# LIQUID SWINE MANURE FOR THE CONTROL OF *MELOIDOGYNE JAVANICA* (TREUB) CHITWOOD

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## ABSTRACT

Heck, D. W., I. Santos, R. Dallemole-Giaretta, and E. A. Lopes. 2014. Liquid swine manure for the control of *Meloidogyne javanica* (Treub) Chitwood. Nematropica 44:93-100.

Liquid swine manure (LSM) is produced on a large scale in swine production and can be useful for the control of plant diseases. The effect of incorporation of LSM (0, 5, 10, 15, or 20%; v:v) into the soil for the control of *Meloidogyne javanica* on tomato plants was studied at soil pH levels of 5.0, 5.9, and 7.5 under greenhouse conditions. In a separate experiment, the interaction of three pH levels and five doses of LSM, with or without biofumigation (covering amended soil with plastic for seven days), was investigated. Increasing doses of LSM up to 20% increased shoot mass of tomato at all three pH levels. Amending the soil with 15.84% and 18.17% of LSM resulted in the lowest number of galls and eggs on tomato roots at soil pH 5.0. At soil pH of 5.9 and 7.5, the greatest reduction in gall and egg numbers varied from 5.26 to 5.55% and 3.85 to 8.33% of LSM amendment. There was a significant interaction for soil pH levels × LSM doses on the number of galls. A low level of galling occurred on plants from soil amended with LSM at doses of 17.22% (pH 5.0), 16.85% (pH 5.9), and 16.20% (pH 7.5). Increasing levels of LSM reduced the number of eggs in non-biofumigated plots at all pH levels. Soil amendment with 20% of LSM reduced the number of eggs of the nematode by more than 80%, and maximum suppression of nematode reproduction was observed in soils amended with 19.78% (pH 5.0), 16.08% (pH 5.9), and 15.33% (pH 7.5) of LSM. Soil microbial activity increased linearly in both biofumigated and non-biofumigated treatments with increasing levels of LSM at all pH levels. This study provides evidence that soil amendment with 12 to 14% of soil volume with liquid swine manure can reduce the population of *M. javanica* and improve tomato plant growth.

*Key words:* alternative control, ammonia, biofumigation, organic residue, pH levels, root-knot nematode.

## RESUMEN

Heck, D. W., I. Santos, R. Dallemole-Giaretta, and E. A. Lopes. 2014. Utilização do chorume líquido de suinos no controle de *Meloidogyne javanica* (Treub) Chitwood. Nematropica 44:93-100.

O chorume líquido de suínos (CLS) é produzido em larga escala na suinocultura e pode ser útil para o controle de doenças de plantas. O efeito da incorporação de CLS (0, 5, 10, 15, ou 20%; v:v) ao solo para o controle de *Meloidogyne javanica* em tomateiro foi estudado em solos com níveis de pH de 5,0; 5,9 e 7,5 em condições de casa de vegetação. Em um experimento separado, a interação dos três níveis de pH, cinco doses de CLS e com ou sem biofumigação (cobrindo o solo com plástico por sete dias). Doses crescentes de CLS até 20% aumentaram a massa da parte aérea de tomateiro em todos os três níveis de pH. A incorporação ao solo das doses 15,84% e 18,17% de CLS resultaram nos menores números de galhas e ovos nas raízes de tomate em pH do solo igual a 5,0. Nos solos com níveis de pH de 5,9 e 7,5, as maiores reduções no número de galhas e de ovos variaram com a incorporação de CLS nas doses entre 5,26 a 5,55% e 3,85 a 8,33%, respectivamente. Houve interação significativa entre os níveis de pH do solo x doses de CLS em relação ao número de galhas. O número mínimo de galhas nas plantas foi formado em solo tratado com doses de 17,22% (pH 5,0), 16,85% (pH 5,9) e 16,20% (pH 7,5). Doses crescentes de CLS reduziram o número de ovos do nematoide em solos não biofumigados em todos os níveis de pH. A incorporação de 20% de CLS ao solo reduziu mais de 80% o número de ovos do nematoide, com redução máxima em solos contendo 19,78% (pH 5,0); 16,08% (pH 5,9) e 15,33% (pH 7,5) de CLS. A atividade microbiana do solo aumentou linearmente com a aplicação de doses crescentes de CLS em solos biofumigados e não biofumigados em todos os níveis de pH do solo. Este estudo apresenta evidências que a incorporação com doses de 12 a 14% de chorume líquido de suínos ao solo pode reduzir a população de *M. javanica* e melhorar o crescimento do tomateiro.

*Palabras clave:* controle alternativo, amônia, biofumigação, resíduo orgânico, os níveis de pH, nematóide das galhas.

## INTRODUCTION

Liquid swine manure (LSM) is an organic residue, produced on a large scale in swine production and is mainly used as an organic fertilizer although there have been reports of using LSM for the control of plant diseases (Conn *et al.*, 2005; Assmann *et al.*, 2007; Morales *et al.*, 2007). Suppression of soil-borne pathogens with LSM involves multiple modes of action. When applied to the soil, LSM undergoes several alterations during its degradation, releasing different compounds as a function of the soil pH. In alkaline soils, there is greater release of ammonia, and in acidic soils, there is a release of nitrous acid and volatile fatty acids, which is responsible for the control of soil-borne plant pathogens (Conn and Lazarovits, 2000; Tenuta *et al.*, 2002; Conn *et al.*, 2005). Liquid swine manure improves the physical and chemical characteristics of the soil, alters its pH and electrical conductivity, and increases microbial activity, also contributing to control the plant-parasitic organisms (Tenuta *et al*., 2002; Santos *et al*., 2009).

Despite the great potential of LSM for the sustainable management of plant diseases, there are few studies on the control of phytonematodes (Mahran *et al.*, 2008), especially regarding the genus *Meloidogyne* Goeldi. Therefore, in the present study we evaluated the influence of different doses of LSM in soil with different pH levels for the control of *Meloidogyne javanica* on tomato.

## MATERIALS AND METHODS

Four experiments were carried out under greenhouse conditions at the Universidade Tecnológica Federal do Paraná, Campus Pato Branco, Paraná, Brazil. A mixture of soil and sand (1:1, v:v) was used in the experiments. The soil was obtained from a managed field subjected to no tillage for 15 yr, classified as typic Dystrophic Red Latosol (Bhering *et al.*, 2008), containing 75.0% of clay, 23.6 of silt, and 1.4 of sand, with a pH of 5.13. Liquid swine manure was obtained from a finishing pig farm, located in the municipality of Itapejara d'Oeste, PR, Brazil. Chemical analysis of the mixture of soil and sand (Table 1), hereafter referred as soil, and LSM (Table 2) used in the present study were performed according to methodology described by Tedesco *et al*. (1995) and Pavan and Miyazawa (1996), respectively.

In order to obtain different predetermined pH levels (pH 5.0 – experiment I; pH 5.9 - experiment II; pH 7.5 – experiment III), lime was incorporated into the soil substrate, corresponding to doses of 0.0, 12.0 and 45.0 tons per ha, respectively. Soil samples were analyzed in order to check and confirm the pH after liming.

In the experiments I to III, 4,000 eggs of *M. javanica* were added to 0.5 kg of the soil in 0.7 kg-plastic pots. Then, LSM (sample I) was incorporated to the soil at concentrations of 0, 5, 10, 15, or 20% (v:v). Nematode inoculum was composed of eggs collected from the roots of tomato plants kept in pots in a greenhouse, and extracted by the technique of Hussey and Barker (1973), modified by Bonetti and Ferraz (1981). One seedling of the tomato Santa Cruz 'Kada' (10.0 cm tall) was transplanted to each pot 1 wk after soil infestation. Tomato biomass and the numbers of galls and eggs per gram of tomato root were evaluated 45 d after seedling transplanting. Nematode reproduction and plant damage were measured by

Table 1. Chemical characteristics of the soil (a mixture of sand and Dystrophic Red Latosol, 1:1, v:v) before initiating the experiments.

| pH | O.M[x] | $Al^{3+}$ | Al | Ca | Mg | K | P | Cu | Zn | V[y] | SMP[z] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $CaCl_2$ | g dm$^3$ | ------------ Cmolg$^{(+)}$ dm$^3$ ------------ | | | | | ------ mg dm$^3$ ------ | | | % | |
| 5.13 | 58 | 0.56 | 0.75 | 3.73 | 1.37 | 0.37 | 8.68 | 1.00 | 3.50 | 35.66 | 6.3 |
| Sand (%) | | | | Clay (%) | | | | Silt (%) | | | |
| 50.1 | | | | 37.5 | | | | 12.4 | | | |

[x]O.M.= Organic material.
[y]V(%) = Base saturation.
[z]SMP = Shoemaker, McLean, and Pratt (1961)

Table 2. Chemical characteristics of the liquid swine manure (LSM) used in the experiments.

| Liquid Swine Manure | N | P | K | Ca | Mg |
|---|---|---|---|---|---|
| | -------------------- % -------------------- | | | | |
| Sample I | 3.26 | 0.46 | 0.25 | 1.05 | 0.31 |
| Sample II | 3.76 | 0.61 | 0.34 | 1.48 | 0.37 |

counting the number of galls and eggs per whole root system, expressed as number of galls or eggs per gram of root. Nematode eggs were extracted from tomato roots according to Hussey and Barker (1973) and were counted under a dissecting microscope at 40× magnification. The experiments were carried out under greenhouse conditions and performed in completely randomized design with seven replications per treatment.

In experiment IV, the same soil substrates and doses of LSM (sample II) were used. However, this experiment was performed in completely randomized design with a 2 × 3 × 5 factorial arrangement (with and without biofumigation × different substrate pH levels × LSM doses) with seven replications. After soil infestation with the nematode and incorporation of LSM to the soil, half of the pots were hermetically sealed in 3-kg capacity plastic bags and then stored on a bench of a greenhouse at 25ºC ± 2ºC for 7 d. The sealed treatments were considered the biofumigation treatment, while those unsealed were considered to be without biofumigation. Treatments without biofumigation were also maintained in the same environment but without the plastic bags. After 7 d, the plastic bags were removed from the pots with biofumigation, and all the pots were taken to the greenhouse, where one seedling of the tomato Santa Cruz 'Kada' was transplanted per pot.

The experiment was carried out for 55 d and the same variables were evaluated as in the previous tests. In this experiment, the microbial activity was estimated according to the methodology described by Grisi (1978), based on the absorption of $CO_2$ released from 100 g of soil into a sealed headspace chamber for 15 d using KOH 0.5 N. Maximum and minimum temperatures were recorded during the period of the experiments. The average temperatures in the experiments I, II, III, and IV were 28.6°C, 27.8°C, 23.7°C and 26.9°C. Data were subjected to analysis of variance and, when significant, to regression analysis at 5% ($P < 0.05$) probability of the type I error by the F-test, using the statistical software GENES version 5.1 (Cruz, 2013).

## RESULTS

Increasing doses of LSM up to 20% increased shoot mass of tomato at all three pH levels. Soil amendment with 14.3% of LSM resulted in maximum root mass of tomato plants at soil pH of 5.0 (Fig. 1).

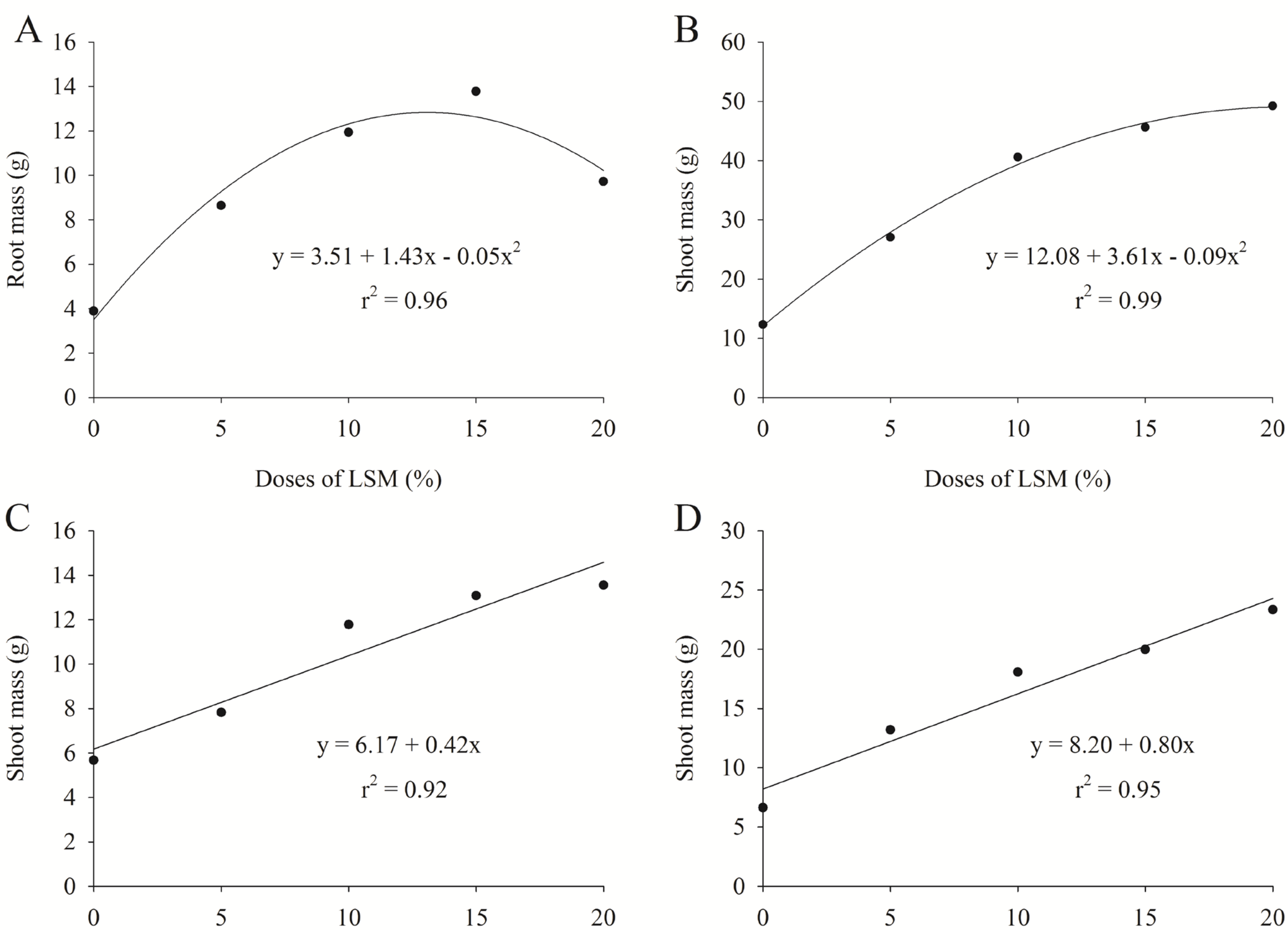


Fig. 1. Tomato root and shoot mass grown in soils amended with different doses of liquid swine manure (LSM) at soil pH levels of 5.0 (A and B), 5.9 (C) and 7.5 (D). Pato Branco, PR, Brazil, 2011.

However, root mass was not influenced by different doses of LSM at soil pH of 5.9 and 7.5 (data not shown).

The relationship between doses of LSM and nematode galling severity and reproduction was best described by quadratic (soil pH 5.0) or exponential models (soil pH 5.9 and 7.5) (Fig. 2). Amending the soil with 15.84% and 18.17% of LSM resulted in the greatest reduction in the number of galls and eggs on tomato roots, respectively, when soil pH was 5.0. On the other hand, critical doses for suppressing galling and reproduction at soil pH of 5.9 and 7.5 were much lower than at soil pH of 5.0, range of 5.26 to 5.55% and 3.85 to 8.33%, respectively (Fig. 2).

Root mass was influenced by the interaction soil pH × LSM doses (Fig. 3A) and between biofumigation × LSM doses (Fig. 3B). Maximum root mass was observed with soil amendment with 16.67% (pH 5.0) and 12.17% (pH 5.9) (Fig. 3A). The maximum root mass was recorded in plants cultivated in soil

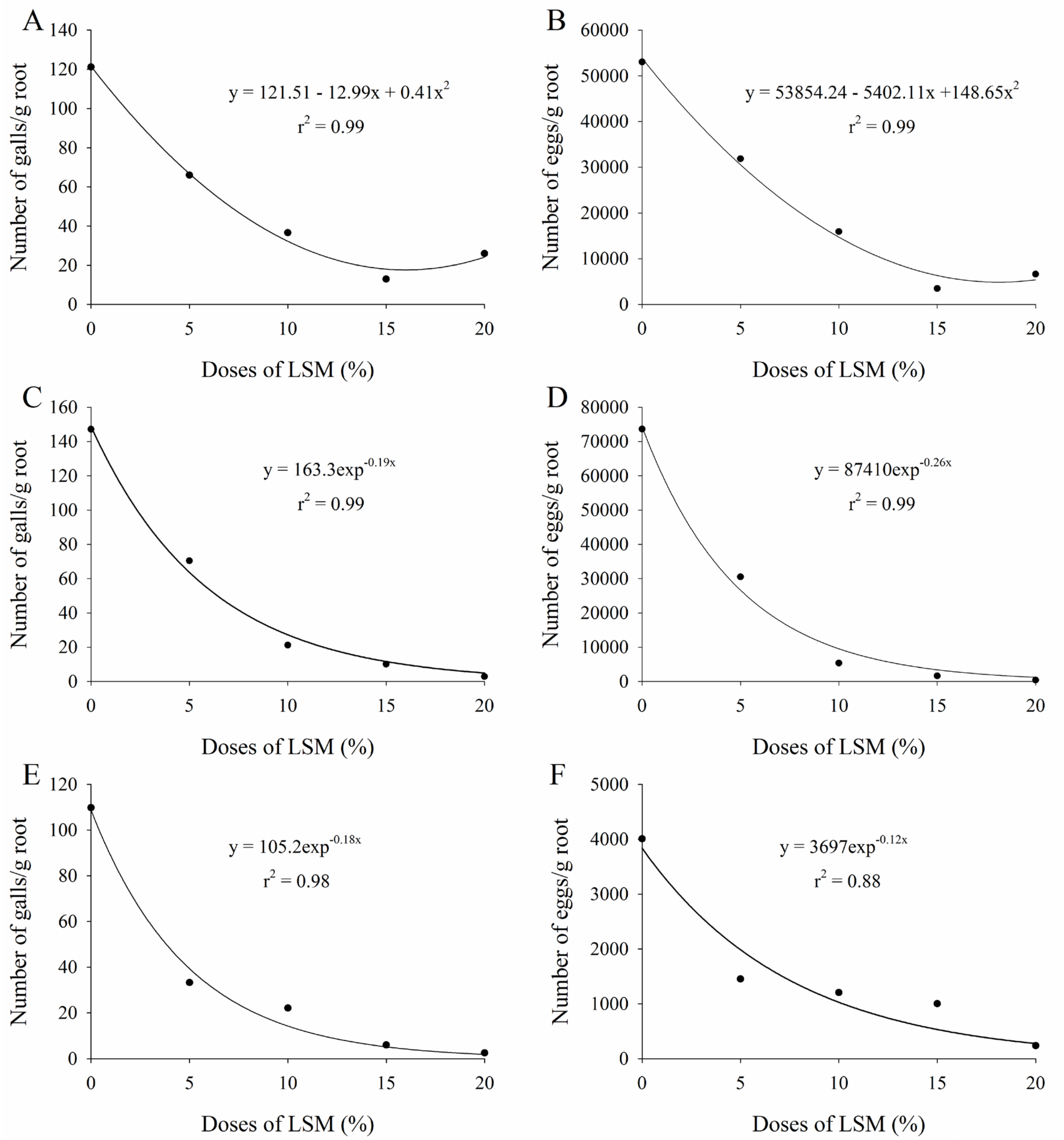


Fig. 2. Number of galls and eggs per g of roots of tomato grown in soils amended with different doses of liquid swine manure (LSM) at soil pH levels of 5.0 (A and B), 5.9 (C and D), and 7.5 (E and F). Pato Branco, PR, Brazil, 2011.

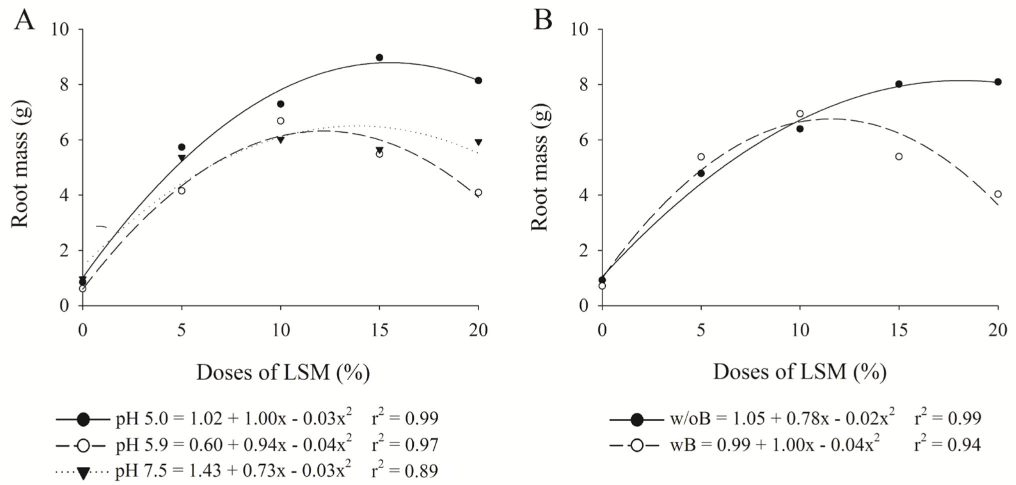


Fig. 3. Root mass of tomato plants grown in soils amended with different doses of liquid swine manure (LSM) at soil pH levels of 5.0, 5.9, and 7.5 (A) with (wB) or without biofumigation (w/oB) (B). Pato Branco, PR, Brazil, 2011.

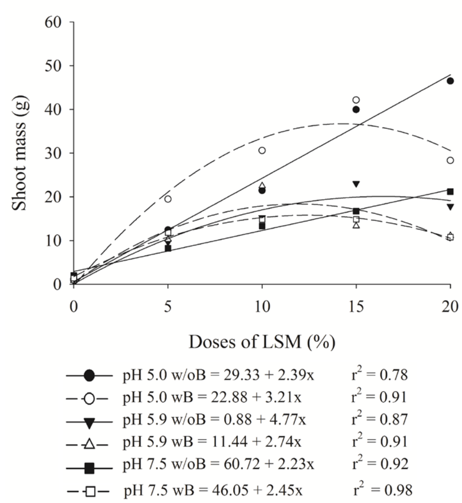


Fig. 4. Shoot mass of tomato plants cv. Kada grown in soils amended with different doses of liquid swine manure (LSM) at three soil pH levels (5.0, 5.9, and 7.5) with (wB) or without biofumigation (w/oB). Pato Branco, PR, Brazil, 2011.

amended with 12.38% and 19.5% of LSM, with or without biofumigation, respectively (Fig. 3B).

There was significant interaction for biofumigation × pH levels × LSM doses for shoot mass of tomato plants. In treatments without biofumigation, shoot mass was increased at increasing doses of LSM up to 17.4% (pH 5.9) and 20% (pH 5.0 and pH 7.5). Soil amendment with LSM at rates of 14.53% (pH 5.0), 11.69% (pH 5.9), and 12.61% (pH 7.5) resulted in maximum shoot mass of plants grown in biofumigated soils (Fig. 4).

There was significant interaction between soil pH levels and LSM doses for the number of galls. A minimum number of galls was formed on plants from amended soil with LSM doses of 17.22% (pH 5.0), 16.85% (pH 5.9), and 16.20% (pH 7.5). Reductions in the number of galls were higher than 85% at all pH levels (Fig. 5A).

There was a significant interaction for biofumigation × pH levels × LSM doses for the number of eggs of *M. javanica* (Fig. 5B). Increasing doses of LSM reduced the number of eggs of the nematode in non-biofumigated plots at all pH levels, according to linear models. Soil amendment with 20% of LSM reduced the number of eggs of the nematode by more than 80%. Quadratic models were the best fit for the relationship between LSM doses and number of eggs of the nematode in biofumigated soils at all three pH levels (Fig. 5B). Maximum reduction in nematode reproduction was observed in soils amended with 19.78% (pH 5.0), 16.08% (pH 5.9), and 15.33% (pH 7.5) of LSM. Soil microbial activity increased linearly in biofumigated and non-biofumigated plots by increasing doses of LSM at all soil pH levels (Fig. 6).

## DISCUSSION

This study provides evidence that soil amendment with LSM can reduce the population of *M. javanica* and improve tomato growth. In general, doses of LSM varying from 12 to 14% of soil volume reduced the number of galls and eggs on tomato roots without causing apparent phytotoxicity. Besides the potential of LSM for the management of *M. javanica*, this organic amendment can suppress other soil-borne pathogens by the production of toxic levels of ammonia, nitrous acid, and short-chain volatile fatty acids (Tenuta *et al.*, 2002; Conn *et al.*, 2005; Mahran *et al.*, 2008;

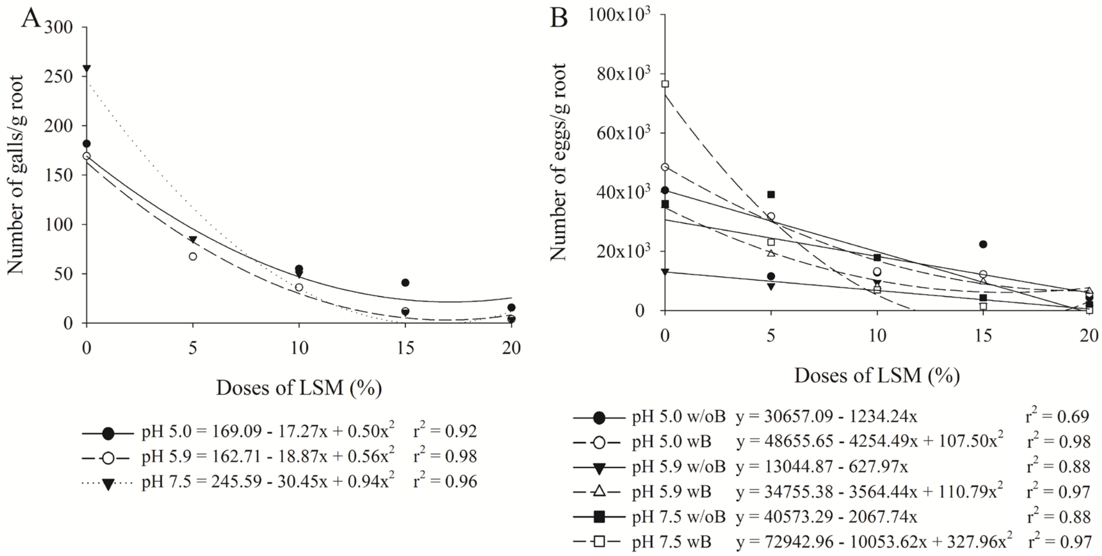


Fig. 5. Number of galls (A) and eggs (B) of *Meloidogyne javanica* per gram of tomato cv. Kada roots grown in soils amended with different doses of liquid swine manure (LSM) at three soil pH levels (5.0, 5.9, and 7.5) with (wB) or without biofumigation (w/oB). Pato Branco, PR, Brazil, 2011.

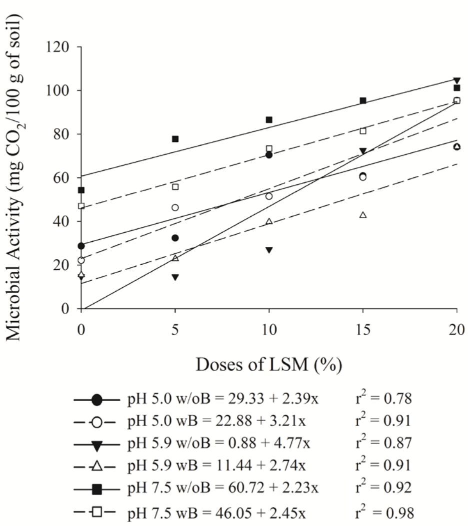


Fig. 6. Microbial activity in soils amended with different doses of liquid swine manure (LSM) at three soil pH levels (5.0, 5.9, and 7.5) with (wB) or without biofumigation (w/oB). Pato Branco, PR, Brazil, 2011.

Xiao *et al*., 2008). Doses higher than 12 to 14% can reduce nematode populations, but might also cause phytotoxicity, as indicated by decreased root or shoot weights at higher dosage levels. This phytotoxicity could be a result of the accumulation of ammonia and nitrates in the soil during the decomposition process (Mian and Rodríguez-Kábana, 1982; Oka, 2010). In practical terms, this effect can be eliminated by increasing the time between soil amendment and transplanting the seedlings and (or) by using lower concentrations (Oliveira *et al*., 2011). Taking into account the feasibility for field application, the dose of 14% represents field application rate of 280 tons per ha, assuming incorporation of the amendment to a depth of 20 cm, a rate that is very high for application to the entire field (Lopes *et al*., 2009). However, this could be viable for nematode control when applied to small areas on high-value crops or for disinfestations of artificial substrates (Lopes *et al*., 2009; Dallemole-Giaretta *et al*., 2010). The actual quantity needed could also be reduced if the amendment is incorporated less deeply and only the affected portion of the field is treated, a practice commonly used for nematode management (Lopes *et al*., 2009).

Ammonia is one of the most commonly released substances during the decomposition of N-rich organic amendments such as LSM in the soil and can control different plant-parasitic nematodes. The production of free ammonia ($NH_3$), which is toxic to nematodes, will depend on the pH of the soil. High levels of $NH_3$ are commonly found under alkaline conditions of the substrate (Rodríguez-Kábana, 1986). Doses of LSM required to reduce the number of galls and eggs of *M. javanica* at soil pH of 7.5 were lower than at pH of 5.0, implying that the production of nematicidal ammonia and (or) other volatile compounds during the decomposition of the organic amendment occurred (Tenuta and Lazarovits, 2002; Tenuta *et al*., 2002; Conn *et al*., 2005). Variability in our results may have been due to the difference in the composition of LSM samples that were used (Conn *et al*., 2007) and the soil temperatures among the experiments.

Biofumigation enhanced the reduction in the number of eggs of *M. javanica*, especially at soil pH of 7.5. The combination of incorporation of organic amendments and solarization (biofumigation) is an effective method for nematode control (Bello *et al*., 2004; López-Pérez *et al*., 2007; Anita, 2012) and it could be used effectively with lower doses of LSM. The plastic sheeting elevates soil temperatures as a result of the improved thermal conductivity in moist soil, exothermic microbial activity or a combination of both (Gamliel *et a*l., 2000). High temperatures accelerate the decomposition of the organic amendments and reduce the viability of the propagules of the nematode. Besides, ammonia and toxic nematicidal volatile compounds accumulate under the plastic mulch and, consequently, enhance the vulnerability of nematodes to soil heating (Gamliel *et al.*, 2000). However, the excess of ammonia in the soil can reduce plant development such as observed in soils with pH 7.5 and 5.9 (Fig. 4).

The application of LSM also had a positive effect on soil microbial activity in all treatments when compared to the control treatment (Fig. 6). Increased release of $CO_2$ was observed as increasing doses of LSM applied to the soil, particularly in treatments containing soil with pH 7.5 and the treatments without biofumigation, except when the soil (pH 5.9) was biofumigated with LSM doses greater than or equal to 10%. Organic materials, when incorporated to the soil, are sources of energy that permit increased diversity and microbial activity (Widmer *et al*., 2002). Although quantification and identification of the major groups of microorganisms present in each treatment were not performed in this study, it is possible that bacteria were the main microorganisms responsible for control of nematodes in treatments containing pH 7.5. This hypothesis is suggested because of the large relationship between the soil pH and microorganisms since prokaryotes grow better at neutral pH than fungi (Moreira and Siqueira, 2006).

It is possible that the effect of non-phytotoxic doses of liquid swine manure (12 to 14%) on the control of *M. javanica* is a result of the combination of the release of nematicidal compounds during decomposition of LSM (Rodríguez-Kábana, 1986), enhanced microbial activity (Widmer *et al*., 2002), and the improvement in the nutritional status of the plants (Stirling, 1991).

## ACKNOWLEDGMENTS

The authors wish to thank the National Council for Scientific and Technological Development (CNPq) for the scholarship.

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*Received:* *20/IX/2013* *Accepted for publication:* *06/V/2014*
*Recibido:* *Aceptado para publicación:*