International Journal of Agricultural Technology 2013 Vol. 9(4): 953-961
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Effect of agricultural pesticides on the growth and sporulation of nematophagous fungi

Sumalee Mensin[1], Kasem Soytong[2], Robert J McGovern[1] and Chaiwat To-anun[1*]

[1]Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand, [2]Biocontrol Research Unit & Mycology Section, Plant Pest Management Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Ladkrabang, Bangkok, Thailand.

Sumalee Mensin, Kasem Soytong, Robert J McGovern and Chaiwat To-anun (2013). Effect of agricultural pesticides on the growth and sporulation of nematophagous fungi. International Journal of Agricultural Technology 9(4):953-961.

Abstract The soil application of nematophagous fungi represents a potentially useful component for the sustainable management of plant parasitic nematodes. Little is currently known about the effect of agrochemicals on these fungi. Fourteen commonly used agricultural pesticides (insecticides, fungicides and herbicides) were incorporated into Potato Dextrose Agar at 1/3x, 1/2x, 1x and 2x the recommended rate to determine their *in vitro* effect on eight isolates of nematophagous fungi; *Arthrobotrys oligospora* (DLO1-001), *Arthrobotrys oligospora* (MTI2-001), *Arthrobotrys conoides* (API3-001), *Arthrobotrys thaumasium* (JDI1-001), *Arthrobotrys thaumasium* (MPI1-003), *Arthrobotrys musiformis* (MSO1-001), *Pochonia* sp. *(*KJO1-003) and *Paecilomyces* sp. (WJI1-003). The isolates were indigenous to Thailand and were parasitic to root-knot nematodes. All insecticides at all rates affected the development of all fungi to some extent. The insecticides dazomet, carbaryl and chlorpyrifos, the fungicides metalaxyl mixed with mancozeb, fosetyl aluminium and quintozene mixed with etridiazole, and the herbicides paraquat dichloride and oxyfluorfen caused high mycelial growth and sporulation inhibition. The insecticides lambda-cyhalothrin, dinitrotefuran and methomyl, the fungicides toclofos methyl and propamocarb hydrochloride and the herbicide glyphosate-isopropylammonium were less inhibitory to the fungi examined. *Paecilomyces* sp. and *Pochonia* sp. appeared to be less sensitive to the pesticides tested than *Arthrobotrys* species.

Key words: nematophagous fungi, biological control, root-knot nematodes, *Meloidogyne incognita*, pesticides, pesticide sensitivity, abiotic factors

Introduction

Nematophagous or trapping fungi are microfungi that can capture, kill and digest nematodes (Nordbring-Hertz *et al*., 2006). They represent a

[*] **Corresponding author:** To–anun, C.; **e-mail:** chaiwat.toanun@gmail.com

potentially effective alternative to chemical nematicides for sustainable farming systems (Martin, 2003). Abiotic factors are non-living chemical or physical factor in the environment and ecology. They are known as "density independent factors" (Biology online, 2008b). The capacity of a soil ecosystem to prevent or reduce the spread of a pathogen, parasite, or other deleterious agent in soils is called antagonistic potential. It includes disease suppressiveness, fungistasis, antiphytopathogenic potential and biological buffering. In addition, management of antagonists in the soil requires an understanding not only of the intricate interrelationships between host-parasite and parasite-antagonist, but also of the interactions among these relationships, crop production practices and abiotic factors (Richard, 1992). The interaction of rhizosphere microorganisms and their physiological factors influence fungal growth and sporulation. Each organism generally reacts in different ways related to their survival and establishment characteristics.

Conventional agricultural pesticides may have long-term toxicity that can affect beneficial soil microorganisms including nematophagous fungi especially their growth and sporulation. Even though research on utility of nematophagous fungi as biological control agents has occurred worldwide for decades, data on the potential negative effects of agricultural pesticides on these fungi is extremely rare. Jacobs *et al*. (2003) found that two other fungicides, fenpiclonil and tolclofos methyl, slowed or partially inhibited the growth of *Paecilomyces lilacinus*, *Plectosphaerella cucumerina* and *Pochonia chlamydosporia, in vitro*. Kerry *et al*. (2009) reported *P. chlamydosporia* and *P. lilacinus* showed different levels of sensitivity to fungicides; mancozeb+propamocarb hydrochloride, imazail+pencycuron and azoxystrobin but were tolerant to herbicides (bentazone, pendimethalin and metribuzin). *P. chlamydosporia* was more tolerant to high concentrations of fungicides than *P. lilacinus* in liquid culture.

Root-knot nematodes *(Meloidogyne* spp.) severely affect plant root systems through gall formation that can lead to stunting, wilting and/or yellowing. It is the most economically important nematode pests in the Pacific (Plant Protection, 2005). Nematophagous fungi may provide an important component in a sustainable approach to manage these important soil-borne pathogens. The objective of this study was to examine the *in vitro* effect of agricultural pesticides (insecticides, fungicides and herbicides) commonly used in vegetable production in Thailand on the growth and sporulation of indigenous nematophagous fungi parasitic to root-knot nematodes.

Materials and methods

The *in vitro* morphological sensitivity of eight nematophagous fungi recovered from Thailand including *Arthrobotrys oligospora* DLO1-001 and isolate MTI2-001, *Arthrobotrys conoides* isolate API3-001, *Arthrobotrys thaumasium* isolate JDI1-001 and isolate MPI1-003, *Arthrobotrys musiformis* isolate MSO1-001, *Pochonia* sp. isolate KJO1-003 and *Paecilomyces* sp. isolate WJI1-003 were assessed on 14 different pesticides. These pesticides with their trade names and recommended rates included (1) six insecticides, dazomet (Basamid-G 98 % GR®, 2,450 ppm a.i.), dinotefuran (Starkle-G 1% GR®, 40 ppm a.i.), lambda-cyhalothrin (Karate 2.5 % W/V CS®, 62.5 ppm a.i.), methomyl (Lannate 40 % SP®, 700 ppm a.i.), carbaryl (Sevin 85 % WP®, 2,975 ppm a.i.) and chlorpyrifos (Lorsban 40 % W/V EC®, 1,500 ppm a.i.) (2) five fungicides, quintozene mixed with etridiazole (Terraclor Super X 30% W/V EC®, 900 ppm a.i.), fosetyl aluminium (Aliette 80 WG®, 8,000 ppm a.i.), metalaxyl-M mixed with mancozeb, (Ridomil Gold MZ 65 WG®, 1,700 ppm a.i.), toclofos methyl (Rizolex 50 % WP®, 1000 ppm a.i.) and propamocarb hydrochloride (Previcur - N 72.2 % W/V SL®, 722 ppm a.i.) and 3) three herbicides, paraquat dichloride (paraquat 27.6 % W/V SL®, 1,725 ppm a.i.), glyphosate-isopropylammonium (Glyphosate 48 % W/V SL®, 3000 ppm a.i.) and oxyfluorfen (Goal 2 E 23.5 % W/V EC®, 587.5 ppm a.i.). Each pesticide was tested at 1/3x, 1/2x, 1x and 2x the recommended rate. A stock solution of each chemical was prepared in sterilized distilled water and appropriate quantities were added under aseptic conditions into 250 ml flasks, containing PDA, to achieve the required final concentrations. The amended media were poured into 9-cm-diameter sterilized Petri dishes, under aseptic conditions and allowed to cool. Petri dishes containing non-amended medium served as the control. A fungal culture agar plug (5-mm-diameter) from the colony edge of each fungal isolate was placed in the middle of the Petri dishes. Four Petri dishes of each isolate of each treatment were used as replicates. The inoculated Petri dishes were incubated at room temperature (27±3℃). Diameters of the resulting colonies were measured at 3, 5, 7 and 10 days. The percentage fungal growth inhibition was calculated and analyzed for statistical comparison. To determine sporulation, five colonized fungal agar plugs (0.4-cm-diameter) were removed from each plate after 10 days incubation and the sporulation assessment methods of Liu & Chen (2002) were followed. Values of sporulation were transformed to log (base 10) to improve homogeneity of variance before being subjected to analysis of variance (ANOVA). Original values of colony diameter were used in variance (ANOVA) "Factorial in Completely Randomized Design". Duncan's New Multiple Range Test (DMRT) Test was used for comparison of means of each treatment.

Results

Insecticide, rate, fungal isolate and their interactions significantly (*P<0.01*) affected fungal growth and reproduction. All insecticides at all rates affected the development of all fungi. All rates of dazomet , carbaryl and chlorpyrifos caused high mycelial growth inhibition (Fig. 1). Growth of most fungi except *Paecilomyces* sp. isolate WJII-003 was inhibited 100% by dazomet at all rates; however, *Paecilomyces* sp. isolate WJI1-003, *A. thaumasium* isolate JDI1-001 and *A. musiformis* isolate MSO1-001 were completely inhibited by dinotefuran treatment after 5 days (data not shown). At 10 days after inoculation, all rates of carbaryl caused 60-100% inhibition of all fungal isolates especially *A. conoides* isolate API3-001. Lambda-cyhalothrin and methomyl had a lower effect on growth of all isolates with the exception of *A. thaumasium* isolate MPI1-003 which was highly sensitive to all rates of methomyl.

Sporulation of most fungi was correlated with growth; non production of conidia was detected in many cases such as with all fungi at all rates of dazomet, five fungal isolates except *A. conoides* isolate API3-001, *Paecilomyces* sp. isolate WJI1-003 and *Pochonia* sp. isolate KJO1-003 at all rates of dinotefuran, all fungal isolates except *Pochonia* sp. isolate KJO1-003 at all rates of carbaryl and chlorpyrifos (data not shown). On the other hand, the half-strength (1/2x) and one-third strength (1/3x) recommended rate for lambda-cyhalothrin induced *Paecilomyces* sp. (WJI1-003) to produce higher number of conidia than the non-treated control.

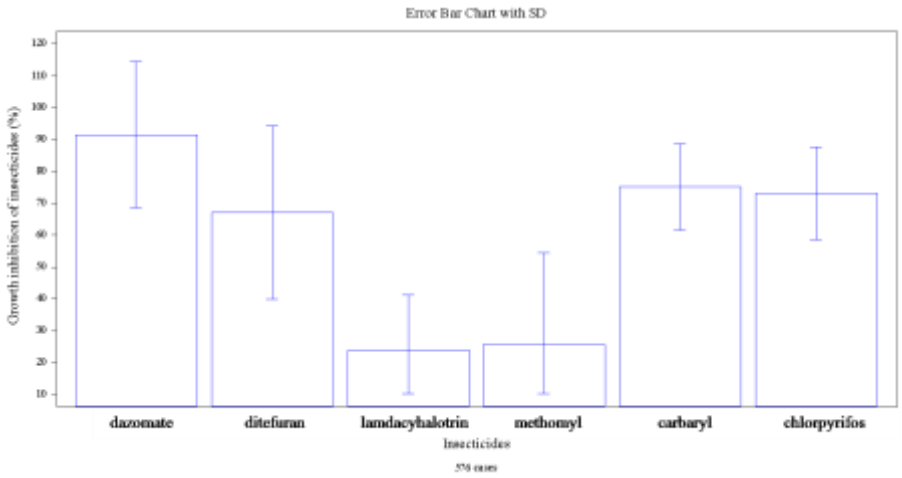

Fig. 1. Effect of various insecticides on growth of nematophagous fungi on Potato Dextrose Agar at 10 days after inoculation. Graph based on the pooled effect of 4 levels (1/3x, 1/2x, 1x, 2x) of the recommended insecticide rate on eight isolates of nematophagous fungi (*P<0.01*).

The analysis of variance using factorial indicated significant effects and interaction of fungal growth inhibition which affected growth and sporulation. These sources were fungicide, usage rate, fungal isolate and all of their interactions.

Metalaxyl mixed with mancozeb caused almost complete inhibition of fungal mycelial growth at all tested concentrations in comparison to the non-treated control (Fig. 2). All rates of fosetyl aluminium caused 100% inhibition at all rates of all fungal isolates except *Paecilomyces* sp. (WJI1-003) and *Pochonia* sp. (KJO1-003). Quintozene mixed with etridiazole (900 ppm a.i.) caused 100% inhibition of most fungi at the double and recommended rates. Only isolate *A. musiformis* (MSO1-001) was significantly inhibited by toclofos methyl; its mycelial growth was inhibited by 65-89% by all rates of the fungicide. Propamocarb hydrochloride had the least effect on the nematophagous fungi; all isolates except *A. conoides* (API1-001) showed little or no sensitivity to the fungicide at 10 days after inoculation. All fungicides at all concentrations affected sporulation by causing partial or complete inhibition and closely paralleled growth inhibition results. Metalaxyl mixed with mancozeb caused non-sporulation of all fungi at all rates as did fosetyl aluminium except for *Paecilomyces* sp. (WJI1-003) (data not shown). Five fungal, *A. oligospora* isolate DLO1-001 and isolate MTI2-001, *A. conoides* (API3-001), *A. thaumasium* isolate JDI1-001 and isolate MPI1-003 did not produce conidia at all rates of quintozene mixed with etridiazole. Propamocarb hydrochloride and toclofos methyl generally decreased sporulation to a lesser extent than the other fungicides.

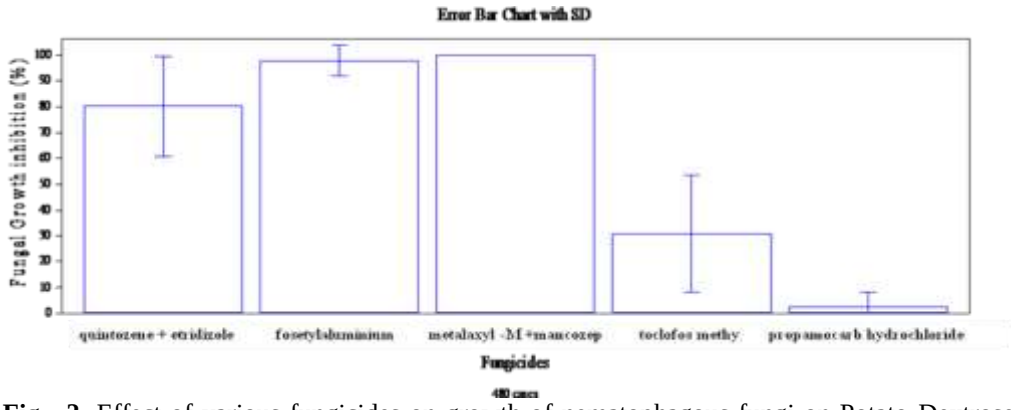

Fig. 2. Effect of various fungicides on growth of nematophagous fungi on Potato Dextrose Agar at 10 days after inoculation. Graph based on the pooled effect of 4 levels (1/3x, 1/2x, 1x, 2x) of the recommended fungicide rate on eight isolates of nematophagous fungi. ($P<0.01$).

Herbicide, usage rate, fungal isolate and all their interactions significantly (*P<0.01*) affected the growth and sporulation at a high probability. Radial mycelia of *A. oligospora* isolate DLO1-001 and isolate MTI2-001, *A. conoides* isolate API3-001, *A. musiformis* isolate MSO1-001, *A. thaumasium* isolate JDI1-001 and isolate MPI1-003 had the highest sensitivity to paraquat dichloride (Fig. 3). Their mycelia were inhibited by 100% at the double and recommended rate. Oxyfluorfen exerted the second strongest growth inhibition effect among herbicides involving reduction of mycelial growth. Only the double rate of glyphosate-isopropyl ammonium caused extensive mycelial growth reduction of *Arthrobotrys* spp. Fungal isolates of *Paecilomyces* sp. and *Pochonia* sp. were generally less sensitive to all rates of the three herbicides than the *Arthrobotrys* spp. tested. Non-sporulation of fungi was detected with at least 90% of all treatments except for *Pochonia* sp. isolate KJO1-003, which only showed a decrease of conidial production. In general, all herbicides caused abnormal mycelial morphology that was directly related to their concentration (Fig. 4).

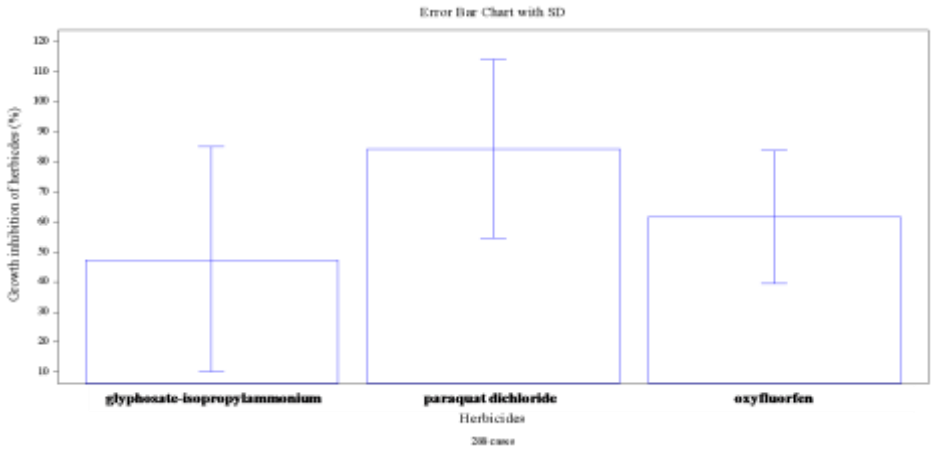

Fig. 3. Effect of various herbicides on growth inhibition of nematophagous fungion Potato Dextrose Agar at 10 days after inoculation. Graph based on the pooled effect of 4 levels (1/3x, 1/2x, 1x, 2x) of the recommended herbicide rate on eight isolates of nematophagous fungi (*P<0.01*).

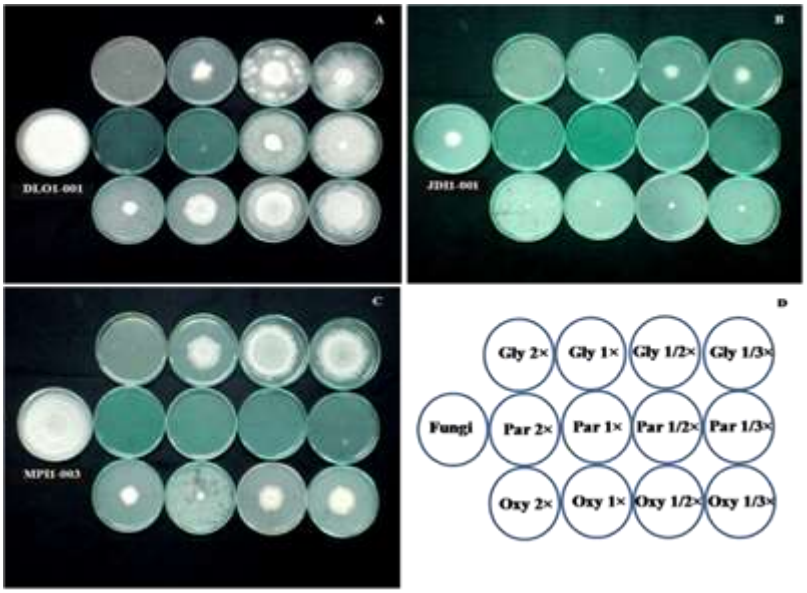

Fig. 4. Sampling of colony characterizations of three nematophagous fungi comparing effect from herbicides on PDA after 10 days incubation. A) *Arthrobotrys oligospora* isolate DLO1-001 B) *Arthrobotrys thaumasium* isolate JDI1-001 C) *Arthrobotrys thaumasium* isolate MPI1-003 and D) Location of tested Petri dish, Gly= glyphosate-isopropylammonium, Par= paraquat dichloride, Oxy= oxyfluorfen, 2x= Double rate, 1x= Recommended rate, 1/2x= Half of recommended rate and 1/3x= One-third of recommended rate.

Discussion

In worldwide agriculture, chemical products may detrimentally affect biocontrol agents. Most pesticides including fungicides, insecticides and herbicides affect growth and development causing abnormalities of many non-target organisms (Wikipedia, 2012i). Goltapeh *et al.* (2008) determined that the fungicides formalin, benomyl, thiophanate methyl and carbendazim and insecticides diflubenzuron and malathion caused 28-100% inhibition of radial mycelial growth of *Arthrobotrys oligospora* in Petri dishes to varying degrees. This research showed that isolates of nematophagous fungi from Thailand also varied with respect to their response to different agrochemicals.

Priority of effect was observed in fungicide phenylamide (metalaxyl mixed with mancozeb) followed by ethyl phosphonate (fosetyl aluminium), but a wide range of pesticide classes including a dithiocarbamate (dazomet), carbamate (carbaryl) organophosphate (chlorpyrifos), aromatic hydrocarbon mixed with thiadiazole (quintozene mixed with etridiazole), bipyridylium (paraquat dichloride) and diphenyl ether (oxyfluorfen) were moderately to highly inhibitory of the growth and sporulation of most fungi tested.

On the other hand, low inhibition by pesticides was often observed in *Paecilomyces* sp. (WJI1-003) and *Pochonia* sp. (KJO1-003) in comparison with the *Arthrobotrys* species examined. This finding is similarly to the result of Jacobs *et al*. (2003), who also observed only weak growth inhibition of these fungi by toclofos methyl. Both fungi rapidly produce numerous small conidia and also have thick, velvety and cottony colonies, respectively, which may act to lessen pesticide inhibition compared to *Arthobotrys* spp. which has a fuzzy or powdery texture. However, no reports indicate that *Paecilomyces* sp. or *Pochonia* sp. release enzymes or metabolites that inactivate toxicants.

Our research results indicate that application of nematophagous fungi as biological agents against root-knot nematodes in plantations using chemicals should be timed with respect to conventional chemical application to avoid inactivation of biological agents concordantly to the conclusion of Kim & Riggs (1998). Furthermore, increasing the amount of bio-agent formulations, by adding a sporulation promoter and/or deploying an inundative approach through frequent bio-control reapplications may help to reduce the effect of agrochemical usage. Other strategies to help ensure the effectiveness of nematophagous fungi as components of an integrated approach to root knot nematode control could also include the use of pesticides which are less inhibitory, reduction of the pesticide rate if feasible and selection of fungal bio-agent isolates that are the least pesticide-sensitive.

Acknowledgements

This work was financially supported by the Thailand Research Fund (DBG5380011).

References

Biology online. (2008b). Biotic factors. Available: http://www.biology-online.org/dictionary/biotic_factor. (June 30, 2012).

Goltapeh, E.M., Shams-bakhsh, M. and Pakdaman, B.S. (2008). Sensitivity of the Nematophagous Fungus *Arthrobotrys oligospora* to Fungicides, Insecticides and Crop Supplements Used in the Commercial Cultivation of *Agaricus bisporus.* J. Agric. Sci. Technol. 10:383-389.

Jacobs, H., Gray, S.N. and Crump, D.H. (2003). Interactions Between Nematophagous Fungi and Consequences for Their Potential as Biological Agents for the Control of Potato Cyst Nematodes. Mycol. Res. 107:47-56.

Kerry, B., Davies, K. and Esteves, I. (2009). Managing Potato Cyst Nematode Through Maximising Natural Decline and Population Suppression. Final Report: Managing PCN Decline. pp. 68.

Kim, D.G. and Riggs, R.D. (1998). Effects of Some Pesticides on the Growth of ARF18 and Its Pathogenicity to *Heterodera glycines*. J Nematol. 30(2):201-205.

Liu, X.Z. and Chen, S.Y. (2002). Nutritional Requirements of the Nematophagous Fungus *Hirsutella rhossiliensis.* Biocontrol Science and Technology, 12:381-393.

Martin, F.N. (2003). Development of Alternative Strategies for Management of Soilborne Pathogens Currently Controlled with Methyl Bromide. Annu. Rev. Phytopathol. 41:325-350.

Nordbring-Hertz, B., Jansson, H.B. and Tunlid, A. (2006). Nematophagous Fungi. Encyclopedia of Lift Sciences, John Wiley & Sons, Ltd. www.els.net. pp. 11.

Plant Protection Service Secretariat of the Pacific Community (2005). Root-Knot Nematodes. Pest Advisory leaflet, 9: pp. 4.

Richard, A.S. (1992). Management of the Antagonistic Potential in Agricultural Ecosystems for the Biological Control of Plant Parasitic Nematodes. Annu. Rev. Phytopathol, 30:245-270.

Wikipedia, the free encyclopedia. 2012i. Environmental Impact of Pesticides. Available: http://en.wikipedia.org/wiki/Environmental_impact_of_pesticides. (October 4, 2012).

(Received 23 April 2013; accepted 30 June 2013)