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Mycoparasitic and Antagonistic Inhibition on *Phytophthora cinnamomi* Rands by Microbial Agents Isolated from Manure Composts

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Abstract: We isolated bacteria and fungi from composted chicken, sheep, cow and horse manure and screened each isolate for their ability to suppress *P. cinnamomi* in dual-culture *in vitro* assays. Of the 180 isolates, including 31 actinomycetes, 64 fungi, 44 fluorescent pseudomonads and 41 endospore-forming bacteria, 45 isolates significantly inhibited the growth of *P. cinnamomi* on plates. The inhibitory microbes included 24 fungi (including *Trichoderma* sp., *Gliocladium penicillioides* and *Fusarium* sp.), 10 actinomycetes (all *Streptomyces* sp.), 7 fluorescent pseudomonads (*Pseudomonas* sp.) and 4 endospore-forming bacteria (*Bacillus* sp.). The most common mode of action observed was antibiosis, although mycoparasitism, indicated by parallel hyphal growth, hyphal coiling, appressorium formation and direct penetration, was also observed with one isolate of *Trichoderma*. These results help to explain the role of microbes in the suppression and biological control of *P. cinnamomi* by composted manures.

Key words: Antagonism, antibiosis, mycoparasitism, biocontrol, *phytophthora cinnamomi*, bacillus, pseudomonas, actinomycete

INTRODUCTION

A number of study shows that antagonistic microbes are potential as biocontrol agent against plant pathogen. Sid *et al*. (2003) found ten out of more than 500 isolates from sweet pepper rhizosphere area were found to be significant to suppress Phytophthora root rot. While, four out of 231 tomato phylloplane and rhizosphere isolates were antagonistic to late blight Phytophthora (Lourenco *et al*., 2004). In addition, mycoparasitic bacteria collected from aerial parts of the cocoa plant have shown great promise in the control of black pod, caused by Phytophthora palmivora (Hoopen *et al*., 2003). Okamoto *et al*. (2000) reported that three bacterial strains isolated from rhizosphere of angelica trees strongly inhibited mycelial growth of *P. cactorum*. Recently, twelve isolates of fluorescent pseudomonads from rhizosphere of pepper significantly inhibit Phytophthora blight (Rajkumar *et al*., 2005)

Other studies on pot trials also indicate some potential biocontrol agents against Phytophthora. Composted chicken manure was found strongly suppressive to root rot caused by *P. cinnamomi* (Aryantha *et al*., 2000). While, composted cow manure was significantly suppressive to *P. capsici* (Khan *et al*., 2004). Whether the reduced disease is due to decreased pathogen activity or to induced resistance in the plant, or whether there are other mechanisms operating to suppress the pathogen, is still not fully understood. Antagonistic microorganisms found in composts and manures, including *Streptomyces* (Cornell, 1991), *Aspergillus flavipes* (Sztejnberg and Tsao, 1986), *Penicillium janthinellum* (Ownley and Benson, 1992), *Chaetomium globosum*, *Gliocladium virens* and *Trichoderma viride* (Heller and Theiler-Hedtrich, 1994) were found to inhibit the growth of *P. cinnamomi* in dual culture assays. Present study examines the role of antagonistic microorganisms isolated from composted manures that were shown to suppress *P. cinnamomi* in a previous study (Aryantha *et al*., 2000).

MATERIALS AND METHODS

Isolation of antagonists: Antagonistic microorganisms were isolated from composted manures used in a previous

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study in 1996 (Aryantha *et al*., 2000). Serial dilution and plating on selective media separated four groups of microorganisms, actinomycetes, endospore forming-bacteria, fluorescent pseudomonads and fungi. Actinomycetes were isolated on chitin agar (Lingappa and Lockwood, 1962), endospore forming bacteria were isolated after incubating dilutions of compost in nutrient agar amended with 1% Nystatin at 80°C for 10 min (Weste and Vithanage, 1978), fluorescent pseudomonads were isolated on novobiocin-penicillin-cycloheximide (NPC) medium (Sands and Rovira, 1970) and fungi were isolated on PDA amended with 1% chloramphenicol (Booth, 1971). Colonies were identified on the basis of morphology, colour and growth rate and were transferred onto fresh medium into pure culture. All pure bacterial isolates were transferred onto Nutrient Agar (NA), while actinomycetes and fungi were grown on potato dextrose agar (PDA). Isolates were maintained in test tubes and kept at 5°C.

Testing of antagonists on agar plates: Isolates from each group of microbes-actinomycetes, endospore-forming bacteria, fluorescent pseudomonads and fungi-were tested for their ability to inhibit the growth of *P. cinnamomi*. Four agar discs, colonised for 3 days by one of the fungi, or four streaks of a bacterium or actinomycete, were placed on the surface of a Petri dish containing PDA, arranged around a central disc of *P. cinnamomi*. Uninoculated agar discs of the same medium served as controls. The diameter of the *P. cinnamomi* colony was measured after 5 days incubation at 20°C in the dark and organisms that inhibited colony growth by at least 39% were selected for further study.

Examination of antagonistic and mycoparasitic mechanisms: Each isolate was tested for both antibiotic and mycoparasitic activities against *P. cinnamomi*. Antibiosis was observed directly by light microscopy of paired cultures. Mycoparasitism was observed in dual cultures of the pathogen and antagonist on a thin film of agar, prepared by pouring approximately 1 mL molten PDA on a sterile glass slide. Each agar-covered slide was inoculated with an agar disc colonised by *P. cinnamomi* placed in the centre of the slide, while 2 days later each end was inoculated with an agar disc colonised by the antagonist. Slides were placed on moist sterile tissue paper in sealed petri dishes and examined three, four and five days later. One to three drops of 70% ethanol were added to the surface of the colonised agar, followed by two to three drops of lactophenol cotton blue. The slides were covered with cover slips before being examined under the light microscope. Hyphal interactions including coiling, parallel hyphal growth, appressoria formation or direct penetration were examined under light microscope. Freeze-dried slides were examined using scanning electron microscopy (Philips XL30 FEG Field Emission Scanning Electron Microscope).

Identification: Fungal and actinomycete isolates antagonistic to *P. cinnamomi* were identified on morphological characters (conidia, conidiophore, hyphae) observed after staining with lactophenol cotton blue (Rifai, 1969; Carmichael *et al*., 1980; Rehner and Samuels, 1994). Gram differentiation of bacterial isolates used a rapid method described by Suslow *et al*. (1982). Two drops of 3% (w/v) potassium hydroxide (KOH) solution were placed on a clean glass slide. With a flat wooden toothpick, bacterial cells were transferred from agar plates to the drop of KOH. After rapidly agitating the solution in a circular motion for 5-10 seconds the toothpick was raised and lowered to detect a stringing effect. If the stringing effect occurred in 15 sec the isolate was considered as Gram negative.

Data analysis: Significant inhibition of *P. cinnamomi* in the Petri dish screening assays was identified using a one-way ANOVA (Minitab.11, Minitab® Inc, Pennsylvania, USA).

RESULTS

Isolates: More than 180 actinomycetes, endospore-forming bacteria, fungi and fluorescent pseudomonads were isolated from chicken manure and sheep composts (Table 1). The most frequently isolated group, mostly isolated from chicken manure compost, was the fungi (64 isolates). The second most frequent group isolated from composted manure was the fluorescent pseudomonads (44 isolates), followed by endospore-forming bacteria (41 isolates) and actinomycetes isolated mostly from sheep manure compost (31 isolates).

Table 1: Number of isolates of each microbial group (actinomycete, endospore-forming bacteria, fungi and fluorescent pseudomonads) recovered from Mt Derimut soil 5 weeks after the addition of composted manures

	Non treated	Chicken manure	Cow manure	Horse manure	Sheep manure	Total
Actinomycetes	2	2	7	5	15	31
Endospore-forming bacteria	12	6	2	15	6	41
Fungi	9	20	11	12	12	64
Fluorescent pseudomonads	3	18	8	8	7	44
Total	26	46	28	40	40	180

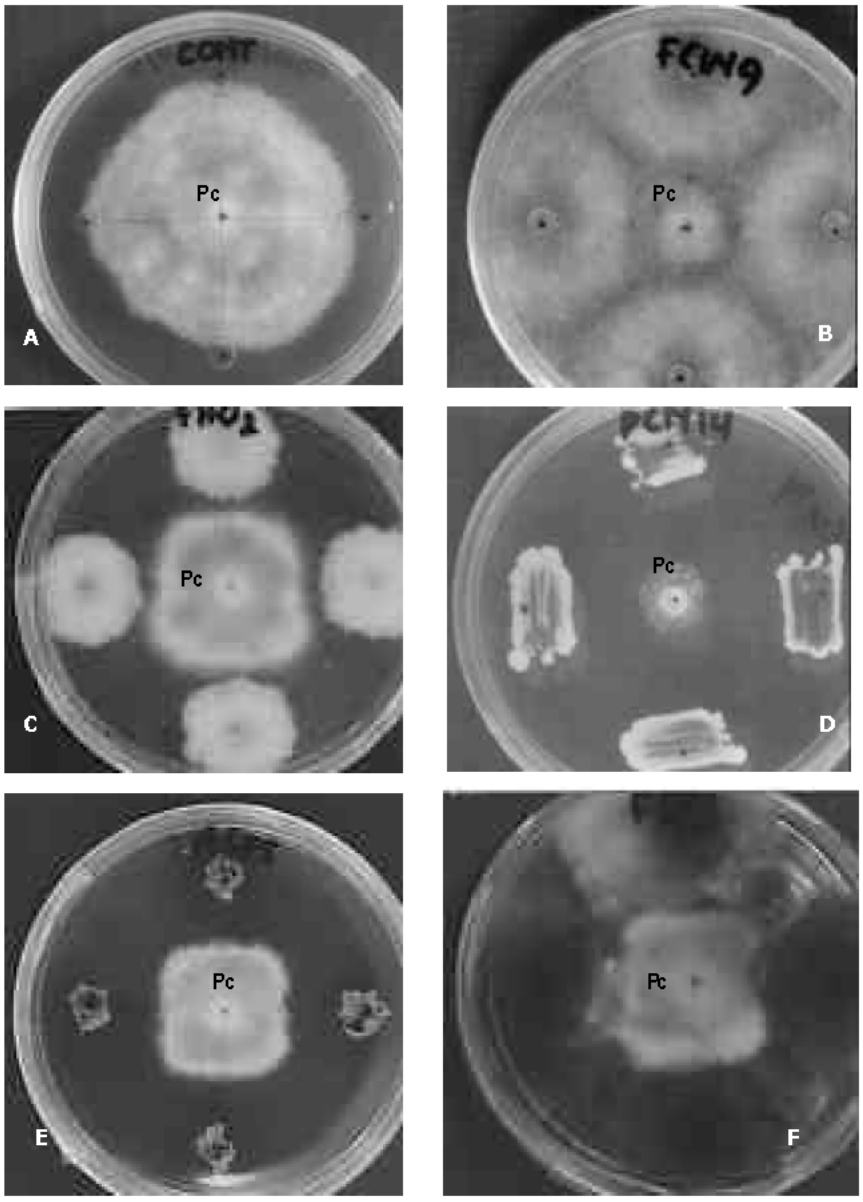

Fig. 1: Some positive isolates against *P. cinnamomi* [Pc] (at centre) identified as inhibition zone of *in vitro* test. (A) Control; (B) *Trichoderma* sp. (fcw9); (C) *Gliocladium penicillioides* (fho2); (D) *Pseudomonas* sp (pcn14); (E) *Streptomyces* sp. (asp5); (F) *Fusarium* sp. (fsp6)

***In vitro* inhibition test:** Forty five isolates, including 24 fungi, 10 actinomycetes, 7 fluorescent pseudomonads and 4 endospore-forming bacteria significantly inhibited the growth of *P. cinnamomi* on plates (Table 2). Figure 1 shows some examples of isolates (bacteria, actinomycetes and fungi) that inhibit the growth of *P. cinnamomi* in culture. The most common mode of action observed was antibiosis, which appeared in co-inoculated plates as an inhibition zone. Lysis of *P. cinnamomi* hyphae was also associated with some bacterial, fungal and actinomycete isolates. A very strong growth inhibition was observed with some isolates (Fig. 1 C-E).

Differences in antibiotic reactions were observed with different isolates. Some bacteria and fungi induced abnormal stunted, highly branched hyphal tips and swollen hyphae at the edge of *P. cinnamomi* colonies.

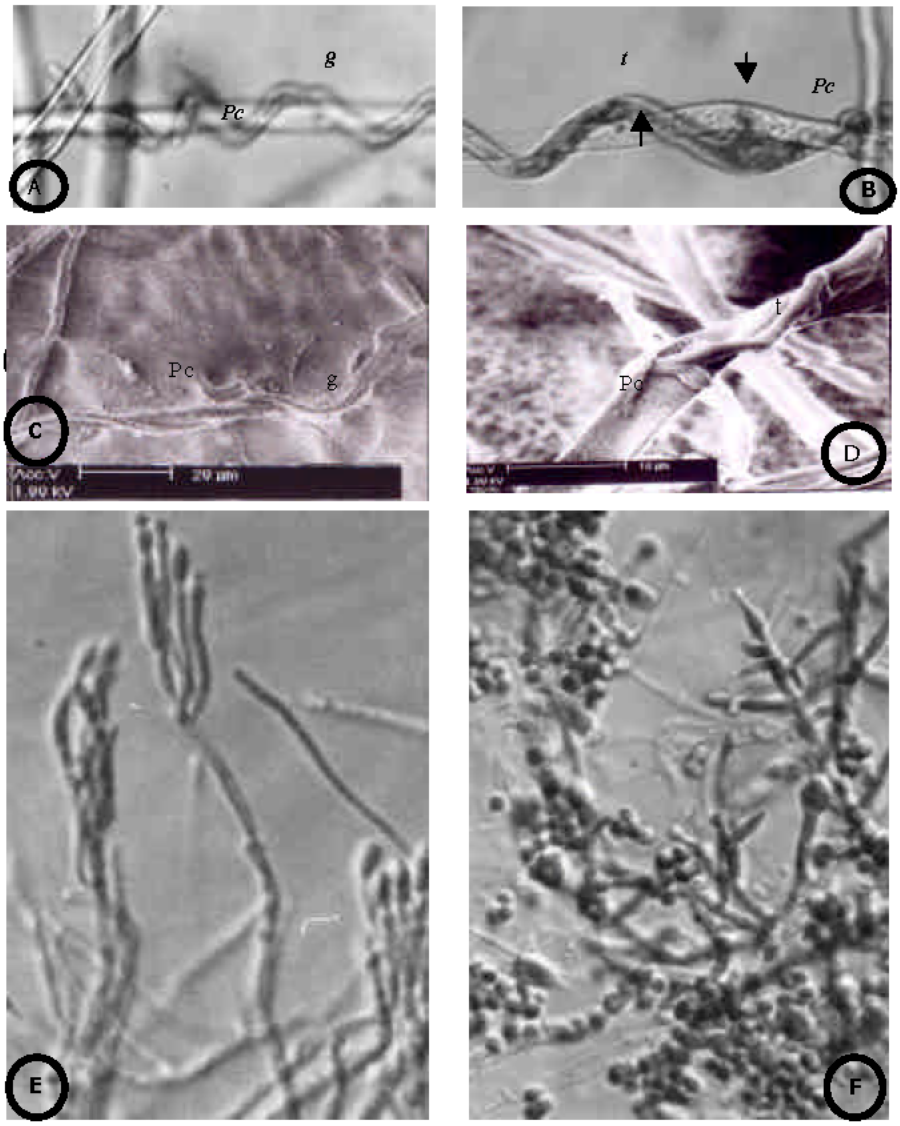

Fig. 2: Mycoparasitic interaction of fungal isolates with *P. cinnamomi* (Pc). (A) Coiling by *Gliocladium* (g); (B) Penetration by *Trichoderma* sp. (t) with appressorium (arrow); (C) coiling observed with SEM; (D) Hyphal penetration by *Trichoderma* sp. observed with SEM; (E) Reproductive structure of *Gliocladium penicillioides*; (F) Reproductive structure of *Trichoderma* sp.

This led to stunting of the colony and ultimately pathogen growth stopped. Stunting and prolific chlamydospore formation was also observed in the presence of actinomycetes.

Mycoparasitism by fungal isolates was also observed (Fig. 2) and both antibiosis and mycoparasitism were observed in the presence of some fungi (Fig. 1B and F). Some isolates of *Gliocladium* sp. displayed either antibiosis or mycoparasitism, but rarely together and only in dual culture slides. Mycoparasitism was observed as coiling, penetration, direct contact and parallel growth alongside host hyphae. Host hyphae were seen to absorb a pigment apparently originating from the antagonist, becoming red, before collapsing and dying (Fig. 2). Clear evidence of appressorial attachment and hyphal penetration was only observed with one isolate of *Trichoderma* sp. (Fig. 2B and D).

Table 2: Fourty five (45) isolates found to be antagonistic to *P. cinnamomi in vitro* out of total 180 isolates.

Type	Source	Code	Percent inhibition of colony diameter after 5 days
Actinomycetes	Cow	acw1	40
	Horse	aho3	39
	Horse	aho5	40
	Sheep	asp4	40
		asp5	42
		asp8	40
		asp9	39
		asp12	40
		asp14	40
		asp15	41
Endospore-forming bacteria	Untreated	bcl6	40
		bcl9	40
		bcl11	54
		bcl12	45
Fluorescent pseudomonads	Chicken	pcn9	45
		pcn14	76
	Cow	pcw1	49
	Horse	pho5	50
		pho7	45
		pho8	46
	Sheep	psp5	47
Fungi	Chicken	fcn4	70
		fcn5	49
		fcn7	45
		fcn8	50
		fcn9	42
		fcn14	48
		fcn15	45
		fcn18	70
	Cow	fcw5	45
		fcw7	68
		fcw9	57
	Horse	fho1	53
		fho2	60
		fho5	45
		fho8	47
		fho10	50
		fho11	47
		fho12	47
	Sheep	fsp1	47
		fsp2	44
		fsp4	50
		fsp6	45
		fsp7	44
		fsp10	60

Identification of the antagonistic isolates: Only the isolates which strongly antagonised *P. cinnamomi* were identified to genus level. All the actinomycetes were filamentous mycelial, sporulating or conidia-producing, aerobic, isolated from soil and produced antibiotics. Based on these features they are classified as *Streptomyces* group (Collins, 1964).

Bacteria were classified as *Bacillus* and *Pseudomonas* based on their growth on selective media, Gram staining, cell shape and aerobic growth (Weste and Vithanage, 1978). All antagonistic bacteria were rod shaped, the endospore-forming bacteria were Gram positive, while the fluorescent pseudomonads were Gram negative.

Based on the colour, shape and structure of conidia, sterigma and conidiophore, growth rate, pigmentation and colony morphology, the antagonistic fungi were identified as *Fusarium*, *Gliocladium*, *Penicillium* and *Trichoderma*. Two of these are shown in Fig. 2 E and F. *Fusarium* is typified by canoe shaped conidia and the production of red pigment on PDA (Fig. 1F). *Gliocladium* is very similar to *Penicillium* in conidia, phialide, sterigma and conidiophore structure. Both have phialides, however, *Gliocladium* typically produces mucilaginous liquid droplets on the surface of sporulating colonies (Petch, 1938). Two species of *Gliocladium* were identified as *G. penicillioides* and *G. roseum*. *G. penicillioides* produces long, slender, dark green conidia while those of *G. roseum* are oval and rosy (Rehner and Samuels, 1994; Fig. 2E), but not on *G. roseum*. Instead of sterigma, *Trichoderma* bears light green conidia on short bottle-like phialides (Fig. 2F) and has a fast growth rate (Rifai, 1969).

DISCUSSION

Microbial isolations were made during the examination of microbial populations recovered from manure compost (Aryantha *et al*., 2000). The isolation of microbes was facilitated by the dilution plate method and transfer of single colonies to fresh medium. This technique provided a single step of isolation and combined with selective media, enabled specific groups of microorganisms to be isolated directly. Inhibitory organisms were isolated from untreated soil and from manure-compost amended soils, with no apparent correlation to the relative ability of these treatments to suppress *P. cinnamomi* in glasshouse trials (Aryantha *et al*., 2000).

One aspect of antagonism observed in the *in vitro* trials was mycoparasitism. Most interactions between *P. cinnamomi* and the antagonists (*Gliocladium* and *Trichoderma*) observed in this study involved coiling and parallel growth. Dennis and Webster (1971) also found that the majority of 80 *Trichoderma* isolates coiled around hyphae of *Fomes* and *Rhizoctonia*. *Trichoderma* sp. also produced an infection or penetration structure similar to the appressorium described by Elad *et al.* (1980). Chambers and Scott (1995) also found that *Trichoderma hamatum* and *T. pseudokoningii* inhibit *P. cinnamomi,* displaying parallel growth, hyphal coiling and appressorium formation. Appressoria are produced by other mycoparasitic fungi including *Stachybotris elegans* parasitising *Rhizoctonia solani* (Benyagoub *et al*., 1994) and *Piptocephalis* (Manocha, 1991).

A few minutes before hyphal contact with either *Gliocladium* or *Trichoderma*, *P. cinnamomi* hyphae lyse. Dennis and Webster (1971) reported the same phenomenon occurring on *Fomes* and *Rhizoctonia* when challenged by *Trichoderma* sp. Antibiosis of *Botrytis cinerea* by *Trichoderma harzianum* results from degradation of fungal cell walls by chitinolytic, cellulolytic, glucanase and xylanase activity (Benhamou and Chet, 1993; Belanger *et al*., 1995). *Gliocladium virens* also produces endochitinase, 1,4 β-chitobiosidase, glucan N-acetyl-β-D-glucosaminidase and glucan 1,3-β-glucosidase (DiPietro *et al*., 1993).

In this study hyphae of *Gliocladium penicillioides* were never observed to overlap the *P. cinnamomi* colony. In all cases *P. cinnamomi* stopped growing before direct contact was made, presumably in response to diffusible inhibitors released by the antagonist. However, when *P. cinnamomi* colonies were inoculated with conidia of *G. penicillioides* or *G. virens*, young mycelia of G. *penicillioides* aggressively parasitised the host using both antibiosis (DiPietro *et al*., 1993) and mycoparasitism (Tu and Vaartaja, 1981). Antibiotics from *Trichoderma* and *Gliocladium* were first reported by Weindling and Nelson (1936). Kelley and Kabana (1976) found that the presence of *Trichoderma* sp. reduced the development of *P. cinnamomi* as indicated by β-glucosidase and phosphatase activities in a semi-*in vivo* study of soil substrate. Enzymatic activities of cellulose and laminarinase incorporated in mulch were demonstrated to be effective against *P. cinnamomi* lately (Downer *et al*., 2001). *Trichoderma viride* and *Gliocladium virens* were also found to inhibit *P. cinnamomi in vitro* (Heller and Theiler-Hedtrich, 1994). There is no previous report that *Fusarium* sp. inhibits *P. cinnamomi*, nevertheless *Fusarium proliferatum* significantly reduces downy mildew incidence on grape, caused by another oomycete, *Plasmopara viticola* (Falk *et al*., 1996).

Stirling *et al.* (1992) found that three fluorescent pseudomonads, nine actinomycetes and *Serratia* sp. were antagonistic to *P. cinnamomi* in plate assays out of 164 isolates. One isolate of fluorescent pseudomonads (PCn14) gave a very strong inhibition to *P. cinnamomi* up to 76% (Table 2). Other studies reported that *Pseudomonas cepacia* and *P. fluorescens* were significant to suppress *P. cinnamomi* growth *in vitro* and *in vivo* (Turnbull *et al*., 1992; Yang *et al*., 2001). Meanwhile, recent report indicates that *Pseudomonas* sp. is a successful biocontrol agent to control take-all disease applied in Washington State (Weller *et al*., 2002).

Present results show that actinomycetes isolates ranks the second best after fungi in total number of isolates which significantly inhibit *P. cinnamomi*. Previous study discovered that *Streptoverticillium albireticuli* (Park *et al*., 2002) and *Streptomyces* (Shimazu *et al*., 2000) significantly inhibited *P. cinnamomi in vitro*. In addition, You *et al.* (1996) isolated 1600 isolates of actinomycetes and found that all of them inhibit *P. cinnamomi* growth *in vitro* by at least 50%.

Further studies are required to evaluate the potential use of these antagonistic and mycoparasitic isolates in biological control and to determine the most active isolates or combinations, application frequency and amounts. Nevertheless, these results provide an explanation for the activity of composted manures and illustrate their potential for biological control of *P. cinnamomi*, especially in nursery situations.

REFERENCES

Aryantha, I.P., R. Cross and D.I. Guest, 2000. Suppression of *Phytophthora cinnamomi* Rands in potting mixes amended with uncomposted and composted animal manures. Phytopathology, 90: 775-782.

Belanger, R.R., N. Dufour, J. Caron and N. Benhamou, 1995. Chronological events associated with the antagonistic properties of *Trichoderma harzianum* against *Botrytis cinerea* -indirect evidence for sequential role of antibiosis and parasitism. Biocontrol Sci. Technol., 5: 41-53.

Benhamou, N. and I. Chet, 1993. Hyphal interactions between *Trichoderma harzianum* and *Rhizoctonia solani*: Ultrastructure and gold cytochemistry of the mycoparasitic process. Phytopathology, 83: 1062-1071.

Benyagoub, M., S.H. Jabaji-Hare, G. Banville and P.M. Charest, 1994. *Stachybotrys elegans* a destructive mycoparasite of *Rhizoctonia solani*. Mycol. Res., 98: 493-505.

Booth, C., 1971. Fungal Culture Media. In: Methods in Microbiology. Booth, C. (Eds.). (Academic Press: London). 4: 67

Carmichael, J.W., W.B. Kendrick, I.L. Conners and L. Sigler, 1980. 'Genera of Hyphomycetes' (University of Alberta Press: Edmonton), pp: 211, 220, 277

Chambers, S.M. and E.S. Scott, 1995. *In vitro* antagonism of *Phytophthora cinnamomi* and *P. citricola* by isolates of *Trichoderma* sp. and *Gliocladium virens*. J. Phytopathol., 143: 471-477.

Collins, C.H., 1964. Microbiological Methods: Mycelial Bacteria-the Actinomycetes. (Butterworths: London), pp: 228-239

Cornell, L.G., 1991. Actinomycete interactions with *Phytophthora cinnamomi* Rands. Phytophthora Newslett., 17: 31-32.

Dennis, C. and J. Webster, 1971. Antagonistic properties of species-group of *Trichoderma*. III Hyphal interactions. Trans. Br. Mycol. Soc., 57: 363.

DiPietro, A., M. Lorito, C.K. Hayes, R.M. Broadway and G.E. Harman, 1993. Endochitinase from *Gliocladium virens*: Isolation, characterisation and synergistic antifungal activity in combination with gliotoxin. Phytopathology, 83: 308-313.

Downer, A.J., J.A. Menge and E. Pond, 2001. Effects of cellulytic enzymes on *Phytophthora cinnamomi*. Phytopathology, 91: 839-846

Elad, Y., I. Chet and J. Katan, 1980. *Trichoderma harzianum*: A biological control agent effective against *Sclerotium rolfsii* and *Rhizoctonia solani*. J. Phytopathol., 70: 119-121.

Falk, S.P., R.C. Pearson, D.M. Gadoury, R.C. Seem and A. Sztejnberg, 1996. *Fusarium proliferatum* as biological control agent against grape downy mildew. Phytopathology, 86: 1010-1017.

Heller, W.E. and R. Theiler-Hedtrich, 1994. Antagonism of *Chaetomium globosum*, *Gliocladium virens* and *Trichoderma viride* to four soil-borne *Phytophthora* species. J. Phytopathol., 141: 390-394.

Hoopen, G.M.T., R. Reese, P. Aisa, T. Stirrup and U. Krauss, 2003. Population dynamics of epiphytic mycoparasites of the genera Clonostachys and Fusarium for the biocontrol of black pod (Phytophthora palmivora) and moniliasis (*Moniliophthora roreri*) on cocoa (*Theobroma cacao*). Mycol. Res., 107: 587-596.

Kelley, W.D. and R.R. Kabana, 1976. Competition between *Phytophthora cinnamomi* and *Trichoderma* sp. in autoclaved soil. Can. J. Microbiol., 22: 1120-1127.

Khan, J., J.J. Ooka, S.A. Miller, L.V. Madden and H.A.J. Hoitink, 2004. Systemic resistance induced by *Trichoderma hamatum* 382 in cucumber against *Phytophthora crown* rot and leaf blight. Plant Dis., 88: 280-286.

Lingappa, Y. and J.I. Lockwood, 1962. Chitin media for selective isolation and culture of Actinomycetes. Phytopathology, 52: 317-325.

Lourenco, V., L.A. Maffia, R.S. Romeiro and E.S.G. Mizubuti, 2004. Isolation and selection of antagonists to *Phytophthora infestans* on tomato. Phytopathology, 94: No. 6.

Manocha, M.S., 1991. Physiology and Biochemistry of Biotrophic Mycoparasitism. In 'Handbook of Applied Mycology. Volume 1: Soil and Plants'. Arora, D.K., B. Rai, K.G. Mukerji and G.R. Knudsen (Eds.). (Marcel Dekker Inc.: Hongkong), pp: 273-300.

Okamoto, H., M. Sato, Y. Miyata, M. Yoshikawa and M. Isaka, 2000. Biocontrol of Phytophthora root rot of Angelica trees by *Enterobacter cloacae* and *Serratia ficaria* strains. J. General Plant Pathol., 66: 86 - 94.

Ownley, B.H. and D.M. Benson, 1992. Evaluation of *Penicillium janthinellum* as a biological control of *Phytophthora* root rot of azalea. J. Am. Soc. Hortic. Sci., 117: 407-410.

Park, J.O., K.A. El-Tarabily, E.L. Ghisalberti and J. Sivasithamparam, 2002. Pathogenesis of *Streptoverticillium albireticuli* on *Caenorhabditis elegans* and its antagonism to soil-borne fungal pathogens. Lett. Applied Microbiol., 35: 361-365.

Petch, T., 1938. Gliocladium. Trans. Br. Mycol. Soc., 22: 257-263.

Rajkumar, M., W.H., Lee, K.J. Lee, 2005. Screening of bacterial antagonists for biological control of *Phytophthora* blight of pepper. J. Basic Microbiol., 45: 1 55 - 63.

Rehner, S.A. and G.J. Samuels, 1994. Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. Mycol. Res., 98: 625-634.

Rifai, M.A., 1969. A revision of the genus *Trichoderma*. Mycol. Papers, 116: 1-56.

Sands, D.C. and A.D. Rovira, 1970. Isolation of fluorescent pseudomonads with a selective medium. Applied Microbiol., 20: 513-514.

Shimazu, M., Y. Nakagawa, Y. Sato, T. Furumai, Y. Igarashi, H. Onaka, R. Yoshida and H. Kunoh, 2000. Studies of endophitic actinomycetes (I) Streptomyces sp. Isolated from Rhododendron and its antifungal activity. J. General Plant Pathol., 66: 360-366

Sid, A., M. Ezziyyani, C. Egea-Gilabert and M.E. Candela, 2003. Selecting bacterial strains for use in the biocontrol of diseases caused by *Phytophthora capsici* and *Alternaria alternata* in sweet pepper plants. Biol. Plant., 47: 569 - 574.

Stirling, A.M., A.C. Hayward and K.G. Pegg, 1992. Evaluation of the biological control potential of bacteria isolated from a soil suppressive to *Phytophthora cinnamomi*. Aust. Plant Pathol., 21: 133-142.

Suslow, T.V., M.N. Schroth and M. Isaka, 1982. Application of a rapid method for Gram differentiation of plant pathogenic and saprophytic bacteria without staining. Phytopathology, 72: 917-918.

Sztejnberg, A. and P.H. Tsao, 1986. Evaluation of certain hyphomycetes as biocontrol agents for azalea root rot caused by *Phytophthora cinnamomi*. Phytopathology, 76: 847.

Tu, J.C. and O. Vaartaja, 1981. The effect of the hyperparasite (*Gliocladium virens*) on *Rhizoctonia* root rot of white beans. Can. J. Bot., 59: 22.

Turnbull, L.V., H.J. Ogle, A.M. Stirling and P.J. Dart, 1992. Preliminary investigations into the influence of *Pseudomonas cepacia* on infection and survival of proteas in *Phytophthora cinnamomi* infected potting mix. Sci. Hortic., 52: 257-263.

Weindling, R. and O.H. Nelson, 1936. The isolation of toxic substances from the culture filtrate of *Trichoderma*. Phytopathology, 26: 1070.

Weller, D.M., J.M. Raaijmakers, B.B.M. Gardener and L.S. Thomashow, 2002. Microbial populations responsible for specific soil suppressiveness to plant pathogen. Ann. Rev. Phytopathol., 40: 309-348

Weste, G. and K. Vithanage, 1978. Effect of *Phytophthora cinnamomi* on microbial populations associated with the roots of forest flora. Aus. J. Bot., 26: 153-167.

Yang, C., D.E. Crowley and J.A. Menge, 2001. 16S rDNA fingerprinting of rhizosphere bacterial communities associated with healthy and *Phytophthora* infected avocado roots. FEMS Microbiol. Ecol., 35: 129-136.

You, M.P., K. Sivasithamparam and D.J. Kurtboke, 1996. Actinomycetes in organic mulch used in avocado plantations and their ability to suppress *Phytophthora cinnamomi*. Biol. Fert. Soils, 22: 237-242.