J. Agric. Sci. Mansoura Univ., 27(8): 5295 - 5301, 2002

EFFECT OF CULTURE FILTRATES OF CERTAIN *Fusarium culmorum* ISOLATES ON EGG HATCHING OF *Meloidogyne incognita.*

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ABSTRACT

The role of culture filtrate of five Egyptian pathogenic isolates of *F. culmorum* isolated from crown and root rot disease of wheat plant were evaluated on egg hatching of *Meloidogyne incognita*. Result showed that all culture filtrates reduced number of hatched juveniles and inhibition percentage ranged from 93.5 to 97.7 % .The highest decrease in number of hatched juveniles (97.7%) was obtained at 100 % concentration of culture filtrates of aggressive isolate, and the lowest decrease (86.3%) was found at 10 % concentration of culture filtrate in the weak isolate. All concentrations of standard zeralenone toxin, 300, 200, 100, 50, and10 pmm., gave significant reduction in hatched juveniles compared with control and no significant difference between concentrations ranged from 300 –50 ppm., in the percentage of alive juveniles that it was zero. Three toxins were isolated from *F. culmorum* isolates and identified as zearalenone, deoxynivalenol and unknown toxin . The effect of these toxins were studied on egg hatching and the result obtained showed that zearalenone and deoxynivalenol toxins gave the highest decrease, but the unknown toxin gave the lowest. No significant difference between zearalenone and deoxynivalenol toxins in the percentage of alive juveniles that it was zero.

Keywords: *Fusarium culmorum* ,isolates filtrates, egg- hatching, *Meloidogyne incognita*

INTRODUCTION

Plant-parasitic nematodes especially, root-knot nematodes are considered among the most important factors limiting crop production all over the world (Antoon, 1999). The management of these pests relies mainly on the repeated use of the chemical nematicides, which maintain yield 50 % greater than that of untreated plantations (Timmer and French, 1979 and Gowen and Quénéhervé, 1990). However, the use of these chemical compounds has many drawbacks among which are the potential residues in fruits, ground water pollution, effect on non-target organisms, and their higher toxicity. Therefore, alternative control strategies, with byproducts and microorganisms, are urgently needed.

Several investigators used fungal filtrates in biological control of plant-parasitic nematodes and they found that toxic metabolites produced by plant-pathogenic fungi may cause deterioration of giant cells, reduce hatching, and immobilize the second-stage juveniles of root-knot nematode (Ciancio *et al.*, 1988; Fattah *et al.*, 1989; and James *et al.*, 1999).

Cayrol,(1989) studied the role of culture filtrates of *Fusarium* spp. against plant-parasitic nematodes and found that the culture filtrates which produced in liquid media by *Fusarium* spp., *Aspergillus niger* and *Paecilomces lilacinus* were active against eggs, larvae and adults of *Meloidogyne* spp. Siddiqui and Hnsain, (1991) used culture filtrate of *Fusarium solani* to control *M. incognita* on chickpea. Hallmann and Sikora, (1994) found that tomato roots treated with non-pathogenic mycelium of *F. oxysporum* or its culture filtrate lowered root penetration with *M. incognita* and gave 50 % control of *M. incognita* in pot experiment. They also found that the culture filtrate was highly nematicidal *in vitro* and may be a source for new active substances important for nematode control. Zaki, (1994) showed that, in laboratory experiments, culture filtrate of *P. lilacinus* inhibited egg hatching of *M. javanica in vitro.*

The objective of this work was to study the role of culture filtrate of *Fusarium culmorum* on egg- hatching of *M. incognita.*

MATERIALS AND METHODS

Five Egyptian pathogenic isolates of *Fusarium culmorum* which isolated from crown and root- rot disease of wheat plants collected from different governments, isolate 2, 3, 7, 19 and B were used. Isolate 2 was weak, isolate 19 was moderate and isolates 3, 7 and isolate B were aggressive to wheat plant (Gamal, 1997). These isolates were grown on 100 ml of steam-sterilized Czapek-Dox liquid media in 250-ml flasks. The medium was inoculated with 5 mm in diameter agar disc of *F. culmorun* isolates taken from two weeks old cultures. The inoculated flasks were incubated for 4 weeks at 25°C without shaking, then the fungal cultures were filtered through Whatman No. 1. Filter paper. The culture filtrates were sterilized using Zietes filter. Five concentrations i.e., (100, 75, 50, 25 and 10 %) of the culture filtrates of the tested isolates were prepared and used. Silica gel bands, which identified as Zearalenone, Deoxynivalenol and unknown (Gamal, 1997) were scraped, dissolved in acetone and tested against egg hatching of *M. incognita.* Five concentrations of standard Zearalenone toxin (300, 200, 100, 50 and 10 ppm) were tested . *M. incognita* was cultured in the greenhouse on eggplant c.v. Black beauty . Egg-masses of *M. incognita* were harvested from infected roots (Hussey and Barker, 1973). Ten egg-masses of *M. incognita* were suspended in each test tube containing 3 ml of each culture filtrate or toxins concentration. Another 10 egg-masses were suspended in sterilized distilled water to serve as a check. Tubes were incubated at 25°C in an incubator for 14 days. Number of hatched juveniles, percentage of inhibition, and the percentage of alive and dead juveniles in each treatment were counted. Each treatment was replicated four times. The obtained data were statistically analyzed using a computer software program of statistical analysis system, SAS (SAS Institute, 1988).

RESULTS

Results of culture filtrates of *F. culmorum* isolates on egg hatching of *M. incognita* were presented in (Table 1). Data showed that all isolates (weak, moderate and aggressive) reduced number of hatched juveniles and

the percentage of inhibition ranged from 93.5 to 97.7 % in all tested isolates. All hatched juveniles were also found dead. Table (2) show the following results:

1. All tested concentrations, showed no siificant differences among weak or aggressive isolates on numbers of hatched juveniles;
2. The highest decrease in numbers of hatched juveniles (97.7%) was obtained at 100 % concentration in culture filtrate of aggressive isolate, and the lowest decrease (86.3 %) was found at 10 % concentration in culture filtrate of week isolate ; and
3. In aggressive isolate, no significant differences among all the tested culture filtrate concentrations, all the hatched juveniles were found dead.

Table 1: Effect of culture filtrates of *F. culmorun* isolates on egg hatching of *M. incognita*.

Isolates	No. of hatched juveniles /10 egg masses	Decrease %	Alive juveniles %
Isolate 2	158 b	93.7	0.0
Isolate 19	162 b	93.5	0.0
Isolate 3	144 b	94.3	0.0
Isolate 7	111 b	95.6	0.0
Isolate B	57 b	97.7	0.0
Control	2508 a	0.0	100

Data are average of 4 replicates
Means followed by the same letter are not significantly different at p = 0.05.

Effect of standard concentrations of zerarlenon toxin were studied and the data presented in Table(3)show that:1.All tested concentrations gave significant reduction in the number of hatched juveniles compared to control; and

2. No significant differences between toxin concentrations ranged from 50 to 300 ppm in the percentage of alive juveniles.

The effect of the three toxins, produced by *F. culmorum* on egg hatching were studied (Table 4) data show the following results:

1- The unknown toxin showed the lowest decrease in the number of hatched juveniles, and the highest effect was found in doexynivalenol and zearalenone toxins;
2- No significant differences between deoxynivalenol and zearalenone toxins on numbers of hatched juveniles. The percentages decrease of culture filtrate in aggressive and weak isolates were 96.1 and 75.9 %, respectively, in zearalenone toxin, while, it was 97.7 and 87.8 %, in deoxynivalenol toxin; and
3- In the aggressive isolate no significant difference among zearalenone or deoxynivalenol toxins in the percentage of alive juveniles.

Table 2: Effect of culture filtrates of weak and aggressive isolates of *F. culmorum* at different concentrations on egg hatching of *M. incognita*

Tested Isolate	Concentration of culture filtrates														
	100 %			75 %			50 %			25 %			10 %		
	No. of hatched juveniles	Decrease %	Alive juveniles %	No. of hatched juveniles	Decrease %	Alive juveniles %	No. of hatched juveniles	Decrease %	Alive juveniles %	No. of hatched juveniles	Decrease %	Alive juveniles %	No. of hatched juveniles	Decrease %	Alive juveniles %
Isolate 2 (Weak isolate)	160 b	93.4	0.0	163 b	93.3	0.0	207 b	91.5	0.0	256 b	89.5	25.7	334 b	86.3	57
Isolate B (aggressive isolate)	57 b	97.7	0.0	63 b	97.4	0.0	126 b	94.8	0.0	48 b	93.9	0.0	169 b	93.0	0.0
Control	2431 a	0.0	100	2431 a	0.0	100	2431 a	0.0	100	2431 a	0.0	100	2431 a	0.0	100

Data are average of 4 replicates
Means followed by the some letter are not significantly different at $p = 0.05$.

Table 3: Effect of standard Zearalenone toxin at different concentrations on egg hatching of *M. incognita*

Tested Isolate	Concentration of culture filtrates														
	300 ppm			200 ppm			100 ppm			50 ppm			10 ppm		
	No. of hatching juveniles	Decrease %	Alive juveniles %	No. of hatching juveniles	Decrease %	Alive juveniles %	No. of hatching juveniles	Decrease %	Alive juveniles %	No. of hatching juveniles	Decrease %	Alive juveniles %	No. of hatching juveniles	Decrease %	Alive juveniles %
Standar Zearalenone	0.0 b	100	0.0	0.0 b	100	0.0	27 b	98.9	0.0	63 b	97.4	0.0	184 b	92.4	17.4
Control	2431 a	0.0	100	2431 a	0.0	100	2431 a	0.0	100	2431 a	0.0	100	2431 a	0.0	100

Data are average of 4 replicates
Means followed by the some letter are not significantly different at P= 0.05.

Table 4: Effect to toxins produced by *F. culmorun* isolates on egg hatching of *M. incognita*

Toxin	*Fusarium culmorum* isolate					
	Isolate B (aggressive isolate)			Isolate 2 (weak isolate)		
	No. of hatched juveniles / 10 egg masses	Decrease %	Alive juveniles %	No. of hatched juveniles / 10 egg masses	Decrease %	Alive juveniles %
Toxins No. 1 (zearalenone)	98 c	96.1	0.0	602 c	75.9	44.7
Toxins No. 2 (deoxynivalenol)	57 c	97.7	0.0	305 c	87.8	38.4
Toxins No. 3 (unknown)	1533 b	38.9	78.1	1559 b	37.8	64
Control	2508 a	0.0	100	2508 a	0.0	100

Data are average of 4 replicates
Means followed by the same letter (s), in each column, are not significantly different at p = 0.05.

DISCUSSION

The search for new methods to use as sources of biological product is an important goal for those seeking to reduce the significant economic damage caused by plants parasitic nematodes. Culture filtrates of fungi exhibit a range of specificity and modes of action in their antagonistic activity toward nematodes. Khan and Khan (1992) studied the effect of culture filtrates of soil fungi on the hatching and mortality of root knot nematode, *M. incognita* and found that fungal filtrates of 15 different fungi showed nematicidal properties against *M. incognita*. The obtained results show that culture filtrates of *F. culmorum* isolates decrease the percentage of egg hatching from 93.5 to 97.7 % and inhibited the immobilized second stage jnveniles of *M. incognita*. All the isolates tested also inhibited the percentage of a live jnveniles to zero. Similar results were observed by Hallman *et al.*, (1996) who found that culture filtrates of *F. oxysporum* inactivated juveniles of *M. incognita* 100 % after 2 hours.

In vitro studies, the effect of culture filtrates concentrations ranged from 100 to 10% were studied, and results revealed that the culture filtrates affected egg hatching and the decrease ranged from 93.4 to 86.3% in weak isolate and 97.7 to 93.0 % in aggressive isolate. The aggressive isolate also showed no significant difference among the tested concentrations in the percentage of a live juveniles since it was zero. These findings agreed with those of Khan and Khan, (1992) who found that the percentage mortality and inhibition of hatching of *M. incognita* was directly proportional to the concentrations of the culture filtrates of the tested fungi. The effect of standard concentrations of zearalenone toxin were tested for inhibition the egg hatching as well as and a live juveniles of *M. incognita* and the results showed that no differences between the concentrations ranged from 300-50 ppm. On the other hand 10 ppm gave a 17.4 % of alive juveniles.

The isolates of *F. culmorum* produced three type of toxins (zearalenone, deoxynivalenol and unknown) in both weak or aggressive isolates. These toxins were tested for inhibition the egg hatching and a live

juveniles of *M. incognita*. Results showed that the unknown toxin had weak effect, but zearalenone and deoxynivalenol have strong action on egg hatching, the decrease percentage was 96.1 and 97.7% in aggressive isolate and 75.9 and 87.7% in weak isolate, respectively. Similar effect was observed by Savard *et al.*, (1990) who found that among the array of metabolites produced by *Fusarium* spp., some are known to be mycotoxins that are also nematicidal components. Moreover, Mani *et al.* ,(1986) also mentioned that nonpolar, long-chain alkanes were tentatively identified as the nematicidal components of the *Fusarium solani* culture filtrate.

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تأثير راشح بعض عزلات الفطر *Fusarim culmorum* على فقس بيض نيماتودا تعقد الجذور *Meloidogyne incognita*

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تم دراسة دور راشح مزارع خمس عزلات ممرضة من الفطر *F. culmorum* والتي عزلت من مرض عفن الجذور التاجي لنباتات القمح في مصر على فقس بيض نيماتودا تعقد الجذور *M. incognita* وأظهرت النتائج المتحصل عليها أن لراشح جميع هذه العزلات أثر في التقليل من نقص البيض وخروج اليرقات وكانت نسبة هذا التثبيط تتراوح من ٩٣,٥ إلى ٩٧,٧ % وكان أعلى تأثير لتقليل عدد خروج اليرقات هو (٩٧,٧ %) عند تركيز ١٠٠ % من العزلة الشرسة بينما كان اقل نقص عند تركيز ١٠ % لراشح العزلة الضعيفة . اتضح أيضا أن جميع التركيزات القياسية المختبرة من السم الفطري زيرالينون وهي ٣٠٠ , ٢٠٠ , ١٠٠ , ٥٠, ١٠ جزء في المليون قد أعطت نقص معنوي في عدد خروج اليرقات من البيض وذلك عند مقارنتها بالكنترول . ولا يوجد إي فروق معنوية بين التركيزات من ٥٠ إلى ٣٠٠ جزء في المليون في تأثيرها على نسبة اليرقات الحية بعد خروجها من البيض إذ كانت تقضى عليها جميعا . تم عزل ثلاثة سموم من عزلات الفطر *F. culmorum* وقد عرفت هذه السموم على أنها سم الزيرالينون وسم الدى اوكسى نيفالينول وسم لم يعرف بعد مجهول . وعند دراسة تأثير هذه السموم على فقس بيض هذه النيماتودا اتضح أن السم الفطري زيرالينون و الدى اوكسى نيفالينول أعطيا أعلى معدل لنقص فقس البيض بينما السم المجهول فقد أعطى اقل نقص . وقد اتضح انه لا يوجد فرق معنوي بين سم الزيرالينون وسم الدى اوكسى نيفالينول في تأثيرهما على نسبة اليرقات الحية إذ قضيا عليها جميعا.