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# Potential of Sucrose and *Pennisetum purpureum* cv. Cameroon Mulch on the Management of *Meloidogyne javanica* and *M. incognita*

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#### **ABSTRACT**

The effects of sucrose on motility and infectiveness of juveniles of <u>Meloidogyne javanica</u> were evaluated under laboratory and greenhouse conditions, using brown sugar and crystal sugar. Results showed that crystal and brown sugar solutions reduced motility and promoted morphological alterations of juvenile nematodes in vitro. The increasing dosages of sucrose reduced the number of galls and egg masses in tomato root by reducing the number of infective juveniles. The efficiency of granular sucrose, sucrose solution, with and without elephant-grass mulch, in the reduction of reproduction and final population of <u>M. incognita</u> race 2, were also evaluated. Granular sucrose induced decrease on the numbers of galls and eggs masses per root system as well as on juveniles in the soil. For sucrose solution assay, the best results for reproductive reduction were obtained with 300g of granular sucrose per liter of soil, applied at seven days intervals, associated with elephant-grass mulch.

Key words: Control, root knot nematode, sugar, elephant-grass

#### INTRODUCTION

The nematicides used in controlling nematodes present several inconveniences such as: high cost, toxicity to the environment and the microbial population (Thomason 1987; Noling and Becker, 1994), persistence in the soil and environmental pollution, besides representing risks to human health (Jatala, 1985). Control through genetic resistance is limited by the paucity of resistant cultivars in most economically important crops (Huang and Silva, 1980; Ferraz and Valle, 1995). Other alternatives, such as cultural practices, have been attempted. Crop rotation with antagonistic or

non-host plant species is one of the most widely used methods (Mauch, 1988; Mauch and Ferraz, 1996). *Pennisetum purpureum* Schum. cv. Cameroon (elephant grass) has demonstrated high potential in controlling phytonematodes and the use of its residues, as organic green manure, has been suggested due to the high amount of biomass produced (D'Angieri Filho and Ferraz, 1995a,b; Matsumoto et al., 2002).

Experimental results on this subject, however, are scarce. The use of non-toxic substances aiming at altering the osmotic potential of the soil solution, such as of sucrose, also widens a new field of investigation on nematodes control (Jenkins, 1964).

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Studies carried out with different organic additives, possessing the potential of increasing the populations of free-living nematodes in the soil as well as of fungi associated with nematodes capture, have demonstrated that sucrose also has reduce populations potential phytonematodes. Once better studied, farmers might use this technique with no harm to health and to the environment, but as an efficient plantprotecting agent against phytonematodes attack. Feder (1960), conducted studies in Florida in order to evaluate the influence of sugars on the populations of nematodes in the soil. This author observed a decrease in the nematodes population and concluded that the method could be a new option in controlling these pathogenic agents. He also found that under high infestation conditions, death of nematodes occurred in less than 24 h when 1 to 5% of sucrose or glucose, in relation to soil weight, were incorporated to the soil. Caveness and Jensen (1955) observed that nematodes maintained in a sugar solution for 10 -15 min., recovered shape and motility when washed under tap water. They did not survive, however, when maintained in the sugar solution for longer periods.

The effect of different concentrations of sucrose and other sugars on the control of nematodes has also been confirmed by Monteiro and Suzuki (1962). Falanghe and Dias Neto (1962) achieved 100% control of M. incognita on soybean (Glycine max L. Merr.) cv. Abura using molasses, and 89.5% control using crystal sugar applications. These authors stated that addition of sucrose to the soil efficiently reduced the population of nematodes. They suggested that the nematicide effect was due to alteration of the osmotic potential of the soil solution where the nematodes lived. Monteiro and Suzuki (1962) reported that molasses was a promising nematicide in nematode-infested soils, especially in plant nurseries, with the advantage of being non-toxic to man. However, Vawdrey and Stirling (1997) reported that the suppressive effect of the molasses upon the nematode probably occurred due to the antagonism of microorganisms that used it as substrate.

Feder (1960) added sugar solutions to the soil at concentrations between 30 and 40% of sucrose or 10% glucose and achieved 100% death of nematodes within one hour. The effect was considered due to the high osmotic potential of these solutions, which resulted in nematodes dehydration. The same effect was observed under

different environments (Feder at al., 1960).

The objectives of the present study were: 1) to evaluate the effect of aqueous solutions at different sucrose concentrations on motility and infectivity of M. javanica juveniles  $in\ vitro$ ; and 2) to evaluate, under greenhouse conditions, the efficiency of granular sucrose (non-dissolved crystal sugar) and of sucrose solutions (H<sub>2</sub>O dissolved crystal sugar) plus soil coverage with P.  $purpureum\ cv$ . Cameroon mulch on the reduction of reproduction and final population of M.  $incognita\ race\ 2$ , on tomatoes.

## **MATERIAL AND METHODS**

For the experiments carried out under greenhouse conditions, 1.5 L capacity clay pots containing a methyl bromide-treated (150 mL/m<sup>3</sup>) substrate composed of sand, clay soil and composted bovine manure (1:1:1 v/v) were used and cv. Rutgers tomato (Lycopericon esculentum Mill.) seedlings were transplanted. The tomato seedlings for this study were obtained from pre-selected seeds, which were pre-germinated at 28°C in moistened paper towels in a seed germinator and then transferred (1 plant/pot) to the pots after 15 days germination. Inoculum of M. javanica and M. incognita race 2, which were respectively multiplied on cv. Rutgers tomato and cv. California Wonder pepper plants, was provided by the Agronomical Institute of Paraná, IAPAR, Londrina, Paraná. Nematode eggs were extracted using the technique described by Hussey and Barker, modified by Bonetti and Ferraz (1981). The inoculum was quantified using a Peters counting chamber, under light microscope. Inoculation of seedlings was performed five days after transplantation by adding 5 mL of a suspension containing 5000 M. javanica and M. incognita eggs and/or juveniles into two 1 - 2 cm deep holes made in the soil around each seedling, using an automatic pipette.

# First experiment

The deleterious effect of sucrose solutions at increasing concentrations of brown sugar and crystal sugar on second stage *M. javanica* juveniles was evaluated under laboratory and greenhouse conditions. The treatments with brown sugar solutions and crystal sugar solutions were evaluated in two different assays.

# Brown sugar assay

The treatments evaluated in this experiment were: 0.0% (no-sugar = control), 6.66% (1.09 density), 10.0% (1.11 density), 13.3% (1.12 density), 16.6% (1.14 density) and 20.0% (1.15 density). Brown sugar solutions were obtained by dissolving sugar in distilled- $H_2O$ , followed by vacuum filtration through  $n^o$  1 Whatmann filter paper to eliminate impurities that would turn nematode evaluations under microscope troublesome. A completely randomized experimental design, with six treatments and five replications, was used.

# Crystal sugar assay

The treatments used were: 0.0% (no-sugar – control), 10.0% (1.10 density), 20.0% (1.12 density), 30.0% (1.14 density) and 40.0% (1,17 density). A completely randomized experimental design, with five treatments and five replications, was used.

## **Production of juveniles**

M. javanica eggs were extracted from cv. Rutgers tomato roots according to the methodology described by Bonetti and Ferraz (1981). In order to obtain second stage juveniles, the modified Baermann funnel method (Hooper, 1970) was used. Water suspensions of M. javanica eggs were poured onto paper tissue pads placed on top of plastic screens, which were then supported into H<sub>2</sub>O-filled plastic containers, where they remained for five days at 27°C, forming eclosion chambers. The suspensions containing the second stage juveniles were then poured through 500 mesh screens and recollected into 100 mL Becker flasks, with the aid of a pisseta. Juveniles were quantified by counting under light microscope and the nematode suspensions for inoculation were calibrated to 1100 juveniles/mL of water. A lidded 'eppendorf-type' plastic vial, with 1.5 mL capacity, constituted each replication of the assay. After adding 1 mL of each sucrose solution to each one of the vials, a 0.5 mL aliquot of nematode suspension, containing approximately 550 M. javanica second stage juveniles, was transferred to each one for a final volume of 1.5 mL. The nematodes remained immerse in the respective sucrose solutions for 24 h at room temperature. After that the nematodes were transferred to distilled water for 24 h before evaluation. This evaluation aimed at determining if the effect of the different sucrose concentrations on motility of nematode juveniles was permanent or temporary.

## In vitro evaluation

After remaining immersed in distilled water for 24 h, the proportion of immobilized nematodes in relation to the total juveniles was determined using a Peters chamber under light microscope. A linear regression analysis was performed with the differences among motility means data resulting from brown and crystal sugars treatments. Data for this analysis were transformed to arcsine p where p was the proportion of immobilized juveniles in relation to the total juveniles.

#### **Bio-test evaluation**

Infectivity and reproduction of the nematodes on tomato roots, after undergoing sucrose treatments, were also evaluated under greenhouse conditions. For that, the nematode juveniles from the first experiment were inoculated on 15-days old tomato seedlings grown in clay pots. Forty-five days after inoculation, the number of galls as well as the number of egg masses in each root system were evaluated. After removing, the roots were immersed in a floxine B solution (15 mg/L H<sub>2</sub>O), for 15 minutes. The same completely randomized experimental design with six treatments and five replications for brown sugar and five treatments and five replications for crystal sugar, used in the in vitro evaluation, was applied to this experiment. Data were submitted to linear regression analysis after  $\sqrt{x+0.5}$  transformation.

# **Second experiment**

# Granular sucrose assay (non-dissolved crystal sugar)

In the first assay, increasing dosages of granular sucrose per liter of soil (20, 40, 60, 80 and 100 g) were mixed to the substrate, before pot filling. To each pot, a tomato seedling was then transplanted and at the fifth days after this procedure, a 5 mL suspension containing 5000 *M. incognita* eggs and juveniles was added into two 2-cm deep small holes made around the seedlings. A completely randomized experimental design, with six treatments and five replications, was used.

# Sucrose solutions assay ( $H_2O$ -dissolved crystal sugar)

In the second assay, the soil of each pot was covered with a layer of *P. purpureum* green stems and leaves, immediately after transplanting the tomato seedling into the pot. Each pot received 300 g (fresh weight) of stems and leaves. Inoculation of

*M. incognita* was performed adding 5 mL of a suspension containing 5000 nematode eggs and juveniles/pot. After five days, the application of 50 mL dosages of sucrose solutions (100, 200 and 300 g of crystal sugar / L H<sub>2</sub>O) was started. The regimes of applications were: a) every two days during two weeks; and b) every seven days during three weeks. A randomized block experimental design with 13 treatments and five replications was used. The control was represented by cv. Rutgers tomato seedling inoculated with the nematode suspensions without addition of sucrose solutions.

At the 40<sup>th</sup> day after inoculation the evaluations performed were: 1) root systems fresh matter weight; 2) number of galls and egg masses per root system; 3) second stage juveniles in the soil; and 4) reproduction factor (RF). For the evaluations, the plants were removed from the pots under controlled water flow in order to maintain the integrity of the root systems. After washing under tap water for removal of organic debris, the root systems were placed on top of absorbing paper pads for 30 minutes to eliminate excess water. Fresh matter weight was then determined. For determination of number of galls and egg masses per root system, these were immersed in a Floxin B (15 mg/ L H<sub>2</sub>O) solution for 15 minutes. For eggs extraction as well as for RF determination, the roots were processed according to the technique described by Hussey and Barker, modified by Bonetti and Ferraz (1981). For determination of the number of juveniles in 100 mL soil samples, the method described by Jenkins (1964) was used and counting was performed using a Peters Chamber. Data were subjected to Anova and, when necessary, the Tukey test at 5% probability was used to compare means.

#### RESULTS AND DISCUSSION

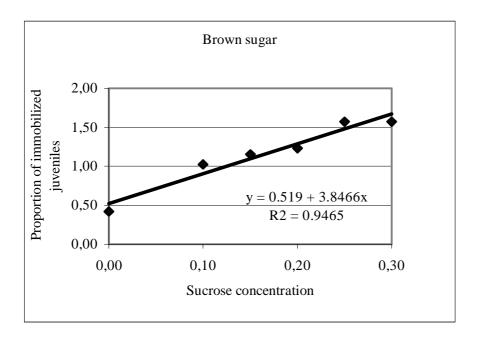
# First experiment

#### In vitro results

Increasing dosages of sucrose for both brown sugar and crystal sugar negatively affected motility of *M. javanica* juveniles.

# Brown sugar assay results

Regression analysis showed a direct correlation between increasing dosages of brown sugar (y=0.519+3.846x; x=arcsine $\sqrt{p}$ ) and the proportion of immobilized juveniles (Fig. 1). There was an accretion of 3.846 in the arcsine $\sqrt{p}$  as an effect of each point of percentage increase in the brown sugar concentration.

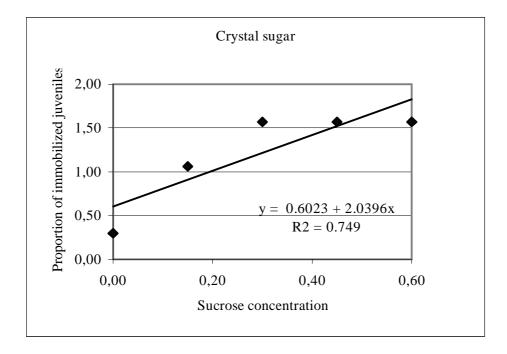


**Figure 1 -** Proportion of immobilized juveniles (data transformed to arcsine√p), exposed to sucrose concentrations (dissolution of brown sugar in water).

# Crystal sugar assay results

For crystal sugar, the arcsine $\sqrt{p}$  equation was y=0.6023+2.0396x and showed a direct correlation between increasing dosages of sucrose and arcsine  $\sqrt{p}$  (Fig. 2), where the increment of one percent unit of sucrose caused and increase of 2.0396 in

the arcsine  $\sqrt{p}$ . The total immobility was reached starting from the 20.0% sucrose concentration. From the 10.0 and 20.0% sucrose concentrations for brown sugar and crystal sugar, respectively, deformations in the cuticle of the juveniles were observed.



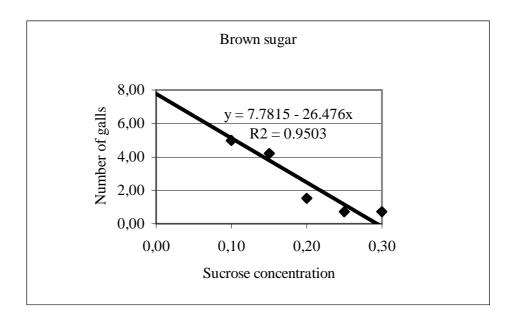
**Figure 2** - Proportion of immobilized juveniles (data transformed to arcsine√p), exposed to sucrose concentrations (dissolution of crystal sugar in water).

These results indicated that with the addition of sucrose to the water suspension containing M. javanica juveniles, an increase in the osmotic potential of the solution occurred, killing the nematodes by dehydration through the process of osmosis. According to Feder (1960), sugar exerted nematicide action due to the increase of the concentration in the solution where the nematodes lived which favored osmosis from inside out. Thus losing water, the nematodes died due to dehydration. Vawdrey and Stirling (1997), using sucrose at 1% concentration, did not observe any effect in the proportion of immobilized juveniles but reported that the use of molasses was efficient in immobilizing M. javanica juveniles. The effect antagonized microorganisms, which were present in the molasses, and had their population substantially increased.

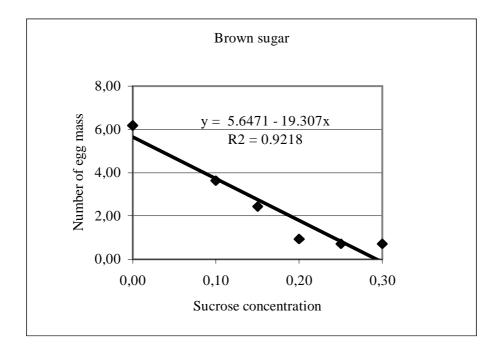
# **Bio-test results**

Increasing dosages of sucrose (crystal and/or brown sugar) significantly reduced the number of galls and egg masses produced by *M. javanica* in the tomato roots, negatively affected the number of infecting juveniles. Sucrose solutions starting from concentrations of 16.6% and 20.0% for brown sugar and crystal sugar, respectively had nematicide effect. Juveniles from these treatments did not recover their infectivity when inoculated on tomato plants. This effect was probably due to the increase of the osmotic potential induced by the addition of sucrose to the soil solution and not to antagonistic effect of soil microorganisms.

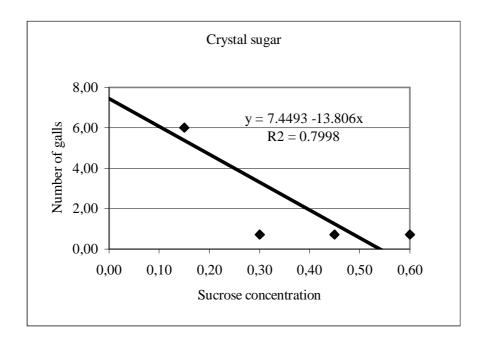
The regression equation obtained for number of galls in the brown sugar assay was y=7.7815–26.476x, showing an inverse correlation between increasing dosages of sucrose and number of galls (Fig. 3). A decrease of 26.476 galls for each percent increment in the sucrose dosage was observed.



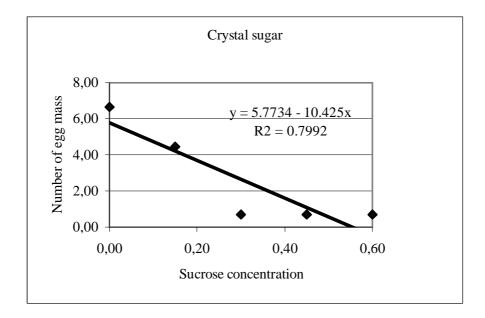
**Figure 3** - Effect of sucrose concentrations (brown sugar) on the number of galls (data transformed in  $\sqrt{x+0.5}$ ) formed by *M. javanica* in tomato roots, under greenhouse conditions.



**Figure 4** - Effect of sucrose concentrations (brown sugar) on the number of egg masses (data transformed in  $\sqrt{x+0.5}$ ) formed by *M. javanica* in tomato roots, under greenhouse conditions.



**Figure 5** - Effect of sucrose concentrations (crystal sugar) on the number of galls (data transformed in  $\sqrt{x+0.5}$ ) formed by *M. javanica* in tomato roots, under greenhouse conditions.



**Figure 6** - Effect of sucrose concentrations (crystal sugar) on the number of egg masses (data transformed in  $\sqrt{x + 0.5}$ ) formed by *M. javanica* in tomato roots, under greenhouse conditions.

For the number of egg masses, the regression equation was y=5.6471-19.307x (Fig. 4). A decrease of 19.307 egg masses for each increase in the percent unit of sucrose was observed for this

variable. In the crystal sugar assay the interpretation was similar. The regression equation for the number of galls was y=7.4493-13.806x (Fig. 5) and for the number of egg masses it was

#### 5.7734–10.425x (Fig. 6).

Concerning feasibility of practical use of sugars, Feder et al. (1960) achieved significant results working, under greenhouse conditions, with pots containing substrate artificially infested with different phytonematodes as well as under field conditions. Vawdrey and Stirling (1997) obtained significant reductions on the number of galls in the roots as well as in the rate of reproduction of M. *javanica* on tomatoes, through regular applications of solutions of 10 g molasses/L H<sub>2</sub>O to infested soils. Identical results were obtained in this study in relation to nematodes reproduction after being submitted to different dosages of sucrose. It was also observed in this study that the effect of brown and crystal sugars was permanent, since the juveniles were dead. This indicated that a nematicide effect and not a stimulating effect on competitor microorganisms occurred. Sucrose exerted an inhibitory effect on the nematodes independently of the presence of antagonistic microorganisms.

# **Second experiment**

# Granular sucrose assay (non-dissolved crystal sugar)

Best control was achieved with the dosage of 80 g of non-dissolved crystal sugar per pot. This values was confirmed by the smaller values for number of galls and egg masses per root system as well as by the smaller number of second stage juveniles in the 100 mL soil samples (Table 1). The remaining dosages did not statistically differed from the control, although presenting promising results. Increasing dosages of sucrose possibly induced a higher predisposition of the plants to nematodes attack.

Although inducing decrease in the number of galls and egg masses per root system and number of second stage juveniles in the soil, the crystal sugar treatment, even in the dosage that rendered the best result, was not able to eliminate the nematodes. Falanghe and Dias Neto (1962), incorporating dry crystal sugar to the soil to control *M. incognita* on soybean, obtained similar results and found that only 10.5% of the plants were attacked. Feder (1960) reported that nematodes died by dehydration due to the increase in the osmotic potential in the soil solution induced by the addition of sugars. In this study, one factor that might have contributed to less positive results was the delay in the dissolution of

the crystal sugar, which was not instantaneous, since the amount of water added to the pots was only sufficient to maintain field capacity in the soil.

# Sucrose solution assay (H<sub>2</sub>O-dissolved crystal sugar)

Decrease in the number of galls per root system, in relation to the control, was observed starting from the 100 g crystal sugar /L H<sub>2</sub>O dosage applied every two days intervals together with P. purpureum green stems and leaves applied immediately after inoculation. However, best results on reduction of reproduction were obtained with the 300 g dosage, applied at seven days intervals together with P. purpureum green stems and leaves (Table 2). According to Daykin and Izlussey (1958), the number of egg masses per root system was an appropriate measurement of nematodes reproduction, although the number of galls could also be used as good indicator of symptoms. Statistically significant differences in the number of egg masses were observed even at the lowest sucrose concentrations. The best result, however, was achieved with the 300 g dosage together with P. purpureum mulch for both application intervals. For second stage juveniles in the soil, all treatments significantly differed from the control and the lowest population of juveniles was observed at the 300 g dosage, applied at every seven days intervals, integrated with the P. purpureum mulch applied immediately after inoculation. All dosage of sucrose used affected development of the tomato plants root systems. For the reproduction factor mean values lower than 1 were observed. This result indicated the efficiency of treatments in reducing the nematode population in the soil (Table 2).

The highest reduction was observed at 300 g crystal sugar dosage, applied at both intervals with *P. purpureum* mulch. The positive results attained with the treatments in which *P. purpureum* green stems and leaves was added were probably due to the release of toxic compounds from those residues. That release might have amplified as a function of the presence of sucrose as well as higher microbial activity in the soil.

These results confirmed the nematicide effect of sugars as previously observed by Caveness and Jensen (1955), Feder (1960) and Feder et al. (1960) under greenhouse as well as under field conditions.

granular sucrose	root system		juveniles	root weight	RF
(g/L)	galls	egg masses	(100 mL soil)	(g)	( <b>Pf / Pi</b> )
0	$435.14^1 a^2$	360.24 a	897.60 a	8.21 a	24.69
20	328.70 b	321.13 ab	453.26 b	12.31 a	24.38
40	295.15 bc	299.29 abc	392.83 c	10.90 a	18.91
60	224.40 c	222.01 c	362.90 d	9.50 a	15.07
80	73.27 d	70.22 d	202.78 e	9.23 a	6.11
100	237.16 bc	244.92 bc	341.88 d	9.65 a	9.54
DMS	96.35	92.37	23.59	6.61	

**Table 1** - Means of the numbers of galls and egg masses per root system, second stage juveniles of *Meloidogyne incognita*, root weight and reproduction factor, under different concentrations of granular sucrose (crystal sugar).

1.34

9.17

**Table 2** - Means of the numbers of galls and of egg masses per root system, second stage juveniles of *Meloidogyne incognita*, root weight and reproduction factor, under different concentrations of sucrose solution (crystal sugar diluted in water), with and without elephant-grass mulch, under two application regimes.

sucrose	Elephant grass	Application regime	Root system		Juveniles	Root weight	RF
			galls	egg masses	(100 mL soil)	<b>(g)</b>	( <b>Pf / Pi</b> )
0			$533.15^1 a^2$	460.53 a	951.11 a	16.87 a	25.40
100	without	two	296.87 b	139.71 b	184.14 b	14.37 ab	8.45
100	without	seven	199.94 bc	67.90 c	89.49 de	13.51 ab	1.62
200	without	two	188.70 bcd	79.57 c	160.20 bc	16.61 ab	6.50
200	without	seven	54.17 cde	26.32 d	65.45 defg	8.18 b	0.95
300	without	two	135.49 cde	70.22 c	115.99 cd	11.92 ab	3.82
300	without	seven	52.42 de	14.75 d	45.16 efg	10.45 ab	0.40
100	with	two	60.22 cde	15.44 d	74.30 def	12.43 ab	0.38
100	with	seven	35.40 e	6.00 d	36.00 fg	9.97 ab	0.22
200	with	two	22.47 e	8.64 d	65.77 defg	12.06 ab	0.28
200	with	seven	9.42 e	2.76 d	31.25 fg	9.87 ab	0.12
300	with	two	8.41 e	3.57 d	41.99 efg	9.62 ab	0.12
300	with	seven	3.72 e	1.93 d	22.18 g	9.14 ab	0.08
DMS			147.39	38.55	51.24	8.48	
CV (%)			40.33	18.81	11.92	28.30	

<sup>&</sup>lt;sup>1</sup> Original data are averages of five repetitions. <sup>2</sup>Means followed by the same upper case letter, in the same column and the same lower case letter in the same row, did not differ at the 5% level of probability by the Tukey test.

These authors were also unanimous in attributing the death of nematodes to the loss of water by plasmalyses. However, Vawdrey and Stirling (1997) suggested that this suppressiveness of sugar to nematodes was not an osmotic effect, but was probably due to antagonism of nematodes by microorganisms that utilized it as a substrate, to example of fungi, bacterial and other nematodes. The effect of sugar on the free-living nematode community, which are important in cycling nutrients in the soil and improvement on agronomic crop performance, mould be less

CV (%)

9.11

#### injurious.

Results obtained as well as those obtained by D'Angieri Filho and Ferraz (1995a, 1995b) and Matsumoto et al. (2002) indicated that *P. purpureum* could be used in controlling *M. incognita* and *M. javanica*, respectively, either as an alternative crop plant in infested areas or as plant residues added to the soil as composted organic matter. The use of antagonistic plants in crop rotation systems aiming at nematodes control has been widely studied and has provided good results. On the other hand, the specific use of *P.* 

33.93

Original data are averages of five repetitions. <sup>2</sup>Means followed by the same upper case letter, in the same column and the same lower case letter in the same row, did not differ at the 5% level of probability by the Tukey test.

purpureum either as an antagonistic plant in crop rotation systems or as organic residue covering in the control of *Meloidogyne* sp. has not yet been fully studied.

#### **RESUMO**

Os efeitos da sacarose sobre a motilidade e a infectividade de juvenis de Meloidogyne javanica foram avaliados em condições de laboratório e casa-de-vegetação, usando açúcar mascavo e açúcar cristal. Os resultados indicaram que as soluções de açúcar mascavo e cristal reduziram a motilidade e promoveram alterações morfológicas em juvenis dos nematóides in vitro. A eficiência da sacarose em grânulos e da sacarose em solução, com e sem a cobertura com matéria vegetal de capim elefante, na redução da reprodução e da população final de M. incognita raça 2 também foi avaliada. A sacarose em grânulos diminuiu os números de galhas e massas de ovos por sistema radicular e também de juvenis no solo. Para o ensaio com sacarose em solução, os melhores resultados para a redução da reprodução foram obtidos com a dose de 300 g de sacarose por litro de solo, aplicada a intervalos de sete dias, juntamente com a cobertura do solo com cobertura de matéria verde de capim-elefante.

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