MANAGEMENT OF CARNATION AND GERBERA TO CONTROL THE ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA*, IN COMMERCIAL POLYHOUSES

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Summary. Studies on the management of carnation and gerbera to control root-knot nematode, *Meloidogyne incognita*, in commercial polyhouses using pre-plant (dazomet) and post-plant (chlorpyriphos, carbosulfan and carbofuran) chemicals in a comparison with various combinations of and with bio-agents (*Paecilomyces lilacinus, Pochonia chlamydosporia*) and neem cake were made. Pre-plant treatment of beds with dazomet followed by the application of neem cake (1 kg/m^2, 15 days later) along with *P. lilacinus* or *P. chlamydosporia* significantly reduced populations of *M. incognita* and the mortality of plants, and suppressed the nematode infection for nearly 2 years. The antagonistic fungi established themselves better in the beds treated with dazomet than in untreated beds. Chlorpyriphos and carbofuran (each applied twice in 6 months) significantly reduced nematode populations in roots and soil. However, there was a build-up of nematode populations in beds treated with these two chemicals after 1 year. On a long term basis, soil management with pre-plant treatment of dazomet, followed by the application of oil cakes plus antagonistic fungi, was more effective against *M. incognita* than post-plant treatment with carbofuran, carbosulfan and chlorpyriphos on carnation and gerbera grown in polyhouses.

The root-knot nematode, *Meloidogyne incognita* (Kofoid *et* White) Chitw., is a serious limiting factor for the production of carnation (*Dianthus caryophyllus* L.) and gerbera (*Gerbera chinensis* L.) in commercial polyhouses. Exotic varieties of these plants from Europe suffered 40-60% mortality due to root-knot nematode infection and its interaction with other soil-borne pathogens (Nagesh and Parvatha Reddy, 1996). In India, yield losses due to *M. incognita* in carnation and gerbera were estimated at 26 and 30%, respectively (Nagesh and Parvatha Reddy, 2000). Substrate soil/media used in hi-tech polyhouses under Indian conditions are rarely treated chemically to eliminate or reduce soil-borne pathogens. Furthermore, crop and polyhouse sanitation is poor, which makes the entry and establishment of nematodes easy. Although methyl bromide was an excellent soil fumigant, its use as a soil fumigant is now prohibited due to its undesirable effects on the ozone layer. Zunke (1981) recommended steam sterilization of soil to at least 30 cm depth or application of dazomet (40 g/m^2 on light and 50 g/m^2 on heavy soil) or aldicarb 10G (5 g/m^2) for the control of four species of root-knot nematodes in gerbera. However, in India, steam sterilization of soil is a costly proposition (working and capital costs) and not practicable. Dazomet, a relatively new soil sterilant to India, has been only sparingly tested and is used for controlling soil-borne fungal pathogens in high value crops. The research described here evaluated pre-plant application of the chemical dazomet, along with the application of neem cake (*Azadirachta indica* Juss.) and parasitic fungi (*Paecilomyces lilacinus* (Samson) Thomson and *Pochonia chlamydosporia* (Goddard) Zare, Gams *et* Evans) and compared these treatments with post-plant soil pesticides, viz. carbofuran, carbosulfan and chlorpyriphos, for the long term control of *M. incognita* in carnation and gerbera.

MATERIALS AND METHODS

The experiment was carried out in naturally *M. incognita*-infested commercial polyhouses in which carnation and gerbera were grown during 1997-2000. Beds of carnation and gerbera (2 m × 1 m) were established separately for each treatment with 3 replicates. Thirty day-old terminal cuttings of carnation and side suckers of gerbera were planted before or after treatment as per the treatments in their respective beds. The spacing adopted for carnation was 20 cm × 15 cm, accommodating 30 plants/m^2 bed, while the spacing adopted for gerbera was 30 cm × 20 cm, accommodating 16 plants/m^2. Foliar sprays of pesticides were made according to recommended schedules in all the beds to control mites, thrips, leaf miner and foliar diseases.

Local strains of *P. lilacinus*, isolate IIHR 96-1, and *Pochonia chlamydosporia* (Zare *et al.*, 2001), isolate IIHR 97-1, isolated from the root-knot nematode-infested rhizospheres of tomato and eggplant, respectively,

IIHR contribution No. 122

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were used in the study. Stock cultures were maintained at 4 °C on potato dextrose agar (PDA) and corn meal agar (CMA), respectively, and mass produced before use on rice grain at 27 ± 1 °C. The respective fungal inocula were prepared by washing the rice grain (laden with the fungal spores and propagules) with water and passing through a 100-mesh sieve. The spore suspensions were collected separately and examined with the aid of a microscope (100 ×). The conidiospore count per ml of filtrate was estimated in a haemocytometer. Aqueous spore suspensions of *P. lilacinus* and *P. chlamydosporia* were incorporated into the beds at 1 litre/m^2 (2 × 10^{12} spores) after the application of neem cake at the rate of 1 kg/m^2 bed.

The treatments evaluated (Tables I-III) were: dazomet; carbofuran; carbosulfan; chlorpyriphos; neem cake + *P. lilacinus*; neem cake + *P. chlamydosporia*; dazomet + neem cake + *P. lilacinus*; dazomet + neem cake + *P. chlamydosporia*; control (untreated check). Dazomet as a pre-plant treatment was applied at 40 g/m^2 30 days before planting, while carbofuran 3G (10 g/m^2), carbosulfan (2 litres of 0.03%/m^2) and chlorpyriphos (2 litres of 0.03%/m^2) were applied to the beds separately, 30 days after planting as a post-plant treatment. The application of post-plant chemicals was repeated 6 months after planting. The application of post-plant chemicals was coordinated with weeding and earthing-up operations. Applications of biocontrol agents made at planting and in combination with pre-plant treatment of dazomet were at the same doses as given above. Three replications were maintained for each treatment.

The overall mean initial population level of *M. incognita* before treating the beds was estimated at 178 ± 9.5 infective juveniles/100 cm^3 soil (five random soil samples/bed). Soil samples (100 cm^3 each) from the beds designated for each treatment and the control were processed and active nematode juveniles were extracted by combining Cobb's sieving and decanting method with the Baermann technique. Juveniles of root-knot nematodes per ml of aqueous suspension were counted with a microscope. The number of the nematodes per 100 cm^3 soil from each replicate was pooled and the mean value for each treatment was calculated. The stem length, number of flower-bearing stems and number of flowers per m^2 were measured on the carnation crop and spike length and number of flowers per m^2 were measured in the gerbera crop in treated and untreated beds. Plant mortality (%) was calculated by dividing the number of plants alive at 1$^1/_2$ and 2$^1/_2$ years by the total number of plants planted, including the plants used for gap filling.

Root-gall index (on a 1-5 scale, according to Heald *et al.*, 1989) and nematode population levels in root (Byrd *et al.*, 1983) and soil (Trudgill *et al.*, 1972) were recorded in each bed at the end of the first and second years after planting. Nematode multiplication rate was calculated by dividing the nematode population levels at harvest by the mean initial nematode population level. The fungal propagules of *P. lilacinus* and *P. chlamydosporia* in rhizospheres of carnation and gerbera were recorded separately on root and in soil (two samples per replicate per treatment), using their respective semi-selective media (Mitchell *et al.*, 1987; Kerry *et al.*, 1993), and expressed in terms of colony forming units (CFU) per unit

Table I. Effects of treatments on root galling and multiplication rate of *Meloidogyne incognita* in carnation and gerbera.

Treatment and dose per m^2	Root gall index (RGI) (0-5 scale)				Nematode multiplication rate (NMR)			
	Gerbera		Carnation		Gerbera		Carnation	
	Year I	Year II	Year I	Year II	Year I	Year II	Year I	Year II
Dazomet (40 g)	0 a	1.8 c	1.6 d	2.0 c	0a	1.8 b	0.6 ab	2.2 b
Carbofuran 3G (10 g)	1.8 d	2.9 f	2.3 f	3.2 g	1.6 cd	2.4 cd	2.4 e	3.2 c
Carbosulfan (1 litre of 0.03%)	1.0 b	1.8 c	1.2 c	2.2 d	1.0 b	2.4 cd	1.3 c	2.4 b
Chlorpyriphos (1 litre of 0.03%)	1.4 c	2.0 d	1.8 e	2.4 e	1.2 b	2.0 b	1.6 c	2.8 bc
Neem cake (1 kg) + *P. lilacinus* (2 ×10^{12} spores)	2.0 e	2.6 e	2.5 fg	2.8 f	1.4 bc	2.2 bc	1.8 cd	2.6 b
Neem cake (1 kg) + *P. chlamydosporia* (2 × 10^{12} spores)	1.8 d	2.6 e	2.4 f	2.8 f	1.4 bc	2.0 b	1.8 cd	2.5 b
Dazomet (40 g) + neem cake (1 kg) + *P. lilacinus* (2 × 10^{12} spores)	0 a	1.4 b	1.0 b	1.6 b	0 a	0.8 a	0.4 a	1.4 a
Dazomet (40 g) + neem cake (1 kg) + *P. chlamydosporia* (2 × 10^{12} spores)	0 a	0.4 a	0.2 a	0.4 a	0 a	0.5 a	0.3 a	1.0 a
Control	2.7 f	3.8 g	3.4 h	4.2 h	2.4 e	3.6 e	3.0 f	4.2 d
F-Test	*	*	*	*	*	*	*	*
SEM ±	0.04	0.05	0.03	0.05	0.07	0.08	0.05	0.12
CD (P = 0.05)	0.11	0.19	0.14	0.18	0.25	0.33	0.20	0.42

* Significant at P = 0.05. Means followed by the same letter within a column are not significantly different.

weight of root or soil. The native fungal populations in soil in carnation and gerbera beds were recorded twice at yearly intervals on potato dextrose agar medium (Jones *et al.*, 1948). The numbers of parasitized and healthy egg masses were counted in order to calculate the percentage of egg masses parasitized. The parasitized egg masses were treated in 0.2% NaOCl for one minute, the total number of eggs per egg mass counted, and the proportions parasitized by the respective fungi (*P. lilacinus* and *P. chlamydosporia*) estimated by placing the surface sterilized egg masses and released eggs on the semi-selective media as described earlier.

Data collected over a period of 3 years in two polyhouses were subjected to analysis of variance and significance assessed by means of a modified Duncan's multiple range test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Significant reductions in the root-gall index and nematode multiplication rate were observed in treated beds compared to the untreated control at the end of the first and second years (Table I). Root-gall index (RGI) and nematode multiplication rate (NMR) were least in plant beds that received dazomet + neem cake + *P. chlamydosporia,* followed by dazomet + neem cake + *P. lilacinus*. Pre-plant treatment with dazomet gave lower RGI and NMR compared to post-plant treatments (carbofuran, carbosulfan and chlorpyriphos). Among the post-plant chemical treatments, carbosulfan gave the lowest RGI and NMR, followed by chlorpyriphos and carbofuran. Chlorpyriphos and carbosulfan (each applied twice in 6 months) significantly reduced nematode populations in root and soil compared to the control but there was nematode population build up in these two treatments after one year.

Pre-plant treatment of beds with dazomet followed by application of neem cake along with *P. lilacinus* or *P. chlamydosporia* significantly reduced the mortality of plants (Table II), and controlled the nematode infection for nearly 2 years.

Stem length and number of flowers per m^2 of carnation and spike length and number of flowers per m^2 of gerbera were maximum in beds treated with dazomet + neem cake + *P. chlamydosporia*, closely followed by those treated with dazomet + neem cake + *P. lilacinus* (Table III). Carnation and gerbera beds that received other treatments (dazomet; carbofuran; carbosulfan; chlorpyriphos; neem cake + *P. lilacinus*; neem cake + *P. chlamydosporia*) yielded significantly greater stem length/spike length and number of flowers/m^2 compared to untreated beds of carnation and gerbera. These yield parameters were, however, significantly smaller when compared to the above-mentioned two treatments. These treatments did not differ significantly in their effects on spike length and flower yield/m^2. Lamberti (1972) observed that the use of various nematicides not only controlled the *M. incognita* and *M. javanica* populations on carnation but also increased flower yields. Aldicarb, applied to gerberas at 3 g/m^2 twice in 6 months, showed good nematicidal effect

Table II. Effect of treatments on the mortality of gerbera and carnation at different stages of growth in soil infested with *M. incognita*.

Treatment and dose per m^2	Plant mortality (%)			
	End of 1 years		End of 2 years	
	Gerbera	Carnation	Gerbera	Carnation
Dazomet (40 g)	25 bc	28 c	28 bc	32 c
Carbofuran 3G (10 g)	20 b	28 c	32 c	30 b
Carbosulfan (1 litre of 0.03%)	28 cd	32 d	30 c	33 cd
Chlorpyriphos(1litre of 0.03%)	22 b	34 d	25 b	38 e
Neem cake (1 kg) + *P. lilacinus* (2×10^{12} spores)	20 b	24 b	25 b	31 bc
Neem cake (1 kg) + *P. chlamydosporia* (2×10^{12} spores)	25 bc	27 bc	28 bc	29 b
Dazomet (40 g) + neem cake (1 kg) + *P. lilacinus* (2×10^{12} spores)	11 a	7 a	15 a	11 a
Dazomet (40 g) + neem cake (1 kg) + *P. chlamydosporia* (2×10^{12} spores)	9 a	6 a	12 a	12 a
Control	34 e	42 e	40 d	52 f
F-Test	*	*	*	*
SEM ±	0.99	0.88	1.02	0.44
CD (P = 0.05%)	3.88	3.79	4.11	1.84

* Significant at P = 0.05. Means followed by the same letter within a column are not significantly different.

Table III. Effect of pre- and post-plant treatments on carnation and gerbera yield parameters, in soil infested with *M. incognita*.

Treatment and dose per m^2	Carnation		Gerbera	
	Stem length (cm)	Number of flowers/m^2	Spike length (cm)	Number of flowers/m^2
Dazomet (40 g)	96.0 c	42.5 b	66.5	44.0
Carbofuran 3G (10 g)	92.0 b	40.0 b	60.8	42.5 a
Carbosulfan (1 litre of 0.03%)	96.5 c	47.5 de	65.5 b	44.0 a
Chlorpyriphos(1 litre of 0.03%)	95.0 b	45.5 d	63.5 b	42.5 a
Neem cake (1 kg) + *P. lilacinus* (2×10^{12} spores)	95.5 bc	43.0 bc	62.0 b	43.0 a
Neem cake (1 kg) + *P. chlamydosporia* (2×10^{12} spores)	98.0 c	46.0 d	64.2 b	44.0 a
Dazomet (40 g)+ neem cake (1 kg) + *P. lilacinus* (2×10^{12} spores)	104.8 d	63.0 f	72.5 c	55.5 b
Dazomet (40 g) + neem cake (1 kg) + *P. chlamydosporia* (2×10^{12} spores)	116.0 e	68.5 g	75.9 c	58.0 b
Control	75.5 a	37.5 a	48.5 a	40.5 a
F-Test	*	*	*	*
SEM ±	0.86	0.44	1.04	1.22
CD (P = 0.05)	3.43	1.66	4.41	5.11

***** Significant at P = 0.05. Means followed by the same letter within a column are not significantly different.

Table IV. Fungal populations (CFU) on root and in soil in the rhizosphere of carnation and gerbera as influenced by the treatments.

Treatment	(Carnation) CFU/10 g				Gerbera CFU/10 g			
	Root		Soil		Root		Soil	
	Year I	Year II	Year I	Year II	Year I	Year II	Year I	Year II
P. lilacinus + neem cake	1998 a	2230 a (11.6)[#]	2244 a	2450 a (9.2)[#]	1424 a	2230 a (56.6)[#]	1892 a	2450 a (29.5)[#]
P. chlamydosporia + neem cake	2338 b	2936 b (25.5)[#]	2670 b	2830 b (6.0)[#]	2440 b	2888 b (18.3)[#]	2370 b	2830 b (19.4)[#]
Dazomet + neem cake + *P. lilacinus*	5332 c (1.66)[##]	7448 c (39.7)[#] (2.33)[##]	6088 c (1.71)[##]	7232 e (18.8)[#] (1.95)[##]	3132 c (1.19)[##]	4928 c (57.3)[#] (1.20)[##]	5088 c (1.68)[##]	7232 c (42.1)[#] (1.95)[##]
Dazomet + neem cake + *P. chlamydosporia*	6728 d (1.87)[##]	7666 d (13.9)[#] (1.61)[##]	7468 d (1.79)[##]	8394 d (12.4)[#] (1.96)[##]	4728 d (0.93)[##]	5922 d (25.2)[#] (1.05)[##]	6168 d (1.60)[##]	8494 d (37.7)[#] (2.00)[##]
Control**	6624 e	8004e (20.8)[#]	9112 e	1.12×10^4 e (22.9)[#]	6992 e	8244 e (17.9)[#]	9346 e	1.1×10^4 e (17.7)[#]
F-Test	*	*	*	*	*	*	*	*
SEM ±	10.92	9.56	8.98	10.88	11.22	22.10	18.92	23.12
CD (P = 0.05)	43.62	38.24	35.92	43.42	44.88	88.40	72.29	92.22

* Significant at P = 0.05. Means followed by the same letter within a column are not significantly different.

** The data given are for the native fungal populations per g in control plots.

[#] Figures in parentheses are per cent increase over the previous year.

[##] Figures in parentheses are the corresponding factors by which increase was recorded in Dazomet treated (neem cake + *P. lilacinus*/*P. chlamydosporia*) beds over Dazomet-untreated (neem cake + *P. lilacinus*/*P. chlamydosporia*) beds.

against root-knot nematodes, better plant growth at the end of the first year and higher yields than untreated controls (Zunke, 1981).

The antagonistic fungi *P. lilacinus* and *P. chlamydosporia* established themselves better in the beds treated with dazomet compared to the untreated beds in terms of CFUs (Table IV). The numbers of CFUs per 10 g of root/soil of both fungi were higher, in both years of observation, in dazomet-treated beds compared to the untreated beds. Application of neem cake with the fungi increased fungal establishment and growth, suppressed nematode populations and provided organic

matter in the crop rhizosphere. Application of dazomet not only reduced nematode populations but also those of native soil-borne fungi and bacteria, thus reducing/eliminating the competition of these populations for the establishment and proliferation of the introduced fungi, viz. *P. lilacinus* and *P. chlamydosporia*.

The numbers of CFUs of *P. lilacinus* on roots were 1.66 times higher in dazomet-treated carnation beds compared to beds not treated with dazomet (i.e. *P. lilacinus* + neem cake) in the first year, and 2.33 times higher in the second year, while in soil the numbers of CFUs increased by 1.71 and 1.95 times, respectively, in the first and second years. Similarly, the numbers of CFUs of *P. chlamydosporia* on root increased by 1.87 times in the first year and by 1.61 times in the second year, while in soil the numbers of CFUs increased by 1.79 and 1.96 times, respectively, in the first and second years due to the pre-plant treatment with dazomet (Table IV).

Similar effects on numbers of CFUs of *P. lilacinus* were found in dazomet-treated gerbera beds: on roots they increased by 1.19 and 1.20 times compared to gerbera beds not treated with dazomet in the first and second years, respectively, and, in soil, by 1.68 and 1.95 times, respectively, in the first and second years (Table IV). Numbers of CFUs of *P. chlamydosporia* on root were 0.93 times those in dazomet-untreated beds at the end of the first year of observation, and 1.05 times in the second year; in soil, the increase in CFUs was 1.6 and 2 times over dazomet-untreated beds in the first and second years, respectively (Table IV). The native fungal populations in control plots also increased in the second year compared to the first year in both gerbera and carnation beds.

Of the two fungi, *P. chlamydosporia* established better and more quickly than *P. lilacinus* both in carnation and gerbera in both years. Also, the parasitization of eggs and egg masses of *M. incognita* by *P. lilacinus* and *P. chlamydosporia* increased in both years when preplant treatment of dazomet was integrated with neem cake and the fungi (Figs. 1 A-D), indicating that the dazomet aided the introduced fungi in effectively parasitizing and subsequently reducing the nematode populations/infection.

Earlier studies showed that application of neem cake in combination with antagonistic fungi not only helped the fungi establish in the crop rhizosphere but also reduced nematode infection significantly (Nagesh and Parvatha Reddy, 1996). Further, by integrating neem cake and antagonistic fungi with fenamiphos, reduction in *M. incognita* populations in gerbera and carnation was achieved at lower doses of the individual components. However, these treatments did not prevent initial plant loss or mortality in the first 6-8 months due to root-knot nematode infection (Nagesh and Parvatha Reddy, 1996). Zunke (1981) also found that, when aldicarb was applied to gerberas infected with *Heterodera trifolii* Goffart at 3 or 6 g/m^2 twice in 6 months, the 3-g treatment gave better plant growth at the end of the first year and higher yields than untreated controls or the 6-g treatment. Application of M.10 (an experimental product) granules, oxamyl as granules or liquid, and aldicarb granules considerably reduced root-knot nematode populations in a glass-house, resulted in improved root systems, and increased numbers of cut-flowers of gerbera compared with plants in untreated

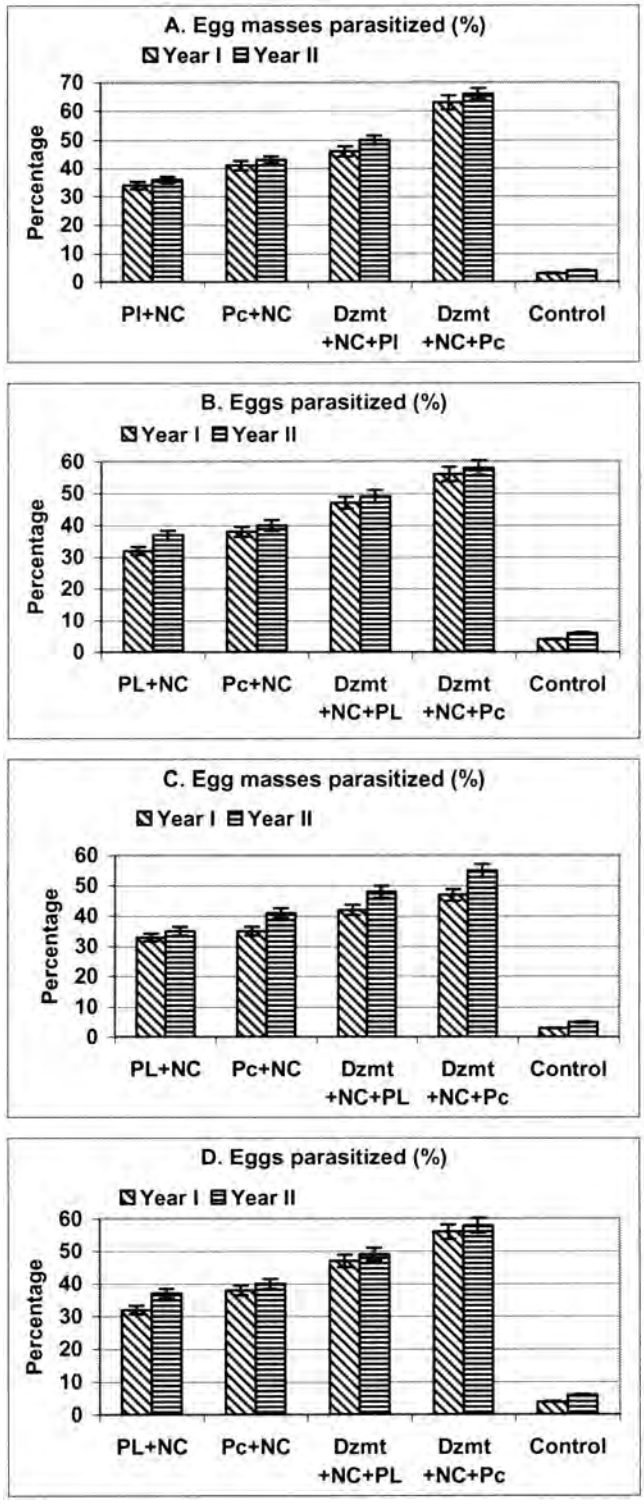

Fig. 1. Fungal parasitization of egg masses and eggs of *Meloidogyne incognita* in carnation (A and B) gerbera (C and D) rhizospheres (Dzmt: Dazomet; NC: neem cake; PL: *P. lilacinus* and PC: *P. chlamydosporia*).

infested soil (Hoogstrate, 1982).

Martinez and Pinzon (1999), in an experiment on the bio-control of *Fusarium oxysporum* f.sp. *dianthi* in carnation, observed a clear interaction between the fungus and the nematode. These authors opined that, for successful biological control of *F. oxysporum* f.sp. *dianthi*, it was necessary to control the nematode. When the nematode was controlled by the use of a promising isolate of *Trichoderma* spp., it was possible to grow susceptible varieties of spray carnations in areas heavily infested with *F. oxysporum* f.sp. *dianthi* without the need for chemical disinfestation of the soil. Also, they suggested that, after any steam treatment or chemical disinfestation of soil, an antagonist of *F. oxysporum* should be applied as soon as possible to prevent the rapid reinfestation of the soil by the pathogen. On this basis, it should be possible to limit plant mortality of carnation and gerbera in root-knot nematode infested polyhouse beds, even at early stages, if beds are treated with dazomet + neem cake + *P. lilacinus/P. chlamydosporia* before planting. Thus, protection of plants in polyhouses against nematode infection needs to begin before planting, with a soil sterilant such as dazomet, followed by establishment of antagonistic potential in the substratum soil/media using fungi like *P. lilacinus* and *P. chlamydosporia.*

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Accepted for publication on 24 July 2005.