RESEARCH/INVESTIGACIÓN

EFFECT OF *AZOSPIRILLUM BRASILENSE*, STIMULATE® AND POTASSIUM PHOSPHITE TO CONTROL *PRATYLENCHUS BRACHYURUS* IN SOYBEAN AND MAIZE

Claudia Regina Dias-Arieria*, Patricia Meiriele Marini, Lais Fernanda Fontana, Miria Roldi and Tiago R. Benetoli da Silva

State University of Maringa, Umuarama Regional Campus, Department of Agriculture, Umuarama, PR, Brazil. *Corresponding author:crdarieira@uem.br

ABSTRACT

Dias-Arieira, C. R., P. M. Marini, L. F. Fontana, M. Roldi and T. R. B. Silva. 2012. Effect of *Azospirillum brasilense,* Stimulate® and potassium phosphite to control *Pratylenchus brachyurus* in soybean and maize. Nematropica 42:170-175.

The aim of the study was to evaluate the effect of *Azospirillum brasilense*, Stimulate® and potassium phosphite, both alone and in combination, on the control of *Pratylenchus brachyurus* in soybean and maize. The maize and soybean were subjected to *A. brasilense*, Stimulate®, potassium phosphite, *A. brasilense*+Stimulate®, *A. brasilense*+potassium phosphite, Stimulate®+potassium phosphite, and *A. brasilense*+Stimulate®+potassium phosphite. Untreated plants, inoculated and uninoculated with *P. brachyurus*, were used as controls. Stimulate® and *A. brasilense* were applied directly to the seeds and the potassium phosphite sprayed fortnightly onto the leaves. After 60 days, in maize, the population of *P. brachyurus* was significantly reduced with potassium phosphite, whereas in soybean, the seeds treatment with Stimulate® resulted in plants that maintained significantly lower populations of *P. brachyurus*. These treatments resulted in increased plant height.

Key words: bioregulators, biostimulants, lesion nematode, management

RESUMO

Dias-Arieira, C.R., P.M. Marini, L.F. Fontana, M. Roldi and T.R.B. Silva. 2012. Efeito de *Azospirillum brasilense*, Stimulate® e fosfito de potássio no controle de *Pratylenchus brachyurus* em soja e milho. Nematropica 42:170-175.

O objetivo do trabalho foi avaliar o efeito de *Azospirillum brasilense*, Stimulate® e fosfito de potássio, isolados e em combinações, no controle de *Pratylenchus brachyurus* em soja e milho. Para isto, sementes de milho e soja foram submetidas aos tratamentos *A. brasilense*, Stimulate®, fosfito de potássio, *A. brasilense*+Stimulate®, *A. brasilense*+fosfito de potássio, Stimulate®+fosfito de potássio, e *A. brasilense*+Stimulate®+fosfito de potássio. Plantas não tratadas com e sem inoculação foram utilizadas como testemunhas. Stimulate® e *A. brasilense* foram aplicados via tratamento de sementes e o fosfito de potássio foi aplicado quinzenalmente via pulverização foliar. Após 60 dias, no milho, a população de *P. brachyurus* foi reduzida principalmente quando se utilizou o tratamento com fosfito de potássio, enquanto para a soja, os melhores resultados foram obtidos para o tratamento de sementes com Stimulate®, estando os mesmos tratamentos relacionados com o aumento na altura das respectivas plantas.

Palabras clave: biorreguladores, bioestimulantes, nematoide das lesões radicular, manejo.

INTRODUCTION

Maize (*Zea mays* L.) is one of the most important agricultural products in Brazil and according to a survey of the Brazilian grain harvest, conducted by the National Supply Company (Conab, 2010), Brazilian farmers around 13 million hectares of maize in the 2009/2010 growing seasons, with an average yield of 4,412 kg/ha. The national maize production for both crops totaled 55.6 thousand tons during the latter season, which was1.5% higher than the previous harvest.

In addition, maize is the main rotation crop used with soybean (*Glycine max* (L.) Merrill) in Brazil. However, continued use of this farming system has triggered a serious problem for national agriculture, namely a substantial increase in populations of root-lesion nematodes in such cropping systems (Ribeiro, 2008). Currently *Pratylenchus brachyurus* (Godfrey) and Filipjev and Schuurmans Steckhoven and *P. zeae* Graham, are cited as having impact from a national economic perspective on soybean, maize and sugar cane (Ribeiro, 2008; Inomoto, 2010; Severino *et al.*, 2010; Obici *et al.*, 2011). *Pratylenchus brachyurus* is capable of infecting roots of maize and soybeans, and stands out among the nematodes limiting soybean productivity in Brazil (Ribeiro, 2008; Inomoto, 2010). Parasitism by this nematode has caused 10 to 25% yield losses in soybean alone, but when concomitant infection with other important plant-parasitic nematodes occurs, the losses are higher for soybean production (Inomoto, 2010).

Control of nematodes is complex and requires the use of integrated practices. In this sense, some products that act as plant-growth promoters and regulators have been the subject of research to induce resistance against plant pathogens (Jackson, 2000, Siddiqui and Mahmood, 2001; Siddiqui, 2004).

Azospirillum is a plant growth promoting bacteria able to increase nitrogen fixation and biosynthesis of plant growth hormones (Steenhoudt and Vanderleyden, 2000). *Azospirillum brasilense* Tarrand, Krieg and Döbereiner had underperformed in the control of nematodes compared to other bacteria in tomato and chickpea (Siddiqui and Mahmood, 2001; Siddiqui, 2004), however this bacterium has shown very satisfactory results in the growth of grasses (Cavellet *et al.*, 2000), but it is unclear what causes resistance to plant-parasitic nematodes in such plants.

Stimulate® is a phyto-stimulant that contains growth regulating chemicals that increase plant growth and development by stimulating cell division, differentiation and cell elongation (Castro and Vieira, 2001). In turn, the potassium phosphite has also been widely used in agriculture for the many advantages that it offers, especially to increase phosphorus uptake by the plant compared to products based on phosphate (Cohen and Coffey, 1986; Jackson, 2000). In addition, other aspects are interesting in the use of phosphite for increasing microbial activity (Cohen and Coffey, 1986) and activation of plant defense mechanisms (Jackson, 2000).

However, is not known whether the use of these products can help control nematodes. Thus, the purpose of the work was to evaluate the effect of treatment with *A. brasilense*, Stimulate® and potassium phosphite, individually and in combinations on populations of *P. brachyurus* in soybean and maize.

MATERIALS AND METHODS

Maize hybrid DKB 390PRO and soybean cv. CD 206 were treated as follows: *A. brasilense* (Marterfix®), Stimulate®, potassium phosphite (Hortifós® PK), *A. brasilense* + Stimulate®, *A. brasilense* + potassium phosphite, Stimulate® + potassium phosphite, and *A. brasilense* + Stimulate® + potassium phosphite. Untreated plants, inoculated and uninoculated with *P. brachyurus*, were used as controls. Stimulate® and *A. brasilense* were applied as seeds treatment at the recommended dosage i.e. 15 ml/kg of seed and 5 ml/kg of seed, respectively, at planting. Phosphite was applied as a foliar spray every 2 weeks, to the point of runoff at a dosage of 1.5 ml/L of water.

Three maize or soybean seed were placed in individual 2 L pots, containing a mixture of soil:sand (2:1; v:v). The soil was autoclaved (120°C/2 h) prior to planting the trials. Ten days after seed germination, plant thinning was performed leaving one seedling of each crop per pot. Each plant was inoculated with a suspension of approximately 2,000 juveniles and adults of *P. brachyurus*. The inoculum was obtained from a pure population, courtesy of Embrapa-Cenargen, that was reared in roots of maize in a greenhouse and was extracted from the root systems using the methodology proposed by Coolen and D'Herde (1972). The inoculum consisted of all the infectious stages of this nematode species (second, third and fourth stage juveniles and adults). After extraction, the nematode suspension was transferred to a Peters counting chamber and evaluated using an optical microscope. The inoculum density was calibrated for approximately 500 specimens per ml. Four 3-4 cm deep holes, each spaced 3 cm from the stem of each plant and 1 ml of this inoculum was added to each hole inoculation was performed with 4 ml per pot.

Uninoculated plants did not receive any nematode inoculum and also represented the plants that were not treated with any product, i.e. not sprayed with any of the products that were evaluated in these trials. These control treatments are further referred to as the "uninoculated and untreated control". However, the inoculated control plants received nematode inoculation but were also not sprayed with any of the products and is further referred to as the "inoculated and untreated control".

The plants remained in the pots for 60 days and irrigated twice daily. Subsequently, the plants were harvested and each plant's shoot separated from its root. The plant height was determined by measuring the length from the stem base to the last leaf, using a millimeter ruler. The root system of each plant was carefully washed and nematodes extracted following the method of Coolen and D'Herde (1972). Nematodes were extracted from 100 cm^3 of soil from each pot using centrifugation (Jenkins, 1964). The extracted nematode samples were placed in tightly closed individual plastic containers and kept in the refrigerator until the nematodes were counted within a week after extraction. Nematodes extracted for each treatment were counted in a Peters chamber with a microscope, with a 40x magnification.

The experiments were carried out in a greenhouse at the State University of Maringa, at the Umuarama Regional *Campus*, Parana, Brazil. The trial layout represent a randomized complete block design (RCBD), repeated six times for each treatment. Each experiment, for maize and soybean respectively, was also repeated twice, namely the first experiments for both crops, from January/2010 to March/2011 and the second experiments from June/2011 to August/2011, to confirm results obtained. The results were subject to a variance analysis and means were compared using the Duncan test at 5% probability.

RESULTS

Maize

In the first experiment with maize, the nematode/g of root and total population (roots + soil) of nematodes was significantly reduced by the Stimulate®, potassium phosphite, Stimulate® + potassium phosphite and *A. brasilense* + Stimulate® treatments when compared to the inoculated and untreated control. However, where all three products were used in combination (Ab+St+Fp) as well as the Ab+Fp and Ab individual treatments did not differ significantly from the inoculated and untreated control. The efficacy of potassium phosphite in terms of a reduction in *P. brachyurus* numbers was confirmed by data obtained in the second experiment (Table 1). Populations of nematodes in the soil showed no treatment was statistically different from the control for both trials. However, in the first experiment *A. brasilense* and Stimulate® + potassium phosphite reduced nematode population density significantly when compared to potassium phosphite and Stimulate® applied individually.

In the second experiment, treatments that promoted significant reductions in the population density of nematodes in roots were Stimulate®, potassium phosphite, *A. brasilense* + potassium phosphate, potassium phosphate + Stimulate® and a combination of the three products (Table 1).

Maize in the uninoculated and untreated control resulted in the greatest plant height, being statistically equal in value to the treatment with *A. brasilense* and significantly higher than the other treatments. But in the second assessment, there was no significant difference between all the treatments and the uninoculated and untreated control; however, only treatments with *A. brasilense* promoted an increase in plant growth compared to those treated with *A. brasilense* + potassium phosphite (Table 1).

Soybean

In both evaluations, soybean treatment with Stimulate® significantly reduced the total population density of *P. brachyurus* compared to the inoculated and untreated control (Table 2). In the first experiment, potassium phosphate + Stimulate® and *A. brasilense* + potassium phosphite + Stimulate® treatments promoted a reduction when compared with *A. brasilense* and *A. brasilense* + potassium phosphate, but did not differ from the control. Differences between nematode populations in the soil were not significant; the total population density reflected directly values seen in the number of nematodes in the roots.

Significantly taller plants were observed for plants treated with Stimulate® compared to the uninoculated and untreated nematode control (Table 2). On the other hand, reduction in plant height was observed for treatments with potassium phosphite and *A. brasilense* + potassium phosphite. In the second evaluation, no statistical difference between treatments was seen.

Both for maize and soybean, plant height was lower in the second experiment, which coincided with the autumn, year period less favorable to the development of these plants (Tables 1 and 2).

DISCUSSION

In analyzing data obtained in the experiments, it was observed that *A. brasilense* in isolation was not effective in reducing the total population density of *P. brachyurus* in maize, compared to the control, but promoted a significant increase in plant height. According to Gauna (2007), although the reproduction of *Meloidogyne* species (root-knot nematodes) is high in plants treated with *A. brasilense*, the increase in the root system of a crop can compensate against multiplication of nematodes, making the plant more tolerant and resulting in higher crop yield. Seed treatment with *Azospirillum lipoferum* Beijerinck also promoted a significant increase in the development of mungbean [*Vigna radiata* L. (Wilczek)], reducing the adverse effects caused in the host by *Meloidogyne incognita* (Kofoid and White) Chitwood, compared to the control (Khan and Kounsar, 2000), and even revealed an increase in legume nodulation.

Potassium phosphite alone or combined with Stimulate® was effective in reducing the *P. brachyurus* population density in maize. In previous work, similar results were observed for other species of plant-parasitic nematodes (Oka *et al.*, 2007). The efficiency of potassium phosphite to control microorganisms is dependent on its direct microbial activity against phytopathogens (Guest and Grant, 1991). However, in this study, the product was applied to the plant shoots, while nematodes are root parasites, so in this case, the efficiency is possibly related to a second factor, i.e., the ability the phosphite has to stimulate self-defense in plants, such as the production of phytoalexins (Dercks and Creasy, 1989). This reinforces the hypothesis in the work done by Salgado *et al.* (2007) that potassium phosphite increases the hatching of *Meloidogyne exigua* Goeldi and did not cause the death of juveniles, i.e. did not directly affect the parasite. In addition, Oka *et al.* (2007) observed that potassium phosphite applied to the shoot was effective in controlling *H. avenae* and *M. marylandi* in wheat and oats. This result may be due to the ability of phosphite to translocate through the plant via the xylem and phloem (Quimette and Coffey, 1990).

Table 1. Number of *Pratylenchus brachyurus* per gram root (Pb/g of root), 100 cm^3 of soil (Pb soil) and total (total roots + soil) (Pb Total) and plant height of maize, 60 days after inoculation and subjected to different treatments.

Treatment	Pb root	Pb soil	Pb Total	Height (cm)
Experiment 1				
Uninoculated, untreated[1]				66.83 a
Inoculated, untreated[2]	1,310.12 a	208.00 abc	21,099.67 a	60.33 bc
A. brasilense (Ab)	1,045.44 a	51.83 c	17,891.83 ab	65.00 ab
Stimulate® (St)	842.54 b	366.33 a	13,413.00 bc	59.33 bc
Potassium phosphite (Fp)	802.29 c	287.67 ab	12,381.00 c	57.67 c
Ab+St	641.53 c	192.67 abc	10,234.33 c	57.67 c
Ab+Fp	996.69 ab	199.17 abc	15,742.50 ab	57.42 c
Fp+St	735.86 c	45.00 c	11,341.67 c	57.92 c
Ab+St+Fp	977.27 ab	163.83 bc	15,077.17 ab	57.08 c
CV (%)	14.8	15.2	14.2	12.3
Experiment 2				
Uninoculated, untreated[1]				45.00 ab
Inoculated, untreated[2]	173.53 a	770.00^{ns}	2,912.50 a	46.35 ab
A. brasilense (Ab)	120.05 ab	1,068.00	2,248.00 ab	49.00 a
Stimulate® (St)	99.00 b	1,175.75	2,319.75 ab	44.33 ab
Potassium phosphite (Fp)	97.48 b	948.50	1,233.75 b	44.40 ab
Ab+St	188.12 a	918.00	2,569.75 ab	41.80 ab
Ab+Fp	109.22 b	1,600.75	2,940.25 a	40.50 b
Fp+St	102.04 b	952.00	2,718.25 a	45.00 ab
Ab+St+Fp	39.62 c	1,016.00	1,293.33 b	46.38 ab
CV (%)	19.8	14.9	18.9	12.1

Within the same experiment, means followed by the same letter in columns do not differ by the Duncan test at 5% probability

[1]Uninoculated and untreated control

[2]Inoculated and untreated control

CV= coefficient of variation

In general, the *A. brasilense* alone or in combination, was not effective in controlling *P. brachyurus*. Similar results were observed by Siddiqi (2004), evaluating the control of *M. incognita* with different bacteria promoting plant growth, in which it was noted that *A. brasilense* showed lower efficiency in controlling these parasites in tomato, especially when compared with *Pseudomonas fluorescens,* in isolation or combined with organic matter obtained from different sources. When associated with symbiotic bacteria, *A. brasilense* was less efficient than other bacteria at promoting plant growth, both in the control of *Meloidogyne javanica* (Treub) Chitwood and growth of chick-peas (Siddiqui and Mahmood, 2001). *Azospirillum brasilense* has shown more affinity for grasses (Steenhoudt and Vanderleyden, 2000), increasing the absorption of several minerals, improving plant growth and, even causing change in the cell arrangement in the superficial layers of the cortex in maize (Lin *et al.*, 1983). Babu *et al.* (1998) observed that the treatment of maize with *Azospirillum* sp., mycorrhizal fungi, and phosphate-solubilizing bacteria promoted a reduction of *P. zeae* and increased plant productivity.

The fact that potassium phosphite was not effective in reducing *P. brachyurus* populations in soybeans, may be due to a different response that plants exhibit when exposed to the product, as treatment with potassium phosphite was also not effective in controlling *M. incognita* on sugar cane, compared to the control (Assunção *et al.*, 2010).

For the soybean crop, Stimulate® treatment showed the best results both for nematode control and in

Table 2. Number of *Pratylenchus brachyurus* (Pb) per gram root (Pb/g of root), 100 cm^3 of soil (Pb soil) and total (total roots + soil) (Pb Total) and height of soybean plants 60 days after the inoculation and subjected to different treatments.

Treatment	Pb root	Pb soil	Pb Total	Height (cm)
Experiment 1				
Uninoculated, untreated[1]				31.00 cd
Inoculated, untreated[2]	480.80 ab	79.20^{ns}	2,422.20 ab	37.00 ab
A. brasilense (Ab)	603.77 a	72.67	3,931.00 a	38.58 ab
Stimulate® (St)	110.74 c	77.50	801.50 c	39.41 a
Potassium phosphite (Fp)	455.32 ab	62.67	2,052.67 bc	27.33 d
Ab+St	495.69 ab	72.67	3,194.00 ab	39.67 a
Ab+Fp	676.36 a	66.60	3,400.60 ab	29.92 d
Fp+St	398.42 b	85.40	2,265.40 abc	30.42 cd
Ab+St+Fp	369.98 b	9.67	1,964.67 bc	32.55 bcd
CV (%)	18.4	13.8	16.9	9.6
Experiment 2				
Uninoculated, untreated[1]				17.88^{ns}
Inoculated, untreated[2]	941.94 ab	612.00 ab	2,924.50 ab	19.50
A. brasilense (Ab)	704.55 abc	476.80 ab	2,187.80 ab	19.42
Stimulate® (St)	372.45 c	337.50 b	1,230.00 c	19.10
Potassium phosphite (Fp)	920.51 ab	569.75 ab	1,834.75 bc	20.37
Ab+St	459.78 c	433.20 ab	1,913.20 bc	18.50
Ab+Fp	1,393.56 a	251.00 b	3,616.00 a	19.00
Fp+St	544.66 bc	222.20 b	2,595.53 ab	20.30
Ab+St+Fp	1,611.02 a	1,035.80 a	4,450.80 a	18.10
CV (%)	19.8	14.9	18.9	12.1

Within the same experiment, means followed by the same letter in columns do not differ by the Duncan test at 5% probability
[1]Uninoculated and untreated control
[2]Inoculated and untreated control
CV= coefficient of variation

stimulating plant growth. This product is classified as a phyto-stimulante which contains phyto-regulators and trace minerals (Castro *et al.*, 1998). According to these authors the product increases plant growth and development, stimulating cell division, cell differentiation and elongation, and may increase absorption and improved use of nutrients.

In conclusion, plants treated with potassium phosphite resulted in the largest reduction in total *P. brachyurus* population density in maize, while in soybeans the best results were obtained from seed treatment with Stimulate®.

ACKNOWLEDGEMENTS

We would like to thank the program "PIBIC-CNPq/Fundação Araucária/UEM" for the financial support of scientific initiation of the second author.

LITERATURE CITED

Assunção, A., L. C. Santos, M. R. Rocha, A. J. S. Reis, R. A. Teixeira, and F. S. O. Lima. 2010. Efeito de indutores de resistência sobre *Meloidogyne incognita* em cana-de-açúcar (*Saccharum* spp.). Nematologia Brasileira 34:56-62.

Babu, R. S., C. Sankaranarayanan, and G. Jothi. 1998. Management of *Pratylenchus zeae* on maize by biofertilizers and VAM. Indian Journal of Nematology 28:77-80.

Castro, P. R. C., and E. L. Vieira. 2001. Aplicações de Reguladores Vegetais na Agricultura Tropical. Guaíba: Agropecuária, 132 p.

Castro, P. R. C., A. C. Pacheco, and C. L. Medina. 1998. Efeitos de stimulate e de micro-citros no desetnvolvimento vegetativo e na produtividade da laranjeira pêra (*Citrus sinensis* L. Osbeck). Scientia Agricola 55:338-341.

Cavallet, L. E., A. C. S. Pessoa, J. J. Helmich, P. R. Helmich, and C. F. Ost. 2000. Produtividade do milho em resposta à aplicação de nitrogênio e inoculação das sementes com *Azospirillum* spp. Revista Brasileira de Engenharia Agrícola e Ambiental 4:129-132.

Cohen, M. D., and M. D. Coffey. 1986. Systemic fungicides and the control of Oomycetes. Annual Review of Phytopatology 24:311-338.

Conab. Companhia Nacional de Abastecimento. 2010. Acompanhamento de safra brasileira de grãos 2010/2011. Primeiro levantamento, outubro 2010. Brasília: Conab. 44 p.

Coolen, W. A., and C. J. D'Herde. 1972. A method for the quantitative extraction of nematodes from plant tissue. State Agriculture Research Center: GHENT, Belgium.

Derks, W., and L. L. Creasy. 1989. Influence of fosetyl-Al on phytoalexin accumulation in the *Plasmopara viticola* grapevine interaction. Physiological and Molecular Plant Pathology 34:203-213.

Gauna, P. 2007. Alternativas biológicas para el control de *Meloidogyne* sp. en pimiento deinvernadero. Nematropica 37:157-158.

Guest, D., and B. Grant. 1991. The complex action of phosphonates as antifungal agents. Biological Review 66:159-187.

Inomoto, M. M. 2010. Nematoides da Soja. Boletim Passarela da Soja. Luís Eduardo Magalhães, BA, 2:11.

Jackson, T. J. 2000. Action of the fungicide phosphite on *Eucaliptus marginata* inoculated with *Phytophthora cinnamomi*. Plant Pathology 49:147-154.

Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disease Report 48:692.

Khan, M. R., and K. Kounsar. 2000. Effect of seed treatment with certain bacteria and fungi on the growth of mungbean and reproduction of *Meloidogyne incognita*. Nematologia Mediterranea 28:221-226.

Lin, W., Y. Okon, and R. W. F. Hardy. 1983. Enhanced mineral uptake by *Zea mays* and Sorghum bicolor roots inoculated with *Azospirillum brasilense*. Applied and Environmental Microbiology 45:1775-1779.

Obici, L. V., C. R. Dias-Arieira, E. S. Klosowski, L. F. Fontana, T. P. L. Cunha, S. M. Santana, and F. Biela. 2011. Effect of leguminous plants on *Pratylenchus zeae* and *Helicotylenchus dihystera* in naturally infested soils. Nematropica 41:215-222.

Oka, Y., N. Tkachi, and M. Mor. 2007. Phosphite inhibits development of the nematode *Heterodera avenae* and *Meloidogyne marylandi* in cereals. Nematology 97:396-404.

Quimette, D. G., and M. D. Coffey. 1990. Symplastic entry and phloem translocation of phosphonate. Pesticides Biochemistry and Physiology 38:18-25.

Ribeiro, N. R. 2008. Nematoides e sua implicação na agricultura *in* 2º Ciclo de Palestras da Aprosmat, Sorriso, Campo Novo do Parecis, Rondonópolis e Canarana, 8 a 13/9/2008.

Salgado, S. M. L., M. L. V. Resende, and V. P. Campos. 2007. Efeito de indutores de resistência sobre *Meloidogyne exigua* do cafeeiro. Ciência e Agrotecnologia 31:1007-1013.

Severino, J. J., C. R. Dias-Arieira, and D. J. Tessmann. 2010. Nematodes associated with sugarcane in sandy soils in Paraná, Brazil. Nematropica 40:111-119.

Siddiqui, Z. A. 2004. Effects of plant growth promoting bacteria and composed organic fertilizers on the reproduction of *Meloidogyne incognita* and tomato growth. Bioresource Technology 95:223-227.

Siddiqui, Z. A., and I. Mahmood. 2001. Effects of rhizobacteria and root symbionts on the reproduction of *Meloidogyne javanica* and growth of chickpea. Bioresource Technology 79:41-45.

Steenhoudt, O., and J. Vanderleyden. 2000. *Azospirillum*, a free-living nitrogen-fixing bacterium closely associated with grasses: genetic, biochemical and ecological aspects. Microbiology Review 24:487-506.

Received: / *Recibido:* *28/II/2012*

Accepted for publication: / *Aceptado para publicación:* *1/V/2012*