



Antagonistic interactions among different species of leaf litter fungi of Central Luzon State University

Waing KGD^{1*}, Abella EA¹, Kalaw SP¹, Waing FP² and Galvez CT¹

¹ Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines 3120 email krstngcrdlsng@yahoo.com

² Philippine Rice Research Institute, Maligaya, Science City of Muñoz, Nueva Ecija, Philippines 3119

Waing KGD, Abella EA, Kalaw SP, Waing FP, Galvez CT 2015 – Antagonistic interactions among different species of leaf litter fungi of Central Luzon State University. *Plant Pathology & Quarantine* 5(2), 122–130, Doi 10.5943/ppq/5/2/9

Abstract

Fungal decomposers isolated from leaf litters may exhibit antagonistic interactions which can influence the growth of other microorganisms and breakdown of litters. Thus, to identify new species of fungi for biological control, the interactions among different species of leaf litter decomposing fungi in Central Luzon State University were evaluated. Nine species of fungi isolated from leaf litters namely; *Aspergillus flavus*, *A. niger*, *A. niveus*, *Colletotrichum gloeosporioides*, *Fusarium semitectum*, *Neosartorya fisheri*, *Penicillium citrinum*, *P. decumbens* and *P. purpurogenum* were used in this study. Dual culture method was used to determine the interaction between fungal isolates. A 10-mm disc of 7 day old culture of each fungal isolate was placed at one end of the PDA plate and another fungal isolate of the same size and age was placed on the other end. Interactions were described as antagonism and mutual antagonism. Mutual inhibition at a distance, mutual slight inhibition and antagonism were the observed fungal interactions between fungal isolates when paired. A total of 15 pairs showed mutual inhibition at a distance, 11 pairs recorded mutual slight inhibition and 10 pairs exhibited antagonism. Hyphal interactions such as hyphal folding, bending and coiling were observed in fungi that were victims in an antagonistic interaction. Hence, this study revealed that some species of *Aspergillus*, *Penicillium*, *Fusarium* and *Neosartorya* exerted antagonistic effect on other species of fungal isolates. The biological control of pathogens by antagonistic microorganisms can be used as an effective alternative to existing disease management strategies.

Key words – antagonism – dual culture – hyphal coiling – mutual inhibition

Introduction

Fungal organisms are extremely diversified, versatile and are important players in the ecosystems. In addition to their main role as decomposers, fungi established numerous interactions with their hosts, substrates, competitors and even with abiotic factors in the environment (Dix & Webster 1995). Some of these interactions may be beneficial to all partners involved (mutualistic interactions), whereas others are detrimental for at least one partner (antagonistic interactions). Interactions between fungi can be used to determine when a fungus used as a biological control agent against other fungi. Interactions within and among microbial communities are numerous and

they range from synergistic and mutualistic to antagonistic and parasitic. Antagonistic and parasitic interactions have been exploited in the area where biological control of plant pathogenic microorganisms occur (Duffy et al. 2003). The interactions can be dynamic at both ecological and evolutionary time scales, and shift along a natural continuum from mutualism to antagonism, depending on shifting cost/benefit ratios (Kiers & Denison 2008).

Antagonism means any activity of one organism which in some way adversely affects another growing in association with it. This includes antibiosis, competition and exploitation (Khara & Hadwan 2008). Two organisms may interact in the presence of one in some way affects the performance of the other. Mechanisms of fungal antagonism and defence often include the production of biologically active metabolites by one species that exert effects on potential competitors and (or) predators. Studies show that such ecological phenomena leads to the discovery of novel and potentially beneficial bioactive fungal metabolites (Gloer 1995).

Another form of association between microbes is antibiosis. It is an association between two microorganisms which is detrimental to at least one of them caused by the release of metabolites or cell components (Haggag & Mohamed 2007). It is generally recognized as the principal mechanism of interference competition by which fungi may exclude other organisms from resources potentially available to each other. The mutual intermingling growth of two organisms without any zone of inhibition indicates the failure of the production of antibiotics either by the pathogen (or) by the antagonist whereas; formation of zone of inhibition is an indication for the production of antibiotic substances either by the pathogen against antagonistic fungi or vice versa (Gomathi & Ambikapathy 2011). Inhibition by antibiosis is often species-specific and a response only occurs when appropriate species meet. Hence, antibiosis is the production of secondary metabolites, that have an antimicrobial effect even at low concentrations (Howell 1998) by producing volatile components and non-volatile antibiotics that are inhibitory against a range of soil borne fungi (AL-Saeedi & AL-Ani 2014).

Therefore, determination of interaction among fungal isolates would possibly discover fungi as bio-control against other microorganisms. Biological control, being relatively cheaper, less laborious and environmentally friendly, makes an attractive option (Adebola & Amadi 2010). According to Okigbo & Ikediugwu (2000), biological control has proved to be effective in the control of pathogens and has the advantage of non-essential periodic application of chemical fungicides. This may also reduce the diseases caused by certain microorganisms and lessen the effect of undesirable pests for the production of quality crops and less usage of commercially available pesticides. Hence, the biological control of plant diseases is now making an increasing attention, although the potential of biological control via the effect of phyllosphere antagonists has been understood (Evueh & Ogbebor 2008).

Materials & Methods

Fungal organisms

Nine species of fungi isolated from leaf litters of Central Luzon State University (CLSU) were used in the study. These were *A. flavus*, *A. niger*, *A. niveus*, *C. gloeosporioides*, *F. semitectum*, *N. fisheri*, *P. citrinum*, *P. decumbens* and *P. purpurogenum*.

Dual culture method

A 10-mm disc of 7 day old culture of each fungal isolate was placed at one end of the PDA plate and another fungal isolate of the same size and age was placed on the other end. Fungi were allowed to grow for seven days at room temperature and their interaction was determined.

Interactions were described as antagonism and mutual antagonism (Dix & Webster 1995). On antagonism, fungal isolates were classified as aggressor or victim. The mycelium of the aggressor advances on a broad front over the mycelium of the victim (Dix & Webster 1995). For mutual antagonism, interaction was classified as either mutual slight inhibition or mutual inhibition at a distance. For mutual slight inhibition, both fungi approaches each other until almost

in contact and there is a narrow demarcation line of 0.1 mm to 2 mm while mutual inhibition at a distance has a visible distance of more than 2 mm between two opposing fungi (Fakhrunnisa et al. 2006).

Microscopic observation

The strip where the two fungal isolates meet was cut using a sharp scalpel and laid down on a clean glass slide after incubation period. Microscopic analysis of hyphal interactions between fungal isolates was observed under compound light microscope.

Results

Table 1 shows the antagonistic interactions between different species of leaf litter fungi. Mutual inhibition at a distance was observed since the distance between the two fungal species was more than 2 mm. This was exhibited by *A. flavus* when paired with *F. semitectum* and *P. citrinum*; *A. niger* paired with *A. niveus*, *N. fischeri*, *P. citrinum* and *P. decumbens*; *A. niveus* paired with *N. fischeri*, *P. citrinum*, *P. decumbens* and *P. purpurogenum*; *N. fischeri* when paired with *P. citrinum* and *P. decumbens*; *P. citrinum* when paired with *P. decumbens* and *P. purpurogenum*; and *P. decumbens* when paired with *P. purpurogenum*.

Mutual slight inhibition was exhibited when *A. flavus* was paired with *A. niveus* and *A. niger*; *A. niveus* paired with *C. gloeosporioides* and *F. semitectum*; *N. fischeri* when paired with *C. gloeosporioides* and *F. semitectum*; *P. citrinum* when paired with *C. gloeosporioides* and *F. semitectum*; *P. decumbens* paired with *F. semitectum*; and *P. purpurogenum* when paired with *F. semitectum* and *C. gloeosporioides*. In which the two fungal species were almost in contact with a narrow demarcation line of 0.1 mm to 2 mm.

Meanwhile, antagonism was observed when *A. flavus* was paired with *C. gloeosporioides*, *N. fischeri*, *P. decumbens* and *P. purpurogenum* wherein *A. flavus* was the aggressor and the other fungal organisms were the victim. Hyphal folding, coiling and damage were observed on the victim upon observation under the microscope. When *A. niger* was paired with *P. purpurogenum*, *C. gloeosporioides* and *F. semitectum*, hyphal coiling of *P. purpurogenum* and *F. semitectum* and damaged hyphae of *C. gloeosporioides* were observed. Hyphal coiling of *N. fischeri* was observed under the microscope when paired with *P. purpurogenum*. Similarly, *P. decumbens* showed antagonistic interaction when paired with *F. semitectum*. Further hyphal coiling of *F. semitectum* was observed when paired with *C. gloeosporioides*.

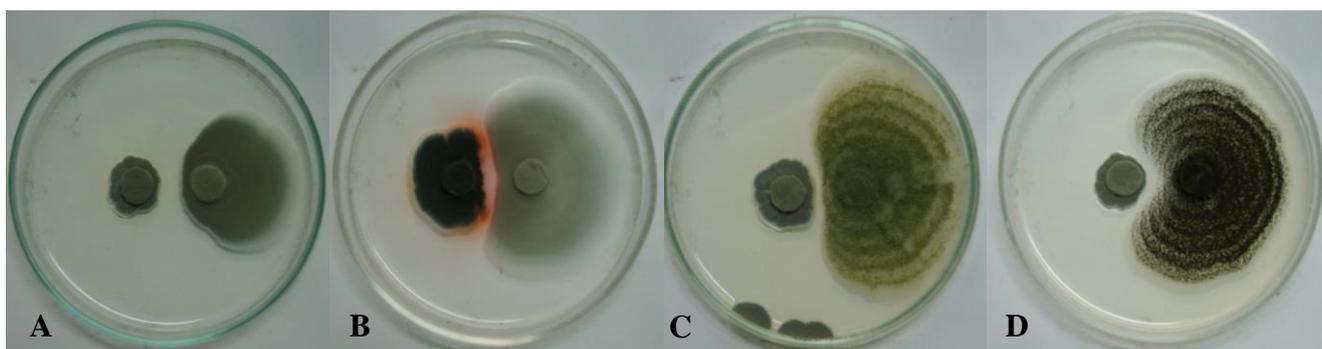


Fig 1 – Mutual inhibition at a distance represented by *P. citrinum* (left) and *P. decumbens* (right) (A), *P. purpurogenum* (left) and *P. decumbens* (right) (B), *P. citrinum* (left) and *A. flavus* (right) (C) and *P. citrinum* (left) and *A. niger* (right) (D).

Discussion

Mutual inhibition at a distance (Fig 1), mutual slight inhibition (Fig 2) and antagonism (Fig 3) were observed between fungal isolates when paired. A total of 15 pairs showed mutual inhibition at a distance, 11 pairs recorded mutual slight inhibition and 10 pairs exhibited

antagonism. *In vitro* inhibition of fungi has been attributed to some factors such as antibiotic production and pH changes in the medium (Dickson & Skidmore 1976). Jeffries & Young (1994) revealed, production of extracellular metabolites (such as antibiotics and lytic enzymes) was one of the mechanisms of antagonism between two fungal isolates. When two opposing species

Table 1 Antagonistic interactions among different species of leaf litter fungi.

Interacting Fungi		Interaction
<i>A. flavus</i> (+)	<i>F. semitectum</i> (+)	Mutual inhibition at a distance*
<i>A. flavus</i> (+)	<i>P. citrinum</i> (+)	Mutual inhibition at a distance*
<i>A. flavus</i> (+)	<i>A. niveus</i> (+)	Mutual slight inhibition*
<i>A. flavus</i> (+)	<i>A. niger</i> (+)	Mutual slight inhibition*
<i>A. flavus</i> (+)	<i>C. gloeosporioides</i> (-)	Antagonism
<i>A. flavus</i> (+)	<i>N. fischeri</i> (-)	Antagonism
<i>A. flavus</i> (+)	<i>P. decumbens</i> (-)	Antagonism
<i>A. flavus</i> (+)	<i>P. purpurogenum</i> (-)	Antagonism
<i>A. niger</i> (+)	<i>A. niveus</i> (+)	Mutual inhibition at a distance*
<i>A. niger</i> (+)	<i>N. fischeri</i> (+)	Mutual inhibition at a distance*
<i>A. niger</i> (+)	<i>P. citrinum</i> (+)	Mutual inhibition at a distance*
<i>A. niger</i> (+)	<i>P. decumbens</i> (+)	Mutual inhibition at a distance*
<i>A. niger</i> (+)	<i>P. purpurogenum</i> (-)	Antagonism
<i>A. niger</i> (+)	<i>C. gloeosporioides</i> (-)	Antagonism
<i>A. niger</i> (+)	<i>F. semitectum</i> (-)	Antagonism
<i>A. niveus</i> (+)	<i>N. fischeri</i> (+)	Mutual inhibition at a distance*
<i>A. niveus</i> (+)	<i>P. citrinum</i> (+)	Mutual inhibition at a distance*
<i>A. niveus</i> (+)	<i>P. decumbens</i> (+)	Mutual inhibition at a distance*
<i>A. niveus</i> (+)	<i>P. purpurogenum</i> (+)	Mutual inhibition at a distance*
<i>A. niveus</i> (+)	<i>C. gloeosporioides</i> (+)	Mutual slight inhibition*
<i>A. niveus</i> (+)	<i>F. semitectum</i> (+)	Mutual slight inhibition*
<i>N. fischeri</i> (+)	<i>P. citrinum</i> (+)	Mutual inhibition at a distance*
<i>N. fischeri</i> (+)	<i>P. decumbens</i> (+)	Mutual inhibition at a distance*
<i>N. fischeri</i> (+)	<i>C. gloeosporioides</i> (+)	Mutual slight inhibition*
<i>N. fischeri</i> (+)	<i>F. semitectum</i> (-)	Mutual slight inhibition*
<i>N. fischeri</i> (-)	<i>P. purpurogenum</i> (+)	Antagonism
<i>P. citrinum</i> (+)	<i>P. decumbens</i> (+)	Mutual inhibition at a distance*
<i>P. citrinum</i> (+)	<i>P. purpurogenum</i> (+)	Mutual inhibition at a distance*
<i>P. citrinum</i> (+)	<i>C. gloeosporioides</i> (+)	Mutual slight inhibition*
<i>P. citrinum</i> (+)	<i>F. semitectum</i> (+)	Mutual slight inhibition*
<i>P. decumbens</i> (+)	<i>P. purpurogenum</i> (+)	Mutual inhibition at a distance*
<i>P. decumbens</i> (+)	<i>F. semitectum</i> (+)	Mutual slight inhibition*
<i>P. decumbens</i> (-)	<i>C. gloeosporioides</i> (+)	Antagonism
<i>P. purpurogenum</i> (+)	<i>F. semitectum</i> (+)	Mutual slight inhibition*
<i>P. purpurogenum</i> (+)	<i>C. gloeosporioides</i> (+)	Mutual slight inhibition*
<i>F. semitectum</i> (-)	<i>C. gloeosporioides</i> (+)	Antagonism

(+) aggressor

(-) victim

(*) mutual antagonism

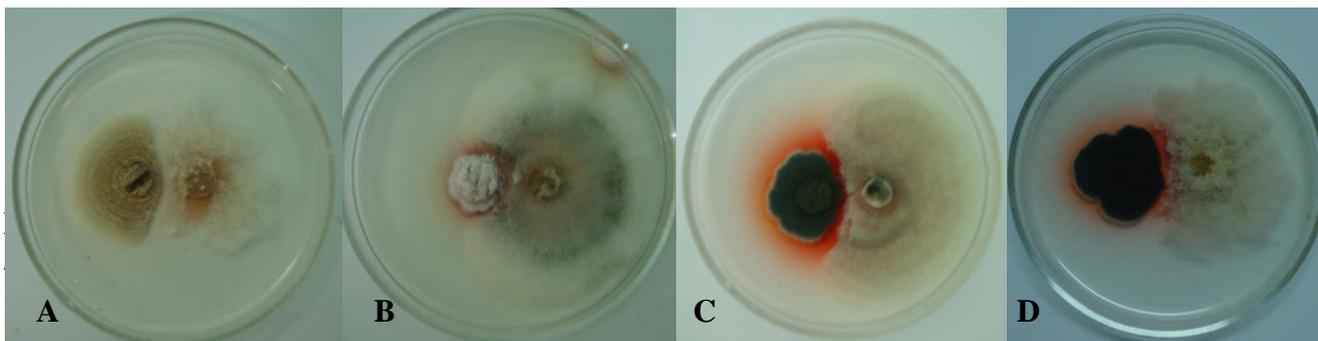


Fig 2 – Mutual slight inhibition represented by *N. fischeri* (left) and *F. semitectum* (right) (A), *A. niveus* (left) and *C. gloeosporioides* (right) (B), *P. purpurogenum* (left) and *C. gloeosporioides* (right) (C) and *P. purpurogenum* (left) and *F. semitectum* (right) (D).

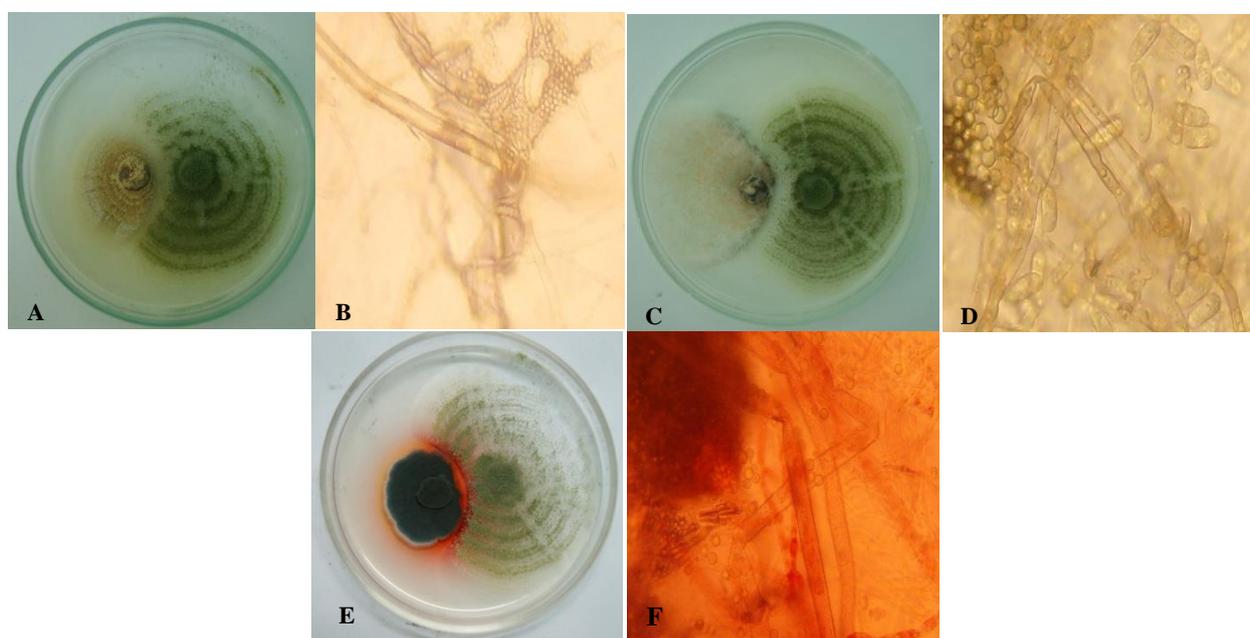


Fig 3 – Antagonistic interaction of representative fungal isolates; *N. fischeri* (left) and *A. flavus* (right) (A), hyphal coiling of *N. fischeri* due to spores of *A. flavus* (400x) (B), *C. gloeosporioides* (left) and *A. flavus* (right) (C), hyphal folding and damage of *C. gloeosporioides* caused by spores of *A. flavus* (400x) (D), *P. purpurogenum* (left) and *A. flavus* (right) (E) and hyphal coiling and folding of *A. flavus* due to presence of spores of *P. purpurogenum* (400x) (F).

produce inhibitory metabolites, mutual inhibition may take place. The presence of an inhibition zone in dual culture without hyphae contact suggests the secretion of diffusible non-volatile inhibitory substance of fungal organisms. Studies have demonstrated that before the interaction of mycelia, (e.g. *Trichoderma* sp.) low quantities of extracellular exochitinases are produced (Kullnig et al. 2000, Brunner et al. 2003). The diffusion of these enzymes dissolves cell fragments of host cells. These cell fragments in turn induce the production of further enzymes and trigger a cascade of physiological changes, stimulating rapid and directed growth of *Trichoderma* sp. (Zeininger et al. 1999).

Aspergillus species, when paired with species of *Fusarium* and *Penicillium* produced a zone of inhibition. The zones of inhibition produced were made by, due to the production of antifungal metabolites (Adejumo et al. 1999) or an indication for the production of antibiotic

substances either by the pathogen against antagonistic fungi or vice versa (Gomathi & Ambikapathy 2011). Antibiotics were used to capture resources already occupied by a competitor, or to secure a resource that may be under threat. The production of antifungal metabolites or antibiotics of fungal organism which can inhibit the other will be very important in the discovery of biocontrol against pathogenic organisms.

Aspergillus spp. had also been reported inhibitory to several plant pathogens (Getha et al. 2005, Gachomo & Kotchoni 2008). Subsequently many works had been reported that *A. japonicus* produces a wide variety of enzymes which may be involved in antifungal activity (Simoes & Tornisielo 2006). Evueh & Ogbemor (2008) were able to observe that *Aspergillus* sp. lysed the cytoplasm of *C. gloeosporioides* on Potato Dextrose Agar. This could be a result of antagonism due to parasitism and (or) antibiosis as lytic activity.

Moreover, Zazzerini & Tosi (1985) reported that species of *Fusarium* and *Penicillium* showed strong antagonistic activity. The interactions of the *Fusarium* spp. with other species to a large extent resulted in mutual inhibition on contact or overgrowth by the *Aspergillus* and *Penicillium* spp., regardless of temperature and growth rates of the species (Marin et al. 1998).

Furthermore, some species of *Penicillium* are well known for their antagonistic activity against pathogen by producing antibiotics and induce resistance in plants by activating multiple defense signals (Hossain et al. 2007). Cook & Baker (1983) stated that *Penicillium* and *Trichoderma* have long been recognized as antagonists to plant pathogenic fungi. The results *in vitro* inhibition assay of Alwathnani et al. (2012) revealed that species of *P. citrinum* could rapidly colonized the medium and found to be effective in inhibiting growth of the *F. oxysporum* f. sp. *phaseoli*. This may be due to fungistatic effect or might be attributed to the secretion of antibiotics by the fungi or other inhibitory substances produced by the antagonists such as viridian, gliovirin, geodin, terricin, terric acid, aspergillic acid, and dermadin etc. (Howell 1998, Mondal et al. 2000, Vey et al. 2001, Landreau et al. 2002, Yan et al. 2006). Studies of Ordentlich et al. (1992), found, 3-(2-hydroxypropyl)-4-(2-hexadienyl)-2(5H)-furanone, a new natural product which is secreted by *Trichoderma harzianum* which is also an inhibitory substance. The degree of effectiveness varies according to the nature, quality, and quantity of antibiotics/inhibitory substances secreted by the antagonists (Kubicek et al. 2001, Harman 2006, Singh 2006, Woo et al. 2006). Studies of Khokhar et al. (2012), demonstrated that, controlling the onion black rot pathogen, *A. niger*, with 14 *Penicillium* species as biological control agents using dual culture agar plate assays. The resulted isolates showed very high antagonistic effects on the growth of *A. niger* mycelium.

Studies of Begashaw (2003), revealed, the antagonistic activity of *Trichoderma*, *Aspergillus*, *Pencillium*, *Neosartorya* and *Fusarium* using dual culture assay showed positive antagonism against *G. candidum* (100%), *F. solani* (100%) and *C. gloeosporioides* (76%). Results of Taboonpong et al. (2014), revealed that *Neosartorya* sp. and *Talaromyces flavus* inhibited 83.9% and 83.3% mycelial growth of *Pyricularia oryzae* and *Alternaria brassicicola*, respectively.

Dual culture technique also showed that *A. niger*, *P. citrinum*, *Penicillium* sp. and *T. harzianum* inhibited the radial colony growth of the *F. oxysporum* f. sp. *lycopersici* (Alwathnani et al. 2012). While mutually intermingled growth of some *Penicillium* species with *Aspergillus* without any zone of inhibition indicates the failure of the production of antibiotics either by the pathogen or by the antagonist (Khokhar et al. 2012). Although many examples of antibiosis in agar culture involve inhibition, fungi which show mutual inhibition in some pairings fail to exhibit in other species. This may imply that there was some reciprocal exchange and recognition of chemical signals between competitors in mutually inhibited pairings, leading to accumulation of mutually inhibiting products. There are some indications that this phenomenon may occur in some inhabitants of leaf litter (Mitchell 1982 as cited by Cooke & Rayner 1984).

The hyphal interactions; hyphal damage, hyphal coiling and folding were observed between paired fungal isolates under the light microscope (Figs 3B, 3D, 3F). The hyphae appear to become intertwined, and then the antagonist takes over the available resource in the medium. In more aggressive forms of fungal behaviour, the hyphae of one competitor advance into the mycelium of the other and destroy it by overgrowth through hyphal interactions. The organisms rely on contact

and cover such phenomena as mycoparasitism and thus, hyphal interference exist (Dix & Webster 1995) which indicates that the fungus acts as a nutrient source over the other. In the study of Inbar et al. (1996), dense coils of hyphae of *T. harzianum* and partial degradation of the *Sclerotinia* cell wall were observed in dual culture method. This concludes that hyphal mycoparasitism, rather than sclerotial parasitism, is the mechanism use of *T. harzianum* which controls *S. sclerotiorum*. But different modes of hyphal interactions vary between paired organism because of a limited host range and different modes of action among organisms. This observation is supported by the research of Lifshitz et al. (1984) in which *Pythium nunn* parasitize several species of *Pythium* but was not mycoparasitic against *Fusarium oxysporum* f. sp. *cucumerinum* or *Trichoderma koningni*.

Based on the study of Ramakrishna et al. (1993), distinct interaction patterns between competing species on the grain surface of barley were identified and determined by rate of hyphal extension and branching, namely (a) faster growth of one species causing progressive inhibition of the slower-growing species; (b) faster growth initially of one species which is then inhibited by the slower-growing species; (c) one species grew faster than the other but with no adverse effects; (d) one species grew faster than the other initially, but growth rates of both declined later during interaction; (e) both species grew at similar rates initially but growth rate of one declined during competition; and (f) both species grew at similar rates initially but later reduced the growth of each other.

Acknowledgement

This study was supported by Commission on Higher Education of the Philippine Government.

References

- Adebola MO, Amadi JE. 2010 – Screening three *Aspergillus* species for antagonistic activities against the cocoa black pod organism (*Phytophthora palmivora*). Agriculture and Biology Journal of North 1(3), 362–365.
- Adejumo TO, Ikotun T, Florin DA. 1999 – Biological control of *Protomyces phaseoli*, the causal agent of leaf smut of Cowpea. Journal of Phytopathology 147, 371–375.
- AL-Saedi SS, AL-Ani BM. 2014 – Study of antagonistic capability of *Trichoderma harzianum* isolates against some pathogenic soil borne fungi. Agriculture and Biology Journal of North America 5(1), 15–23.
- Alwathnani HA, Perveen K, Tahmaz R, Alhaqbani S. 2012 – Evaluation of biological control potential of locally isolated antagonist fungi against *Fusarium oxysporum* under *in vitro* and pot conditions. African Journal of Microbiology Research 6(2), 312–319.
- Begashaw L. 2003 – Utilisation of rhizosphere microflora in the biocontrol of root rot and growth enhancement of lettuce under hydroponic systems. MSc dissertation, University of Pretoria, Pretoria. <http://hdl.handle.net/2263/24478> (accessed 25 May 2015).
- Brunner K, Peterbauer CK, Mach RL, Lorito M, Zeilinger S, Kubicek RL. 2003 – The N-acetylglucosaminidase of *Trichoderma atroviride* is essential for chitinase induction by citin of and major relevance to bio-control. Current Genetics 43, 289–295.
- Cook RJ, Baker KF. 1983 – The nature and practice of biological control of plant pathogens. American Phytopathological Society, St. Paul, MN. pp. 539.
- Cooke RC, Rayner ADM. 1984 – Ecology of saprotrophic fungi. London, UK: Longman. 415 p.
- Dickson CH, Skidmore AM. 1976 – Interaction between germinating spores of *Septoria nodorum* and phyloplane fungi. Transactions of the British Mycological Society 66, 45–56.
- Dix NJ, Webster J. 1995 – Fungal ecology. London. Chapman & Hall.
- Duffy B, Schouten A, Raaijmakers JM. 2003 – Pathogen self-defense: mechanisms to counteract microbial antagonism. Annual Review of Phytopathology 41, 501–538, Doi:10.1146/annurev.phyto.41.052002.095606.

- Evueh GA, Ogbebor NO. 2008 – Use of phylloplane fungi as biocontrol agent against *Colletotrichum* leaf disease of rubber (*Hevea brasiliensis* Muell. Arg.). African Journal of Biotechnology 7(15), 2569–2572.
- Fakhrunnisa M, Hashmi H, Ghaffar A. 2006 – *In vitro* interaction of *Fusarium* spp., with other fungi. Pakistan Journal of Botany 38(4), 1317–1322.
- Gachomo EW, Kotchoni SO. 2008 – The use of *Trichoderma harzianum* and *T. viride* as potential biocontrol agents against peanut microflora and their effectiveness in reducing aflatoxin contamination of infected kernels. Biotechnology 7, 439–447.
- Getha K, Vikineswary S, Wong WH, Seki T, Ward A, Goodfellow M. 2005 – Evaluation of *Streptomyces* sp. for suppression of *Fusarium* wilt and rhizosphere colonization in pot grown banana plantlets. Journal of Microbiology and Biotechnology. 32(1), 24–32.
- Gloer JB. 1995 – The chemistry of fungal antagonism and defense. Canadian Journal of Botany 73, 1265–1274, Doi:10.1139/b95-387.
- Gomathi S, Ambikapathy V. 2011 – Antagonistic activity of fungi against *Pythium debaryanum* (Hesse) isolated from chilli field soil. Advances in Applied Science Research 2(4), 291–297.
- Haggag WM, Mohamed ALA. 2007 – Biotechnological aspects of microorganisms used in plant biological control. American-Eurasian Journal of Sustainable Agriculture 1, 7–12.
- Harman GE. 2006 – Overview of mechanisms and uses of *Trichoderma* spp. Phytopathology 96, 190–194.
- Hossain MM, Sultana F, Kubota M, Koyama H, Hyakumachi M. 2007 – The plant growth-promoting fungus *Penicillium simplicissimum* GP17-2 induces resistance in *Arabidopsis thaliana* by activation of multiple defense signals. Plant Cell Physiology 48, 1724–1736.
- Howell CR. 1998 – The role of antibiosis in biocontrol. In *Trichoderma* and *Gliocladium*, Ed. C. P. Kubicek & G. E. Harman. London; Bristol, PA: Taylor & Francis. pp. 173–184.
- Inbar J, Menendez A, Chet I. 1996 – Hyphal interaction between *Trichoderma harzianum* and *Sclerotinia sclerotiorum* and its role in biological control. Soil Biology and Biochemistry 28, 757–763.
- Jeffries P, Young TWK. 1994 – Interfungal parasitic relationship. CAB International, Wallingford.
- Khara HS, Hadwan HA. 2008 – Sanctions, cooperation, and the stability of plant-rhizosphere mutualisms. Plant Disease Research 2, 144–147.
- Khokhar I, Haider MS, Mukhtar I, Mushtaq S. 2012 – Biological control of *Aspergillus niger*, the cause of Black-rot disease of *Allium cepa* L. (onion), by *Penicillium* species. Journal of Agrobiology 29(1), 23–28, Doi 10.2478/v10146-012-0003-5.
- Kiers ET, Denison RF. 2008 – Sanctions, cooperation, and the stability of plant-rhizosphere mutualisms. Annual Review of Ecology, Evolution, and Systematics 39, 215–236.
- Kubicek CP, Mach RL, Peterbauer CK, Lorito M. 2001 – *Trichoderma*: From genes to biocontrol. Journal of Plant Pathology 83, 11–23.
- Kullnig C, Mach RL, Lorito M, Kubicek CP. 2000 – Enzyme diffusion from *Trichoderma atroviride* to *Rhizoctonia solani* is a prerequisite for triggering of *Trichoderma* ech42 gene expression before mycoparasitic contact. Applied and Environmental Microbiology 66, 2232–2234.
- Landreau A, Pouchus YF, Sallenave-Namont C, Biard JF, Boumard MC, Robiou du PT, Mondeguer F, Goulard C, Verbist JF. 2002 – Combined use of LC/MS and biological test for rapid identification of marine mycotoxins produced by *Trichoderma koningii*. Journal of Microbiological Methods 48, 181–194.
- Lifshitz R, Dupler M, Elad Y, Baker R. 1984 – Hyphal interactions between a mycoparasite, *Pythium nunn*, and several soil fungi. Canadian Journal of Microbiology 30(12), 1482–1487.

- Marin S, Sanchis V, Rull F, Ramos A, Magan N. 1998 – Colonisation of maize grain by *Fusarium moniliforme* and *F. proliferatum* in the presence of competing fungi and their impact on fumonisin production. *Journal of Food Protection* 61(11), 1489–1496.
- Mondal G, Dureja P, Sen B. 2000 – Fungal metabolites from *Aspergillus niger* AN27 related to plant growth promotion. *Indian Journal of Experimental Biology* 38, 84–87.
- Okigbo RN, Ikediugwu FEO. 2000 – Studies on biological control of postharvest rot of yams (*Dioscorea* spp.) with *Trichoderma viride*. *Journal of Phytopathology* 148(6), 351–355.
- Ordentlich A, Wiesman Z, Gottlieb HE, Cojocar M, Chet I. 1992 – Inhibitory furanone produced by the biocontrol agent *Trichoderma harzianum*. *Phytochemistry* 31(2), 485–486.
- Ramakrishna N, Lacey J, Smith JE. 1993 – Effects of water activity and temperature on the growth of fungi interacting on barley grain. *Mycological Research* 97(11), 1393–1402, Doi:10.1016/S0953-7562(09)80175-5.
- Simoes MLG, Tornisielo SMT. 2006 – Optimization of xylanase biosynthesis by *Aspergillus japonicus* isolated from a "Caatinga" area in the Brazilian state of Bahia African. *Journal of Biotechnology* 5, 1135–1141.
- Singh HB. 2006 – *Trichoderma*: A boon for biopesticides industry. *Journal of Mycology and Plant Pathology* 36, 373–384.
- Taboonpong K, Manoch L, Chamswarn C, Piasai O. 2014 – Diversity of microfungi in marine sediments from the Gulf of Thailand and Andaman Sea and the *in vitro* antagonistic activity against plant pathogenic fungi. *Thai Journal of Agricultural Science* 47, 99–108.
- Vey A, Hoagland RE, Butt TM. 2001 – Toxic metabolites of fungal biocontrol agents. *Progress, problems and potential*. CAB international, Brisol. pp. 311–346.
- Woo SL, Scala F, Ruocco M, Lorito M. 2006 – The molecular biology of the interactions between *Trichoderma* spp., phytopathogenic fungi and plants. *Phytopathology* 96, 181–185.
- Yan XS, Quing-Tao S, Shu-Tao X, Xiu-Lan C, Cai-Yun S, Yu-Zhong Z. 2006 – Broad spectrum antimicrobial activity and high stability of trichokonins from *Trichoderma koningii* SMF2 against plant pathogens. *FEMS Microbiology Letters* 260, 119–125.
- Zizzerini A, Tosi L. 1985 – Antagonistic activity of fungi isolated from sclerotia of *Sclerotinia sclerotiorum*. *Plant Pathology* 34, 415–421, Doi:10.1111/j.1365-3059.1985.tb01381.
- Zeininger S, Galhaup C, Payer K, Woo SL, Mach RL, Fekete C, Lorito M, Kubicek CP. 1999 – Chitinase gene expression during mycoparasitic interaction of *Trichoderma harzianum* with its host. *Fungal Genetics and Biology* 26, 131–140.