EVALUATION OF THREE NEMATOPHAGOUS FUNGI IN CONTROLLING ROOT KNOT NEMATODE USING ANIMAL MANURE AND WHEAT GRAIN AS CARRIER SUBSTRATES

BY

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The examining committee consider this thesis satisfactory and acceptable for the award of **Master of Science Degree** in plant protection.

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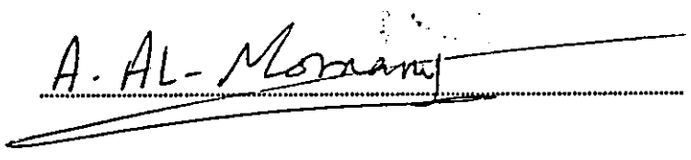

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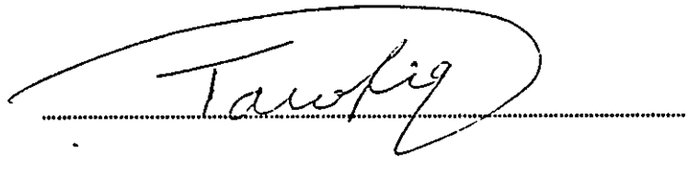

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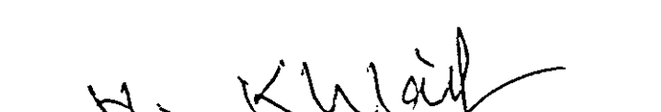

TO MY FAMILY

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I. Introduction

Root-knot nematodes are important plant pathogens affecting crop production throughout the world. In Jordan, the root-knot nematode (*Meloidogyne spp.*) is considered a big problem facing plants in irrigated areas (Abu-Gharbieh, 1988). Recent problems in the use of chemical nematicides have enhanced the development of biocontrol methods for integrated management of plant parasitic nematodes with various types of antagonistic organisms (Cabanillas *et al.*, 1989a).

There are several problems associated with pesticides usage of which the most important is the harmful enviromental effect on humans and natural life . Specifically with nematicide, the effect is short lasting which means an increase in the population of nematodes several months after the application of nematicide (Jatala , 1986) . Also using resistant varieties to nematodes have developed new races of nematodes.

Nematode antagonists as a promising method of nematode control includes a diverse group of fungi, bacteria, viruses, nematodes and others. However, fungi constitute the largest and most promising group including both predacious and parasitic fungi (Qadri, 1989). The parasitic fungi which were found to be successful bioagents against nematodes include *Paecilomyces lilacinus* (Thom) Samson (Jatala,1979), *Verticillium chlamydosporium* Goddard (Kerry, 1986) and *Fusarium oxysporum* (Qadri, 1989).

Method of application of the different formulation and selection of biological control agents play an important role in successful introduction of antagonists and subsequent biocontrol of certain plant parasitic nematodes (Cabanilles *et al.*,1989b). New methods for nematophagous fungi introduction are needed to be used more effectively against *Meloidogyne* and other plant parasitic nematodes. Using of

animal manures as substrate for carrying fungi is one tactic which could be beneficially used to apply biocontrol agent and substitute the traditional methods for delivery of nematophagous fungi, such as infested wheat grains or in aqueous spore suspension. The objectives of this study were to control root knot nematode by fungi through :

a) Evaluation of animal manures for mass production of three nematophagous fungi : *Paecilomyces lilacinus, Fusarium solani and F. oxysporum*.

b) Evaluation of these three fungi in controlling root knot nematode, using animal manures versus wheat grains as carrier substrates.

c) Studying the survival period of these fungi on different substrates during storage.

II. Review of Literature

1. Biological control of nematodes by fungi

Fungal antagonists of nematode consists of a great variety of organisms which include the nematode-trapping or predacious fungi, parasites of nematode eggs, parasites of sedentary female of nematode and fungi that produce metabolic compounds toxic to nematode (Mankau, 1980a).

Over 100 species of predacious fungi that trap and prey on nematodes were known since 1888 when they were first reported by Zopf. (cited by Qadri, 1989).

Cayrol *et al.*, in 1979 were able to prepare two commercial products of predacious fungi for nematode control. "Royal 300" an isolates of *Arthrobotrys robusta* for control of *Ditylenchus myceliophagus* on mushrooms and "Royal 350" different isolate of *Arthrobotrys* for control of *Meloidogyne* in tomato (cited by Jatala, 1986).

Nematode parasitic fungi consists of a great variety of organisms attacking various developmental stages of nematode include vermiform, sedentary females and eggs (Mankau 1980a).

Parasitic fungi on nematode female generally fall into two groups obligate and non- obligate. The obligate parasites are few-namely *Catenaria auxiliaria* (Kuhn) Tribe and *Nematophthora gynophila* and very specialized organisms that are capable of invading the female within few days of exposure of the root surface. Non-obligate parasites are also few but more than obligate group. They include primarly fungi recognized as egg parasites such as *Fusarium oxysporum,Verticillium chlamydosporium, Fusarium solani, Paecilomyces lilacinus* and others (Jatala *et al.*, 1979, Kerry, 1984, Arjun *et al.*, 1982). These parasitic fungi on eggs of plant parasitic nematodes has been recently discovered (Jatala, 1986, Mankau 1980a). The first

noticed of parasitic fungi on nematode eggs by Lyest in 1967. He found that *Fusarium spp* and *Cephalosporium spp* were able to parasitize on nematode eggs. Later the other investigatores discoverd new parasitic fungi on nematod eggs such as *Paecilomyces lilacinus* (Thom.) Samson (Jatala *et al.*, 1979),*Verticillium chlamydosporium* (kerry, 1984) and other fungi.

Nematode eggs of the group Heteroderidae and those deposited in gelatinous matrix are more vulnerable to attack by parasitic fungi than those of migratory parasites. The oviposition nature of these nematode results in total exposure of eggs to fungal attack. However, once fungal invasion takes place the process is progressive (Jatala, 1986).

Kerry (1987) summarized the characteristics required for the organism to become successful biological control agent as the following : organism must be rapid colonizer and persist in soil, virulent, predictable control below economic threshold, easy to be produced and applied to the soil in low cost, good storage, compatable with either agrochemicals and standard farm practicles and to be safe.

Several methods were used for introduction of nematophagous fungi into the soil. The methods can be summarized into three main categories: a- Spore and mycelium suspension of nematophagous fungi (Villanuvea and Davide, 1984 b), b- Coating seeds or tubers with fungus (David and Batino,1985) and c-Mass propagation of nemato-phagus fungi on different substrates (Jatala , 1981). The third method is becoming more used by many investigators. Over 23 papers have been reported the use of various types of substrates for introduction of nematophagous fungi into soil. Introduction of nematophagous fungi on carrier substrate enhanced the fungal establishment in soil and get subsequent protection of crop against plant parasitic

nematode (Cabanillas *et al.*, 1989 a and b) .

2. Fungi as bioagents to control root-knot nematode.

Qadri (1989) reviewd the potential control of nematode by mycoflora. He listed more than thirty reports concerning fungi associated with plant parasitic nematodes including160 fungal species belonging to 70 genera. The most consistant fungal species reported were *Verticillium chlamydosporium* Godd., *Fusarium oxysporum* schlecht, *Cylindocarpon destructans* scholten, *Paecilomyces lilacinus* (Thom.) Samson,*Exophila pisciphila* Carm and*Gliocladium roseum* Bain.

Jatala *et al.* (1979) discovered the fungus *Paecilomyces lilacinus* as bio-control agent for controlling *Meloidogyne incognita.* They isolated the fungus from eggs of *M. incognita* on potato roots in Peru and tested in greenhouse and field. The results showed that the fungus was able to affect populations of *M. incognita* and *Globodera pallida* (Table 1).

Paecilomyces lilacinus is a soil-borne fungus affecting nematode by parasitizing on eggs, juvenile within eggs and adult females of root-knot and cyst nematodes (Mitchill *et al.*, 1987). It produces the antibiotic P-168 which have wide antimicrobial activity against fungi, gram ·positive bacteria and might be detrimental to eggs, juveniles and femals of root-knot nematode (Domsch *et al.*, 1980) .

The nematology group at International Potato Center collaborating with the International Meloidogyne Project (IMP) decided to utilize the IMP as an agent for distrubution of *P. lilacinus* to the nematologists collaboration with that program. They have sent the cultures of *P. lilacinus* to nematologists in 46 countries around the world for testing this fungus against plant parasitic nematodes.

Several workers have evaluated the efficiency of *P. lilacinus* in nematode control in culture media (*in vitro*), or in greenhouse or field (*in vivo*). Out of 21 reports from 9 countries reviewed here (Table 1), approximately all reports listed here proved that *P. lilacinus* was able to control root knot and cyst nematode.

The first note on fungi infected nematode eggs was reported by Lysek in 1967, when he found the eggs of nematode was parasitized by*Fusarium* spp and *Cepholosporium* spp.

Morgan -Jones *et al.* (1981) did a survey of fungi associated with population of cysts of *Heterodera glycines* in soybean field soil in U.S.A. They found 27 different fungi were associated with cyst. Three fungi were found to occur with considerable frequency, namely *Fusarium oxysporum*, *F. solani* and *Stagonospora heterodero* .

In Jordan, Saleh and Qadri (1989) tested the fungi associated with *Heterodera schachtii* in Jerash. They found over 20 fungal species associated with cysts of *H. schachtii.* The fungi frequently isolated were *Verticillium chlamydosporium Fusarium oxysporum* and *F. solani.* Qadri (1989) reported the successful control of *H. schachtii* and *M. javanica in vitro* and greenhouse experiments with *F. oxysporum* , *F. solani* and other fungi (Table 1).

Table 1 : *Fusarium oxysporum* , *F. solani* and *Paecilomyces lilacinus* tested for the ability to attack or suppress several cysts and root-knot nematodes (Results : (+) able to attack nematode, (-): not able to do so)

Fungus	Host	Country	Test *in vitro/in vivo*	Result	*Ref.
Fusarium oxysporum	*Globodera rstochiensis*	Germany	*in vitro*	-	54**
			pot:potato	+	54
	Heterodera glycines	U. S. A	*in vitro*	+	22
	H. schachtii	Jordan	*in vitro*	+	54
			pot : sugar beet	+	54
		U. S. A	*in vitro*	+	51
			pot: sugar beet	+	51
	Meloidogyne arenaria	U. S. A	*in vitro*	-	22
	M. javanica	Jordan	*in vitro*	+	54
			pot: tomato	+	54
F. solani	*G. rostochiensis*	Germany	*in vitro*	-	54
	H. glycines	U. S. A	*in vitro*	-	22
	H. schachtii	Jordan	*in vitro*	+	54
			pot: sugar beet	+	54
	H. zea	India	pot: maize	+	3
	M. arenaria	U. S. A	*in vitro*	-	22
	M. javanica	Jordan	*in vitro*	+	54
			pot : tomato	+	54
Paecilomyces lilacinus	*G. Pallida*	Peru	field : potato	+	33
	G. rostochiensis	Pakistan	field : potato	+	60
		Panama	field : potato	+	31
		Philippine	field : potato	+	13

* Reference

** Cited by Qadri

Fungus	Host	Country	Test *in vitro*/in vivo	Result	Ref.
P. lilacinus	*M. arenaria*	U. S. A	*in vitro*	+	57
			in vitro	+	21
			pot : squash	+	57
			pot : squash	+	11
			pot : tomato	+	11
			pot : squash	+	54
	M. incognita	U. K.	*in vitro*	+	18
		U. S. A	*in vitro*	+	19
		Egypt	pot : corn	+	27
			pot : okra	+	27
			pot : tomato	+	27
		India	pot : brinjal	+	65
		Philippine	pot : cotton	+	13
			pot : potato	+	73
			pot : tomato	+	73
		Pureto Rico	pot : tomato	+	59
P. lilacinus	*M. incognita*	U. S. A	pot : pepper	+	16
			pot : tobacco	+	16
			pot : tomato	+	16
			pot : tomato	+	4
			pot : tomato	+	7
		U. K.	pot : pepper	+	18
		Pakistan	field : okra	+	63
			field : mung	+	63
			field : gram	+	63
			field : okra	+	64
			field : mung	+	64
		Peru	field : potato	+	33
			field : potato	+	35
		U. S. A	field : tomato	+	6

Fungus	Host	Country	Test *in vitro* /in vivo	Result	Ref.
P. lilacinus	*M. incognita*	U.S.A	field : tomato	+	7
	M. javanica	Pakistan	field : mung	+	65
		U.S.A	field : tobacco	-	25

3. Substrates used for nematophagous fungi mass production and soil inoculation .

Several unsuccessful attempts have been made in the past to introduce nematode-trapping fungi into soil for biological control of plant parasitic nematodes (Mankau, 1962).

Linford and Yap in 1939 were the first who used bagasse as organic additive at the rate of 2.5t/ha for propagation of nematode-trapping fungi and introduced it into the soil for biological control of *Meloidogyne* spp. (cited by Kerry 1984).

Recently, wheat and rice grains are becoming common substrates for mass propagation of nematophagous fungi. They serve as a carrier substrates for fungi delivery into the soil to control plant parasitic nematodes (Jatala, 1981, Jatala *et al.*, 1985, Shahzad and Ghaffar, 1987 and1989).

Jatala (1986) conducted greenhouse and field experiments and confirmed that *P.lilacinus* was able to control *Meloidogyne* spp. at the rate of 1.5 kg of fungi infested wheat grains per 40-square meter. Rice grain was used by Sharma and Trivedi (1989) as carrier substrates for *P. lilacinus* . Four inoculum levels of fungus-infested rice grain were used up to 75 g/1.5 kg soil. They found that as the rate of application increase the control of *Meloidogyne incognita* also increase.

Villanueva and Davide (1984 a) evaluated growth of nematode trapping fungus *Arthrobotrys cladodes* and a number of local isolates of *P. lilacinus* on various substrates (Table 2). They found that fungi can be grown extremely on mashed potato and water lily.

Davide and Zorilla (1983) controlled *Globodera rostochiensis* in field soil by using water lily as carrier substrate for *P. lilacinus* at the rate of 175 g water lily

infested by fungi per square meter.

From 14 substrates tested *in vitro* for mass propagation of diffrent species of nematophagous fungi, there were only 5 substrates reported for heavily mass production of fungi. These are rice grain, rye grain, wheat bran, mashed potato and water lily (Table 2).

Several investigators have used different types of substrates for delivery of nematophagous fungi into soil. Fourteen substrates were reported here as carriers of nematophagus fungi to control root-knot and cyst nematode in greenhouse and field (Table 3). From 14 substrates tested, 7 types were found to be successful carriers of fungi to control nematodes . These are wheat grain , rice grain , oat grain, rye grain, rice straw, water lily and diatomaceous earth granules .

4 . Survival of fungi on carrier substrates through storage period

Cabanillas *et al* (1989 a) studied the viabilty of *P. lilacinus* through storage period on different carrier substrates by using alginate pellets, diatomaceous earth granules, soil, soil plus chitin and wheat grains .The viability was determined over a period of 8 weeks. The results showed that the viability remained high in wheat grain and diatomaceous granules over a storage period, while it dropped intermediately in alginate pellets after 7 days of storage and sharply drop after 14 days of storage in soil and chitineous soil.

Papavizas *et al.* (1987) repoted that the *Talaromyces flavus* remained viable at 5 and 15 C for 15 weeks of storage on alginate pellets *(Talaromyces flavus* is antagonistic against several soil-borne plant pathogen as *Verticillium* spp).

Table 2 : Substrates tested for mass propagation of several nematophagous fungi *in vitro* (Results (A) heavily mass production, (B) moderately mass production and (C) weakely mass production).

Fungus	Substrate	Country	Result	Reference
Arthrobotrys cladodes	coir dust	philippine	C	73
	corn grits		B	73
	Ipil-ipil		B	73
	mashed potato		A	73
	rice hull		C	73
	water lily		A	73
Meria coniospira	rye grain	Canada	A	71
Paecilomyces lilacinus	coir dust	Philippine	C	73
	corn grits		B	73
	Ipil-ipil		B	73
	gram husk	India	B	4
	gram grain		B	4
	mashed potato	Philippine	A	73
	molasses	India	C	4
	rice bran		B	4
	rice husk		C	4
	rice hull	Philippine	C	73
	rice grain	India	A	4
	water lily	Philippine	A	73
	wheat bran	India	A	4

Table 3: Substrates tested as carriers of nematophagous fungi for controlling plant parasitic nematodes in soil (+able to attack nematode, - not able).

Fungus	Substrate	Host	Country	Test	Rate	Result	Referenc
Arthrobotrys irregulariis	rye grain	*Meloidogyne* spp.	-	-	140g/m^2	+	7
Dactylaria thaumasia	leaf mold	*Globodera rostochiensis*	-	-	13.6t/ha	+	40
D. candida	vermiculite	*Heterodera avena*	-	-	2.5 t/ha	+	40
Fusarium oxysporum	wheat grain	*H. schachtii*	Jordan	pot : sugar beet	0.5% w/w	+	54
		M. javanica		pot : tomato	0.32% w/w	+	4
F. solani		*H. schachtii*		pot : sugar beet	0.5% w/w	+	4
		M. javanica		pot : tomato	0.32% w/w	+	4
Gliocladium roseum	oat grain	*M. arenaria*	U. S. A	pot : squash	0.5% w/w	+	7
Meria coniospora	rye grain	*M. hapla*	Canada	pot : tomato		+	71
Paecilomyces lilacinus	algenate pellet	*M. incognita*	U. S. A	field : tomato	40 g/m^2	-	7
	diatomaceous granules			field : tomato	35.6 g/m^2	+	7
	oat grain	*M. arenaria*		pot : squash	0.5% w/w	+	56
	rice grain			pot : tomato	0.5% w/w	+	11
				pot : squash	0,5% w/w	+	11
		M. incognita	India	pot : brinjial	5% w/w	+	7
			Pakistan	field : okra	40 g/m^2	+	63

Table 3. Continued

Fungus	Substrate	Host	Country	Test	Rate	Result	Reference
P. lilacinus	rice grain	*M. incognita*	Pakistan	field : mung	40 g/m^2	+	63
				field : gram	40 g/m^2	+	63
				field : okra	40 g/m^2	+	64
		M. javanica		field : mung	40 g/m^2	+	64
				field : mung	50 g/m^2	+	64
				pot : mung	1 % w/w	+	65
	rice straw	*M. incognita*		field : mung	50 g/m^2	-	64
	soil		U.S.A	field : tomato	211 g/m^2	-	7
	soil + chitin			field : tomato	209 g/m^2	-	7
	sorghum grain		Pakistan	field : mung	50 g/m^2	-	64
	water lily	*G. rostochiensis*	Philippine	field : potato	175 g/m^2	+	13
	wheat grain	*G. pallida*	Peru	field : potato	37.5 g/m^2	+	35
		G. rostochiensis	Pakistan	pot : potato	14 g/30cm-d	+	60
		M. incognita	Egypt	pot : corn	37.5 g/m^2	+	27
				pot : tomato	37.5 g/m^2	+	27

Table 3. Continued

Fungus	Substrate	Host	Country	Test	Rate	Result	Reference
P. lilacinus	wheat grain	*M. incognita*	Egypt	pot : okra	37.5 g/m^2	+	27
			Peru	field : potato	37.5 g/m^2	+	30
			U. S. A	pot : tobacco	0.5 % w/w	+	16
				pot : tomato	0.5 % w/w	+	16
				pot : pepper	0.5 % w/w	+	16
				field : tomato	40 g/m^2	+	70
		M. javanica	U. S. A	field : tobacco	245/m^2	-	25
	wheat straw	*M. incognita*	Pakistan	field : mung	50 g/m^2	-	64
Various trapping -fungi	bagasse	*Meloidogyne* spp.	-	- (1)	2.5 t/ha	-	44
	maize grain		-	-	5 t / ha	-	44
Verticilliunm chlamydosporium	attapulgite	*H. avena*	Germany	pot : wheat	2.5 % w/w	-	39
	oat grain			pot : wheat	2.5% w/w	+	39

* No available data .

III. Materials and Methods

1. Nematophagous fungi tested and their origin.

The following fungi were used in this study : *Paecilomyces lilacinus* (Thom.) Samson, *Fusarium solani* (Mart) Sacc. and *F. oxysporum* schlecht. *Paecilomyces lilacinus* was provided by Dr. Jatala from the International Potato Center, Lima, Peru.

Fusarium solani and *F. oxysporum* were isolated by Qadri (1989) from *Heterodera schachtii* from Jerash in Jordan.

2.Substrate types and preparation.

Fresh layer, broiler, cow sheep manures and fermented mix manure (mixture of poultry , sheep and cow manure) were used as substrates for fungal propagation compared with wheat grain as standard media.

Animal manures were air dried, chopped, moistened with tap water at the ratio of 1:1 w/w, mixed gently and placed in proper flasks. The mixture was autoclaved for 30 minutes at 121 °C at 15 PSI.

Wheat grain medium was prepared according to the method described by Jatala (1981). Wheat grains were soacked in water for 12 hours, dried from free water, placed in proper flasks and autoclaved for 30 minutes at 121 °C at 15 PSI.

3. Evaluation of animal manures for mass propagation of nematophagous fungi in the laboratory.

3.1 Media preparation

3.1.1. Wheat grain media

Twenty grams of air dried wheat grains were prepared as previously described (see III. 2). Macerated wheat grain media was prepared by moisting 20 grams of macerated wheat with tap water at the ratio of 1:1w/w. Each medium was placed

into 100 ml screw-cap milk dilution bottles then autoclaved. Three replicates were prepared for each treatment.

3.1.2 Animal manure media .

Twenty grams air dried layer, broiler, cow and sheep manures and fermented mix manure, were prepared as described earlier (See III. 2). Every type of the above mentioned media was filled into 100 ml screw-cap milk dilution bottles and autoclaved .Three replicates were prepared for each treatment.

3.2 Inoculation and growth assessments.

Animal manures and wheat grains, which served as fungal substrates were inoculated with a stock spore suspension of each fungus. Spore suspension was prepared from ten days old culture of each fungus grown on potato dextrose agar (PDA). One ml of each spore suspension was used to inoculate each milk dilution bottle. Each ml of *Paecilomyces lilacinus* spore suspension contained 4 $x10^8$ spores. *Fusarium solani* and *F. oxysporum* were used at the concentration of $4x10^5$ and $1x10^5$ spores per ml, respectively.

All inoculated bottle were incubated at 25C° for ten days. Bottles were shaken daily to obtain uniform growth of the fungus. The suitability of media for each fungus was assessed at the end of the incubation period.

A fungal growth index of five scales was used for all fungi to evaluate the suitability of substrates for each fungus as described by Villanueva and Davide (1984 a).

One gram of each colonized substrate was suspended in 99 ml sterile distilled water to evaluate the concentration of fungal spores. A series of dilutions were used

for direct counting using a Neubauer Haemacytometer. The pH, electrical conductivity, nitrogen (Kijeldahl method), carbon (Walky-Black procedure), phosphorus and potassium percentages were determined as reported by Page *et al.* (1982).

Data were subjected to one way analysis of variance. Mean separation was done according to the Duncan's Multiple Range Test.

4. Effect of nematophagous fungi on *Meloidogyne* population.

4.1 Greenhouse study.

Wheat grain, layer and broiler manures were used as carrier substrates for the following nematophagous fungi : *Paecilomyces lilacinus, Fusarium solani* and *F. oxysporum* and tested on root-knot nematode *Meloidogyne javanica*.

4.1.1 Preparation of fungal inocula.

Wheat grain media was prepared as described by Jatala (1981). Fresh layer and broiler manures were prepared as described earlier (see III. 2). 500 ml flasks were half filled with each of the substrates and the rest of the volume remained empty for ventilation, then autoclaved for 30 minutes at 121 °C.

Each flask of the three types of media, wheat grain, layer manure and broiler manure was inoculated with one centimeter agar discs taken from the respective fungal culture included in the test and incubated at 24°C. Flasks were shaken daily to obtain uniform fungal colonization. Fourteen days later, fungal inocula were ready for soil inoculation.

4.1.2 Preparation of root-knot nematode inoculum

The method described by Barker (1985) to prepare *Meloidogyne* inoculum was used. Tomato roots infested with *Meloidogyne javanica* obtained from greenhouse culture were washed, cut into 1-2 cm segments and shaken vigorously in 200 ml of 0.5% NaOCl solutions for 2-4 minutes. The resulting solution was sieved through 75 and 25 µm sieves, respectively. Materials collected by the last sieve were washed for several times with water to remove traces of NaOCl. Number of eggs per ml was determined using the binocular.

4.1.3 Test on *Meloidogyne javanica*

Three fungi on three different carrier substrates were tested in pot experiment on *Meloidogyne javanica*.

Treatments were as follows :

1. No nematode (Control).
2. Nematode alone.
3. Nematode + Layer manure.
4. Nematode + Broiler manure.
5. Nematode + Wheat grain.
6. Nematode+Layer manure inoculated with *Paecilomyces lilacinus*.
7. Nematode+Layer manure inoculated with *Fusarium solani*.
8. Nematode+Layer manure inoculated with *Fusarium oxysporum*
9. Nematode + Broiler manure inoculated with *Paecilomyces lilacinus*.
10. Nematode + Broiler manure inoculated with *Fusarium solani*.
11. Nematode + Broiler manure inoculated with *Fusarium oxysporum*

12. Nematode + Wheat grain inoculated with *Paecilomyces lilacinus*.

13. Nematode + Wheat grain inoculated with *Fusarium solani*.

14. Nematode + Wheat grain inoculated with *Fusarium* oxysporum.

Plastic pots of fifteen centimeter diameter (1.5 kg soil capacity) were filled with methyl bromide fumigated soil. Each pot was inoculated with 10 grams (0.67% w/w) of colonized or non-colonized layer manure , broiler manure or wheat grain and mixed thoroughly. Each pot was planted with 30 days-old tomato seedling (*Lycopersicon esculentum* cv. Maisara). Pots were inoculated six days later with 5000 eggs per pot of the root-knot nematode *Meloidoygne javanica* (Roman and Marcono, 1985). The treatments were replicated five times in two ways analysis of variance. Plants were grown in the greenhouse, irrigated and fertilized as needed. The experiment was terminated 80 days after nematode inoculation.

Fresh root and oven dry shoot weights of tomato plants were recorded. Number of galls, galling index and percentage of parasitized egg masses were evaluated using the scale of 0-5 (0 = no galling, 1 = 1- 10%. of root galling, 2=11 - 25% root galling , 3=26-75 % root galling , 4=76-90 % root galling, 5=91-100 % root galling) as described by Barker (1985).

The percentage of infected egg masses was measured as described by Cabanillas *et al.* (1989b). Galled roots were washed to remove soil particles. Ten egg masses were taken randomly from galled roots of each plant, surface sterilized with 0.5% NaOCl for 30 seconds, rinsed in sterile tap water and placed on the surface of Petri-dishes containing solidified semi-selective media for *Paecilomyces lilacinus* (Mitchell, 1987) or PDA containing penta-chloro nitro-benzene (PCNB) (400 ppm) for *Fusarium solani* and *F. oxysporum*. . All dishes were incubated at 25 °C for 5 days.

The number of infected egg masses by the fungus were counted as percentage of parasitized egg masses.

4.2 Field study.

The three parasitic fungi *Paecilomyces lilacinus*, *Fusarium solani* and *Fusarium oxysporum* on wheat grain and layer manure were used in the field study.

4.2.1 Preparation of fungal inocula

Wheat grain and layer manure media were prepared as described earlier (see III. 3.1). Substrates were placed in 2000 ml jars. Jars were half filled with substrates, autoclaved and inoculated with three centimeter agar discs taken from each fungal culture. Then jars were incubated at 25 °C and shaken daily. Two weeks later, the wheat grain and layer manure inocula were ready for soil inoculation.

4.2.2 Test on *Meloidogyne javanica*

The experiment was carried out in microplots $2m^2$ (2 x 1) at the Jordan University Farm in the Jordan Valley. Field soil was known naturally heavily infested with the root-knot nematode *Meloidogyne javanica*. Each microplot was inoculated with 100 grams per square meter of colonized or non-colonized wheat grains or with 500 grams per square meter of colonized or non-colonized layer manure. The soil was mixed thoroughly with hoe and covered with 1m-wide black plastic mulch. Each microplots was planted (Nov. 24,1989) six days later with eight tomato seedlings(*Lycopersicon esculentum* cv. Maisara) of 25 days old. Plants were irrigated by drip irrigation. The tomato seedlings were covered wih transparent plastic tunnels for two months to protect them from frost injury. The tomato plants were

irrigated, sprayed with pesticides and fertilized as needed.

In this experiment three fungi and two substrates were used in microplots in a randomized complete block in split plot design with five replicates. Four main plots and two sub-plots were used in the experiment as follows :

Main plots included :

1 -No fungus

2 -*Paecilomyces lilacinus.*

3 -*Fusarium solani*

4 -*Fusarium oxysporum.*

Sub-plots included :

1 -Wheat grain

2 -Layer manure

The experiment was terminated after six months from planting (may 25,1990) . The following parameters were evaluated at the end of the experiment to study the effect of parasitic fungi on *Meloidogyne javanica* : galling index of scale 1-6 (1= no galling, 2 = less than 10% root galling, 3 = 11-30 % root galling, 4 = 31-75 % root galling, 5 = 76-90 % root galling and 6 = 91-100 % root galling), number of juveniles per100 cm^3 soil using Baermann trays method (Barkar, 1985), the percentage of parasitized egg masses (See III. 4.1.3), yield and fresh root weight.

5. Survival of fungi:

5.1 Survival of fungi in the soil :

From the field study mentioned above, soil samples were taken two times, seventy days after planting and at termination time. Soil samples of 1/2 kg from each microplot were taken from 5-15 cm depth, mixed and placed in tightly closed plastic bags.

Soil samples were collected for determination of fungal propagules and number of second stage juveniles of *Meloidogyne javanica*. Fungal propagules were determined using soil dilution method (Menzies, 1963). 10^{-2} dilution was used for *Fusarium* spp. and 10^{-4} dilution for *Paecilomyces lilacinus* using a semi-selective media for *Paecilomyces* and PDA containing PCNB (400 ppm) for *Fusarium* spp.

Petri-dishes were incubated at 25 ° C for 5 days. Fungal propagules were identified and counted . The number of fungal propagules were calculated per one gram oven dry soil.

5.2 Survival of fungi on carrier substrates through storage

Paecilomyces lilacinus, *Fusarium solani* and *Fusarium oxysporum* were propagated on layer manure , broiler manure and wheat grain as described earlier (see III. 3.1) and used to study the viability of these fungi on substrate through storage period. Inoculated wheat grain, layer and broiler manures were placed in paper bags and stored at room conditions (25 $\pm$5 °C) for six months.

Samples were taken after 5, 15, 30, 45, 90, 150 and 180 days of storage to determine the viability of fungal propagules . One gram of stored material was

placed into a milk dilution bottle. Series of dilutions were used until reaching 10^{-8} dilution. Two Petri-dishes were used for each dilution and one ml from the suspension was transfared to each dish containing PDA and incubated at 25°C for 5 days. Colony forming unit/gram oven dry substrate (CFU/g) were counted later.

6- Pathogenicity test

The pathogenicity test of *Fusarium oxysporum* Schlecht and *F. solani* (Mart.) Sacc. was carried out in the greenhouse on the following plants:

1 - Tomato (*Lycopersicon esculentum* cv. Maisara) .

2 -Tomato (*Lycopersicon esculentum* cv. Maramand)

3 -Egg plant (*Solanum melogena* cv. Black beauty)

4 -Melon (*Cucumis melo* cv. Ananas anjar choice).

5 -Bean (*Phaseolus vulgaris* cv. Lolita).

Pots of ten centimeter diameter were filled with methyl bromide fumigated soil. Each pot was planted with one seedling of tomato or eggplant (3 weeks-old), melon or bean (10 days-old). Spore suspensions were prepared from 5 days old cultures of *F. solani* or *F. oxysporum.* Inoculation was done with equally concentrated spore suspension of each inoculum by applying 10 ml of suspension around base of each plant,each ml containing $5x10^6$ spores of *F. solani* or 4×10^6 spores of *F.oxysporum* . Treatments were replicated two times and irrigated as needed. The treatments were as follows.

1- Tomato (Maisara) + F.s.*

2- Tomato (Maisara) + F.o**

3- Tomato (Maisara) alone.

4- Tomato (Maramand) +F.s.*

5- Tomato (Maramand) + F.o.* *

6- Tomato (Maramand) alone

7- Egg plant + F.s.

8- Egg plant + F.o.

9- Egg plant alone

10- Melon + F.s.

11- Melon + F.o.

12- Melon alone

13- Bean + F.s.

14- Bean + F.o.

15- Bean alone

Plants were checked for vascular wilt disease caused by *F. oxysporum* after 20, 40 and 60 days of planting and after 60 days for root rot disease caused by *F. solani.*

* F.s. = *Fusarium solani*

** F.o. = *Fusarium oxysporum*

IV. Results

1 - Evaluation of animal manures for mass production of three nematophagous fungi.

Mycelial growth of *Paecilomyces lilacinus* was very abundant on wheat grain, broiler and layer manures which did not differ in their suitability as a substrate (Table 4). Moderate to abundant mycelial growth were observed on cow and fermented mix manure and poor growth on sheep manure. Growth of *Fusarium solani* was very abundant on wheat macerate, layer and broiler manures with no differences among the three substrates, but significantly lower growth was observed on wheat grain medium. The rest of substrates were less suitable for *F. solani*, though all were able to support moderate fungal growth. Growth of *F. oxysporum* was highest on layer manure and wheat grain , followed with broiler manure, macerated wheat and cow manure. Poor growth was observed on sheep and fermented mix manure (Table 4) .

Sporulation of the three tested fungi was much higher on broiler, layer or cow manures than on wheat grains (Table 5). Except the low sporulation of *P. lilacinus* on sheep manure, the three fungi produced similar numbers of spore on wheat grain, fermented manure and sheep manure. (Appendix A) .

Values of the pH, electrical conductivity (EC), phosphorus (P), potassium (K) nitrogen (N), carbon (C) and C/N ratios for the evaluated substrates (Layer, broiler, cow, sheep and fermented manure and wheat grain) are presented in Table (6) .
Layer and broiler manure have highest nitrogen content and lowest C/N ratios compared with other tested substrates, followed with cow manure and wheat grain.
Layer manure, broiler manur and wheat grain have pH 6.6, 6.8 and 6.3, respecively, while the remained substrate have pH greater than 7.5 (Table 6).

Table 4 : Growth index [1] of three nematophagous fungi on different animal manures media compared to wheat media

Substrates	Growth index		
	Paecilomyces lilacinus	*Fusarium solani*	*Fusaruim oxysporum*
Wheat grain	5.0 a	4.7 b	4.8 ab
Wheat macerate	--[2]	5.0 a	4.0 c
Layer manure	4.7 a[3]	5.0 a	5.0 a
Broiler manure	4.7 a	5.0 a	4.7 b
Cow manure	4.0 b	4.2 c	3.5 d
Sheep manure	2.3 d	3.0 d	2.5 e
Fermented manure	3.2 c	3.5 d	2.2 f
LSD	0.66	0.25	0.247

(1) Growth index : 1 = no growth, 2 = poor growth, 3 = moderate growth, 4 = abundant growth and 5 = very abundant growth

(2) Data not present

(3) Means in the same column followed with similar letter are not significantly different according to the Duncan's Multiple Range Test (DMRT)

Table 5 : Number of spores of three nematophagous fungi produced on animal manures media compared to wheat media .

Substrates	Number of spores per gram x 10^7		
	Paecilomyces lilacinus	*Fusarium solani*	*Fusaruim oxysporum*
Wheat grain	77.3 [1] bc	7.6bc	2.9 d
Wheat macerate	- -[2]	13.3 b	4.5 c
Layer manure	167.2 a[3]	32.3 a	17.2 a
Broiler manure	174.3 a	29.7 a	6.2 b
Cow manure	101.0 b	13.1 b	4.7 c
Sheep manure	45.3 d	6.3 c	3.0 d
Fermented manure	67.0 cd	6.6 c	2.3 d
LSD	24.42	6.31	1.31

(1) All values average of three replicates

(2) Data not present .

(3) Means in the same column followed with similar letter are not significantly different according to the Duncan's Multiple Range Test (DMRT) .

Table 6 . Values of pH, electrical conductivity (EC),% phosphorus (P),% potassium (K),% nitrogen (N),% carbon (C) and C / N ratios of the different substrates.

Substrate	PH	EC	K (%)	P (%)	C (%)	N (%)	C/N
Layer manure	6.6	8.7	1.1	1.9	41.3	5.11	8.03
Broiler manure	6.8	6	1.6	1.9	36.1	4.4	8.2
Cow manure	7.8	13	0.9	0.37	33.9	2.77	12.23
Sheep manure	7.9	13	2.4	1.4	47.9	2.1	22.8
Fermented manure	8	9.5	1.64	4.7	17.4	1.9	9.1
Wheat grain	6.3	1.6	0.38	0.36	52.4	2.67	19.6

2 . Effect of colonized amendments by nematophagous fungi on root-knot nematode population in the greenhouse.

The carrier substrates of three nematophagous fungi affected the protection of tomato against *Meloidogyne javanica* differently. *P. lilacinus*, *F. solani* and *F. oxysporum* formulated on layer manure significantly reduced root galling by 58% , 58% and 53% , respectively compared with non-formulated layer manure and 64% , 64% and 59%, respectively compared with nematode alone treatment (Table 7) .

The galling index in colonized broiler manure was higher than on colonized layer manure in non-significant manner (except for *P. lilacinus*), but significantly lower than non-colonized broiler manure (Table 7) .

Formulating the three nematophagous fungi on wheat grains reduced significantly gall formation by 36% in both *F. solani* and *F. oxysporum* but not significant in *P. lilacinus*.

Number of galls were reduced significantly with layer manure colonized with *F. oxysporum* , *P. lilacinus and F. solani* by 86%, 84% and 70%, respectively. There were no significant differences in number of galls between pots treated with colonized amendments (Tabel 7).

Substrates colonized by *P. lilacinus*, *F. solani* or *F. oxysporum* showed significantly higher percentage of parasitized egg masses compared with non colonized substrates, except for *F. solani* on wheat grain. The highest percentages of infected egg masses were in pots inoculated with *P. lilacinus* and *F. solani* formulated in layer or broiler manure. It varied from 44% to 76 % (Table 7). Also, *F. oxysporum* showed 54% on layer manure and 32% on wheat grain treatments. Organic amendments free from colonized fungi did not affect gall formation, number

of galls or parasitized egg masses when added to pots at the rate of 0.67% w/w. Root and shoot weights were not affected by all treatments, except for the treatments which were inoculated with *P. lilacinus* formulated on organic matter which showed higher shoot weight. Pots free from nematode, fungus and organic amendment showed lowest root weight (Table 7) .

2.1 Correlation between gall index and percentage of parasitized egg masses.

There were significant correlations between gall index and percentage of egg masses parasitized in many treatments . Gall index in treatments of *P.lilacinus, F. solani* and *F. oxysporum* formulated on layer manure were negatively correlated with percentage of parasitized egg masses .The correlation coefficients were significant at 5% probability . Also *F. solani* on broiler manure had significantly negative correlation coefficients at the same probability. Galling index in fungi colonized in wheat grain, *P.lilacinus* and *F. solani* colonized in broiler manure were not correlated with percentage of infected egg masses (Table 8) .

4) Layer alone (No fungi)	3.8 bc	102.8 abc	0 d	13.1 bcd	10.38abc
Nematode +Broiler manure inoculated with					
5) *P.lilacinus*	3.0 cd	77.8 bcd	44.0 bc	17.4 a	10.9 abc
6) *F. solani*	2.0 de	30 de	62.0 b	13.5 bcd	8.44 abc
7) *F. oxysporam*	2.7 de	55 de	28 c	14.86 abcd	9.56 abc
8) Broiler alone (No fungi)	4.0 abc	96.0 abc	0 d	13.2 bcd	13.36 abc
Nematode + wheat grain inoculated with					
9) *P.lilacinus*	4.0 abc	129.0 ab	34.0 c	16.3 ab	14.64 ab
10) *F. solani*	3.2 c	71.6 bcd	12.0 d	14.5 abcd	10.5 abc
11)*F. oxysporam*	3.2 c	67 cd	32 c	14.83 abcd	12.0 abc
12) Wheat alone (No fungi)	5.0 a	139 a	0 d	12.8 bcd	12.2 abc
13) Nematode alone	4.4 ab	130 ab	0 d	11.8 d	16.2 a
14) Soil alone	0 f	0 e	0 d	12.24 d	5.94 c

(1) Galling index (0-5) : 0= 0 (no gall) and 5= 100% root infection (Maximum gall)

(2) Means in the same column followed with similar letter are not significantly different according to DMRT.

Table 8 : Correlation coefficients (r) of different galling index to percentage of parasitized egg masses in greenhouse experiment

Treatments	r	p *
Layer manure formulated by either		
P.lilacinus	- 0.892	0.041
F. solani	- 0.953	0.011
F. oxysporum	- 0.911	0.031
Broiler manure formulated by either		
P.lilacinus	0.154	1.00
F. solani	- 0.942	0.016
F. oxysporum	- 0.3	1.00
Wheat grain formulated by either		
P.lilacinus	0.16	1.00
F.solani	- 0.612	0.272
F. oxysporum	0.46	1.00

* Correlation coefficients significant at $P < 0.05$

3 . Effects of formulated amendments by fungi on *M. javanica*, fungal survival and plant growth in the field.

3.1 *Meloidogyne javanica.*

(a) Galling index

Galling was reduced significantly in treatments inoculated with *P. lilacinus* , *F. solani* and *F. oxysporum* compared with treatments free from fungi. Also the galling index was significantly reduced with layer manure more than with wheat grain. The interaction of nematophagous fungi (X) amendments showed a significant effect. Among the formulations of *P. lilacinus* , *F. solani* and *F. oxysporum* on layer manure galling reduced by 32%, 27% and 27% and on wheat grain by 27% , 19% and 15.4%, respectively (Table 9) .

(b) Percentage of root infection

P. lilacinus and *F. solani* significantly decreased root infection with *Meloidogyne javanica* while there were no differences between *F. oxysporum* and amendments free from nematophagous fungi. Layer manure reduced percentage of root infection significantly compared with wheat grain. Among the interaction of nematophagous fungi (X) organic amendment, colonized layer manure with the three nematophagous fungi significantly decreased percentage of root infection. Colonized wheat grain decreased root infection significantly with *P. lilacinus* and *F. solani* but not with *F. oxysporum* (Table 9).

(c) Second stage juveniles of *Meloidogyne javanica.*

The three nematophgagous fungi were able to reduce the number of *Meloidogyne javanica* juveniles significantly compared with the control. On the other hand Layer manure reduced second stage juveniles significantly than of wheat grain. The

interaction of nematophagous fungi (X) layer manure was significantly affecting on number of second stage juveniles, while interaction with wheat grain did not affect juvenile populations. (Table 10).

(d) percentage of parasitized egg masses .

Plots treated with nematophagous fungi showed suppression to development of nematode eggs compared with plots treated with the carrier alone. Parasitized egg masses of *M. javanica* were significantly higher in plots recieved layer manure than of plots recieved wheat grain. The interaction of nematophagous fungi (X) layer manure was significant, *P. lilacinus* , *F solani* and *F. oxysporum* parasitized egg masses by 74%, 50% and 58%, respectively (Table 10). While there were no significant interaction between nematophagous fungi and wheat grains .

3 . 2 Survival of fungi in soil

Fungal propagules per one gram oven dry soil was determined at 70 days from the incorporation of fungi into soil (Table 11). The number of fungal propagules was influenced by types of carriers. The highest fungal population was found significantly with*P. lilacinus* and *F solani* and the number of propagules per gram oven dry soil was 4. 6 $X10^5$ and 4.5 X 10^4,. respectively. The population of *F. oxysporum* was not significantly different from plots treated with autoclaved amendment alone. Among the organic amendments, populations of fungi in layer manure treatments were significantly higher than of those in wheat grains . The interaction of nematophagous fungi (X) layer manure was significant. The highest colony forming units per gram soil (CFU/g) was recovered in plots treated with *P. lilacinus* , *F. solani* and *F. oxysporum* formulated in layer manure. It was

9.18 X 10^5, 8 X10^4 and 0.9 X 10^4, respectively (Table 11).There were no significant difference in CFU/g in plots treated with fungi formulated in wheat grain. At the end of growing season, populations of nematophagous fungi were decreased. The plots treated with *P. lilacinus* significantly still harboured highest number of propagules than those treated with *F. solani*, *F. oxysporum* or amendment alone. Also at termination CFU/g was significantly higher in layer manure than on wheat grain (Table 11). The interaction of nematophagous fungi (X) organic matter was not significant; except for *P. lilacinus* colonized in layer manure which had the highest number of propagules (Table 11). Among the treatments, CFU/g in plots inoculated with colonized layer manure was higher than plots treated with colonized wheat grain and fungal populations in colonized wheat grain was over the control.

3.3 - Yield and root weights.

Yield and root weights of tomato at termination time showed that there were no significant differences between treatments of the three nematophagous fungi and amendments free from fungi (Table 12). Among the amendment treatmens, yield and root weights were higher in layer manure than of wheat grain but not in significant manner. *P. lilacinus* formulated in layer manure was able to increase the yield significantly while for other treatments the interaction of nematophagous fungi (X) organic matter was not significant. Plots of colonized layer manure with *F. oxysporum* was significantly increased in the root weight over other treatments. In spite of that the interaction of nematophagous fungi (X) amendment was not significant.

Table 9 : Effect of nematophagous fungi, organic amendments and their interaction on galling index and root infection by *M. javanica*.

Treatments	Galling index (1-6) [1]	Root infection (%)
Paecilomyces lilacinus	3.4 b [2]	41.17 b
Fusarium solani	3.7 b	49.73 b
Fusarium oxysporum	3.8 b	54.47ab
Control	4.8 a	75.65 a
Layer manure	3.45 b	43.76 b
Wheat grain	4.4 a	66.75 a
Interaction of Nematophagous Fungi X Organic Amendments		
P. lilacinus formulated on		
layer manure	3.0 d	31.8 d
Wheat grain	3.8bc	50.5 c
F. solani formulated on		
layer manure	3.2cd	36.7 d
Wheat grain	4.2 b	62.8 bc
F. oxysporum formulated on		
layer manure	3.2cd	33.54 d
Wheat grain	4.4 b	75.4 ab
Control		
layer manure alone	4.4 b	73.0ab
Wheat grain alone	5.2 a	78.0 a

(1) Galling index 1 - 6 ; 1= 0 (No galling) and 6 = 100% of root galling

(2) Means in the same column followed with similar letter are not significantly different ($P<0.05$) according to DMRT

Table 10: Effect of nematophagous fungi, organic amendments and their interaction on parasitized egg masses and juveniles of *M. javanica*.

Treatments	Juveniles (1)	Egg masses parasitized (%)
Paecilomyces lilacinus	88.6 b (2)	46.0 a
Fusarium solani	98.7 b	30.0 b
Fusarium oxyspurum	88.2 b	38.0 ab
Control	155.9 a	0 c
Layer manure	80.75b	45.5 a
Wheat grain	134.95a	11.5 b
Interaction of Nematophagous Fungi X Organic Amendments		
P. lilacinus formulated on		
layer manure	40.4 b	74.0 a
Wheat grain	136.8a	18.0 c
F. solani formulated on		
layer manure	65.0 b	50.0 b
Wheat grain	132.4a	10.0 c
F. oxysporum formulated on		
layer manure	55.6 b	58.0b
Wheat grain	120.8a	18.0c
Control		
layer manure alone	162.6a	0 c
Wheat grain alone	149.8a	0 c

(1) Per 100 cm^3 soil.

(2) Means followed by the same letter within each column are not significantly different ($P<0.05$) according to DMRT

Table 11: Fungal populations (CFU/g X 10^3) after 70 and 180 days of soil inoculation in tomato plots

Treatments	Mid-season (70 days)	Termination time (180 days)
Paecilomyces lilacinus	461.1 a [1]	384.1 a
Fusarium solani	45.74 b	21.5 b
Fusarium oxysporum	5.2 c	4. 7 b
Control	0.587 [2] c	0.57 b
Layer manure	252 A	202.8 A
Wheat grain	4.34 b	2.69 b
Interaction of Nematophagous Fungi X Organic Amendments		
P. lilacinus formulated on		
layer manure	918 a	768 a
Wheat grain	4.3 c	0.3 b
F. solani formulated on		
layer manure	80.8 b	36 b
Wheat grain	10.6c	6.4 b
F. oxysporum formulated on		
layer manure	8.98 c	6,2 b
Wheat grain	1.44 c	3.32 b
Control		
layer manure alone	0.23 c	0.42 b
Wheat grain alone	0.95 c	0.73 b

(1) Means in the same column followed with similar letter are not significantly different (P<0.05) according to DMRT

(2) CFU / g * 10^3 for *Fusarium* spp .

Table 12: Effect of nematophagous fungi, organic amendments and their interaction on yield and root fresh weight of tomato.

Treatments	Yield (kg/plot)	Root weight [2] (g/plot)
Paecilomyces lilacinus	21.57 a [1]	539 a
Fusarium solani	16.57 a	526 a
Fusarium oxysporum	21.15 a	548 a
Control	17.24 a	484 a
Layer manure	20.56 a	564.8 a
Wheat grain	17.65 a	486.0 a
Interaction of Nematophagous Fungi X Organic Amendments		
P. lilacinus formulated on		
layer manure	25.38 a	590 ab
Wheat grain	17.93 bc	488 bc
F. solani formulated on		
layer manure	16.67 b	559 abc
Wheat grain	15.8 c	494.0 bc
F. oxysporum formulated on		
layer manure	22.9 ab	610 a
Wheat grain	19.4 ab	486 bc
Control		
layer manure alone	19.0 bc	499 bc
Wheat grain alone	17.47 bc	474 bc

(1) Means in the same column followed with similar letter are not significantly different ($P < 0.05$) according to DMRT.

(2) Air dry weigth.

3 .4 Correlations between results of the field study

(a) Galling index with percentage of egg masses infected.

All plots treated with fungi formulated on organic amendments showed a negative correlation (Table 13). The correlation coefficents were significant in plots with layer manure colonized by *P. lilacinus* , *F solani* or *F. oxysporum.* On the other hand, the correlation was not significant in treatments where wheat grain was formulated by the three nematophagous fungi (Table 13).

(b) Galling index with number of fungal propagules at termination time.

P. lilacinus and *F. solani* on layer manure showed negative correlations between galling index and colony forming units at termination time. The correlation coefficent was significant at the probability of 5%. The correlation coefficient was negative in plots treated by *F. oxysporum* on layer manure and on wheat grain but not significant (Table 13). There was no correlation between galling index and CFU/g in plots treated by amendments free from fungi.

(c) Galling index with yield

Significantly negative correlation coefficeints between galling indexes and yield were found in treatments with *P. lilacinus* , *F. solani* and *F. oxysporum* formulated in layer manure (Table 13). For other treatments, the correlation coeffeicent was negative but not significant .

(d) Percentage of root infection with percentage of parasitized egg masses

The correlation between these variables were significantly in *P. lilacinus* , *F. solani* and *F. oxysporum* colonized in layer manure and *F. solani* on wheat grain (Table 13). The values were approximatly -1 indicating a perfect linear correlation between the two variables. Also *F. oxysporum* and *P. lilacinus* on

wheat showed negative correlations but not in a significant manner (Table 13).

(E) Number of second stage juveniles with percentage of parasitized egg masses .

Negative correlation coefficients were significantly in layer manure formulated with three nematophagous fungi between number of second stage juveniles and percentage of parasitized egg masses, while there were no correlations in plots treated with nematophagous fungi colonized on wheat grain (Table 13).

(F) Percentage of parasitized egg masses with number of fungal propagules .

Positive correlation was found between percentage of parasitized egg masses and CFU/ g in plots recieved layer manure colonized by the three nematophagous fungi, also in plots inoculated by wheat grain colonized by *F. solani* (Table 13) . There were no correlations in remained treatments including *P. lilacinus* and *F. oxysporum* formulated on wheat grain .

Treatments	(1) r	p ***	(2) r	p	(3) r	p	(4) r	p	(5) r	p	(6) r	p
P. lilacinus + LM *	- 0.914	0.029	-0.89	0.047	-0.937	0.021	-0.927	0.023	-0.89	0.024	0.931	0.022
P. lilacinus + WG**	- 0.69	0.31	-0.49	1.0	-0.42	1.00	-0.638	0.23	-0.32	1.00	0.43	1.0
F. solani + LM	- 0.917	0.048	-0.83	0.072	-0.73	0.082	-0.911	0.034	-0.891	0.043	0.890	0.0488
F. solani + WG	- 0.968	0.006	-0.72	0.162	-0.32	1.00	-0.99	0.001	-0.614	1.00	0.921	0.032
F. oxysporum + LM	- 0.923	0.032	-0.812	0.078	-0.893	0.0491	-0.911	0.049	-0.91	0.021	0.911	0.038
F. oxysporum + WG	- 0.327	1.0	-0.43	1.0	-0.612	0.218	-0.320	1.0	-0.43	1.00	0.38	1.00
LM alone			-0.327	1.0	0	1.00						
WG alone			-0.31	1.0	-0.358	1.00						

.ayer manure (LM)

Wheat grain (WG)

Correlation coefficient (r) significant at P '< 5%

Correlation coefficients (r) of different percentages of gall indices to percentage of parasitized egg masses.

Correlation coefficients (r) of different gall indices to CFU/g at termination.

Correlation coefficient (r) of different gall indices to yield.

Correlation coefficient (r) of different percentages of root infections to percentage of parasitized egg masses.

Correlation coefficients (r) of different numbers of second stage juveniles to percentage of parasitized egg masses.

Correlation coefficents (r) of different percentage of parasitized egg masses to CFU/g.

4 . Effects of carrier substrates on viability of nematophagous fungi through storage .

The effect of carriers and length of storage on the viability of *P. lilacinus* , *F. solani* and *F. oxysporum* were determined over a period of six months. The viability of fungal propagules was not affected with length of storage in carrier substrates (Table 14). *P. lilacinus* and *F. solani* remained highly viable on wheat grain, layer and broiler manure over the six months of storage. Also *F. oxysporum* spores remained viable on wheat grain and broiler manure over the storage period, but decrease in layer manure intermidiately after 120 days of storage (Appendix B) .

5- Pathogenicity test

Tested plants cultivars Maisara, Maramand, Black beauty, Ananas anjar choice and Lolita were remained healthy and no infection was caused by ethier *Fusarium solani* or *F. oxysporum* on all tested plants.

Table 14 : Effect of carriers and length of storage at 25 ± 5°C on viability of *P. lilacinus* , *F. solani* and *F. oxysporum* (CFU/g X 10^7) * .

Treatments	Days						
	5	15	30	45	90	120	180
P. lilacinus colonized on :							
Layer manure	780	740*	750	770	780	800	780
Broiler manure	850	810	870	840	850	830	850
Wheat grain	280	240	300	260	270	300	280
F. solani colonized on :							
Layer manure	71*	71	62	63	71	64	62
Broiler manure	52	48	53	52	53	55	48
Wheat grain	7	6	6	7	7	6	6
F. oxysporum colonized on :							
Layer manure	70	65	73	71	73	55	45
Broiler manure	60	60	63	62	64	62	62
Wheat grain	3	3	4	4	4	4	3

* Colony Forming Unit per gram substrate (CFU / g) .

1 . Evaluation of animal manures for mass production of *P. lilacinus, F. solani* and *F. oxysporum* .

The present investigation demonstrated that the layer, broiler and cow manure can be used as a suitable alternatives to wheat grains for mass production of all involved fungi. Mycelial growth index of *P. lilacinus, F. solani* and *F. oxysporum* on these manures and the number of spores produced on these substrates which was exceeded the number produced on wheat grains support this conclusion .

Sheep and fermented mix manures were suitable as wheat grain medium for the spore production of the three nematode antagonistic fungi involved in the evaluation. In addition to these facts, animal manures are cheaper than wheat for using in the mass production of fungi. Animal manure practicaly used over water lily, Grain husk, wheat straw or coconut chair dust, because some of these substrates is not available or can be used as animal food, e.g. grain husk or straw (Davide and Zorilla,1983). Spore production of *P. lilacinus* on wheat grain medium was very similar to those achieved on the same medium by Cabanillas *et al.* (1989 a). The variability in species and genera of fungi used in this study and the consistency in the suitability of layer and broiler manure for their growth might allow to extrapolate results for other fungi that possess similar saprophytic activity.

Layer and broiler manure produced higher spores than other substrates, the low C/N ratios (8.03 and 8.2) and high nitrogen contents of these media lead to stimulate fungal growth and sporulation (Table 6) . Cow manure with the C/N ratio 12.23 produced spores greater in number than of sheep and wheat grain which have C/N ratios 22.8.and 19.6, respectively. Organic matters with low C/N ratios resulted in broad spectrum stimulations of soil microflora (Rodriguez-Kabana *et al.*, 1987). The

suitability of these substrates for establishment of fungi in soil and their consequent potential in nematode control was done in both greenhouse and field tests.

2 - Effect of nematophagous fungi on *Meloidogyne javanica* populations in soil

2 . 1 Greenhouse study

Results of greenhouse showed that the types of carrier substrates used in the introduction of nematophagous fungi into soil influnced the degree of protection of tomato against *M. javanica*.. On the other hand, the three nematophagous fungi tested, showed similar efficiency in controlling *M. javanica* in pot experiment with the same carrier. There were no differences between native bioagents (*F. solani* and *F. oxysporum*) and introduced one (*P. lilacinus*) in controlling *M. javanica* .

The three nematophagous fungi formulated on layer manure, resulted in decreasing galling and increasing percentage of parasitized egg masses, galling and number of galls were reduced by 55% and 78%, respectively and the percentage of egg masses infected varied between 54-76%. The above results indicated that galling was reduced due to parasitism of *P. lilacinus*, *F. solani* and *F. oxysporum* on eggs within egg masses of *M. javanica*. Therefore eggs were destroyed and the number of hatching eggs for new generation was decreased.

The three nematophagous fungi formulated on broiler manure caused different reductions in galling index by 25-50% and by 19-69% in gall number, the percentage of infected egg masses was varied between 28% to 62%. The data in table 7 showed that there was a relation between percentage of parasitized egg masses and galling. In case of *F. solani* on broiler manure, the percentage of parasitized egg masses was

62% and this was the highest percentage of parasitism for fungi formulated on broiler manure, while the galling index was 2 and it was the lowest index in these treatments. Inspite of this, there were no significant differences in the efficiency of three fungi on broiler manure in nematode control.

Wheat grains were used by many investigators as a carrier substrates (Table 3). Wheat grain, in this study, showed significant variations in nematode control between colonized and non-colonized grain in *F. solani* and *F. oxysporum* but not in *P. lilacinus*. The reduction in galling ranged from 25% to 36%.

Correlations between galling index and percentages of parasitized egg masses are presented in table 8. Negative correlations were noted in pots treated by three fungi formulated on layer manure or *F. solani* on broiler manure which indicates that, as the percentage of infected egg masses increased the galling was decreased. Also, if the percentage of infected egg masses decrease the galling increase and this happened in the fungi formulated in wheat grain.

Wheat have similar mycelial growth index in layer and broiler manure (Table 4), but different in spore production (Table 5). One gram of *P. lilacinus* colonized on layer, broiler manure or wheat grain contained $7.7X10^9$, 8×10^9 and $3x10^9$ propagules. One gram of colonized *F. solani* contained 6.3×10^8, 5×10^8 and 0.7×10^8 propagules and for *F. oxysporum* contained 7.3×10^8, 6×0^8 and 5×10^8 propagules, respectively. These are the concentrations of fungi which were incorporated into the soil. Pots received similar inoculum levels (Ten grams of colonized organic amendments) but different inoculum densities. The density of fungal propagules on layer manure was higher by 2.6 times in *P.lilacinus* , 14.5 times in *F. oxysporum* and 9 times in *F. solani* than on wheat grain. Therefore fungi formulated on poultry

manure caused a more reduction in galling than fungi colonized on wheat grain. The results of the efficacy of *F. solani* and *F. oxysporum* formulated on wheat grain to control *M. javanica* agreed with the findings by Qadri (1989).

Plant growth was not affected significantly in all treatments in spite of using fungal inoculum at the rate of 0.67% w/w. Results of Shahzad *et al*, (1990) in controlling *M.javanica* showed that plant growth was not significantly different, but the population of *M. javanica* was reduced at the rate of application 1% w/w infected rice grain.

Another important drawback in using wheat grain was noticed in our experiment. It was the losses of wheat grain after introduction into soil by field rats due to feed on the wheat kernels. This decreased inoculum level of fungi which might have negatively effect on nematode control.

2 . 2 . Field study.

The results obtained from the field test showed that *P. lilacinus, F. solani* and *F. oxysporum* could be used as active agents for biological control of the root-knot nematode *Meloidogyne javanica* (Table 9). The activity of nematophagous fungi against nematode populations depend on method used for bioagents introduction into soil, soil conditions and other factors (Kerry, 1987). Kerry (1984) was able to control *Heterodera avenae* by *Verticillium chlamydosporium* and other parasitic fungi by introduction of bioagents formulated on oat grains, but not as spore suspension .

The field results showed that the type of carrier used in introduction of nematophagous fungi into soil influnced fungal survival in soil, establishment and subsequent protection of plants against root-knot nematode. The introduction of the three fungi into soil on layer manure was more effective in controlling root-knot nematode than on wheat grain.

Layer manure was proved to be a suitable substrate for mass production of various nematophagous fungi. The fungi cultured on layer manure produced heavily mycelium growth and high spore numbers (Tables 4 and 5).

In case of biological control of nematodes by parasitic fungi, spore numbers are important. The ability of the fungus to proliferate abundantly in the soil after its addition is important for its activity (Kerry , 1984). Therefore *P. lilacinus, F. solani* and *F. oxysporum* colonized on layer manure increased the percentage of parasitized egg masses of *M. javanica* and decreased nematode populations significantly than that of wheat grain (Table 10). This fact was ensured by the negative correlations found between the number of second stage juveniles and

percentage of parasitized egg masses in plots which recieved nematophagous fungi formulated on layer manure (Table 13).

In addition to that, the decomposition of organic matter by microorganisms in soil might result in an increased enzymatic activity of amended soil and accumulation of specific end product compounds which might have nematicidal effect (Rodriguez- Kabana, 1987). The magnitude of organic matter activity against the pathogen depends on the nature of organic amendment or chemical composition and the species of microogranisms. Kabana (1987) reported that the materials which have typical low C/N ratios and high protein or amine contents (N contents) might have nematicidal effect. In our experiment, plots treated by fungi-free layer manure showed less galling than of plots treated with wheat grain alone. This could be due to the contents of layer manure which have 5.1% nitrogen, while wheat grain contains 2.67% nitrogen only (Table 6).

Another factor which increased the efficacy of layer manure over wheat grain in nematode control is the inoculum level. Wheat grains infesed by *P. lilacinus, F. solani* and *F. oxysporum* introduced into the soil at the rate of100 g/m^2. This rate exceeded by two and half times of what was recommended by Jatala (1981). He was successful in controlling *M. incognita* and *G. rostochiensis* by *P. lilacinus* introduced to soil at the rate of 37.5 gram of inoculated wheat grain per square meter (Jatala, 1986). Several investigators used different rates of fungi infested cereal grains. They found that by increasing the rate of application, the control of nematode was increased (Table 3).

Cabanillas *et al.* (1989 a) reported that wheat grain could be used as a carrier substrate at an economical level, but the rate of application should not exceed 100 -

200 Kg/ha. This rate is low compared with a recommended rate (400 Kg/ha) by Jatala (1981) and too low compared with the rate used in this experiment (1000 Kg/ha). The rate of application 1000 Kg/ha is not practical and not economical to use in Jordan or other countries. In addition to that, the activity of three nematophagous fungi in controlling *M. javanica* was greater in layer manure than of wheat grain as previously mentioned.

Up to our knowledge, there are few reports dealing with biocontrol of *M. javanica* by nematophagous fungi. Hewlett *et al.* (1988) failed to control *M. javanica* in field soil by *P. lilacinus* in spite of using 245 g of infested wheat per square meter.

Addition of animal manure to soil for crop improvement is an old practice in agriculture. In Jordan, most farmers use the poultry manure more than other manures. They apply manures at the rate of 5-20 tons/ha for improving crop production. Use of 500 g/m^2 layer manure infested with nematophagous fungi (5 tons/ha) for delivery into soil, showed significant results in reduction of nematode population. Introduction of bioagents formulated on organic manure, could be consider practical , economical and compatible with standard farming practices . Survival and establishment of *P. lilacinus, F. solani* and *F. oxysporum* in field soil were better in formulated layer manure than that of wheat grain after 70 days of inoculation. This is important for continuity of biological control of nematode. Reduction of CFU/g at harvesting time may be due to reduction in nutrition or other soil factors.

Yield and root weights were not different between all treatments. Similar results were observed by Shahzad *et al.* (1990) and Qadri (1989). These reports showed significant control of *M. javanica* by some types of fungi but does not affect

plant growth. Also, some of the plants were infected by late blight and tomato yellow leaf curl virus, which might have a negative effect on the yield.

Interactions between fungal antagonists and organic amendments were expressed by different correlations factors. *P. lilacinus, F. solani* and *F. oxysporum* colonized in layer manure showed negative correlations between galling index and percentage of parasitized egg masses, percentage of root infection and percentage of parasitized egg masses, galling index and CFU/g, number of second stage juveniles and percentage of parasitized egg masses (Table 13) indicated that reduction in nematode population was due to fungal antagonists but not to other factors. Positive correlation was found between percentage of parasitized egg masses and CFU/g which confirms the preceding conclusion

Higher percentage of infected egg masses could reduce the initial level of nematode inoculum in the subsequent growing season. The development of optimum delivery system for biocontrol agents should aid in the advancement of biocontrol research and its integration into managment systems.

3. Survival of fungi on carrier substrates through storage.

P. lilacinus and *F. solani* remained viable in carrier substrates (Layer, Broiler and wheat grain) at the same level through six months of storage at 25 ± 5°C. Similar viability was observed by Cabanillas *et al.* (1989 a) in wheat grain and diatomaceous earth granules for *P. lilacinus*, which remained viable on these carriers through 56 days of storage. The results showed that *F. oxysporum* remained viable on wheat grain and broiler manure through six months of storage, but it was reduced slightly on layer manure after 120 days .

In general, the differences in survival on organic media may be attributed to fungal species, availability of nutrients, length of storage, favourable temperature and other growth factors. Temprature is an important factor in fungal survival in different formulations. Conidia of *Talaromyces flavous* in alginate pellets stored for 15 weeks survives better at 5 and 15 than at 25°C (Papavizas *et al.*, 1987). The length of survival period is improtant for the practical use of a biocontrol agent in the future works. It is necessary to test the efficacy of fungal bioagents formulated on organic amendments to control plant parasitic nematodes after several storage periods. There is no experimental evidence to indicate the relationship between survival of *P. lilacinus* or other nematophagous fungi and the efficacy in nematode control (Cabanillas *et al.*, 1989 a)

4. Pathogenicity test

All plant cultivars tested remained free from wilt or root rot disease which indicated that the *Fusarium oxysporum* is not one of the following formae speciales:

1 - *F. oxysporum* Schl. f.sp. *lycopersici.*

2 - *F. oxysporum* Schl. f.sp. *phaseoli*

3 - *F. oxysporum* Schl. f.sp. *melonis.*

4 - *F.oxysporum* Schl. f.sp. *melongena*

Also, the pathogenicity test indicated that the *Fusarium solani* is not one of the following formae specialis:

1. *F. solani* (Mart.) Sacc. f. sp. *cucurbitae*:
2. *F. solani* (Mart.) Sacc. f. sp. *phaseoli*

The pathogenicity test showed that the *F. solani* and *F. oxysporum* which served as a nematophagous fungi are not pathogenic to tomato, eggplant, bean and melon . These fungi were isolated by Qadri (1989) from cysts of *Heterodera schachtii* . They may be new formae speciales of *F. oxysporum* and *F. solani.* Due to many difficulties we were unable to classify them.

VI- Conclusions

1 - Layer and broiler manures were mostly satisfactory substrates for mass production of *Paecilomyces lilacinus, Fusarium solani and F. oxysporum.*

2- High nitrogen content in the substrate stimulated mycelial growth and sporulation of *P.lilacinus* , *F. solani* and *F. oxysporum.*

3- *P. lilacinus, F. solani* and *F. oxysporum* were effective bioagents against the root-knot nematode *Meloidogyne javanica.*

4- The three nematophagous fungi formulated on poultry manures were significantly more effective on *M. javanica* than on wheat grain.

5- *P. lilacinus and F. solani* remained viable on layer, broiler manure and wheat grain through six months of storage at 25 ± 5°C. *F. oxysporum* remained viable on broiler manure and wheat grain but viability decreased on layer manure after 120 days of storage .

6- *F. solani* and *F. oxysporum* included in the test were not pathogenic to tomato (Maisara and Maramand), eggplant (Black beauty), bean (Lolita) and melon (Ananas anjar choice).

VII- Summaries

English summary

This research was conducted in laboratory, greenhouse and field .

Five types of animal manure (Layer,broiler, cow, sheep and fermented mix manure) were compared with wheat grain and evaluated as media for mass production of three nematode egg-parasitic fungi (*Paecilomyces lilacinus, Fusarium solani & F. oxysporum*) for control of root-knot nematode *Meloidogyne javanica* . Mycelial growth indices of *P. lilacinus, F. solani* and *F. oxysporum* on layer and broiler manure were similar or higher than on wheat grains, but was moderate on cow manure, or poor on sheep and fermented manure . Spore production of the three nematophagous fungi on layer and broiler manure was 22-590 % higher than on wheat grain.

Layer and broiler manure compared with wheat grain were used as carrier substrates of *P. lilacinus, F. solani* and *F. oxysporum* for studying their efficacy on controlling *Meloidogyne javanica* under greenhouse conditions. *P. lilacinus, F. solani* and *F. oxysporum* formulated on layer manure and delivered into soil at the rate of 0.67w/w significantly reduced galling on the tomato roots by 58%, 59% and 53%, of formulated on broiler manure by 25%, 50% and 32.5%, respectively, While the three fungi formulated on wheat grain reduced galling by 25-36%. Higher percentage of parasitized egg masses were showed in treatments of nematophagous fungi colonized on layer manure. It varied by 54-74% then on broiler manure it varied by 28-62%. The lowest percentages of infected egg masses was in pots treated by fungi colonized on wheat grain it and

varied by 12 -34%.

Results of field experiment showed that the introduced bioagent *P. lilacinus* from Peru was able to control the root-knot nematode *M. javanica*. It was able to survive and establish in the Jordan Valley soil. Locally obtained fungi *F. solani* and *F. oxysporum*, which isolated in Jordan, significantly reduced *Meloidogyne javanica* population in the Jordan Valley . Also, the study showed that the layer manure was able to affect root-knot nematode populations and fungal survival and establishment. *P.lilacinus F.solani* and *F. oxysporum* colonized on layer manure reduced galling by 56% ,50% and 54%, but that colonized wheat grain reduced galling by 35% , 20% and 38%, respectively. The highest percentage of parasitized egg masses (50-74%) was found in nematophagus fungi formulated on layer manure .

Survival of the three parasitic fungi on the three carrier substrates was studied through six months of storage. *P. lilacinus* and *F.solani* remained viable on layer manure, broiler manure and wheat grain through storage period *F. oxysporum* remained viable on broiler manure and wheat grain but decreased on layer manure after 120 days of storage.

Pathogenicity test of *F.solani* and *F. oxysporum* showed that these fungi were not pathogenic to tomato, melon, eggplant and bean.

الملخص العربي

تقييم ثلاثة انواع من الفطريات المتطفلة على النيماتودا في مكافحة نيماتود تعقد الجذور باستعمال روث الحيوانات والقمح كمواد حاملة للفطر

أجري هذا البحث في المختبر والبيت الزجاجي وفي الحقل . حيث تم تقييم خمسة أنواع من روث الحيوانات وهي روث الدجاج البياض وروث الدجاج اللاحم وروث الابقار وروث الاغنام والروث المخمر ومقارنتها بالقمح من أجل إستعمالها كبيئات لنمو وتكاثر ثلاثة أنواع من الفطريات (*Paecilomyces lilacinus , Fusarium solani & F. oxysporum*) التي تتطفل على بيوض وإناث النيماتود لإستعمالها في مكافحة نيماتود تعقد الجذور *M. javanica*. أظهرت نتائج المختبر أن الفطريات التي تم إختبارها نمت على روث الدجاج البياض وروث الدجاج اللاحم بشكل مشابه لنموها على حبوب القمح ، بينما كانت درجة نمو الفطر متوسطة على روث الأبقار وضعيفة على روث الأغنام والروث المخمر. كذلك كانت كمية الأبواغ الفطرية المنتجة من الفطريات الثلاثة على روث الدجاج البياض وروث الدجاج اللاحم تزيد بمعدل ٢٢ ـ ٥٢٠ ٪ مقارنة بالأعداد المنتجة على القمح ، بينما كانت متشابهة على كل من روث الأغنام والروث المخمر وحبوب القمح .

لقد أختير روث الدجاج البياض وروث الدجاج اللاحم للمقارنة بحبوب القمح كبيئات لحمل الفطريات الثلاثة التي تتطفل على النيماتود إضافتها الى التربة من أجل دراسة تأثيرها على نيماتود تعقد الجذور *M. javanica* تحت ظروف البيت الزجاجي . أدت إضافة كل من الفطريات التالية *P. lilacinus* و *F. solani* و *F. oxysporurm* المحمولة على روث الدجاج البياض الى التربه بمعدل ٦٧ر. ٪ وزن : وزن الى تقليل العقد الجذرية الناتجه عن نيماتود تعقد الجذور في البندورة بشكل واضح بمعدل ٥٨ ٪ و ٥٨ ٪ و ٥٣ ٪ والمحمولة على روث الدجاج اللاحم بمعدل ٢٥ ٪ و ٥٠ ٪ و ٥ر٣٢٪ والمحمولة على القمح بمعدل ٢٥ ٪ و ٣٦ ٪ و ٣٦ ٪ على التوالي ، كذلك كانت أعلى نسبة لتطفل الفطريات على أكياس بيض النيماتود في المعاملات التي عولجت بروث الدجاج البياض المحمل بالفطريات ، حيث تراوحت نسبة

روث الدجاج البياض وروث الدجاج اللاحم وحبوب القمح . وبينت الدراسة ان الفطريين *P. lilacinus & F.solani* بقيا بصوره حيه خلال فترة التخزين على البيئات الثلاث وكذلك فقد بقي الفطر *F.oxysporum* بصورة حيه على روث الدجاج اللاحم وحبوب القمح ، بينما قلت حيوية الفطر بشكل بسيط على روث الدجاج البياض بعد مرور ١٢٠ يوم من التخزين .

أظهرت نتائج دراسة أمراضية السلالات الفطرية *(F.oxysprum & F.solani)* المستعملة في البحث ان هذه السلالات لا تسبب امراض للنباتات التالية البندورة والشمام والباذنجان والفاصوليا .

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IX - Appendices

Appendix A

Fig . 1 . Mycelial growth of *P. lilacinus*, *F. solan.*

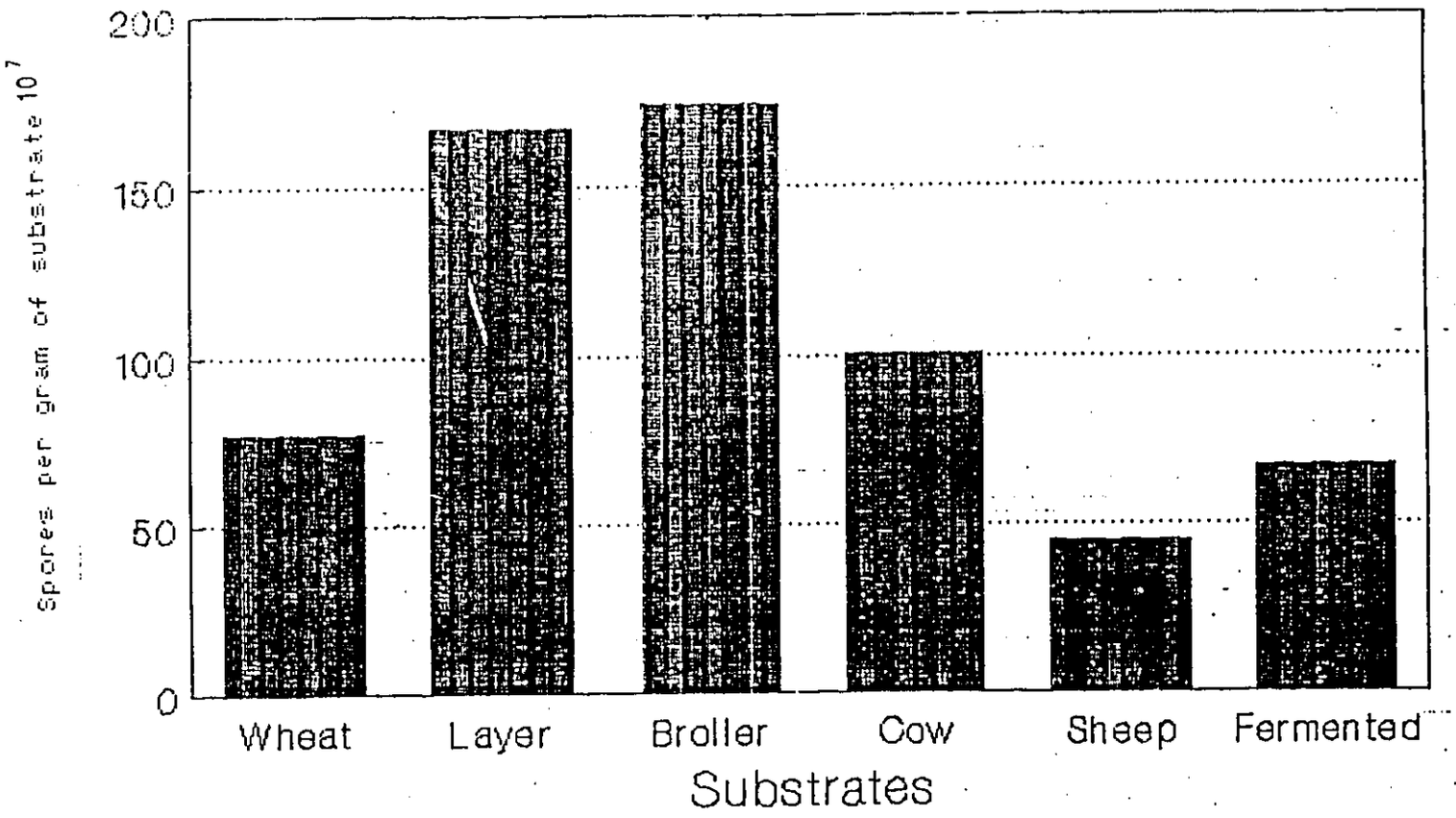

Fig . 2 . Number of spores produced by *Paecilomyces lilacinus* on different substrates.

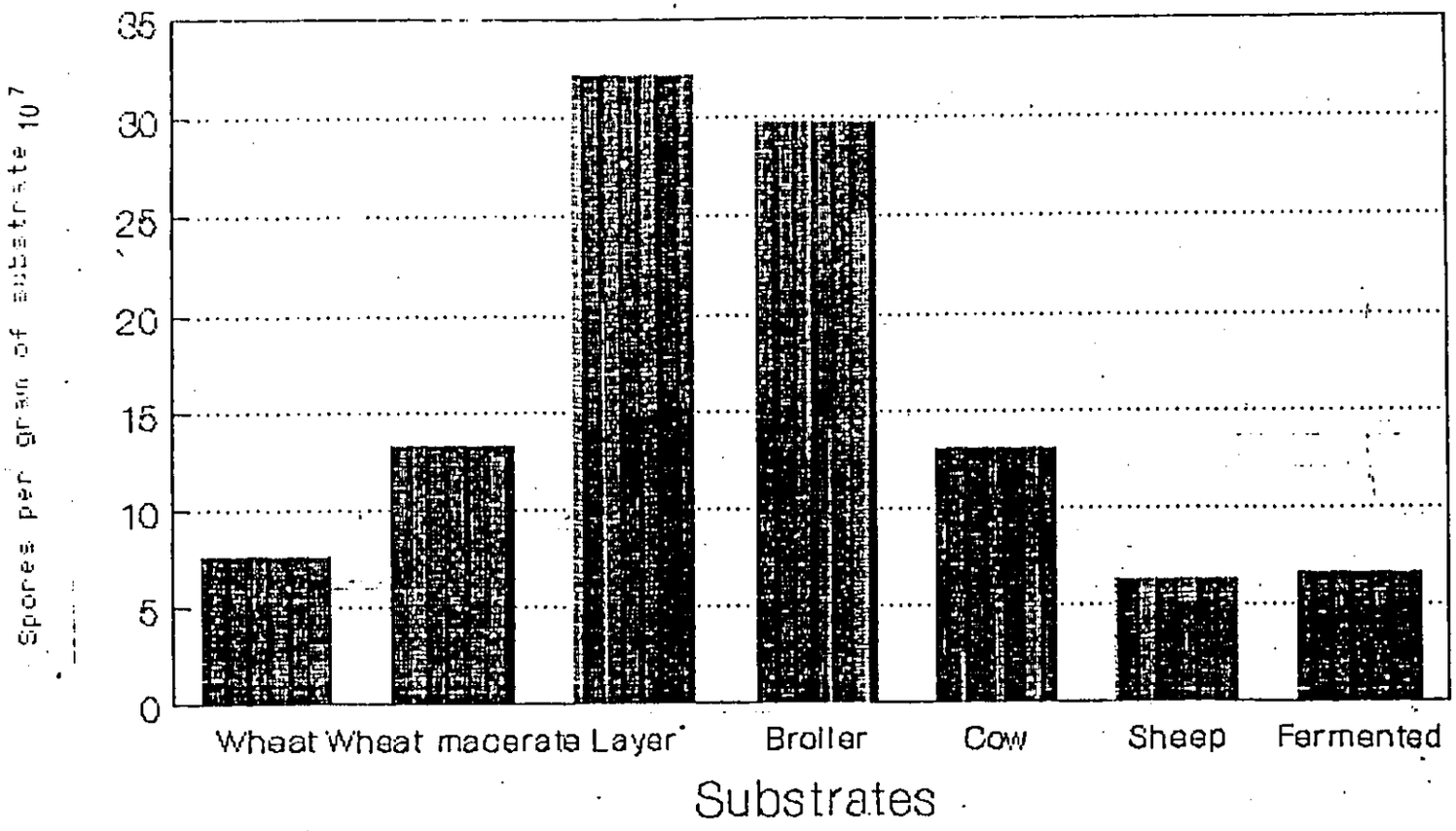

Fig . 3 . Number of spores produced by *Fusarium solani* on different substrates.

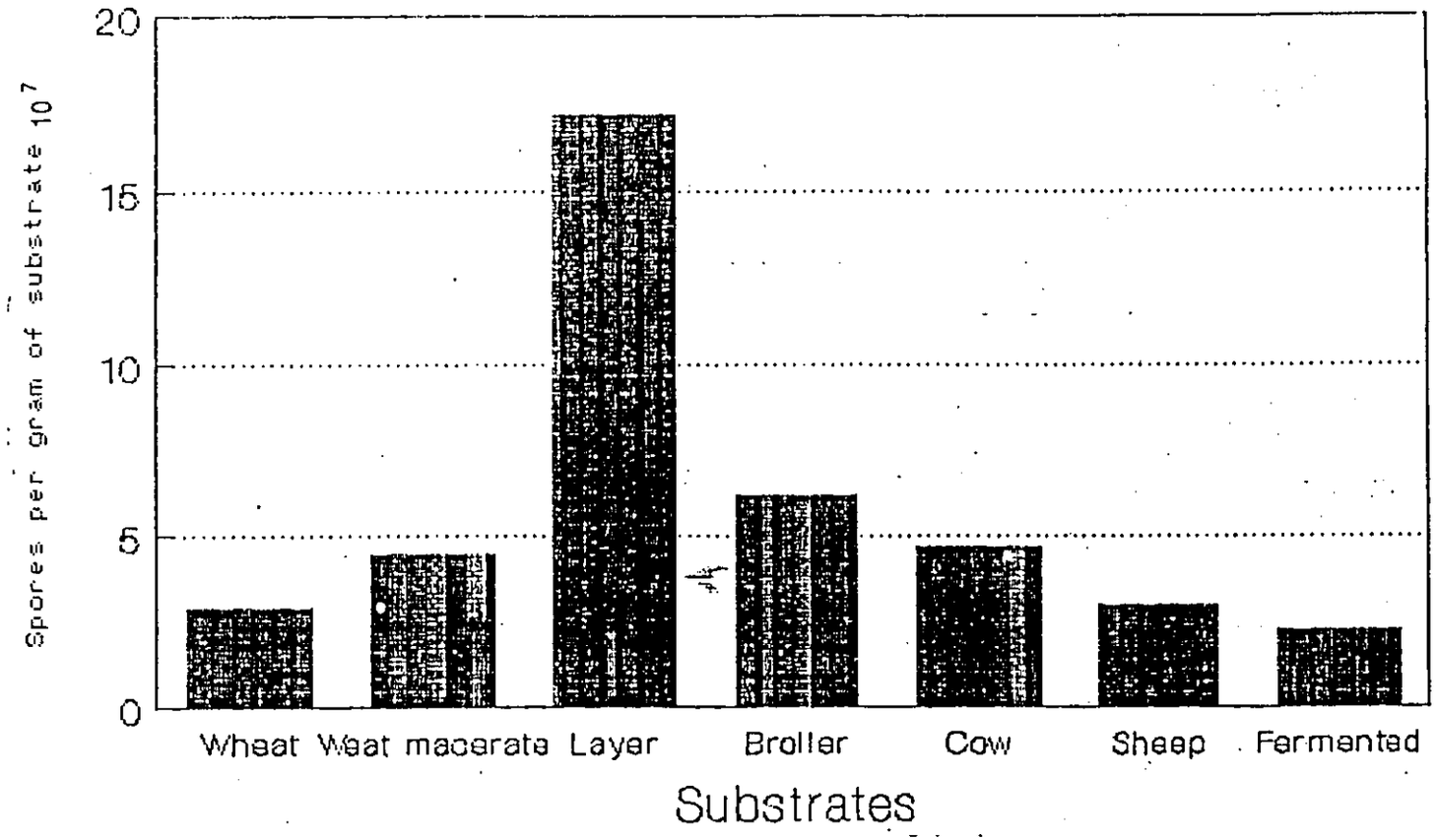

Fig . 4 . Number of spores produced by *Fusarium oxysporum* on different substrates.

Appendix B

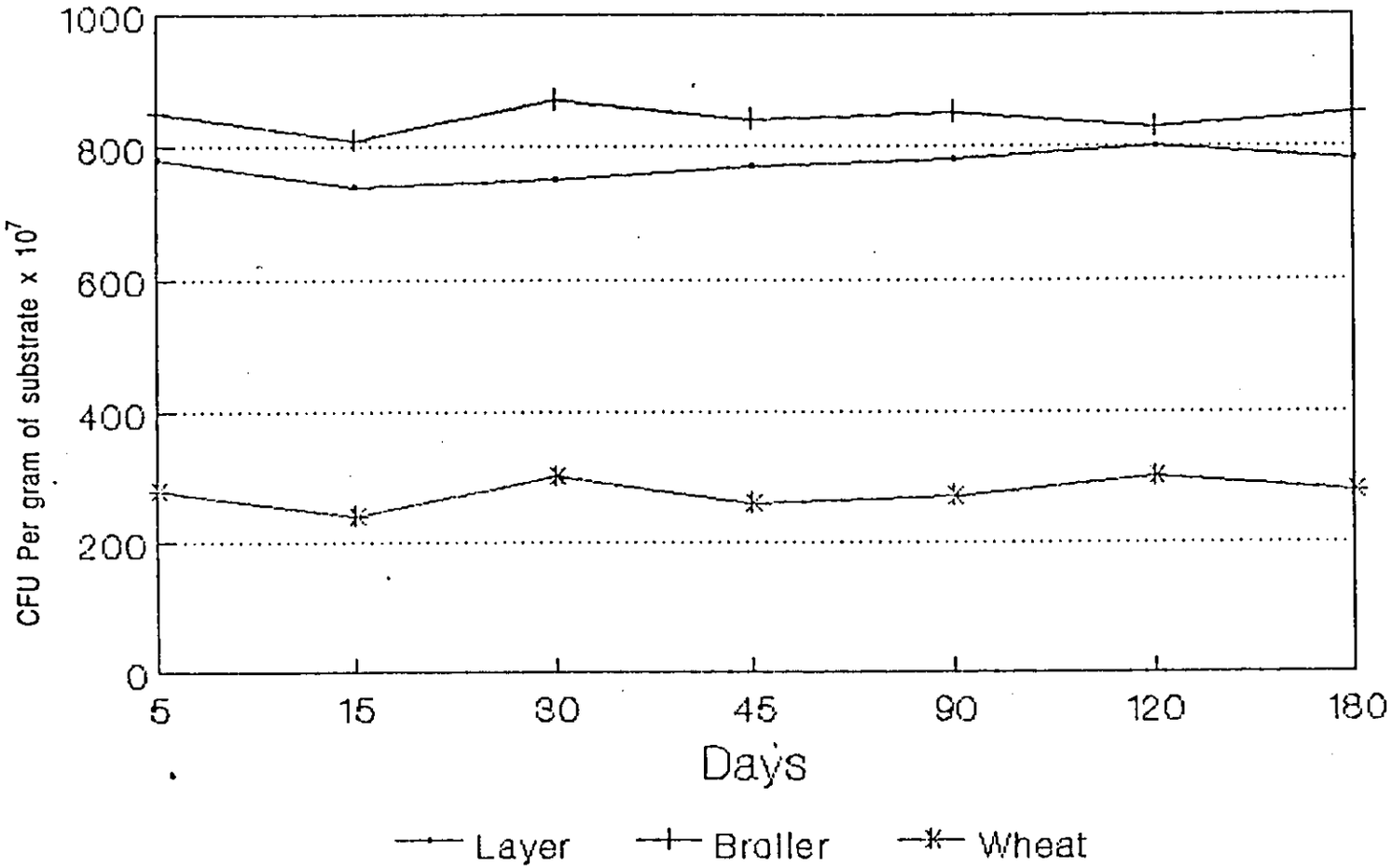

Fig . 1 Effects of carriers and length of storage on viability of *Paecilomyces lilacinus*.

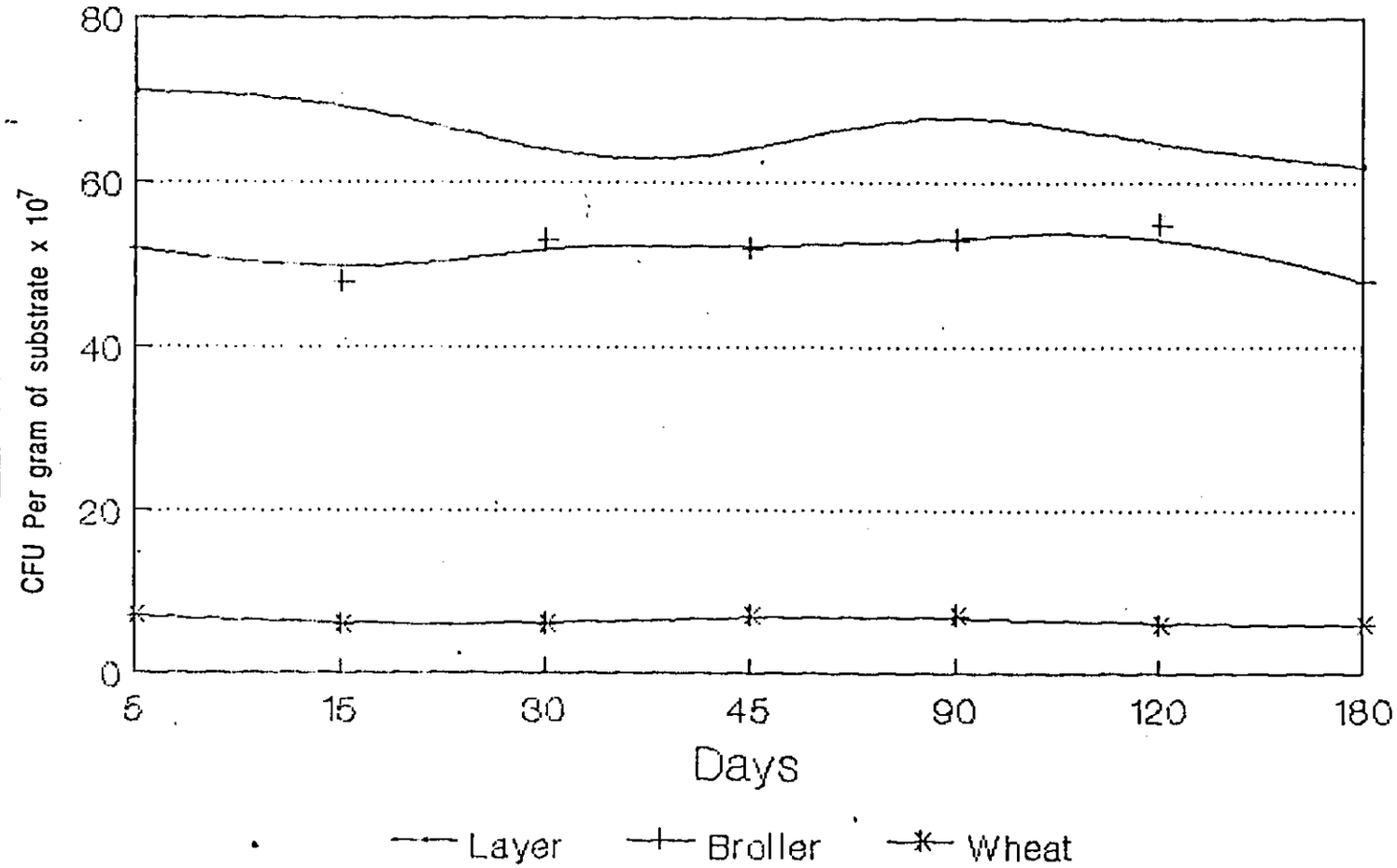

Fig . 2 Effects of carriers and length of storage on viability of *Fusarium solani*.

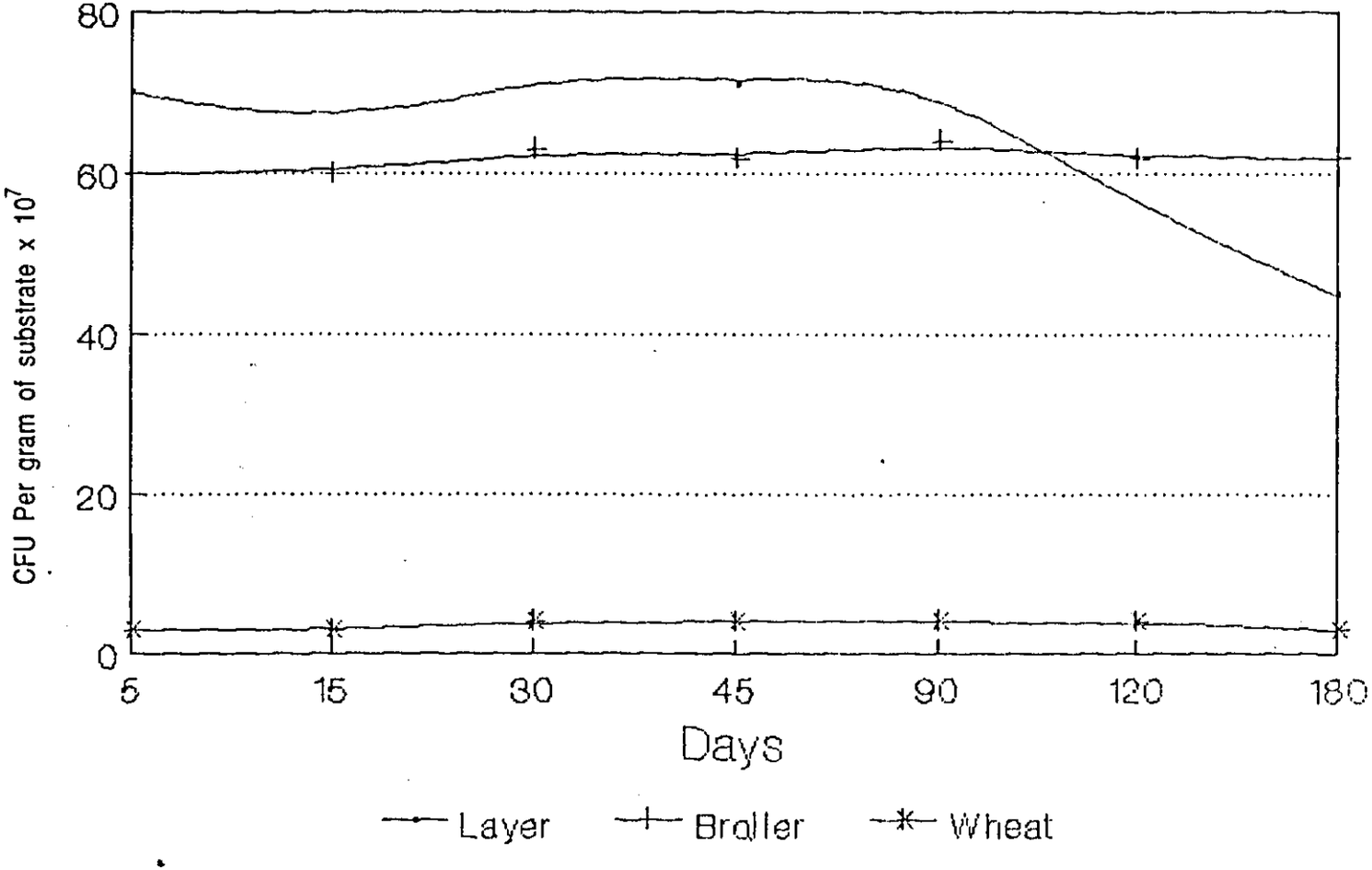

Fig. 3 Effects of carriers and length of storage on viability of *Fusarium oxysporum*.