *C. spirale* ND35 against valsa canker of apple, results of dual culture on PDA plate showed that *C. spirale* ND35 was capable of strong antagonism against *Valsa*

ceratosperma, and for inhibiting mycelial growth, the crude extract of liquid culture of corn steep powder broth was more effective than that of Malt extract broth (MEB) (Xin and Shang, 2005). *C. globosum* occurring as an entophyte and acting as a biocontrol agent has not been reported so far. Thus, this endophytic isolate can be utilized as natural bioagent against post harvest pathogens..

Authors' affiliations:
NEETA SHARMA, Department of Botany, Mycology and Plant Pathology Division, University of Lucknow, LUCKNOW (U.P.) INDIA

REFERENCES

Adaskaveg, J.E., Forester, H. and Sommer, N.F. (2002). Principles of post harvested pathology and management of decay of edible horticulture crops, p. 163-195: In A.A. Kader (Ed.). *Post harvested technology of horticulture crops*. 3rd. University Calif. Agr. Natural Resource. Pub. 3311.

Aggarwal, R., Tewari, A.K., Srivastava, K.D. and Singh, D.V. (2004). Role of antibiosis in the biological control of spot blotch (*Cochliobolus sativus*) of wheat by *Chaetomium globosum*. *Mycopathologia,* **157** : 369–377.

Aggarwal, R., Tiwari, A. K., Dureja, P. and Srivastava, K.D. (2007). Quantitative analysis of secondary metabolite produced by *Chetomium globosum* kunze. ex fr., *J. Biol. Control,* **21**(1):163-168

Copping, L.G. and Menn, J.J. (2000). Biopesticides: a review of their action, applications and efficacy. *Pest Manag. Sci.,* **56** : 651–767.

Di Poetro, A., Gut-Rella, M., Pachlatko, J.P. and Schwinn, F.J. (1992). Role of antibiotics produced by *Chaetomium globosum* in biocontrol of *Pythium ultimum*, a causal agent of damping-off. *Phytopathology,* **82** : 131–135.

Ellis,M.B.(1971). *Dematiaceous hyphomycetes common wealth mycological institute*. Kew, Surry, Englend.

Habbard, J. P., Harmen, G. E. and Eckernode, C.J. (1982). Interaction of a biological control agent *Chetomium globosum* with the seed coat microflora. *Canadian J. Microbial.,* **28**:431-437

Istvan, U. (2002). Transforming natural products into natural pesticides – experience and expectations. *Phytoparasitica,* **30** : 439–442.

Khol, J., Molhoek, W.H.L., Vander, Plas, C.H. and Fokkema, N.T. (1995). Effect of *Ulocladium atrum* and other antagonistics on sporulation of *Botrytis cineria* on dead lily leaves to field condition. *Phyopathology,* **85**:393-401.

Kim, S. B. and Hwang, K. B. (2007). Microbial fungicide in the control of plant disease. *J. Phytopathology,* **155** (11-12) : 641-653.

Kommendahl, T. and Chand, M. Z. (1975). Biocontrol of corn root infection in the field by seed treatment with the antagonist. *Phytopathology,* 65 : 296-300.

Mandal, S., Srivastava, K.D., Aggarwal, R. and Singh, D.V. (2004). Mycoparasitic action of some fungi on spot blotch pathogen (*Drechslera sorokiniana*) of wheat. *Indian Phytopath.*, **52**: 39–43.

Misaro, T., Ko, K. and Yamagachi, I. (1977). Use of antibiosis in the agriculture. *Adv. Appl. Microbiol.,* **21**:53-88.

Rodrigues, K. F., Costa,G. L. and Carvalho, M. P. (2005). Evolution of extract produces by some tropical fungi as potential cholinesterase inhibiters. *World J. Microbiol. & Biotechnol.,* **21**(8-9) : 1671-1621.

Soytong, K. (1992). Antagonism of *Chetomium Cupreum* to *Pyricularia Oryzae*. *J. Plant Prat. Tropics,* **9**: 17-24.

Soytong, K. (1995). *Chetomium,* as a biocontrol agent against plant pathogens. Proceedings of the XIII International Plant Protection Congress, 2-7 July, 1995. The Hague-The Netherlands.

Tveit, M. and Moore, M.B. (1954). Isolates of *Chatomium* that protects oat from *Helminthosporium Victoriae*. *Phytopathology,* **44**: 686-689.

Usuwan, P. and Sotong, K. (1998). Biological control of Phytophthora rots of citrus in the field. Proceedings of the 24th Congress on Science and Technology, 19-20 October, 1998, Thailand.

Vannacci, G. and Harman, G.E. (2004). Biocontrol of seed borne *Alternaria raphani* and *A. brassicicola. Canadian. J. Microbiol.*, **34**: 850– 856.

Walther,I.D. and Gindrat, D.(1988). Biological control of damping-off of sugarbeet and cotton with *Chetomium globosum* or a fluorescent *Pseudomonas* sp. *Canadian J. Microbiol.,* **34** : 631-637.

Xin, Y. F. and Shang, J.J. (2005). Biocontrol trial of *Chetomium spirle* ND 35 against apple cancer. pp. 436-437.

****