ALTERNATIVES TO CHEMICAL NEMATICIDES FOR THE CONTROL OF *MELOIDOGYNE INCOGNITA* INFESTING BEANS

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Summary. Studies were carried out to assess the effect of crystallized and brown sugars, elephant-grass cv. Cameroon, filter cake, sugarcane juice and *Pinus elliottii* sawdust for the control of *Meloidogyne incognita* infesting the bean (*Phaseolus vulgaris*) cv. Carioquinha. Observations were made on their effect on nematode natural enemies, such as yeasts, actinomycetes, sporulating bacteria and cellulolytic and phosphate solubilizing fungi. Filter cake (30,000 kg/10 m^2) and *P. elliottii* (10,000 kg/10 m^2) were the best treatments for the control of *M. incognita* population. The sawdust soil cover resulted in greater yield weight averages and in healthier root systems. The soil amendments influenced soil microflora and soil chemical properties in the field.

Chemical nematicides have been used to reduce or prevent losses caused by nematodes but their effectiveness can be impaired by adverse environmental effects (Thomason, 1987; Noling and Becker, 1994) and by microbial degradation, which renders them ineffective (Stirling *et al.*, 1992). These observations have resulted in renewed interest in assessing alternative methods for plant parasitic nematode management.

Several authors have reported the value of organic amendments for the control plant parasitic nematodes and improved crops yields (Sing and Sitaramaiah, 1973; Muller and Gooch, 1982; Novaretti and Nelli, 1985; Novaretti *et al.*, 1989; Jonathan *et al.*, 1991).

Filter cake, also known as filterpress or pressmud (a waste byproduct of sugarcane processing), rich in organic matter (70%) and other nutrients, are methods used by small farmers for nematode control in India (Sing and Sitaramaiah, 1973; Muller and Gooch, 1982).

Sawdust has also proved efficient in controlling nematodes in soils and plant roots of many important economic crops (Stirling, 1989; Stirling *et al.*, 1995). Vawdrey and Stirling (1997) also assessed the suppressive effect of applications of sawdust and filter cake on a *Meloidogyne javanica* population in tomato plants. The roots were practically free of galls and the soil nematode populations were lower in the treatment with sawdust spread on the soil surface.

The use of non-toxic substances to alter the osmotic potential of the soil solution opens a new field of investigations in nematode control. However, detailed assessments are needed for each condition before it can be used in efficient nematode control and management (Feder, 1960).

The objective of this study was to assess the effect of several soil amendments (1) crystallized sugar, (2) brown sugar, (3) napier grass, (4) filter cake, (5) sugar cane juice and (6) *Pinus elliottii* sawdust on *M. incognita* infesting bean, and their effect on nematode natural enemies, such as yeasts, actinomycetes, sporulating bacteria and cellulolytic and phosphate solubilizing fungi.

MATERIAL AND METHODS

The study was carried out under field conditions in a purple Latosol (EMBRAPA, 1984) containing 67% clay, 13% silt and 20% sand at Londrina, Paraná State, Brazil. The experimental area was previously cultivated with *Lolium multiflorum* Lam. and lupin (*Lupinus albus* L.) with a history of severe *M. incognita* (Kofoid *et* White) Chitw. infestation. The local climate is Cfa (sub-tropical) type (Köpen classification) with an average temperature of 30 °C, annual precipitation 1,645 mm, without a defined dry season and a relative humidity approximately 71% (Corrêa *et al.*, 1982).

Table IV shows the chemical properties of the soil before and after treatment application.

A randomized complete block design with eight treatments of five replicates was used. Each 4 m x 2.5 m (10 m^2) plot was formed by 5 four-meter rows of plants spaced 0.5 m apart.

An initial assessment of the root-knot nematode population in the soil (Jenkins, 1964) was made on 31 March, 1999 before application of the treatments. A second sampling was made on 15 June, 1999 to assess second stage juvenile (J_2) numbers and to analyze the soil microbiota. A third evaluation was made on 30 August, 1999 on soil samples and also on the roots of ten bean plants from each plot. The plants were analyzed for weight and number of galls on the roots, height and number of pods.

The following treatments were applied per hectare: 1) 5,000 kg of crystallized sugar; 2) 5,000 kg of crystallized sugar + 20,000 kg of incorporated napier grass (*Pennisetum purpureum* Schum.) cv. Cameroon; 3) 6,000 kg of brown sugar; 4) 20,000 kg of incorporated

napier grass; 5) 30,000 kg of incorporated filter cake; 6) 20,000 liters of sugar cane juice; 7) 10,000 kg of dry *Pinus elliottii* Engelm. sawdust; and, 8) untreated control.

Twenty days after the application of the treatments, 12 seeds/row (meter) of the bean (*Phaseolus vulgaris* L.) cv. Carioquinha, were sown with a hand-sowing device. Nematodes were quantified in soil samples collected at depths of 0-25 cm and 25-50 cm from ten different locations in each plot before and after sowing. Nematodes were extracted (Jenkins, 1964) from 100 ml of composted soil samples per plot. The root system was removed from the above ground portion, washed in running water to remove organic debris and placed on absorbent paper for 30 minutes to remove excess moisture. Fresh weight and number of galls per root system were determined. Plant height and number of pods per plant were also determined.

The microbiological assessment of the soil (yeasts, actinomycetes, sporulating bacteria and cellulolytic and phosphate solubilizing fungi) was made on five samples collected at the 0-25 cm depth from each plot. One kg of soil was collected per treatment to obtain each sample. The samples were sieved through 2 mm and 20 mesh screens before processing. After sieving, 10 g soil sub-samples were taken from each sample and placed in closed flasks, and 90 ml distilled water added. The subsamples were then centrifuged at 200 rpm for 15 minutes and submitted to serial dilution according to the microorganism group, 10^{-2} for yeasts, 10^{-3} for sporulating bacteria, 10^{-3} for actinomycetes and 10^{-1} for cellulolytic and 10^{-2} for phosphate solubilizing fungi. From each dilution a 0.1 ml aliquot was transferred to individual plates with the respective culture medium. Three replicates were used for each soil sample. Culture media were: a) YMA + C + T (yeast extract, malt extract and agar + Chloramphenicol + tetracycline) for yeasts; b) NA (nutrient agar) for sporulating bacteria; c) ACA (starch, casein and Agar) for actinomycetes; d) CAA (Cellulose, asparagine and agar) for cellulolytic fungi; and, e) GES (glucose, soil extract and organic salts) for phosphate solubilizing fungi (Valarini, 1998).

Tubes containing sporulating bacteria at 10^{-3} dilution were heat treated in a water bath at 85 °C for 15 minutes before plating. The heat treatment for actinomycetes was 50 °C for 10 minutes. The plates were incubated upside down at 25 °C in the dark. The number of developed colonies was independently assessed after the corresponding incubation period for each microorganism group yeasts (3 to 5 days), sporulating bacteria (24 to 48 hours), actinomycetes (5 to 7 days) and cellulolytic and phosphate solubilizing fungi (10 to 15 days). Only the cellulolytic and phosphate solubilizing fungi colonies showing transparent halos were considered.

Data were statistically analyzed by an analysis of variance and treatment means were compared by the Tukey test at the 5% level of probability.

RESULTS AND DISCUSSION

Filter cake and *P. elliottii* were the best treatments for the control of reproduction of *M. incognita* population infesting the bean (Table I). A homogeneous distribution of the *M. incognita* juvenile population in the ex-

Table I. Effect of treatments on the *Meloidogyne incognita* second stage juveniles under field conditions.

Treatments	Sampling (depths)	J_2 /100 ml soil		
		First assessment [1 2 3 *] (31 March)	Second assessment (16 June)	Third assessment (30 Aug)
Crystal sugar	0-25 cm	14.33 (205.35) A a	16.31 (266.02) C a	17.38 (302.06) C a
Crystal sugar	25-50 cm	7.85 (61.62) A b	8.37 (70.06) AB b	8.79 (77.26) AB b
Crystal sugar + napier grass	0-25 cm	13.80 (190.44) A a	14.45 (208.80) E a	12.63 (159.52) D a
Crystal sugar + napier grass	25-50 cm	7.92 (62.73) A b	8.34 (69.56) B b	6.97 (48.58) C b
Brown sugar	0-25 cm	14.12 (199.37) A a	20.82 (433.47) B a	22.65 (513.02) B a
Brown sugar	25-50 cm	7.85 (61.62) A b	8.55 (73.10) AB b	9.40 (88.36) A b
Napier grass	0-25 cm	14.31 (204.78) A a	15.27 (233.17) D a	10.63 (113.00) E a
Napier grass	25-50 cm	7.92 (62.73) A b	8.00 (64.00) B b	7.18 (51.55) CD b
Filter cake	0-25 cm	14.25 (203.06) A a	10.77 (115.99) F a	9.83 (96.63) E a
Filter cake	25-50 cm	8.04 (64.64) A b	6.82 (46.51) C b	5.89 (34.69) C b
Sugar cane juice	0-25 cm	14.31 (204.78) A a	20.75 (430.56) B a	22.20 (492.84) B a
Sugar cane juice	25-50 cm	8.03 (64.48) A b	8.54 (72.83) AB b	8.78 (77.09) AB b
Pinus elliottii saw dust	0-25 cm	14.26 (203.35) A a	10.34 (106.92) F a	6.10 (37.21) F a
Pinus elliottii saw dust	25-50 cm	6.25 (39.06) B b	2.60 (6.76) D b	2.47 (6.10) D b
Control	0-25 cm	14.37 (206.50) A a	23.49 (554.78) A a	28.56 (815.67) A a
Control	25-50 cm	7.98 (63.68) A b	9.12 (83.17) A b	9.97 (99.40) AB b
LSD treatment/depth		0.99	0.76	1.72
LSD depth/treatment		0.63	0.78	1.10
CV (%)		8.22	11.30	15.57

[1] Means transformed to $\sqrt{x}$; [2] original means in parenthesis; [3] means followed by the same upper case letter in the columns and lower case letter in the rows did not differ at the 5% level of probability by the Tukey test; * upper case letters (comparison within depths) and lower case letters (comparison among depths).

perimental area in the first nematode assessment was detected. No significant differences among the juveniles number were detected at either depth in the plots. Generally, the population density of *M. incognita* second stage juveniles collected at the 0-25 cm depths was greater than at 25-50 cm depth. In the second assessment, the treatments differed statistically from the control at 0-25 cm depth, demonstrating the substrate incorporation effects on the average number of juveniles. However, the juvenile population increased as the bean plant grew and developed, except in the filter cake and *P. elliottii* sawdust treatments which showed a reduction in juveniles numbers (Table I). These results are in line with those of Jonathan *et al.* (1991) who detected reductions in nematode populations in sugar cane roots cultivated in an area, which had received similar treatments. An identical situation was observed in the third assessment, where the filter cake and the *P. elliottii* sawdust were also the best treatments to reduce the nematode numbers. The results confirm the potential of these materials for the control of *Meloidogyne* soil populations.

All treatments showed a statistically significant reduction in the number of galls per root system compared to the control, with best results observed for the filter cake and *P. elliottii* sawdust soil applications (Table II). The suppressive effect of sawdust against nematodes was significant enough to be of commercial interest. However, our results suggest that if sawdust is to be utilized as a soil amendment, careful attention will have to be given to prevent nitrogen deficiency problems. Thus appropriate nitrogen fertilization regimes will have to be developed for crops planted immediately after sawdust is applied, and for subsequent crops. The results obtained agree with those by Stirling (1989) and Stirling *et al.* (1995) who observed significant reductions in the number of galls in ginger and apple plants cultivated in soil treated with sawdust. Vawdrey and Stirling (1997) showed that tomato plants growing in sawdust-amended plots were almost free of galls and had the lowest populations of root-knot nematode.

No attempt was made to determine the mode of action of sawdust, but the increase in numbers of free-living nematodes suggested that it was a biological phenomenon. Perhaps the activity of nematophagous fungi increased in response to sawdust, as Barron (1992) noted that these fungi were cellulolytic and lignolytic and were commonly associated with rotting wood. Carneiro (1986), Tokeshi (1991) and Altieri *et al.* (1996) observed a direct correlation among organic matter levels and the predatory and parasitic fungi population, nitrogen fixing microorganisms, phosphate solubilizing fungus and mycorrhizas in the soil. Bacteria, actinomycetes and fungi are able to use cellulose as a carbon and energy source. These microorganisms offer advantages in phytonematode control (Kubicek *et al.*; 1993, Haran *et al.*, 1996).

Root fresh weight of the bean plants treated with crystallized sugar + napier grass differed from the control (Table II). The brown sugar and *P. elliottii* sawdust treatments, although showing the greatest means, did not differ statistically from the control for plant height. The highest number of pods per plant was obtained with the brown sugar treatment (Table II).

In relation to soil microflora in the field, the highest number of phosphate solubilizing fungus colonies was observed in the incorporated crystallized sugar + napier

Table II. Effect of treatments on the galls and growth of bean cv. Carioquinha in a field infested by *M. incognita*.

Treatments	Gall number/ root system	Root system [1 2 3] weight (g)	Plant height (m)	Pod number/ plant
Crystal sugar	10.48 (109.83) b	29.58 ab	0.22 bc	4.74 (22.47) bc
Crystal sugar + napier grass	7.75 (60.06) d	23.46 b	0.21 bc	4.73 (22.37) bc
Brown sugar	10.40 (108.16) b	26.10 ab	0.25 a	4.91 (24.11) a
Napier grass	8.36 (69.89) c	28.56 ab	0.22 b	4.69 (22.00) c
Filter cake	3.81 (14.52) e	27.98 ab	0.20 c	4.77 (22.75) b
Sugar cane juice	10.50 (110.25) b	33.02 a	0.22 bc	4.81 (23.14) b
Pinus elliottii saw dust	2.27 (5.15) f	33.30 a	0.24 a	4.80 (23.04) b
Control	11.28 (127.24) a	32.54 a	0.25 a	4.81 (23.14) b
LSD	0.33	8.00	0.0145	0.08
CV (%)	6.63	44.63	10.42	2.76

[1] Means transformed to $\sqrt{x}$; [2] original means (in parenthesis); [3] means followed by different letters differed significantly at the 5% level of probability by the Tukey test.

grass treatment and the lowest in the control. The highest number of sporulating bacteria colonies was observed in the incorporated napier grass treatment and the lowest in the control. Similar results were obtained for actinomycetes. The incorporated brown sugar treatment presented the lowest number of actinomycete colonies. The greatest cellulolytic fungus population was observed in the incorporated *P. elliottii* sawdust treatment (Table III). The yeast population analyses showed that the filter cake and crystallized sugar treatments were distinct from the others, with a higher number of colonies (Table III). No differences in colony number were detected in the control between the control actinomycetes and cellulolytic fungus, or between phosphate solubilizing fungus or sporulating bacteria (Table III).

Table IV shows that the lowest soil phosphorus (P) content was observed in treatments with incorporated brown sugar, napier grass and the control. The lowest carbon contents in the soil (C) was detected in the *P. elliottii* sawdust treatment, while the lowest soil pH was observed in the control. Soil analysis carried out after sowing bean showed that all treatments had a tendency to increase soil acidification and soil aluminium (Al)

Table III. Effect of treatments on soil microflora in the field[1] [2] [3].

Treatments	Microorganism groups/culture media									
	Phosphate solubilizing (GES)		Sporulating bacteria (NA)		Actinomycetes (ACA)		Cellulolytic (CAA)		Yeasts (YMA)	
Crystal sugar	3.63 (13.18) B	b	2.58 (6.66) D	e	5.19 (26.94) C	a	3.20 (10.24) B	c	2.93 (8.58) A	
Crystal sugar + napier grass	12.43 (154.50) A	a	2.24 (5.02) EF	d	3.81 (14.52) E	b	2.92 (8.53) C	c	2.22 (4.95) B	
Brown sugar	2.96 (8.76) D	a	2.19 (4.80) F	c	1.85 (3.42) G	d	2.62 (6.86) D	b	2.30 (5.29) B	
Napier grass	3.00 (9.00) CD	c	7.25 (52.56) A	a	6.59 (43.43) A	b	2.51 (6.30) D	d	2.19 (4.80) B	
Filter cake	2.47 (6.10) E	d	3.54 (12.53) B	b	5.19 (26.94) C	a	2.94 (8.64) C	c	2.96 (8.76) A	
Sugar cane juice	3.00 (9.00) CD	c	3.23 (10.53) C	b	5.84 (34.11) B	a	3.32 (11.02) B	b	1.67 (2.79) C	
Pinus elliottii saw dust	3.15 (9.92) C	c	3.11 (9.67) C	c	4.38 (19.18) D	a	4.05 (16.40) A	b	1.70 (2.89) C	
Control	1.79 (3.20) F	b	1.85 (3.42) G	b	2.65 (7.02) F	a	2.49 (6.20) D	a	1.41 (1.99) D	
LSD microorganism/treatment = 0.1781 (1.30)										
LSD treatment/microorganism = 0.2074 (1.52)										

[1] Means transformed to $\sqrt{x}$; [2] original means (in parenthesis); [3] means followed by the same upper case letter in the columns and lower case letter in the rows did not differ at the 5% level of probability by the Tukey test.

Table IV. Chemical analysis of soil collected before and after treatment application.

Treatments	mg/dm³	mg/dm³	pH	cmol$_c$/dm³					%			
	P	C		Al	H + Al	Ca	Mg	K	S[1]	T[2]	V[3]	Al[4]
				Before application								
First sampling	1.9	17.01	4.70	0.12	6.68	4.41	1.32	0.23	5.96	12.64	47.15	1.97
				After application[5]								
Crystallized sugar	4.1	20.57	4.40	0.54	9.00	4.15	1.28	0.30	5.73	14.73	38.90	8.61
Crystallized sugar + napier grass	4.4	21.17	4.50	0.42	9.00	4.29	1.40	0.44	6.13	15.13	40.51	6.41
Brown sugar	3.3	19.97	4.40	0.60	9.00	3.82	1.08	0.16	5.06	14.06	35.98	10.60
Napier grass	3.5	18.68	4.50	0.39	7.75	3.87	1.20	0.27	5.34	13.09	40.79	6.80
Filter cake	5.6	20.85	4.50	0.34	8.35	3.93	1.24	0.33	5.50	13.85	39.71	5.82
Sugar cane juice	5.7	20.61	4.40	0.54	9.00	3.31	1.08	0.44	4.83	13.83	34.92	10.05
Pinus elliottii saw dust	4.7	17.96	4.40	0.64	9.00	3.56	1.16	0.30	5.02	14.02	35.80	11.30
Control	3.4	19.80	4.30	0.74	9.00	3.20	1.04	0.16	4.40	13.40	32.83	14.39

[1] Sum of bases; [2] cation exchange capacities; [3] base saturation; [4] aluminium saturation; [5] 120 days after application.

content. Higher potassium content (K) in the soil was detected in crystallized sugar + napier grass and sugar cane juice treatments. Lowest K content was detected in the brown sugar and control treatments. Robinson and Chenery (1958) observed that napier grass cv. Cameroon used as dead soil cover caused an increase in soil potassium levels. The base saturation (V) was lowest in the control, while aluminium saturation (AL) was highest (Table IV). These results are partially in line with those obtained by Buenda and Purcini (1973), who reported that under fertile soil conditions plant cover can increase the carbon level, the pH value and the available calcium, phosphorus and potassium contents, and reduce the exchangeable aluminium and toxic manganese levels.

our results suggest a potential benefit from organic amendments in reducing nematode damage on bean, more work will be required before these materials can be reliably used under commercial conditions to obtain nematode control. Single application of substrates to the soil was promising for nematode control and suggests that subsequent applications could bring greater benefits and lead to the suppression of phytonematodes. The application rates used in our experiments were very high and strategies involving repeated applications of smaller quantities of organic materials over longer periods should be tested.

The long term costs and benefits of applying organic amendments also require thorough evaluation, while optimum application rates, performance in soil other than fertile clay loams, mechanisms of action, rates of development and decline of suppressiveness and impacts of organic amendments on other soil-borne pathogens are some of the other areas requiring further research. Likely factors conditioning the nematode reduction would be the changes in soil temperature and moisture and in the biological flora that control plant material transformation and, consequently, alter the macro and micro nutrient soil content (Lal, 1974; Flaig *et al.*, 1975).

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