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FEASIBILITY OF STORED *Pasteuria penetrans* ENDOSPORES ISOLATE P-100 IN ROOT POWDERS ON *Meloidogyne incognita* INFECTION TO CERTAIN PLANTS.

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ABSTRACT

The feasibility of stored *Pasteuria penetrans* isolate P-100 with tomato root powders for long-term period (7 years) at room temperature on the development of *Meloidogyne incognita* on sunflower in comparison with datura was investigated under greenhouse conditions. Results indicated that contradicting data was evident with respect to number of eggmasses and galls on roots of either plant hosts due to the tested storage conditions of endospores. However, the presence of *P. penetrans* under this tested storage conditions showed better increase in length and fresh weight of shoot for sunflower and datura plants with values of percentage increase of 25.29 & 21.82% and 35.29 & 64.18%, respectively. Moreover, an improvement of root growth parameters of both host plants was also obtained without any significant differences except that of datura root length (18.6%).

Keywords: *Pasteuria penetrans* P-100, *Meloidogyne incognita*, sunflower, datura, storage in root powder.

INTRODUCTION

Infestation of root-knot nematodes, *Meloidogyne* spp. to agricultural crops showed a great economic damage and yield losses for Egyptian national income.

Chemical control of these nematodes has successfully limited their detrimented effect. 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 endoparasitic bacterium, may provide an alternative or supplement to chemical control.

The effect of storage conditions of spores on the attachment and development of *P. penetrans* on second stage juveniles (j2's) of *Meloidogyne* spp. has been studied by many workers, i.e.; Hatz and Dickson (1992), Dickson *et al.* (1994), Orui (2001 & 2002) and Refaei (2003). *Pasteuria penetrans* endospores developed more quickly within its host (*M. javanica*) at 30°C. and 35°C. than at 25°C. or below (Hatz and Dickson, 1992). Endospores of *Pasteuria* spp. appear to survive long periods under dry, adverse conditions, thus giving them the potential for long shelf life. *Pasteuria* spp. endospores have been noted on root-knot nematode (j2's) collected from soil 120 cm deep (Dickson *et al.*, 1994).

Moreover, storage conditions of spores i.e. – 20, 5 or 25°C for 0, 20 or 40 days and 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 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).

Recently, Refaei (2003) indicated that when the endospores of *P. penetrans* isolate P-20 were stored in a freezer for 7 years and reused as biological agent against *M. incognica* infecting sunflower plants showed a relative improvement of certain growth parameters of sunflower and significantly reduced number of galls and eggmasses.

Therefore, since the frozen endospores of *P. penetrans* isolate P-20 as a safety agent in nematode management was considered to be as a practical technique for long-term period (Refaei, 2003), an attempt was carried out to determine the feasibility of stored *P. penetrans* endospores isolate P-100 in tomato root powders for long-term period (7 years) at room temperature on *Meloidogyne incognita* j2's infection to certain plant hosts under greenhouse conditions.

MATERIALS AND METHODS

Impact of *Pasteuria penetrans* isolate P-100 endospores stored in tomato root powders for long-term period (7 years) on the root-knot nematode, *Meloidogyne incognita* infecting sunflower (susceptible plant) in comparison with datura (resistant plant):-

Bacterial culture: In order to obtain the bacterial culture of *P. penetrans* P-100, naturally infected galls with females of root-knot nematodes reared on roots of tomato plants (*Lycoperscion esculentum* Mill cv. Rutgers) in a greenhouse of Nematology Division at the University of Florida, Gainesville, Florida, U.S.A. were collected, air-dried, grounded as root powders, stored in a brown glass bottle, tight and kept in the Nematology laboratory at room temperature for 7 years. The cover of this glass bottle removed from time to another for aeration.

The total number of the endospores of *P. penetrans* P-100 used in this experiment was determined to be approximately 15 millions spores for all P-100 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. Newly hatched juveniles of *M. incognita* were collected by Baermann-pan technique (Goodey, 1957).

The stored endospores of *P. penetrans* P-100 in tomato root galls powder (5 gm), added to *M. incognita* j2's in 1000 ml flask, with 600 ml tap water, mixed well and after 2 hrs at room temperature, each inoculum contained 1000 j2's with endospores of P-100 was added into three holes around the root system of either sunflower (*Helianthus annus* L.) or datura (*Datura stramonium*) seedlings which were separately planted into plastic pots (300 cm) 9-cm-d filled with sterilized (1:1) clay: sandy soil. Each tested

plant cultivar was replicated three times as well as the control (nematode without bacterium). Each seedling was 15 cm height at the time of adding the second stage juveniles (1000 j2's/pot), irrigated with water as needed during the course of the experiment that lasted 45 days.Mite and 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

Results on the impact of grounded tomato roots heavily infected with *Meloidogyne* spp. containing *Pasteuria penetrans* endospores isolate P-100 stored at room temperature as powder for 7 years on *Meloidogyne* incognita infection to sunflower and datura plants under greenhouse conditions are shown in Tables (1 and 2). It was evident that the presence of *P. penetrans* with j2's obviously behaved differently in relation to suppressing nematode infection according to storage conditions for long-term period in root powders at room temperature (Table 1). It was clear that the presence of *P. penetrans* with j2's did not reveal any significant differences in number of eggmasses and galls on roots of either sunflower or datura plants except for the latter one with respect to number of galls (9.3) when compared to that of the control (14.3). However, number of galls on sunflower roots with percentage increase was amounted to 8.5%, whereas number of eggmasses on datura roots with percentage increase of 98.5% in the presence of such bacterium (Table 1). Apparently, these reverse results of *P. penetrans* P-100 on development and reproduction of *M. incognita* on sunflower (susceptible host) as well as datura (resistant host) clearly due to the way of stored endospores P-100 in root powders at room temperature for 7 years. Such results are accordance with the findings of Brown and Smart (1984) who reported that endospore attachment (*P. penetrans*) decreased if endospore infested soil was air-dried before adding nematodes and water.

Concerning the impact of dried root powder storage endospore of *P. penetrans* P-100 at room temperature for 7 years with *M. incognita* under the above condition on plant growth response of sunflower as well as datura, data are presented in Table (2). It was evident that the presence of *P. penetrans* endospores that were stored in root powders at room temperature for 7 years relatively showed better increase in length and fresh weight of shoots for sunflower and datura plants with values of percentage increase of 25.29 & 21.82%; and 35.29 & 64.18%, respectively (Table 2). However, the presence of *P. penetrans* under this storage conditions also improved root growth parameters of sunflower and datura plants without any significant differences except that of datura root length which revealed the value of 18.6% decrease in treatment with this bacterium (Table 2).

Table (1): Effect of *Pasteuria penetrans* endospores P-100 stored in tomato root powders for 7 years at room temperature on development of *Meloidogyne incognita* (j2's) infecting sunflower as well as datura under greenhouse conditions.

Treat-ments	Galling and repoducation of *M. incognita***			
	No. of galls	% increase	No. of eggmasses	% increase
	Sunflower			
P-100	168.3 a	8.5	60.0 a	-
N free of P-100	155.0 a	-	61.7 a	-
F-test	NS	-	NS	-
	Datura			
P-100	9.3 b	-	1.33 a	98.5
N free of P-100	14.3 a	-	0.67 b	-
F-test	*	-	*	-

N = *M. incognita*
** Each figure is the mean of three replicates.

Table (2): Influence of stored *Pasteuria penetrans* P-100 in tomato root powders mixed with *Meloidogyne incognita* (j2's) on plant growth response of sunflower and datura under greenhouse conditions.

Treat-ments	Plant growth parameters**							
	Shoot length (cm)	% of incre-ase	Shoot fresh wt. (gm)	% of incre-ase	Root length (cm)	Root fresh wt. (gm)	Shoot dry weight (gm)	% of incre-ase
	Sunflower							
P-100	53.0 a	25.29	5.5 a	21.82	17.2 a	4.2 a	1.6 a	77.7
N free of P-100	42.3 b	-	4.3 b	-	16.2 a	3.5 a	0.9 a	-
F-test	*	-	*	-	N.S	N.S	N.S	-
	Datura							
P-100	25.3 a	35.29	11.0 a	64.18	15.3 b	5.7 a	2.3 a	64.28
N free of P-100	18.7 b	-	6.7 b	-	18.3 a	4.9 a	1.4 b	-
F-test	*	-	**	-	*	N.S	*	-

** Each figure is the mean of three replicates
N = *M. incognita* (j2).

In conclusion, the tested storage conditions of *P. penetrans* endospores P-100 negatively affected the abilities of this bacterium isolate P-100 on *M. incognita* infection either to sunflower (susceptible host) or datura (resistant host) when compared with the freezing storage of the same bacterium isolate P-20 (Refaei, 2003) that proved to be the best way of such spore storage and was considered as a practical method for long shelf life of such biological agent in nematode management. Moreover, the present investigation reported reverse results in suppressing nematode development, a situation which can be explained by the unsuitable tested spore storage conditions.

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مدى ملائمة تخزين جراثيم بكتيريا "باستيريا بنترانز" سلالة ب-١٠٠ فى مسحوق جذور على إصابة نيماتودا "ميليدوجين إنكوجنيتا" لبعض النباتات.

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تم دراسة مدى ملائمة تخزين جراثيم البكتيريا "باستيريا بنترانز" سلالة ب-١٠٠ لمدة ٧ سنوات على درجة حرارة الغرفة والتى توجد فى مسحوق جذور الطماطم على تطور نيماتودا "ميليدوجين إنكوجنيتا" فى نباتات عباد الشمس بالمقارنة بنباتات الداتورا تحت ظروف الصوبة.

وأسفرت الدراسة على نتائج معاكسة لما هو معروف عن هذه البكتيريا كعامل حيوى فعال فى تقليل عدد العقد الجذرية وكتل البيض لنيماتودا تعقد الجذور تحت ظروف تخزين جراثيم هذه البكتيريا لمدة ٧ سنوات فى درجة حرارة الغرفة.

واظهرت النتائج أيضا أن وجود البكتيريا المخزنة بهذه الطريقة أعطى زيادة فى طول ووزن المجموع الخضرى بنسبة مئوية: ٢٩ر٢٥، ٨٢ر٢١% لنباتات عباد الشمس، وكذلك بقيم: ٢٩ر٣٥، ١٨ر٦٤% لنباتات الداتورا لهذه القياسات السابقة على التوالى. هذا بالإضافة إلى تسجيل زيادة غير معنوية فى باقى القياسات النباتية لكل من نباتات عباد الشمس والداتورا فى وجود البكتيريا فيما عدا طول الجذر لنباتات الداتورا الذى كان أقل بنسبة ٦ر١٨% بالمقارنة بالمعاملة الضابطة.