Minufiya J. Agric. Res. Vol. 32 No.4: 1193 - 1202 (2007)

IMPACT OF DIFFERENT NUTRIENTS ON SOME BIOLOGICAL ASPECTS OF *MELOIDOGYNE JAVANICA* IN RELATION TO THE OBLIGATE HYPERPARASITE *PASTEURIA PENETRANS*

M.E. Mahdy [1]; Y. A. Rjoub[2] and A. M. Kella[3]

1- Fac. of Agric., Minufiya Univ., Dept. Agric. Botany, Shebin El-Kom, Egypt.

2- Al- Balqaa Applied Univ., Al- Husen Polytechnic College, Irbid , Jordan.

3- Plant Pathology Research Institute, Agic. Res. Centre, Giza, Egypt.

(Received: June. 6 , 2007)

ABSTRACT: In this research two experiments were conducted under laboratory and greenhouse conditions. Laboratory experiment was carried out in Petri dishes to study the effect of different nutrient solutions at different forms on the percentages of infected juveniles (J_2) of Meloidogyne javanica with Pasteuria penetrans endospores and the mean number of endospores /J_2 of M. javanica at two times and three concentrations of P. penetrans endospores 10^4, 10^5 and 10^6 endospores / ml. Results of experiment show that all used nutrients enhanced the percentage of infected J_2 of M. javanica by P. penetrans endospores as well as the mean number of endospores / J_2 of M. javanica compared to the nutrient untreated Petri dishes. The highest % of infected J_2 with P. penetrans endospores was obtained with Hogland solution without Fe treatment at all used concentrations followed by all Hogland solution structures. The same trend of results was obtained for the mean attached number of endospores / J_2 as the highest mean number of attached endospores / J_2 were obtained when Hogland solution applied without Fe. Laboratory results reported that also increasing the concentration of endospores / ml increased the percentage of infected J_2 of M. javanica with P. penetrans endospores as well as in mean number of attached endospores / J_2. Results of greenhouse experiment show that treating the plants with all Hogland solution structures increased the mean number of attached endospores / J_2 and the percentage of infected juveniles with P. penetrans endospores compared with the nutrients untreated control. The highest mean number of attached endospores / J_2 and the percentage of infected J_2 with P. penetrans was obtained with Hogland solution either with or without Fe element. Results also indicate that treating the plants with Hogland nutrient solution in presence of P. penetrans was enhanced the fresh shoot and root weights.

Key Words: Tomato plants (Lycopersicon esculentum Miller cv. Rutgers); Micro and macro nutrients; Meloidogyne javanica; Pasteuria penetrans.

INTRODUCTION

Macro and micro nutrients which enrich the rhizosphere are derived from a variety of sources and have a complex physical and chemical nature. These materials play very important role in the growth and development of both plants and soil microorganisms including nematodes and bacteria. The effect of some soil environmental factors on plant parasitic nematodes and bacteria was investigated: soil moisture (Stirling and Wachtel, 1980); high and low temperature (Dutky and Sayre, 1978; Stirling *et al.*, 1986; Williams *et al.*, 1989; Bird *et al.*, 1990; Oostendorp *et al.*, 1990 and Davies *et al.*, 1991). However the effect of macro and micro nutrients on the biological activity of plant parasitic nematodes infected with *Pasteuria penetrans* is unknown. A very little works were attempted to study the role of fertilizers on the attachment rate of *P. penetrans* endospores to *Meloidogyne* spp. juveniles. So, the aim of this research is a trial to discover the role of Hogland solution on the development of *M. javanica* and *P. penetrans* under laboratory and greenhouse conditions.

MATERIALS AND METHODS

Nematode population: The nematode population used in this research originated from greenhouse culture maintained at the Faculty of Agriculture, Minufiya University, where *Meloidogyne javanica* (Treub) Chitwood was reared in a greenhouse on tomato plants (*Lycopersicon esculentum* Miller cv. Rutgers). Eggs of *M. javanica* were extracted from roots in 0.5% sodium hypochlorite (Hussey and Barker, 1973) and caught on a 25 µm sieve. Second stage juveniles (J_2) were hatched from these eggs on Baermann funnels and only J_2 less than 2 days old were used for experimentation.

Bacterial cultures: *Pasteuria penetrans* (Pp) isolate Japan used in this study was obtained from Dr. Simon Gowen, Dept. of Agriculture, Reading University, Early Gate, Reading, RG6 6AT, Berkshire, UK. *P. penetrans* Sayre and Starr (1985) was reared in a greenhouse on tomato plants infected with *M. javanica*. Endospores of Pp were extracted from parasitized females of *M. javanica* growing on tomato roots (Oostendorp *et al.*, 1990).

Nutrional solutions: Different nutritional solutions were prepared according to Hogland and Arnon (1938).

Laboratory experiments: The attachment of *P. penetrans* was studied in the laboratory in sterile sandy soil under different Hogland solution structures. Five treatments were applied as follows: complete Hogland solution; Hogland solution without iron (Fe); Hogland solution without manganese (Mn); Hogland solution without zinc (Zn) and check without any nutrients. Twenty gram of sterile sand was placed in each Petri dish (6 cm in diam.) and was received 1 ml of each nutrional solution except check which received only water. Spore suspension of *P. penetrans* isolate Japan was prepared by adding 0.1 gram of Pasteuria root powder to small amount of distilled water in a pestle and mortar. Root debris was removed after mixing

the powder with water thoroughly by pouring the suspension through a 25µm sieve (Stirling and Wachtel, 1980). The different concentration levels of spore suspension were measured with a hemicytometer slide and adjusted to three levels: 1000,000 (10^6); 100,000 (10^5) and 10,000 (10^4) per Petri dish. One day later all treatments were received 500 freshly J_2 of *M. javanica*. Treatments were replicated four times and kept under laboratory conditins (24-26°C) for three days. Juveniles were extracted from the soil using the sugar flotation technique (Jenkins, 1964). The numbers of endospores attached to J_2 and the percentages of infection were counted for 20 J_2 per replicate using an inverted microscope at a magnification of 600 X as shown in Fig. (1). The *in vitro* experiments were replicated two times as two tests (Experiment1 and Experiment 2).

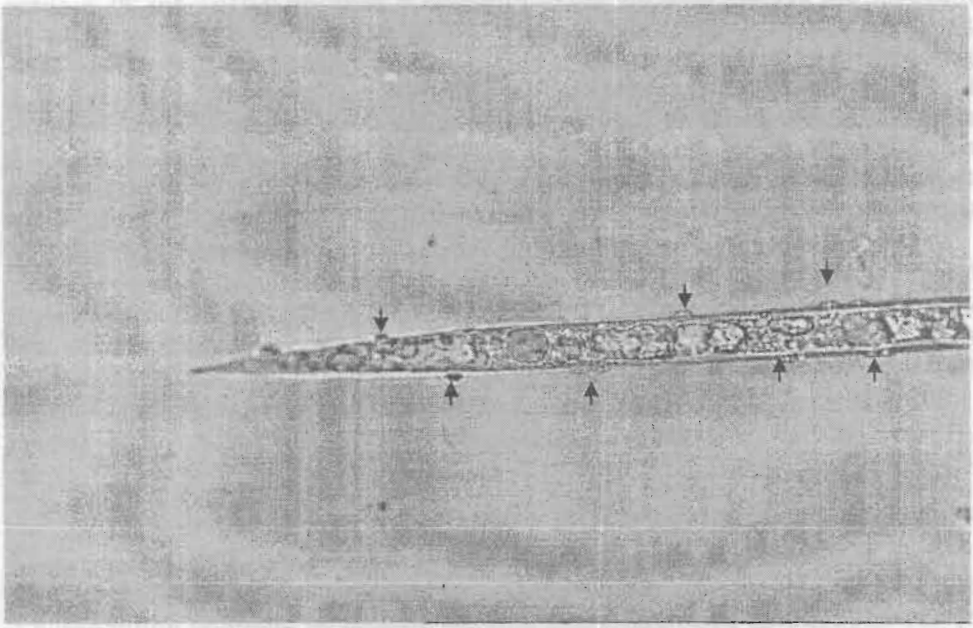

Fig. (1) : *P. penetrans* isolate Japan endospores attached to the cuticle of J_2 of *M. javanica* (arrows point to attached spores) .

Greenhouse experiments: Three weeks old tomato seedlings cv. Rutgers were transplanted into clay pots (10 cm in diam.) filled with 500 gram sand-soil mixture (3:1; v/v). Treatments consisted of two groups, the first group included four treatments: completely Hogland+*M. javanica* (Mj)+*P. penetrans* (Pp); Hogland without Fe+Mj+Pp; Hogland without Mn+Mj+Pp; Hogland without Zn+Mj+Pp, the second group included the same above mentioned

treatments without Pp endospores inoculation; in addition to the treatments of Mj+Pp as well as the check one (control).

Five ml of *P.penetrans* endospore suspension (10^6 spore/ml) were inoculated into four holes around the root system and after two days 500 J_2 of *M. javanica* were inoculated by pipetting into the same holes around the roots. Plants were irrigated and sprayed by the different Hogland solution structures by using handle sprayers once a week. Pots were kept under greenhouse conditions (25±5°C) in randomized block design and allowed to grow for two months. Sixty days after nematode inoculation, plants were uprooted and washed with tap water and the following parameters were determined: fresh shoot and root weight; mean number of attached endospores/J_2 by using the same procedures in laboratory tests; the percentage of Pp infected J_2; number of galls/root system; number of egg masses/root system with the aid of Phloxine B solution 0.015% for 20 minutes as described by Daykin and Hussey (1985); number of eggs/egg mass by pressing 10 egg masses separately from each replicate on a clean glass slide in a drop of sodium hypochlorite (NaOCL) under a cover slip to separate and determine the number of eggs/egg mass under a light microscope. As well as number of Pp infected females was counted by cutting and dipping the root system of each replicate in a beaker full with tap water for at least 4 days depending on the host type at room temperature until they become softened.

Roots washed through 500µm and 250µm sieves to separate the females from the root debris and 20 of It per replicate were examined for the infection with endospores by transferring one female to a glass slide and squashing it with a cover slip. The squashed females were examined under the microscope to determine the percentages of infected females per replicate.

Statistical analysis: Data were subjected to the analysis of variance (ANOVA), and the treatments were compared by Duncan's multiple range test (P=5%).

RESULTS AND DISCUSSION

Data in Table (1) revealed that all treatments with Hogland solutions were encouraged significantly the percentage of infection of *M. javanica* juveniles by the endospores of *P. penetrans* and the number of attached endospores / J_2 when compared with the nutrients untreated Petri dishes under laboratory conditions. Results showed that Hogland solution without Fe was the best one in increasing the percentage of Pp infected J_2 and the number of attached endospores / J_2 at the three concentrations of Pp endospores when compared with the other treatments.

Results showed no significant differences between the other Hogland solution structures in enhancement the percentage of infected juveniles by the endospores and the mean number of attached endospores/J_2. Results showed also that the low concentration of endospores 10^4 endospores/ml

was the lowest level in increasing the percentage of Pp infected J_2 and the mean number of attached endospores / J_2 of *M. javanica* compared with others.

Table (1): Effect of nutrients on the attachment of *Pasteuria penetrans* endospores to *Meloidogyne javanica* juveniles in two tests and three endospore concentrations under laboratory conditions.

Treatments	% of Pp infected J_2 of *M. javanica*						Mean no. of endospores/J_2 of *M. javanica*					
	Experiment 1			Experiment 2			Experiment 1			Experiment 2		
	High	medium	Low	High	medium	Low	High	medium	Low	High	medium	Low
Hogland	55.0 b	47.5 ab	26.3 b	57.5 b	45.0 b	37.5 b	2.2 b	1.3 ab	0.7 b	2.5 b	1.3 b	1.0 b
Hogland without Fe	60.0 a	55.0 a	47.5 a	67.5 a	61.3 a	56.3 a	2.7 a	1.5 a	1.2 a	2.8 a	1.8 a	1.8 a
Hogland without Mn	45.0 c	35.0 b	27.5 b	52.5 b	32.5 c	23.8 c	1.3 d	0.8 b	0.7 b	1.5 c	0.9 b	0.6 b
Hogland without Zn	51.3 bc	42.5 ab	26.3 b	53.8 b	37.5 bc	30.0 bc	1.7 c	0.8 b	0.6 b	1.6 c	0.9 b	0.7 b
Hogland Free (control)	46.3 bc	40.0 b	22.5 b	52.5 b	37.5 bc	27.5 c	1.7 c	1.0 ab	0.5 b	2.1 b	1.1 b	0.7 b

*High= 10^5 endospores/ml *Medium= 10^5 endospore/ml *Low=10^4 endospores/ml
*Means in columns followed by the same letters are not significantly different according to Duncan's multiple range test (P=5%).

Data in Table (2) show that treating plants with nutrient solutions increased the mean number of attached endospores/J_2 of *M. javanica* and the percentage of infected J_2 with Pp endospores compared with the nutrient untreated plants (check). The highest % of Pp endospores infected juveniles and mean number of attached endospores/J_2 was obtained with the treatment of Hoagland solution either completely or without Fe element.

Results in Table (2) show also that all used nutrient solutions enhanced the vegetative plant growth characters i. e. fresh shoot and root weight of tomato plants either in presence or in absence of *P. penetrans* compared with the check.

Table (2): Effect of nutrients on the growth; number of attached endospores of *Pasteuria penetrans*/J_2 and the percentage of Pp infected J_2 of tomato plants infected with *Meloidogyne javanica*, under greenhouse conditions.

Treatments	Fresh weight (gm)		No. endospores/J_2 of *M. javanica*	(%) Infected J_2
	Shoot	Root		
Pp+Mj+Hogland	25.3 a	10.8 a	2.1 b	52.5 a
Pp+Mj +Hogland without Fe	25.1 a	9.9 ab	2.8 a	52.5 a
Pp+Mj+Hogland without Mn	19.4 bc	7.7 bc	0.8 c	35.0 c
Pp+Mj+Hogland without Zn	21.2 abc	6.5 c	0.9 c	41.3 b
Pp+Mj	11.8 e	5.0 cd	0.9 c	42.5 b
Mj+Hogland	19.5 bc	9.9 ab	0.0 d	0.0 d
Mj+Hogland without Fe	14.3 de	6.0 cd	0.0 d	0.0 d
Mj+Hogland without Mn	22.5 ab	9.3 ab	0.0 d	0.0 d
Mj+ Hogland without Zn	17.1 cd	9.6 ab	0.0 d	0.0 d
Check	3.7 f	3.6 d	0.0 d	0.0 d

*Pp= *Pasteuria penetrans* *Mj= *Meloidogyne javanica*

*Means in columns followed by the same letter are not significantly different according to Duncan's multiple range test (P=5%).

Results in Table (3) revealed that the lowest number of galls, egg masses/ root system as well as number of eggs/egg mass were obtained in the presence of the endoparasitic bacterium *P. penetrans* compared to the bacterium untreated plants. The highest significant reduction in related nematode parameters was noticed with plants treated with *P. penetrans* plus Hogland solutions without Fe element followed by the treated plants with Pp with completely Hogland solution. Results also revealed that the highest number in related nematode parameters was obtained with the treated plants with

Hogland solution without Zn in absence Pp endospores. Results also show that all examined females were 100% infected with Pp endospores with all Hogland solution structures.

Table (3): Effect of nutrients on number of galls; number of egg masses/root system; eggs/egg mass and % of *Pasteuria penetrans* infected females of *Meloidogyne javanica* in tomato plants under greenhouse condition.

Treatments	No. galls/ Root system	No. egg masses/ Root system	No. eggs/ egg mass	(%) Infected Females
Pp+Mj+Hogland	46.5 cd	22.8 bc	127.0 d	100.0 a
Pp+Mj+Hogland without Fe	23.8 cd	20.5 bc	66.8 e	100.0 a
Pp+Mj+Hogland without Mn	61.5 bc	22.8 bc	117.8 d	100.0 a
Pp+Mj+Hogland without Zn	69.8 abc	48.8 b	104.5 d	100.0 a
Pp+Mj	39.5 cd	31.8 bc	100.8 d	100.0 a
Mj+Hogland	111.5 ab	96.5 a	212.5 c	0.0 b
Mj+ Hogland without Fe	59.0 c	48.0 b	202.8 c	0.0 b
Mj+Hogland without Mn	66.5 bc	55.8 b	365.5 b	0.0 b
Mj+ Hogland without Zn	130.5 a	100.8 a	407.8 a	0.0 b
Check	00.0 d	00.0 c	00.0 f	0.0 b

*Pp= *Pasteuria penetrans* *Mj= *Meloidogyne javanica*

Means in columns followed by the same letter are not significantly different according to Duncan's multiple range test (P=5%).

These results are confirmed with those of (Siddiqui and Shaukat, 2003; Siddiqui, *et al.*, 2002; Hue, *et al.*, 2004) as they found that iron and zinc had important role in the relation between fungi and bacteria attacking plant parasitic nematodes.

Finally, it could be concluded that the iron element play an important role in the biological control process of *Meloidogyne javanica* using *P. penetrans*, where the decrease of the rate of iron concentration in the soil increase the percentage of J_2 infection with *P. penetrans* endospores, this may be due to that the deficiency of iron element eliminate the ability of nematodes to

protect their cuticle bodies from the bacterial attack and at the same time increased the activity of bacteria spores.

REFERENCES

Bird, A. F.; P. G. Brisbane, S. G. McClure and W. L. Kimber (1990). Studies on the properties of the spores of some populations of *Pasteuria penetrans*. J. Invertebrate Pathology, 55: 169-178.

Davies, K. G., V. Laird and B. R. Kerry (1991). The motility root invasion and infection of *Meloidogyne incognita* encumbered with spores of the obligate hyperparasite *Pasteuria penetrans*. Revue de Nematologie, 14: 611-618.

Daykin, M. E. and R. S. Hussey (1985). Staining and histopathological techniques in Nematology. Pp. 39-48 *in* Barker, K. R.; Carter, C. C. and Sasser, J. N., eds. An advanced treatise in Meloidogyne, Vol.II Methodology, Raleigh: North Carolina State University Graphics.

Dutky, E. M. and R. M. Sayre (1978). Some factors affecting infection of nematodes by the bacterial spore parasite *Bacillus penetrans*. J. Nematol., 10: 285 (Abstract).

Hogland, D. R. and D. I. Arnon (1938). Agricultural Experiment Station. Circular 347; Berkeley, California.

Hue, N. V., M. Serracin, D. P. Schmitt and H. C. Bittenbender (2004). Nutrient and nematode status of coffee and soils from orchards in Hawaii. Communications in Soil Science and Plant Analysis, 35(13/14): 2023-2036.

Hussey, R. S. and K. R. Barker (1973). A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique for separating nematodes from the soil. Plant Dis. Rep. 57: 1025-1028.

Jenkins, W. R. (1964). A rapid centrifugal flotation technique for separating nematodes from the soil. Plant Dis. Rep. 48: 692.

Oostendorp, M., D. W. Dickson and D. J. Mitchell (1990). Host range and ecology of isolates of *Pasteuria* spp. from the southeasteren United States. J. Nematol., 22: 525-531.

Sayre, R. M. and M. P. Starr (1985). *Pasteuria penetrans* (ex Thorne, 1940) nom. Rev., comb. n., sp. n., a mycelial and endospore-forming bacterium parasitic in plant-parasitic nematode. Proc. Helminth. Soc. Wash. 52: 149-165.

Siddiqui, I. A. and S. S. Shaukat (2003). Role of iron in rhizobacteria mediated suppression of root infecting fungi and root-knot nematode in tomato. Nematologia-Mediterranea, 31 (1): 11-14.

Siddiqui, I. A., S. S. Shaukat and M. Hamid (2002). Role of zinc in rhizobacteria mediated suppression of root infecting fungi and root-knot nematode in tomato. J. Phytopathol., 50 (10): 569-575.

Stirling, G. R. and M. F. Wachtel (1980). Mass production of *Bacillus penetrans* for the biological control of root-knot nematodes. Nematologica, 26: 308-312.

Stirling, G. R., A. F. Bird and A. B. Cakurs (1986). Attachment of *Pasteuria penetrans* spores to the cuticles of root-knot nematodes. Revue de Nematologie, 9: 251-260.

Williams, A. B., G. R. Stirling, A. C. Hayward and J. Perry (1989). Properties and attempted culture of *Pasteuria penetrans* a bacterial parasite of root-knot nematode, *Meloidogyne javanica*. J. Applied Bacteriology, 67: 145-146.

تأثير العناصر الغذائية على بعض الصفات البيولوجية المؤثرة علي تطفل بكتيريا الباستريا بينيترانس علي نيماتودا تعقد الجذور ميلودوجينا جافنيكا

مجدي السيد مهدي[١] ياسين عبد الرؤوف رجوب[٢] عاطف محروس كيله [٣]

١- قسم النبات الزراعي – كليه الزراعـة – جامعة المنوفية – شبين الكوم ــ مصر

٢- جامعه البلقاء التطبيقية – كليه الحصن – أربد – المملكة الأردنية

٣- معهد بحوث أمراض النباتات – مركز البحوث الزراعية – الجيزة – مصر

الملخص العربي

تمت هذه الدراسة للكشف عن دور العناصر الغذائية على كفاءة تطفـل جـراثيم بكتيريـا الباسـتريا بينيترانس علي نيماتودا تعقد الجذور ميلودوجـينا جافنيكا حيث تمت هذه الدراسـة تحت الظروف المعملية وظروف الصوبه الزجاجية.

تحت الظروف المعملية تمت الـدراسة باستخدام أطـباق بـتري حـيث تـم استخـدام ثلاث تركيزات من جـراثيم البكتيريا وهي ١٠٤ , ١٠٥ , ١٠٦ جرثومة لكل مللـي . أوضـحت النتائج أن استخدام كل العناصر الغذائية قد أدى إلى زيـادة نـسبة إصـابة يرقـات نيمـاتودا ميلودوجينا جافنيكا بجراثيم البكتيريا وكذلك زاد التصاق عدد جـراثيم البكتيريـا علـي يرقـات النيماتودا بالمقارنة بالكنترول . ودلت النتائج أيضا أن استخدام العناصـر الغذائية بدون الحديد قد أدى إلى زيـادة نسـبة إصابة يرقات النيمـاتودا بجراثيم البكتيريـا وذلك عنـد المـستوي ١٠٦ جرثومة /مللي وكذلك زاد مـتوسط عدد الجراثيم علي يرقه النيماتودا.

أما تحت ظروف الصوبه الزجاجية فقـد تمت الدراسة في أصص فخار وتـم اسـتخدام معلق من جراثيم البكتيريا بتركيز ١٠٦ جرثومة لكل مللي وأوضحت النتائج أن معاملة نباتـات الطماطم بالمحـاليل المغذية قد أدي إلي زيـادة متوسـط عـدد جراثيم البكتيريا المتطفلة علي يرقات نيماتودا تعقد الجذور وكذلك زادت نسـبة إصابة يرقات النيمـاتودا بجـراثيم البكتيريـا بالمقارنة بالكنترول وعند استخدام المحلول المغذي بدون عنصر الحديد أدي إلى زيـادة نـسبة إصابة يرقات النيماتودا بجراثيم البكتيريـا وكذلك زاد متوسط عدد جراثيم البكتيريـا المتطفلـة علي يرقات النيماتودا . أظهرت النتائج أيضا أن معاملة النباتات بالمحلول المغذي فـي وجـود جراثيـم البكتيريا يكون مشجع للنمو الجذري والخضري للنبات.