J. Agric. Sci. Technol. (2008) Vol. 10: 383-389

RESEARCH NOTES

Sensitivity of the Nematophagous Fungus *Arthrobotrys oligospora* to Fungicides, Insecticides and Crop Supplements Used in the Commercial Cultivation of *Agaricus bisporus*

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ABSTRACT

The effect of various pesticides (diflubenzuro, malathion, mancozeb and carbendazim), disinfectants (calcium hypochlorite and formaldehyde) and oil cakes (sunflower and soybean oil cakes) commonly used as supplements in mushroom cultivation on the growth of the nematophagous fungus, *Arthrobotrys oligospora*, was studied under *in vitro* conditions. Carbenazim caused 99% inhibition of radial mycelial growth in Petri dishes at all concentrations tested (10-40 µg a. i. ml^{-1}) in comparison to non treated dishes. Mancozeb caused 43% and 23% inhibition at 250 and 500 µg a. i. ml^{-1} respectively and 99% inhibition at concentration of 1000 µg a. i. ml^{-1} and above. Diflubenzuro and malathion at 10-40 µg a. i. ml^{-1} caused 30-41% and 24-54% inhibition, respectively. Formalin (0.5-2.0% v/v) inhibited growth of *A. oligospora* completely. However, calcium hypochlorite, sunflower and soybean oil cake at concentrations of up to 2.0% w/v caused less than 3.5% inhibition.

Keywords: *Agaricus bisporus*, *Arthrobotrys oligospora*, Biocontrol, Disinfectant and Oil cakes.

INTRODUCTION

White button mushroom [*Agaricus bisporus* (Lange) Singer], an edible fleshy fungus, is a commercially cultivated crop with a total world production of more than two million metric tonnes (Chang and Miles, 2004; Mohammadi Goltapeh and Pourjam, 2005). The annual production in Iran is approximately 30,000 metric tonnes (Mohammadi Goltapeh and Pourjam, 2005). Mushroom cultivation is adversely affected by many pests and diseases caused by various organisms, namely bacteria, fungi, viruses, insects and nematodes. Among all these natural enemies, nematodes are distinguished as the most destructive and harmful ones and, once introduced, they become the main limiting factors in the successful cultivation of the mushroom crop (Cayrol, 1967). Several cases have been reported where nematodes have limited mushroom productivity or resulted in total crop failures (Sharma *et al*., 1984; Garcha *et al*., 1986; Mohammadi Goltapeh and Rezaei Danesh, 2006; Sharma *et al*.,

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[DOR: 20.1001.1.16807073.2008.10.4.10.8]

2002; Staunton, 1996; Fletcher *et al*., 1986; Heydari *et al*., 2006).

There is increasing awareness about the harmful effects of pesticides to human health, non target organisms and the environment. Furthermore the efficacy of the pesticides can be compromised by the development of resistance in pests. Pesticides can also lead to residue problems in food, especially in mushrooms where they have been shown to be translocated to the mushrooms from treated composts (Bahl and Agnihorti, 1989). There is therefore a need to reduce pesticide inputs and develop an alternative strategies, such as cultural or biological control, such as with nematophagous fungi. *A. oligospora* is the most frequently associated nematophagous fungus encountered in the casing soils used in the commercial production of white button mushroom (Grewal, 1993). Species of *Arthrobotrys* have been commericially developed for the control of *Ditylenchus myceliophagus* in mushroom cultivation in France (Cayrol and Frankowski, 1979). *Arthrobotrys* forms adhesive hyphal networks to catch and kill the nematodes and can parasitize various nematodes parasitic on the mushroom crop, namely *D. myceliophagus, Aphelanchoides composticola, A. bicaudatus, Aphelenchus avenae*, as well as saprophagous species like *Rhabditis* and entomoparasitic nematodes of the genus *Steinernema* (Grewal, 1993). Therefore, the fungus may have potential for incorporation into integrated control programs developed for mushroom cultivation. *A. oligospora* produces a range of nematicidal compounds, including linoleic acid (Anke *et al*., 1995) and oligosporons (4', 5'-dihydro-oligosporon, hydroxyoligosporon, and 10', 11'-epoxyoligosporon) (Anderson *et al*., 1995). However, for it to be successfully integrated into mushroom cultivation, there is a need to assess the compatibility of the nematophagous fungus *A. oligospora* with the various pesticides, disinfectants and crop supplements commonly used in a commercial mushroom production.

MATERIALS AND METHODS

The sensitivity of *A. oligospora* was tested *in vitro* to the insecticides diflubenzuron (Dimlin 25% WP) and malathion (Malathion 50% EC), the fungicides carbendazim (Bavistin 50% WP) and mancozeb (Indofil M-45) and the disinfectants formaldehyde (Formalin 37%) and bleaching powder (calcium hypochlorite). In addition, *A. oligospora* was tested against sunflower cake and soybean cake which are commonly used for compost supplement in mushroom crop production. Carbendazim was tested at 10, 20, 30 and 40 µg a. i. ml^{-1}, Indofil M-45 at 500, 1,000 and 2,000 µg a. i. ml^{-1}, diflubenzuron at 10, 20, 30 and 40 µg a. i. ml^{-1} and Malathion at 10, 20, 30 and 40 µg a. i. ml^{-1}. Stock solutions of the chemicals were prepared in sterilized distilled water and volumes added under aseptic conditions into 250 ml flasks, containing half-strength CMA (corn meal agar), so as to achieve the required final concentrations. Formalin was tested at 1, 2, 3 and 4% v/v and calcium hypochlorite at 0.5, 1, 1.5 and 2% w/v, and the required amount of the chemical was directly added to the medium (CMA) in the flasks. For the sunflower and soybean oil cakes, either 0.5, 1, 1.5 and 2.0g of the crushed cake was added to 100 ml media (corn meal broth), and this was then churned in a waring blender for 2-3 minutes. Agar was added to the churned media and autoclaved at 15 psi for 45 minutes. The amended media were poured into 9 cm sterilized Petri dishes, under aseptic conditions and allowed to cool. Plates were inoculated in the center with a 5 mm mycelial disc, taken from the edge of an actively growing three day-old *A. oligospora* culture. Petri dishes containing non-amended medium served as the control (check). For each concentration and treatment there were three replicate Petri dishes. The inoculated Petri dishes were incubated at 25±1°C. The colony diameter was recorded daily until colonies had grown to the edge of the Petri dish. The diameter of growth zone was measured in treated and untreated cultures, and inhibi-

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[DOR: 20.1001.1.16807073.2008.10.4.10.8]

tion percentages were recorded daily (every 24 hours) by comparing the growth rates according to the following equation (Vincent, 1947):

Percentage of fungal growth inhibition= (C-T)/C×100

Where: C= Growth of the fungus in control and T= Growth of the fungus in treatment

Data were subjected to transformation (arcsin+0.1) and analysed by analysis of variance using MSTATC, SAS and means separated by Duncan's multiple range test.

RESULTS AND DISCUSSION

Effect of Pesticides on the Growth of *A. oligospora*

At all concentrations tested, the pesticides diflubenzuron, malathion, mancozeb and carbendazim caused significant (P= 0.05) growth inhibition of *A. oligospora* in comparison to the control (Table1). Carbendazim at all the concentrations tested (10-40 µg a. i. ml^{-1}) caused almost complete inhibition of fungal growth, with significantly (P= 0.05) greater inhibition than all other pesticides except for mancozebat 1,000 and 2,000 µg a. i. ml^{-1}. Diflubenzuron and malathion caused the maximium inhibition of 41.2% and 54.1%, respectively at the highest concentration tested (40 µg a. i. ml^{-1}) and mancozeb caused 49.4% inhibition at the lowest concentration tested (250 µg a.i. ml^{-1}). These results suggest that low concentrations of carbendazim (10 µg a. i. ml^{-1}) are almost completely inhibitory to *A. oligospora,* as are higher concentrations of mancozeb (>1000 µg a. i. ml^{-1}). However, concentrations of diflubenzuron and malathion of 40 µg a. i. ml^{-1} caused only moderate inhibition of radial growth (<54%).

The nematicidal effect of some insecticides has been known, and used practically in agriculture. For example, Diazinon is listed as a means of foliar nematode control in greenhouse florist crops (LaMondia, 2004). Furthermore, it has been shown that some fungicides, like Maneb, and Mancozeb are toxic to certain nematode species, such as *Caenorhabditis elegans*, whereas their potentially toxic metabolite, ethylenethiourea (ETU) had of minimal anti-nematode toxicity only at high concentrations (Easton *et al*., 2001). Additionally, there is some information in the literature indicating the anti-fungal activity of some insecticides, such as Thiodan, Diazinon and Basudin that inhibit mycelial growth of the white button mushroom; however, others such as Dimilin, and Trigard do not show any mycotoxic effect on mycelial growth of the mushroom (Dmoch *et al*., 1989). Here, we have indicated the antifungal activity of two insecticides, namely Malathion and Diflubenzuron, of which the former showed a greater inhibitory effect against growth of the biocontrol agent, *A. oligospora*. Dimilin (Benzuran) is believed to inhibit chitin synthase activity through deactivation of its activator proteases in insects, and the same mechanism of action may also be true for protease (B) in fungi. Also, inhibition of DNA synthesis has been attributed to this insecticide that may also be true for fungi (Matsumura, 1985).

Grewal and Sohi (1988) evaluated the effect of commonly used pesticides on the mycelial growth of *Arthrobotryis conoides* and *A. oligospora*, and found that most of the pesticides inhibited the mycelial growth of both of the fungi.

Pullen *et al.* (1990) reported that benomyl at the rates of 5 and 10 µg a. i. ml^{-1} completely inhibited hyphal growth of the nematophagous fungus *Hirsutella rhossiliensis*; similarly, Chauhan *et al*. (2002) reported 80 percent growth inhibition with *A. musiformis* by carbendazim at 2.0 µg ml^{-1} on water agar.

Based on the findings given in Table 1, it seems that the application of diflubenzuron instead of malathion will lead to improved survival of the *A. oligospora* populations, although the population of other fungi, including mushrooms, and parasitic fungi may also increase in the absence of strong chemical growth inhibitors. Also, considering the antifungal impact imposed by the tested insecticides, the application of slightly higher doses of these insecticides can lead to eco-

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Table 1. Effect of various pesticides on the growth of *Arthrobotrys oligospora* on corn meal agar at 10 days after inoculation.

Pesticides	Concentration ($\mu g\ ml^{-1}$)	Radial growth (mm)	Growth inhibition (%)
Diflubenzuron	10	59	30.6(0.60) h
	20	57	32.9 (0.62)g
	30	55	35.3(0.63)f
	40	50	41.2(0.67)e
Malathion	10	65	23.5 (0.55)i
	20	55	35.3(0.63)f
	30	43	49.4 (0.71)d
	40	39	54.1 (0.73)c
Mancozeb	250	43	49.4 (0.71)d
	500	23	72.9 (0.82)b
	1000	1	98.8 (0.91)a
	2000	1	98.8 (0.91)a
Carbendazim	10	1	98.8 (0.91)a
	20	1	98.8 (0.91)a
	30	1	98.8 (0.91)a
	40	1	98.8 (0.91)a
Control	-	85	0.0 (0.31)j

Within column, mean followed by the same letter are not significant at P= 0.05 (Duncan's multiple range test).
Values in parenthesis are arc sin transformed values after adding 0.1 in all values.
Growth inhibition percentage= (C-T)/C ×100.

nomical control of fungal parasites, in addition to the control of other target pests of importance in third world countries where farmers do not have access to most agrochemicals. However, many more studies are needed in relation to the effects of higher doses on human health and the environment. It can be concluded that the first application of a low doses of malathion followed by the inoculation with *A. oligospora* propagules after some days can lead to the suppression of the parasitic nematodes.

Effects of Disinfectants and Oil Cakes on the Growth of *Arthrobotrys oligospora*

Calcium hypochlorite, sunflower oil cake and soybean oil cake caused little inhibition in the growth of *A. oligospora* at all the three concentrations (Table 2) without showing any significant difference among them. However, formalin completely inhibited the growth of the fungus at all the four rates tested. The interactions between disinfectant/oil cakes and their concentrations were not significant.

Kerry and Crump (1998) reported that soil drenching with formalin reduced significantly the population of the nematophogous fungi *Verticilium chlamydosporium* and *Nematopthora gynopohila*.

The disinfectant, calcium hypochlorite was found compatible with *A. oligospora* and oil cakes did not inhibit the growth of *A. oligospora* compared to non-amended controls. The reduction of fungal growth through soil amendment with oil cakes (groundnut, mustard, sesamum, and binola/cotton seed) has been reported with some fungi such as *Fusarium oxysporum* f. sp. *lycopersici*, where a positive correlation is found between the magnitude of fungal growth inhibition and

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Table 2. Effect of disinfectants and oil cakes on the growth of *Arthrobotrys oligospora* at 3 days after inoculation.

	Concentration (%)	Radial growth (mm)	Growth inhibition (%)
Calcium hypochloride	0.5	85	0.0 (0.31)c
	1.0	85	0.0 (0.31)c
	1.5	83	2.35(0.34)b
	2.0	83	2.35(0.34)b
Formalin 37%			
	0.5	1	98.82(0.91)a
	1.0	1	98.82(0.91)a
	1.5	1	98.82(0.91)a
	2.0	1	98.82(0.91)a
Sunflower oil cake			
	0.5	85	0.00(0.31)c
	1.0	85	0.00(0.31)c
	1.5	83	2.35(0.34)b
	2.0	82	3.52(0.36)b
Soybean oil cake			
	0.5	85	0.00(0.31)c
	1.0	85	0.00(0.31)c
	1.5	83	2.35(0.34)b
	2.0	82	3.52(0.36)b
Control		85	0.00(0.31)c

Within column, mean followed by the same letter are not significant at p = 0.05 (Duncan's Multiple Range Test).
Values in parenthesis are arc sin transformed values after adding 0.1 in all values.
Growth inhibition percentage = (C-T)/ C ×100.

the increase in decomposition over a 45 day period (Raj and Kapoor, 1996). Among the four oil cakes, groundnut and mustard at 2.0% concentration of soil (w/w) were the most effective in reducing pathogen population (> 70%) and disease incidence. However, groundnut seed was found superior to mustard seed, as it not only controlled the disease better, but also improved plant growth (Raj and Kapoor, 1996). Similar effects have been reported with other filamentous pathogenic fungi, such as *Thielavipsis basicola*, *Rhizoctonia solani*, and *Sclerotinia sclerotiorum* (Papavizas *et al*., 1970; Singh, 1968).

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حساسیت قارچ نماتد خوار *Arthrobotrys oligospora* به قارچ‌کشها، حشره‌کشها و مکملهای غذایی استفاده شده در کشت تجاری قارچ خوراکی دکمه‌ای *Agaricus bisporus*

ا. محمدی گل تپه، م. شمس بخش و ب. س. پاکدامن

چکیده

تأثیر آفت‌کشهای مختلف دیفلوبنزرو، مالاتیون، مانکوزب و کاربندازیم، ترکیبات ضدعفونی‌کننده(هیپوکلریت کلسیم و فرمالدئید) و کنجاله‌ها (آفتابگردان و سویا) که به‌طور معمول در کشت و پرورش قارچ خوراکی به عنوان مکمل های غذایی به‌کار می روند، بر رشد قارچ نماتد خوار *(Arthrobotrys oligospora)* در شرایط درون شیشه‌ای (*in vitro*) بررسی شد. کاربندازیم در شرایط درون شیشه‌ای و در تمامی غلظتهای کاربردی (۱۰ تا ۴۰ میکروگرم در میلی‌لیتر($\mu g\ a.i.\ ml^{-1}$) در مقایسه با نمونه‌های تیمار نشده سبب ۹۹٪ بازدارندگی از رشد شعاعی میسلیومی قارچ شد. مانکوزب در غلظتهای ۲۵۰ و ۵۰۰ میکروگرم ($\mu g\ a.\ i.\ ml^{-1}$) بترتیب باعث ۴۳٪ و ۲۳٪ بازدارندگی و در غلظت ۱۰۰۰ میکروگرم و بیشتر نیز سبب ۹۹٪ بازدارندگی شد. دیفلوبنزرو و مالاتیون نیز در غلظت ۱۰ -۴۰ میکروگرم ($\mu g\ a.\ i.\ ml^{-1}$) به‌ترتیب باعث ۳۱-۴۱ و ۲۴-۵۴٪ بازدارندگی شدند. فرمالین با غلظت ۰/۵-۲/۰ ٪ (حجمی) نیز به طور کامل سبب ممانت از رشد قارچ *A. oligospora* شد. درحالی‌که، هیپوکلریت کلسیم، کنجاله آفتابگردان و سویا در غلظتهای بیش از ۲/۰٪(w/v) کمتر از ۳/۵٪ بازدارندگی را سبب می‌شدند.

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