J. Agric. Sci. Mansoura Univ., 28(4): 3127 - 3132, 2003

IMPACT OF FREEZING STORAGE ON ATTACHMENT OF *Pasteuria penetrans* ISOLATE P-20 TO *Meloidogyne incognita* AND NEMATODE DEVELOPMENT ON TWO HOST PLANTS

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ABSTRACT

The influence of freezing storage of *Pasteuria penetrans* isolate P-20 endospores for 7 years on the attachment to *Meloidogyne incognita* juveniles (j2's) at 5, 14, 21 and 42 days of exposure at 5°C. as well as nematode development on sunflower in comparison with Datura was studied. Results indicated that the number of endospores attached to second stage juveniles of *M. incognita* increased with increasing the exposure time. Among the exposure times tested, forteen days after exposure achieved the best result of endospores attachment with value of 12.4 endospores/j2. The maximum number of *P. penetrans* P-20 endospores attached to j2's was 17.3 endospores/j2 after 42 days of exposure.

The presence of *P. penetrans* P-20 on *M. incognita* j2's significantly decrease root galling and number of eggmasses with reduction percentages of 30.3 and 51.7% on sunflower roots, respectively, whereas, in the case of Datura roots, the bacterium did not reveal any significant differences in eggmasses number, even though a few number of root-galls was significantly recorded.

Better increase in shoot length for sunflower; and shoot fresh and dry weight of Datura was obtained with increase percentage of 14.2 and 8.9 and 21.4%, respectively.

Keywords: *Pasteuria penetrans* P-20, *Meloidogyne incognita*, Sunflower, Datura, Freezing storage.

INTRODUCTION

Sunflower; *Helianthus annus* L. is considered to be the most important economic cash crop in Egypt. The root-knot nematodes, *Meloidogyne* spp. are serious nematode pests on agricultural crops, mainly, sunflower. Infestation of this nematode to such plant showed a great economic damage and yield losses.

Chemical control of the root-knot nematodes has successfully limited the detrimented effect of these nematodes. However, environmental and health risks caused by these nematicides in addition to high cost have enhanced scientists to find another alternative tactics to manage economic nematodes below damaging levels. Use of *Pasteuria penetrans* group, which is an obligate nematode endoparastic bacterium, may provide an alternative or supplement to chemical control.

The attachment and development of *P. penetrans* on second stage juveniles (j2's) *Meloidogyne* spp. has been studied by many workers, i.e. Stirling (1981), Hatz and Dickson (1992), Refaei (1995), Orui (2001 & 2002) and Freitas *et al.* (2002). *P. penetrans* endospores attached more readily to

M. javanica at 22.5 – 30°C. than at 15°C. (Stirling, 1981). Moreover, the bacterium developed more quickly within its host (*M. arenaria*) at 30 and 35°C. than at 25°C. or below (Hatz and Dickson, 1992). In 1995, Refaei concluded that the low concentration of either 1,3-D (1,3-dichloropropene) or methyl bromide resulted in a high percentage of endospore attachment of *P. penetrans* P-20 on j2's of *M. arenaria* race 1 with values of 86.6% or 88.4% after 28 days from treatments, and the effect of *P. penetrans* P-20 in reducing nematode development was also evident. This reduction ranged from 3 to 10 times less in treatments with bacterium P-20 than those without it (Refaei, 1995).

Moreover, the effect of storage conditions of spores i.e. 20, 5 or 25°C for 0, 20 or 40 days and of sonication of spores for 30 min. in ice bach with ultrasonic transducer on spore attachment of *P. penetrans* isolates (MIA, MAP and MHP) to j2's of *M. incognita, M. arenaria* and *M. hapla*, respectively, indicated that the number of spores attached per j2 in the non-sonicated spores of the three isolates ranged from 0.2 to 2.3 in all storage conditions. Sonication of spores after storage at 5°C. and 25°C. tended to increase number of spores per j2 more than that after storage at –20°C. and before storage, especially significantly in MAP at 25°C., MIP at 5°C. and MHP at 25°C. (Orui, 2001).

Therefore, the present investigation was undertaken in order to determine the influence of freezing storage of *Pasteuria penetrans* isolate P-20 endospores for long-term period (7 years) on the attachment on *Meloidogyne incognita* j2's), consequently, the ability of j2's infection to sunflower (susceptible host) in comparison with Datura (resistant host).

MATERIALS AND METHODS

Impact of freezing storage on the attachment of *Pasteuria penetrans* isolate P-20 endospores to the root-knot nematode *Meloidogyne incognita* as well as its development on certain plants:

Bacterial culture: In order to obtain the bacterial culture of *Pasteuria penetrans* isolate P-20, naturally infected females of *Meloidogyne arenaria* race 1 reared on roots of tomato plants (*Lycopersicon esculentum* Mill cv. Rutgers) in a greenhouse of Nematology Division at the University of Florida, Gainesville, Florida, U.S.A. were collected, crushed in a small amount of tap water and stored in glass tubes; and frozen in the freezer of Nematology Laboratory Fac. of Agric. for 7 years. The total number of the endospores of *P. penetrans* P-20 used in this experiment was determined to be approximately 83 millions spores for all P-20 treatments.

Nematode population: *Meloidogyne incognita* population used in this study originated from a greenhouse culture that was maintained on colleus plants at the Nematology Research unit, Faculty of Agric., Mansoura University, Egypt. *M. incognita* juveniles were newly hatched (less than one-week-old j2's) extracted by Baermann-pan technique (Goodey, 1957).

The frozen endospores of *P. penetrans* P-20 free of ice, added to *M. incognita* j2's in 50 ml-flask, with 30 ml of tap water, after 2 hrs at room temperature the suspension was divided into unequal two portions.

In order to determine the effect of endospore storage of *P. penetrans* on its attachment to *M. incognita* j2's at various periods, the smaller portion was tested by counting the number of endospores/20 j2/one cm of endospore suspension for four times by research microscope. The average number of endospores/20 j2's was recorded at 5, 14, 21 and 42 days after the beginning of the experiment.

The other portion of j2's with endospores of *P. penetrans* P-20 was also examined for the ability of j2's infecting either seedlings of sunflower, *Helianthus annus* L. or datura, *Datura stramonium* which were separately planted into plastic pots (300 cm^3) 9-cm-d filled with sterilized (1:1) clay: sand soil. Each seedling was 15 cm height at the time of adding the second stage juveniles (1000 j2/pot), irrigated regularly with water during the course of the experiment that lasted 45 days. Each tested plant cultivar was replicated three times, as well as the control (without bacterium). Insect management and recommended fertilizers were followed during the course of the experiment. Data dealing with length of shoots and roots, fresh weight of shoots and roots as well as dry weight of shoots were recorded. Infected roots were stained in 0.01 hot acid fuchsin in acetic acid (Byrd *et al.*, 1983) examined and the numbers of galls and eggmasses were recorded.

Data collected were subjected to analysis of variance (ANOVA). Means of treatments were compared by Duncan's multiple-range test (Duncan, 1955).

RESULTS AND DISCUSSION

I- Effect of freezing storage of *Pasteuria penetrans* isolate P-20 endospores Attachment on j2's of *Meloidogyne incognita*:

Results of the effect of 7 years of freezing storage on the attachment of *P. penetrans* isolate P-20 endospores on j2's of the root-knot nematode, *M. incognita* are shown in Table (1). It was evident that the number of endospores attached to j2's was greatly affected according to the time of exposure. The average number of endospores attached to j2's was 3.5, 12.4, 11.3 and 17.3 endospores/j2 after 5, 14, 21 and 42 days of exposure, respectively (Table 1).

Table(1): Impact of freezing storage of *Pasteura penetrans* P-20 endospores for 7 years on the attachment to *Meloidogyne incognita* (j2's) at interval exposure periods at 5°C.

Days	Number of *P. penetrans* endospores P-20 per j2 at interval times			
	R_1*	R_2	R_3	Average
5	4.4	2.2	4.0	3.5 c
14	12.6	10.0	14.7	12.4 b
21	9.3	13.3	11.3	11.3 b
42	16.6	17.2	18.1	17.3 a

L.S.D.= 3.807

* Each replicate is the mean of number of endospores/20 j2's.

Among the exposure times tested, forteen days after exposure achieved the best result of endospores attachment with value of 12.4 endospores/j2. Obviously, the maximum number of endospores attached to j2's was recorded to be 17.3 endospores/j2 after 42 days of exposure at 5°C.

II- Effect of freezing storage of *P. penetrans* P-20 endospores on development and reporduction of *M. incognita* infecting sunflower and datura plants under greenhouse conditions:

The impact of freezing storage of *P. penetrans* P-20 endospores on development and reproduction of *M. incognita* on sunflower and datura as well as plant growth response was shown in Tables (2 & 3). It was clear that the presence of *P. penetrans* attached to j2's significantly decreased root galling and eggmasses when compared to treatment without bacterium with values of 30.3 and 51.7% reduction on sunflower roots, respectively (Table 2).

Table(2): Effect of endospores of *Pasteura penetrans* P-20 stored in freezer for 7 years on development of *Meloidogyne incognita* (j2's) infecting sunflower as well as datura under greenhouse conditions.

Treatments	Development of nematodes on plant roots			
	No. of galls	% reduction	No. of eggmasses	% reduction
	Sunflower			
P-20	108.0	30.3	30.3	51.7
N free of P-20	155.0		61.7	
F-test	**		*	
	Datura			
P-20	8.7	39.3	0.33	50.7
N free of P-20	14.3		0.67	
F-test	*		N.S	

N = *M. incognita.*

On the other hand, datura plant is considered to be resistant host against *M. incognita* infection, since it showed few numbers of galls and eggmasses. Whereas, in the presence of *P. penetrans* P-20 endospores number of galls was significantly reduced as compared to the absence of this bacterium with reduction percentage of 39.3% (Table 2). Moreover, the presence of *P. penetrans* P-20 did not reveal any significant differences in eggmass numbers when compared to that of the control (nematode free of bacterium), since eggmasse number was very low, eventhough its reduction percentage reached to 50.7% (Table 2).

Regarding, the impact of the freezing storage endospores of *P. penetrans* isolate P-20 attached to *M. incognita* (j2's) on plant growth response of sunflower as well as datura, data are presented in Table (3). It was evident that the presence of *P. penetrans* P-20 relatively improved certain growth parameters of sunflower as well as datura exceeded that of the nematode free of P-20 without any significant differences except that of shoot length for sunflower; and shoot fresh and dry weights for Datura plants.

Obviously, the presence of *P. penetrans* P-20 endospores, attached to j2's showed better increase in shoot length for sunflower as well as shoot fresh and dry weights of datura with percentage of increase amounted to 14.2; 8.9 and 21.4%, respectively (Table 3).

Table(3): Influence of freezing storage *Pasteura penetrans* P-20 attached to *Meloidogyne incognita* (j2's) on plant growth response of sunflower and datura under greenhouse conditions.

Treat-ments	Plant Growth Parameters							
	Shoot length (cm)	% of increase	Shoot fresh wt. (gm)	% of increase	Root length (cm)	Root fresh wt. (gm)	Shoot dry wt. (gm)	% of increase
	Sunflower							
P-20	48.3	14.2	4.0		13.5	4.2	0.7	
N free of P-20	42.3		4.3		16.2	3.5	0.9	
F-test	*		N.S		N.S	N.S	N.S	
	Datura							
P-20	21.3		7.3	8.9	16.3	5.2	1.7	21.4
N free of P-20	18.7		6.7		18.3	4.9	1.4	
F-test	N.S		*		N.S	N.S	*	

*Each figure is the mean of three replicates.

Apparently, the frozen endospores of *P. penetrans* P-20 for 7 years that were free of ice when used showed the maximum number of spores attachment on *M. incognita* j2's after 42 days of exposure at 5°C. Such results are in accordance with the findings of Orui (2001) who reported that when storage endospores of *P. penetrans* isolates (MIA for *M. incognita*, MAP for *M. arenaria* and MHP for *M. hapla*) at –20, 5 or 25°C. for 0, 20 or 40 days with sonication for 30 min., sonication of spores stored for 40 days at 5 and 25°C. tended to increase number of spores/j2 than that for 20 days.
It was concluded that the frozen endospores of *P. penetrans* P-20 as a safety biological agent in nematode management was considered to be as a practical technique for long-term of such biological agent.

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تأثير التخزين المجمد لجراثيم باستيريا بنترانز سلالة ب-٢٠ على التصاقها علـى "ميليدوجينى انكوجينيتا" وتطور النيماتودا على عائلين نباتيين٠

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تم دراسة تأثـير التخزين المجمد لجراثيم بكتيريا "باستيريا بنترانز" سلالة ب-٢٠ لمدة ٧ سنوات على التصاقها على يرقات الطور اليرقى الثانى لنيماتودا تعقـد الجـذور "ميليـدوجينى أنكوجينيتا" بعد ٥، ١٤، ٢١، ٤٢ يوماً من تعريض يرقات النيماتودا لجراثيم البكتيريا وذلك عنـد درجة حرارة ٥ °م وكذلك تطور وتكاثر النيماتودا على نبات عبـاد الشـمس بالمقارنـة بنبـات الداتورا٠

أسفرت النتائج أن عدد الجراثيم الملتصقة بكل يرقة زاد بزيادة مدة التعريض وأن مـدة التعريض بعد ١٤ يوماً أعطت أحسن النتائج فى التصاق الجراثيم على اليرقـات بمقـدار ٤ر١٢ جرثومة/يرقة٠ وكان أقصى عـدد من الجراثيم التى التصق بكل يرقة هـو ٣ر١٧ جرثومـة/ يرقة بعد ٤٢ يوم من التعريض٠ كما أدى وجود جراثيم البكتيريا على كيوتيكـل الطـور اليرقى الثانى لنيماتودا تعقد الجذور إلى نقص معنوى واضح فى أعداد العقد النيـماتودية وكتـل البـيض بنسب نقص تصل إلى ٣ر٣٠% و٧ر٥١% على جذور نبات عـباد الشمـس على التوالى، بينما فى حالة جذور الداتورا فإن البكتيريا لم تسبب أى نقص معنوى فى أعداد كتل البـيض حتـى وأن كان هناك عدد قليل من العقد النيماتودية على جذوره٠

كما أن هناك زيادة واضحة فى طول الساق لنبات عباد الشمس والوزن الرطب والجاف للمجموع الخضرى لنبات الداتورا بنسب زيـادة قـدرها ٢ر١٤% و ٩ر٨% و ٤ر٢١% علـى التوالى٠