MEDEIROS JE; SILVEIRA EB; MARIANO RLR; PEDROSA EMR. 2009. Inconsistency of the biological control of *Meloidogyne incognita* race 2 in melon by endophytic and rhizosphere bacteria. *Horticultura Brasileira* 27: 319-324.

# Inconsistency of the biological control of *Meloidogyne incognita* race 2 in melon by endophytic bacteria

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## ABSTRACT

We obtained 61 rhizobacterium isolates from rhizosphere soil samples collected in melon commercial fields located in Mossoró, Rio Grande do Norte State, Brazil. These isolates, along with 56 endophytic bacteria from the Collection of Cultures of the Plant Bacteriology Laboratory of the Universidade Federal Rural de Pernambuco, were tested for controlling *Meloidogyne incognita* race 2 in melon. To infest the soil with nematodes, 1000 eggs of *Meloidogyne incognita* race 2 per plant were placed in pots where seedlings of the yellow-type melon, cultivar AF 682, were growing for 10 days. Two days before, 20 mL of bacterial suspension (0.7 $OD_{570nm}$) were poured into each pot. After 60 days, fresh root biomass, gall index, egg mass, and the nematode reproduction factor were assessed. Among the 117 isolates screened, the endophytic *Bacillus* ENM7, ENM10, and ENM51 were selected because they significantly reduced egg mass and/or gall index. However, when tested again, separately and in mixtures, these isolates nor confirmed their efficiency *in vivo*, neither affected juvenile emergence *in vitro*. These results give evidence on the inconsistency of using endophytic-bacteria in the control of *M. incognita* race 2 in melon.

**Keywords:** *Meloidogyne*, *Cucumis melo*, rhizobacteria, endophytic bacteria, management.

## RESUMO

**Inconsistência do controle biológico de *Meloidogyne incognita* raça 2 em meloeiro por bactérias endofíticas**

A partir de amostras de solo coletadas em plantios comerciais de meloeiro, situados em Mossoró-RN, foram obtidos 61 isolados de rizobactérias que, juntamente com outros 56 isolados endofíticos pertencentes à Coleção de Culturas do Laboratório de Fitobacteriologia da Universidade Federal Rural de Pernambuco, foram avaliados para o controle de *Meloidogyne incognita* raça 2 em melão. Plantas de meloeiro Amarelo, cultivar AF 682, com dez dias de idade tiveram o solo infestado com 1000 ovos de *M. incognita* raça 2 por planta. Dois dias antes, foram depositados em cada vaso 20 mL da suspensão bacteriana ($DO_{570nm}$ = 0,7). Decorridos 60 dias, foram determinados a biomassa fresca das raízes, os índices de galhas e de massa de ovos e o fator de reprodução do nematóide. Dos 117 isolados avaliados, foram selecionados inicialmente os isolados endofíticos ENM7, ENM10 e ENM51, todos pertencentes ao gênero *Bacillus*, que reduziram significativamente a massa de ovos e/ou o índice de galhas. Contudo, quando testados novamente, separadamente ou em misturas, esses isolados não mantiveram a eficiência na redução dessas variáveis e, *in vitro*, não afetaram a eclosão dos juvenis. Os resultados obtidos evidenciam a inconstância da ação das bactérias endofíticas no controle de *M. incognita* raça 2 em meloeiro.

**Palavras-chave:** Meloidoginose, *Cucumis melo*, rizobactérias, bactérias endofíticas, manejo.

**(Recebido para publicação em 7 de março de 2008; aceito em 14 de maio de 2009)**

**(*Received in March 7, 2008; accepted in May 14, 2009*)**

Nematodes are among the major disease agents in melon. In tropical regions, nematodes are responsible for yield losses from 18 to 33% (Lucas & Sorribas, 1994). In northeastern Brazil, Lima *et al.* (1995) cited *Meloidogyne* spp. as a limiting factor for melon production in Açu, Rio Grande do Norte State, with losses of up to 100%. These are disturbing figures if one recalls that the Northeast is the main melon producer and exporter in Brazil: in 2005, about 274 thousand tons were harvested, the equivalent to 93% of the melon production in the country (IBGE, 2005).

The control of nematodes is a complex task due to their high diversity, polyphagia, and easy dissemination. Thus, it is necessary to employ efficient management practices to reduce and keep nematode populations below the damage level (Freitas *et al*., 2001). The biological control is a good example of a practice to be comprehensively investigated and included in nematode management programs. It is especially pertinent for the root-knot nematode management in melon, since there are no nematicides registered for this crop (Andrei, 2005).

Among the biological agents best suited for the management of nematode populations, we can name the plant growth-promoting rhizobacteria, obligatory parasite bacteria, fungi that parasites eggs, female parasite and predator fungi, and endomycorrhizal fungi (Sikora, 1992). The use of bacteria to control nematodes is a promising research field (Freitas *et al*., 2005), and, among these, the rhizobacteria able to at one time hamper nematode penetration in roots (Sikora, 1988) and promote plant growth (Kloepper *et al*., 1985) are particularly interesting.

Zaveleta-Meija & Van Gundy (1982) were pioneers in reporting reductions in damage caused by root-knot nematodes in cucumber and tomato by seed microbiolization with rhizobacteria. Subsequently, several studies were developed using rhizobacteria in nematode management, some with promising results. *Pseudomonas fluorescens* and *P. putida* were efficient in controlling *Meloidogyne* spp. and

*Radopholus similis* in banana (*Musa* sp.), maize (*Zea mays*), and tomato (*Solanum lycopersicum)* (Aalten *et al*., 1998). Hoffmann-Hergarten *et al*. (1998) succeeded in controlling *M. incognita* in tomato and lettuce (*Lactuca sativa*) with the use of *Pseudomonas* sp. and *Bacillus* sp. Siddiqui & Shaukat (2002) reported that *Pseudomonas aeruginosa* IE-6SP and *P. fluorescens* CHAO were efficient in controlling *M. javanica* in tomato. In USA, the products Paecil = Bioact WG, MeloCon and Nemachek WG (*Paecilomyces lilacinus* strain 251) are already commercially available for controlling *Meloidogyne* spp., *R. similis*, *Heterodera* spp., *Globodera* spp., and *Pratylenchus* spp. (EPA, 2005).

Despite the lack of emphasis on research with endophytic bacteria in Brazil, Naves *et al*. (2004) observed that filtrates of these bacteria reduced both the mobility and emergence of *M. javanica* juveniles, causing high mortality. These organisms have the advantage of living inside vegetal tissues, escaping from the competition with soil microorganisms. In addition, even when present in the soil, before reaching the plant cortex and vascular system, due to the consecutive colonization of the rhizosphere and rhizoplane (Kloepper *et al*., 1992), these bacteria may already interfere in juvenile emergence; nematode direction, mobility, recognition, and penetration in the root; as well as in the processes of feeding and reproduction (Freitas, 2001).

The objectives of this study were to isolate bacteria from the melon rhizosphere and select rhizo- and endophytic-bacteria that could be used in the control of *M. incognita* race 2.

## MATERIAL AND METHODS

**Isolation of bacteria from the melon rhizosphere -** Using a methodology adapted from Gomes *et al*. (2005), we collected ten samples of rhizosphere soil (1 kg) from plants without symptoms of nematode infection, in Mossoró, Rio Grande do Norte State, in melon production areas with history of the disease. In the laboratory, after homogenization, 1.0 g of soil from each sample was placed in Erlenmeyer flasks with 99 mL of sterile distilled water. After homogenizing the soil suspension, we took 1-mL aliquot to be used in a serial dilution, base 10, up to $10^{-3}$. After, 0.1 mL of each dilution was pipetted to Petri dishes with the culture medium NYDA (Pusey & Wilson, 1984) and spread uniformly throughout the medium surface with a Drigalsky spatula. The plates were incubated in BOD for 48 hours, at 30°C. Depending on the color and growth in the medium, isolate colonies were purified and preserved using the method of sterile distilled water (De Vay & Schnathorst, 1963). Then, isolates were submitted to the tests of Gram stain and growth on medium King B (Schaad *et al*., 2001). We also used 57 isolates of endophytic bacteria obtained out of different organs of healthy melon plants (Oliveira *et al*., 2006). These isolates came from the Collection of Cultures of the Laboratory of Plant Bacteriology of the Universidade Federal Rural de Pernambuco.

**Obtaining the inoculum of *Meloidogyne incognita* race 2 -** The inoculum of *M. incognita* race 2 was collected in commercial melon fields in Rio Grande do Norte State, and maintained in greenhouse, in melon, cultivar Amarelo Ouro. The inoculum was prepared according to Hussey & Barker (1973). The concentration of the suspension was adjusted to 1000 eggs $plant^{-1}$. The nematode species was confirmed via the perineal configuration of adult females and isozyme pattern, while the race was confirmed using the test for race differentiation of Hartman & Sasser (1985).

**Selection of bacterial isolates for the biological control of *Meloidogyne incognita* race 2 -** Ten-day old seedlings of yellow melon, cultivar AF 682, grown in 100-mL pots filled with soil fumigated with methyl bromide, were infested with 1000 eggs of *M. incognita* race 2 per plant. Eggs were placed around the plant stem, in four 2-cm deep holes. Two days before the soil infestation with nematodes (Reitz *et al*., 2000), 20 mL of bacterial suspension, adjusted by spectrophotometer to $OD_{570nm} = 0.7$, were dispensed into each pot. Three days after infestation, plants and all the soil in the pots were transferred to 500-mL pots containing soil fumigated with methyl bromide. Plants were kept in greenhouse, at an average temperature of 29±3°C.

The experiment was carried out in a completely randomized design, with 119 treatments (117 bacteria and the relative and absolute control treatments, respectively only with nematodes, and without bacteria and without nematodes), and five replications, with 1-plant plots. After 60 days of infesting the soil, we determined root fresh weight, gall index and egg mass, eggs per root system, and the nematode reproduction factor (ratio between the final and initial nematode population), according to Hussey & Barker (1973). The gall index and the egg mass were estimated using the International Meloidogyne Project scale (Taylor & Sasser, 1978).

Three bacterial isolates which reduced the gall index and/or the egg mass were re-tested for biological control, separately and mixed, using the same methodology as described above. The same isolates were tested also for controlling the juvenile emergence of *M. incognita* race 2 *in vitro*. For this test, the bacterial suspension was adjusted in a spectrophotometer to $OD_{570\ nm} = 0.7$, and poured into plastic container with 4-cm diameter. A sieve was placed over the suspension, and over the sieve, we placed a disc of filter paper, touching the bacterial suspension, mimicking the Baermann funnel model. On the top of the filter paper, 500 eggs of *M. incognita* race 2 were deposited. The eggs were surface sterilized beforehand in a laminar flow, through immersion in streptomycin sulfate 0.1% for 5 minutes (Carneiro *et al*., 1998), followed by rinsing for three times in sterile water, flowing through 500-mesh sieves. The containers were covered with aluminum foil and kept under laboratory conditions, at 25°C. The experiment was carried out in a completely randomized design, with five treatments (the three bacterial isolates separately, a mixture of the isolates, and one control), with four replications, and 1-container plots. After 48 hours, we evaluated in stereomicroscope the percentage of J2 emerged in calibrated boxes.

Data from all experiments were subjected to analysis of variance. Means were compared using the Scott-Knott or Duncan tests ($p \leq 0.05$). We also performed the Pearson correlation analysis ($p \leq 0.01$).

## RESULTS AND DISCUSSION

Out of the ten samples of rhizosphere soil collected in melon production fields in Rio Grande do Norte State, 60 bacterial isolates were selected from the colonies that most frequently appeared with characteristic color and growth pattern within each soil. It was found that 48% of the isolates were Gram positive, and none of the Gram negative isolates produced fluorescent pigment on King B medium, which is indicative of the *Pseudomonas* spp. fluorescent group. Fluorescent and non-fluorescent *Pseudomonas*, gram positive bacteria of the genus *Bacillus*, and *Pasteuria penetrans* are often associated with nematode control (Freitas, 2001).

No bacterial isolate increased significantly ($p \leq 0.05$) the fresh weight of roots in comparison to the relative and absolute controls (Table 1). Moreover, 62% of the isolates had a deleterious effect, decreasing the root fresh weight. Reduction in plant biomass can be caused by deleterious or plant pathogenic bacteria, both detrimental to plant development. Some of the isolates obtained in this work might have been of this sort. According to Schippers *et al*. (1987), rhizobacteria are not always beneficial to plants. In several cases, they can be neutral or even prejudicial. Coimbra *et al*. (2005) observed reduction in the dry matter content of tomato plants treated with rhizobacteria. In the other hand, Tomé *et al*. (2000) reported increments of up to 79% in height and 43% in total weight in tomato plants treated with endophytic bacteria isolated from velvet bean (*Stizolobium aterrimum*).

Although all plants have shown high gall index according to the criteria established by Taylor & Sasser (1978), among the 45 isolates that did not hinder plant growth, 11% have significantly reduced the gall index in comparison to the relative control (Table 1), indicating that these isolates altered the nematode life cycle. When only isolates that had detrimental effects over plant growth were taken into account, there was a positive and significant (Pearson, $p \leq 0.01$) correlation of 54% between root fresh mass and gall index. However, this correlation was as low as 14% when isolates that did not affect plant growth were considered. Nevertheless, the bacterial isolates did not prevent the nematodes from penetrating the roots, since all plants were parasitized. Similar behavior was reported by Coimbra *et al*. (2005), who challenged *M. javanica* with 92 rhizobacterium isolates collected from different crops and found only 34 that effectively reduced the number of galls per plant in tomato. Freitas *et al*. (2005) reported that out of 264 isolates of rhizosphere bacteria of tomato tested to control *M. javanica* and *M. incognita*, only six lessened successfully the number of galls of *M. javanica*.

Egg masses were high in all plants studied. However, 38% of the isolates that did not affect root fresh weight, reduced significantly egg masses, particularly the endophytic bacterium ENM51, which decreased egg masses by 64% in relation to the control (Table 1, Figure 1). Reductions of 68 and 77% in the number of galls and egg mass per g of roots, respectively, were reported in tomato seedlings inoculated with *M. incognita* when grown in substrate incubated with *Streptomyces griseus* subsp. *griseus* (Sousa *et al*., 2006). It is supposed that the bacterium itself or its metabolic products can trigger a hypersensitivity reaction in the giant cells, affecting the nematode feeding. Thus, the egg production declines due to the lack of vital reserves in the nematode, impacting negatively its reproduction (Freitas, 2001). Other mechanisms linked to the control of nematodes by bacteria are the induction of resistance and antibiosis. Experiments effective on testing these last two mechanisms would be the split root technique and the application of filtrates of bacterial cultures, respectively (Reitz *et al*., 2000; Naves *et al*., 2004).

Although some isolates have reduced gall index and egg mass, no isolates were effective in dropping significantly the number of eggs per root system. In contrast, some treatments seem to have stimulated egg production. The reproduction factor observed in the experiment ranged from 22.106 (ENM60) to 2,436 (ENM51) eggs per root system, while in the relative control the reproduction rate was 6,712 eggs. Coimbra *et al*. (2005), when studying 49 rhizobacteria to control *M. javanica* in tomato, found that 69 and 6% of the isolates respectively reduced and increased the number of galls per gram of root.

In our first experiment, the endophytic bacterial isolates ENM7 and ENM10 stood out for their potential to control the root-knot nematode infection in melon, since they reduced significantly gall index and egg mass. In addition to those, we observed that isolate ENM51 induced the smallest egg mass (Table 1). These isolates, which reduced gall index and egg mass up to 40 and 64% respectively (Figure 1), are *Bacillus* and came from the Collection of Cultures of the Laboratory of Plant Bacteriology of the UFRPE. *Bacillus* and *Pasteuria* isolates have shown promising results in the control of the cyst nematode (*Heterodera* sp.) in soybean, as well as the root-knot nematode (*Meloidogyne* sp.) in several hosts. Some of the main advantages of using these microorganisms are their prolonged survival in the soil, resistance to heat and desiccation, the safety to humans and other animals, and the possibility of combining their use with cultural practices in an integrated nematode management system (Freitas & Carneiro, 2000).

When the endophytic bacterial isolates ENM7, ENM10, and ENM51 were tested again, separately and mixed, no reduction in gall index or egg mass was observed (Duncan, $p \leq 0.05$) (data not presented), in disagreement with the results observed in the first experiment. These isolates did not affect juvenile emergence *in vitro* either, suggesting the lack of a direct action of toxic metabolites eventually produced by the isolates. According to Freitas (2001), the toxic metabolites would be absorbed by the egg, inactivating it or causing

**Table 1.** Root fresh weight and indexes of gall and egg mass in melon 60 days after soil infestation with eggs of *Meloidogyne incognita* race 2, in pots inoculated with rhyzo- and endophytic-bacterial isolates from melon (biomassa fresca das raízes (BFR), índice de galhas (IG) e índice de massa de ovos (IMO) em melão 60 dias após a infestação do solo com ovos de *Meloidogyne incognita* raça 2, em vasos inoculados com isolados bacterianos da rizosfera (RM) e endofíticos (ENM) de meloeiros). Recife, UFRPE, 2007.

| Treatments | Indexes[1] | | | Treatments | Indexes | | | Treatments | Indexes[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | RFW[2] | GI[2] | EM[2] | | RFW[2] | GI[2] | EM[2] | | RFW[2] | GI[2] | EM[2] |
| Abs. Control[3] | 17.2a | 0.0c | 0.0d | RM39 | 7.6c | 3.6b | 2.8b | ENM21 | 9.0b | 3.4a | 2.4b |
| Rel. Control[3] | 8.6b | 5.0a | 4.4a | RM40 | 7.5c | 4.6a | 3.6a | ENM22 | 9.8b | 4.6a | 3.6a |
| RM1[4] | 8.6b | 4.6a | 3.8a | RM41 | 6.7c | 4.6a | 4.2a | ENM23 | 7.6c | 4.0a | 3.0b |
| RM2 | 7.3c | 4.6a | 4.0a | RM42 | 7.7c | 3.6b | 3.0b | ENM24 | 7.5c | 3.2b | 1.6c |
| RM3 | 7.0c | 3.6b | 3.2b | RM43 | 6.0c | 4.0a | 3.2b | ENM25 | 12.2b | 4.4a | 3.0b |
| RM4 | 6.2c | 3.4b | 2.6b | RM44 | 6.0c | 3.0b | 2.6b | ENM26 | 6.8c | 4.2a | 2.6b |
| RM5 | 7.0c | 4.8a | 3.4a | RM45 | 5.0c | 4.0a | 2.4b | ENM27 | 8.4b | 4.0a | 3.2b |
| RM6 | 6.5c | 3.2b | 2.8b | RM46 | 5.8c | 2.8b | 2.4b | ENM28 | 6.8c | 4.4a | 3.4a |
| RM7 | 4.6c | 2.6b | 2.2b | RM47 | 7.3c | 3.6b | 2.6b | ENM29 | 7.7c | 3.2b | 2.6b |
| RM8 | 5.0c | 2.8b | 2.0c | RM48 | 7.7c | 3.6b | 2.6b | ENM30 | 7.0c | 3.4b | 2.2b |
| RM9 | 11.2b | 4.4a | 3.6a | RM49 | 10.0b | 4.2a | 3.4a | ENM31 | 7.3c | 2.4b | 2.2b |
| RM10 | 9.0b | 4.6a | 3.8a | RM50 | 7.5c | 3.6b | 3.0b | ENM32 | 9.0b | 4.0a | 2.6b |
| RM11 | 10.2b | 4.8a | 4.2a | RM51 | 4.0c | 2.8b | 2.2b | ENM33 | 8.0b | 3.2b | 3.0b |
| RM12 | 8.7b | 3.8b | 3.4a | RM52 | 6.5c | 3.6b | 2.4b | ENM34 | 10.2b | 4.8a | 3.6a |
| RM13 | 7.4c | 4.4a | 4.0a | RM53 | 7.0c | 3.2b | 2.6b | ENM35 | 7.7c | 1.0b | 2.6b |
| RM14 | 10.2b | 5.0a | 4.2a | RM54 | 7.4c | 2.8b | 2.4b | ENM36 | 7.7c | 3.6b | 2.4b |
| RM15 | 6.0c | 4.2a | 2.8b | RM55 | 7.7c | 2.8b | 2.2b | ENM37 | 6.2c | 2.4b | 2.6b |
| RM16 | 6.0c | 2.6b | 2.4b | RM56 | 6.8c | 4.6a | 3.4a | ENM38 | 7.0c | 4.2a | 2.8b |
| RM17 | 7.7c | 3.6b | 3.0b | RM57 | 6.4c | 3.4b | 2.4b | ENM39 | 7.2c | 3.4b | 2.8b |
| RM18 | 8.2b | 3.8b | 3.4a | RM58 | 6.0c | 2.6b | 2.8b | ENM40 | 7.3c | 3.6b | 3.2b |
| RM19 | 5.4c | 3.6b | 2.8b | RM59 | 7.6c | 4.4a | 3.2b | ENM41 | 7.0c | 4.6a | 4.0a |
| RM20 | 5.2c | 4.2a | 3.0b | RM60 | 8.8b | 4.2a | 3.4a | ENM42 | 7.2c | 3.4b | 3.0b |
| RM21 | 7.2c | 4.2a | 2.6b | ENM1[4] | 7.8b | 4.4a | 4.4a | ENM43 | 7.5c | 2.4b | 2.8b |
| RM22 | 6.7c | 2.6b | 2.5b | ENM2 | 10.2b | 4.4a | 4.0a | ENM44 | 5.6c | 3.4b | 2.4b |
| RM23 | 9.2b | 4.4a | 3.4a | ENM3 | 7.4c | 4.0a | 3.8a | ENM45 | 4.6c | 2.6b | 2.2b |
| RM24 | 11.4b | 4.8a | 3.2a | ENM4 | 6.8c | 5.0a | 4.4a | ENM46 | 9.2b | 4.4a | 3.8a |
| RM25 | 7.5c | 4.0b | 2.8b | ENM5 | 10.0b | 4.0a | 3.6a | ENM47 | 7.0c | 3.6b | 2.4b |
| RM26 | 6.2c | 3.4b | 3.0b | ENM6 | 8.6b | 4.4a | 3.8a | ENM48 | 8.6b | 4.2a | 3.6b |
| RM27 | 6.0c | 3.4b | 2.8b | ENM7 | 9.4b | 3.8b | 2.4b | ENM51 | 9.0b | 4.0a | 1.6c |
| RM28 | 6.6c | 2.6b | 2.8b | ENM8 | 10.0b | 4.6a | 3.8a | ENM52 | 11.6b | 4.6a | 3.0b |
| RM29 | 9.2b | 4.6a | 3.6a | ENM9 | 7.5c | 1.6b | 2.2b | ENM53 | 9.4b | 4.6a | 3.6a |
| RM30 | 7.4c | 4.8a | 3.6a | ENM10 | 8.2b | 3.0b | 2.8b | ENM54 | 7.4c | 4.4a | 3.8a |
| RM31 | 7.5c | 4.8a | 3.4a | ENM11 | 7.5c | 4.0a | 3.0b | ENM55 | 9.6b | 4.0a | 3.2b |
| RM32 | 5.2c | 3.2b | 2.4b | ENM12 | 8.0b | 4.4a | 4.2a | ENM56 | 9.4b | 4.6a | 2.8b |
| RM33 | 10.0b | 4.6a | 4.2a | ENM14 | 6.8c | 3.8b | 3.0b | ENM57 | 10.0b | 4.6a | 3.0b |
| RM34 | 7.8b | 4.6a | 3.8a | ENM15 | 8.8b | 5.0a | 3.8a | ENM58 | 9.8b | 4.6a | 2.8b |
| RM35 | 9.6b | 3.8b | 3.4a | ENM16 | 8.2b | 4.2a | 3.2b | ENM59 | 6.2c | 3.6b | 2.6b |
| RM36 | 9.5b | 4.8a | 4.2a | ENM17 | 7.7c | 3.6b | 3.2b | ENM60 | 7.4c | 4.6a | 3.6a |
| RM37 | 7.7c | 4.0a | 3.0b | ENM18 | 5.0c | 3.8b | 2.8b | ENM61 | 10.8b | 4.2a | 2.8b |
| RM38 | 9.0b | 4.0a | 3.4a | ENM20 | 10.4b | 4.8a | 3.2b | - | - | - | - |
| CV (%) | 23.7 | 16.5 | 21.8 | - | | 16.5 | 21.8 | - | 23.7 | 16.5 | 21.8 |

Means followed by the same letter in the columns did not differ significantly from each other, Scott-Knott test, $p<0.05$ (médias seguidas da mesma letra na colunas não diferem estatisticamente entre si, teste de Scott-Knott, $p<0,05$); [1]Indexes calculated according to Taylor & Sasser, 1978 (índices calculados de acordo com Taylor & Sasser, 1978); [2]RFW= root fresh weiht (biomassa fresca das raízes); GI= gall index (índice de galhas); EM= egg mass (índice de massa de ovos) [3]Abs. control (testemunha absoluta): pots nor infested with nematodes, nor inoculated with bacterial isolates (vasos sem nematóides e sem isolados bacterianos; Rel. control (testemunha relativa): pots infested with nematodes, but not inoculated with bacterial isolates (vasos infestados com nematóides, mas não inoculados com isolados bacterianos); [4]RM= melon rizosphere isolate (isolados bacterianos da rizosfera do melão); ENM= melon endophytic isolate (isolado endofítico de melão).

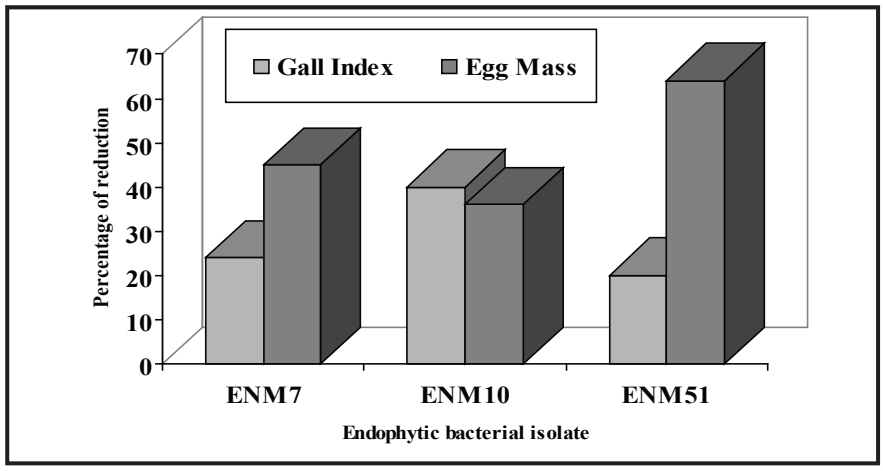


**Figure 1.** Reduction on the indexes of gall and egg mass 60 days after soil infestation with eggs of de *Meloidogyne incognita* race 2 and inoculation with endophytic bacterial isolates - ENM (redução do índice de galhas e índice de massa de ovos 60 dias após a infestação do solo com ovos de *Meloidogyne incognita* raça 2 e inoculação com isolados bacterianos endofíticos - ENM). Recife, UFRPE, 2007.

deformations in the nematode to an extent that would prevent the egg from hatching. Some other studies have also reported a decrease in the number of bacterial isolates effective in controlling nematodes when a second screening was carried out. Habe (1997), upon assessing isolates from the rhizosphere of some Solanaceae to control *M. incognita* race 1, found that around 10% of the bacterial isolates were effective in the first evaluation, while in the second, although carried out only with the most promising isolates, the figure decreased to 4%. Racke & Sikora (1992) also observed a reduction in the number of bacterial isolates which were effective in controlling *Globodera pallida* in potato (*Solanum tuberosum*): in the first assay, 16 out of 179 isolates efficiently controlled the nematode, while in the second assay only six isolates proved to be valuable. It is important to mention that although these are examples of studies with rhizobacteria, this is one of the most important steps in the plant-endophytic bacteria interaction when the source or origin of the endophytics is the soil (Kloepper 1992), like in our study.

The timing of soil inoculation with the bacterial isolates might have been a reason for the lack of growth promotion and *Meloidogyne incognita* control in this study. Two days might have been too short to root colonization and production of toxic substances up to a concentration high enough to inhibit juvenile emergence and viability, just like as observed *in vitro*. In addition, it is necessary to try another mode of inoculating the plants with growth-promoting bacteria, such as seed bacterization. This might be beneficial because during seed germination, exudates that provide selective advantage in colonization and bacterial survival in the roots are released (Kloepper *et al*., 1985).

Although the bacterial isolates were all collected from melon, none was effective in controlling *M. incognita* race 2, corroborating the discussion about the role specificity plays in the efficiency of growth-promoting bacteria (Enebak *et al*., 1998; Shishido & Chanway, 1999). According to Coimbra *et al*. (2005), a broad diversity in the plant species used for the isolating rhizobacteria increases the chances of finding isolates effective in controlling nematodes.

The absence of useful isolates for controlling nematodes among those tested is not uncommon and can be explained also by the number of isolates used. According to Chen *et al*. (1996), the percentage of growth-promoting bacteria is less than 1%. Nevertheless, Racke & Sikora (1992), Habe (1997), and Freitas *et al*. (2005) reported success rates of 2.3, 3.3, and 3.8% respectively when screening bacteria for nematode control. Therefore, if similar rates were found in our work, we would have come across a few promising isolates.

To conclude, this study presented evidence of the complexity of identifying and screening for efficient antagonists to *M. incognita* race 2 in melons among endophytic and rhizosphere bacteria, particularly in relation to the reproducibility and robustness of results. Although the biological control of *M. incognita* race 2 in melon has not been demonstrated in this study, but keeping in mind that there are no nematicides registered for melon (Andrei, 2005), we strongly believe that further studies on the biological control of nematodes are worthwhile, specially combining it to cultural practices in an integrated nematode management. Finally, we would like to draw the attention of those studying the use of bacteria in the biological control of nematodes to the issues of timing and mode of plant inoculation with the bacteria, as well as to the origin and number of isolates.

## ACKNOWLEDGEMENTS

Jeane E de Medeiros thanks the CNPq for the M.Sc. scholarship. Elineide B da Silveira, Rosa de LR Mariano and Elvira MR Pedrosa hold grants for Productivity in Research from CNPq.

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