INTERACTION OF ROOT-KNOT NEMATODE, VAM FUNGUS AND NEEM CAKE IN AMENDED SOILS ON GLADIOLUS

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Summary. Interaction of neem cake, VAM fungus, *Glomus fasciculatum,* and the root-knot nematode, *Meloidogyne incognita*, on floral growth characters, mycorrhizal root colonization and the development of root-knot disease in gladiolus was studied under green-house conditions. Both the neem cake and the VAM fungus increased the spike length, number of florets/spike and the floret diameter whereas the root-knot nematode reduced these floral growth characters. The neem cake at higher doses of 1 and 2% and the VAM fungus significantly increased the floral growth characters and suppressed root galls. These effects were significantly greater in the presence of neem cake/VAM fungus around nematode infected plants. Sporulation and mycorrhizal root colonization was suppressed at higher doses of the cake. Nematode multiplication was suppressed at all cake doses but was greatest at higher doses. Spore viability was lowest and nematode mortality was highest at higher doses of the cake after six weeks of exposure in amended soil. Lower doses of cake in combination with *G. fasciculatum* is suggested as a means to protect gladiolus from root-knot damage.

Gladioli (*Gladiolus* spp.) are commercially very important flowers. They have aesthetic value and medicinal uses. *Meloidogyne* species are the major root-knot problem in this ornamental plant (Gaur *et al.*, 1995) in addition to other pests and diseases. Though the chemicals have proved quite effective in managing nematodes in various crops, environmental health hazards, non-availability and high cost discourage their use.

VA-mycorrhiza and neem cake have been found to suppress root-knot disease in various crops (Hasan, 1992). Besides its pesticidal properties, neem cake serves as a food base for a variety of micro organisms and simultaneously acts as a nutrient source for various crops. Little information is available on interaction of neem cake with VAM fungus. We report on the results of interactions of different doses of neem cake, *Azadirachta indica* A. Juss, VAM fungus, *Glomus fasciculatum* (Thaxter) Gerdemann *et* Trappe emend. Walker *et* Koske and root-knot nematode, *Meloidogyne incognita* (Kofoid *et* White) Chitw. on *Gladiolus* sp. var. 'SS Jubilee'.

MATERIALS AND METHODS

Single egg masses obtained from eggplant, *Solanum melongena* L., were used to initiate a culture of *M. incognita* on tomato, *Lycopersicon esculentum* Mill. A single chlamydospore obtained from sorghum, *Sorghum vulgare* (L.) Moench, was used to culture VAM on sudangrass, *Sorghum sudanense* (Piper) Stapf. Autoclaved soil contained in earthenware pots was used for growing these plants.

Oil-cake, VAM, Nematode Interactions. The experiment was of a factorial design (with three factors: ± nematode, ± VAM and neem cake at five levels of 0, 0.25, 0.5, 1.0 and 2.0%) conducted in a green-house in a completely randomized block design with twenty treatments (Table I). Each treatment was replicated five times. Plants grown in soils not amended with neem cake, nematodes or VAM fungus served as control.

The 25 cm diameter earthenware pots were filled with 3 kg of autoclaved soil (sand 16%, silt 61% and clay 23%) and sand mixture (3:1). The soil used had pH 8.2, EC 2.5 dS/m, ESP 25.1 and organic carbon 0.45% (courtesy Dept. of Soil Science). Powdered neem cake at concentrations of 0.25, 0.5, 1.0 and 2.0% w/w was added and thoroughly mixed into the soil of appropriate pots. Freshly hatched *M. incognita* J_2 and VAM chlamydospores were added, at the rate of 1,000/kg soil, separately and in various combinations (Table I). Following inoculation, an individual sprouted bulb of gladiolus, weighing approximately 30-35 g, was planted in each pot and the soil was watered to allow decomposition of oil-cake. Extractions of *M. incognita* J_2 from egg masses (Barker, 1985) and of VAM chlamydospores (Gerdemann and Nicolson, 1963) were from cultures raised in a glass-house.

Effect of oil-cake decomposition on VAM/Nematode viability. One kilogram of soil contained in 16 cm diameter pots was thoroughly mixed with powdered neem cake at doses of 0.25, 0.5, 1.0 and 2.0%. Unamended soil served as control. Thirty chlamydospores of VAM fungus, contained in nylon mesh, were buried and 1,000 J_2 nematodes were added separately to each pot containing soil amended with varying doses of the cake. There were three replicates for each treatment. Pots were kept moistened to allow proper decomposition of the cake. After 1, 2, 4 and 6 weeks, nematodes were extracted

from soil following a sifting and gravity method and the chlamydospores contained in the nylon screen were removed from the soil, washed in sterile water and transferred under aseptic condition onto moistened filter papers contained in Petri dishes (Koske, 1981). Dishes were incubated for a week and spore germination was determined under a microscope.

On floral emergence, the spike length (from the base of first floret to the tip of spike), the number of florets on each spike and floret diameter were recorded.

Plants were uprooted after three months and roots were washed under running tap water to dislodge adhering soil particles. Galls were indexed on a 0-4 scale (0 = no galls, 1 = 25, 2 = 50, 3 = 75 and 4 = up to 100% of roots galled). To assess mycorrhizal colonization, roots were cleared in near boiling 10% KOH (potassium hydroxide) aqueous solution for 48 hrs. Roots were then stained in Trypan blue following several washings in distilled water to remove the KOH (Phillips and Hayman, 1970). Stained roots were cut into 1 cm segments, and 35 randomly chosen segments were examined using a stereo microscope. Per cent mycorrhizal colonization was determined by Nicolson's formula (1955) as follows:

$$\text{Colonization (\%)} = \frac{\text{No. of root segments colonized}}{\text{Total No. of segments examined}} \times 100$$

After termination of the experiment, 1 kg of soil was processed for nematode extraction by a sifting and gravity method. The nematode suspension was transferred into a counting dish and numbers of juvenile stage were counted using a stereo-microscope. For estimating chlamydospore populations, 1 kg soil was processed by a wet sieving and decanting technique (Gerdemann and Nicolson, 1963).

The data were subjected to factorial analysis of variance and treatments were compared using Duncan's multiple range test (Steel and Torrie, 1980). Nematode and spore count data were transformed to log (X + 1) before analysis (Proctor and Marks, 1974).

RESULTS

Oil cake, VAM, Nematode interactions.

Effect on floral growth characters. The spike length, number of florets/spike and floret diameter were significantly reduced by root-knot nematode but were significantly increased by neem cake (at all doses) and VAM when applied separately to soils. However, the greatest increase was given by neem cake (0.25%) and GF (Table I).

Development of nematode galls. Plants were found to be highly susceptible to *M. incognita* (Fig. 1). The numbers of nematode galls were significantly reduced in the presence of VAM, neem cake (at all doses) or VAM and neem cake combined. But the greatest reduction occurred in treatment involving 2% neem cake or VAM + 2% neem cake (Fig. 1).

Table I. Effect of different doses of neem cake (NC), *Meloidogyne incognita* (MI) and *Glomus fasciculatum* (GF), alone and in various combinations, on floral growth characters of gladiolus var. 'SS Jubilee' grown in pots containing 3 kg soil.

Treatment	Treatment rate	Floral growth characters		
		Spike length (cm.)	No. of florets/spike	Floret diameter (cm.)
NC	0.25%	35 b	13 b	10.0 a
NC	0.5%	36 b	13 b	10.5 b
NC	1.0%	40 e	15 c	11.0 c
NC	2.0%	45 df	18 d	11.0 c
MI	1000 J_2/kg soil	25 c	8 f	9.0 c
GF	1000 spores /kg soil	40 e	16 c	11.5 d
MI+GF	1000 J_2 + 1000 spores/kg soil	36 b	13 b	10.5 b
MI+NC	1000 J_2/kg soil + 0.25%	26 c	8 f	9.5 f
MI+NC	1000 J_2/kg soil + 0.5%	28 c	9 af	9.4 f
MI+NC	1000 J_2/kg soil + 1.0%	34 b	15 c	10.0 a
MI+NC	1000 J_2/kg soil + 2.0%	40 e	17 d	10.5 b
GF+NC	1000 spores /kg soil + 0.25%	50 f	21 e	10.5 b
GF+NC	1000 spores /kg soil + 0.5%	48 f	20 de	10.5 b
GF+NC	1000 spores /kg soil + 1.0%	42 de	13 d	11.0 c
GF+NC	1000 spores /kg soil + 2.0%	43 de	19 e	11.0 c
MI+GF+NC	(1000 J_2 + 1000 spores)/kg soil + 0.25%	46 df	19 e	11.0 c
MI+GF+NC	(1000 J_2 + 1000 spores)/kg soil + 0.5%	45 def	18 de	11.0 c
MI+GF+NC	(1000 J_2 + 1000 spores)/kg soil + 1.0%	41 e	16 c	11.4 cd
MI+GF+NC	(1000 J_2 + 1000 spores)/kg soil + 2.0%	44 e	18 d	11.5 d
Control	(No. MI. No. GF, No NC)	30 a	10 a	10.0 a

Figures followed by similar letters are statistically not significantly different ($P \leq 0.05$).

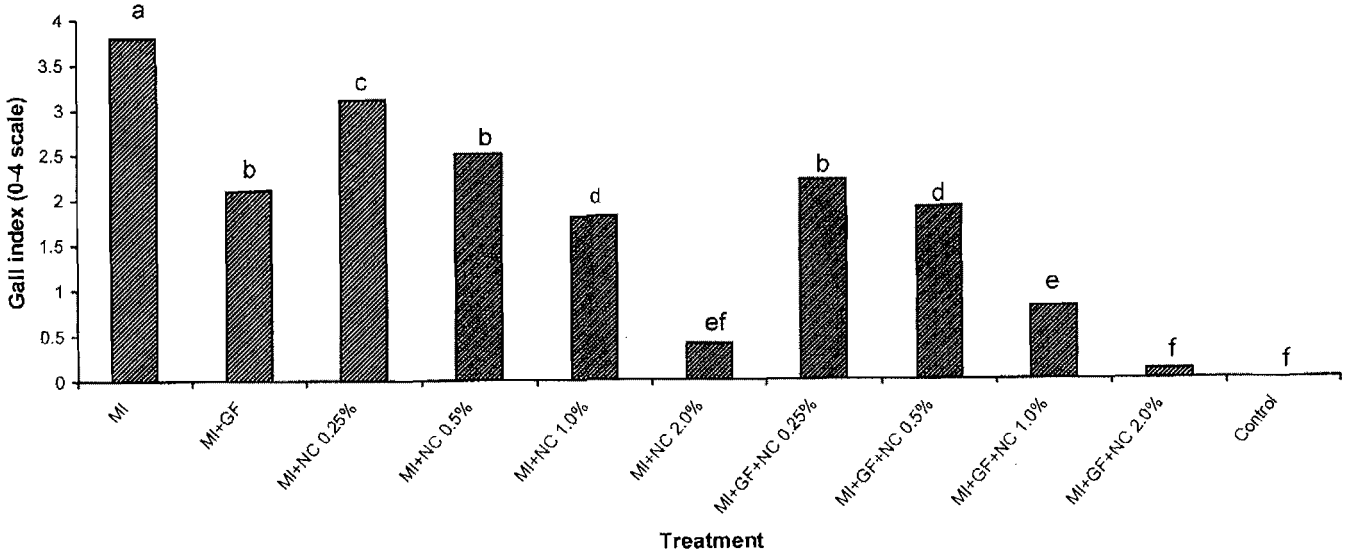

Fig. 1. Effect of interaction of *Meloidogyne incognita* (MI), *Glomus fasciculatum* (GF) and neem cake (NC) on nematode gall development on roots of *Gladiolus* sp. Differences among bars showing a common letter are statistically not significant ($P \leq 0.05$).

Mycorrhizal colonization. Greatest colonization (80 to 88%) of roots by VAM occurred in treatments VAM (GF), VAM + nematode (GF + MI), VAM + neem cake (GF + NC 0.25%) or VAM + nematode + neem cake (GF + MI + NC 0.25%) (Fig. 2). Root-knot nematode or 0.25% neem cake failed to decrease it significantly. On the other hand, colonization was significantly reduced by neem cake at 0.5, 1 or 2% when used alone or in combination with the root-knot nematode, with the greatest reduction in VAM colonization occurring at the highest dose of neem cake (2%).

VAM sporulation and nematode multiplication. Spore production and nematode multiplication were adversely affected by neem cake (Table II), with the effect increasing as dosage of the cake increased. Root-knot nematode had no influence on VAM sporulation but VAM significantly suppressed nematode multiplication.

VAM spore and nematode viability. Spore viability de-

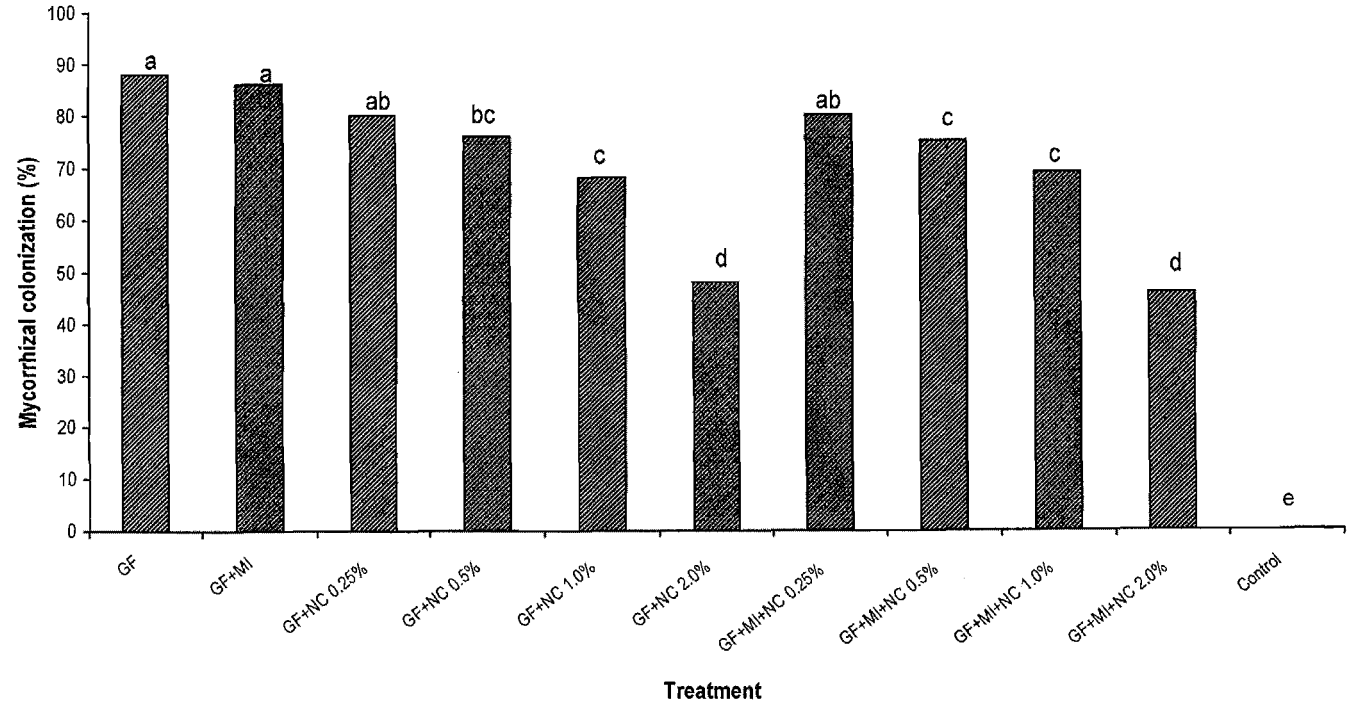

Fig. 2. Effect of interaction of *M. incognita* (MI), *G. fasciculatum* (GF) and neem cake (NC) on mycorrhizal colonization of roots of *Gladiolus* sp. Differences among bars showing a common letter are statistically not significant ($P \leq 0.05$).

Table II. Effect of different doses of neem cake (NC), *M. incognita* (MI) and *G. fasciculatum* (GF) on the development of VAM chlamydospores and root-knot nematodes in soil around gladiolus var. 'SS Jubilee'.

Treatment	Treatment rate	VAM spores/kg soil	Nematodes /kg soil
MI	1000 J_2/kg soil	-	15,450 a
GF	1000 spores /kg soil	11,310 a	-
MI+GF	(1000 J_2 + 1000 spores) /kg soil	10,200 ae	8560 b
MI+NC	1000 J_2/kg soil + 0.25%	-	7215 b
MI+NC	1000 J_2/kg soil + 0.5%	-	4345 c
MI+NC	1000 J_2/kg soil + 1.0%	-	820 d
MI+NC	1000 J_2/kg soil + 2.0%	-	400 e
GF+NC	1000 spores/kg soil + 0.25%	9280 e	-
GF+NC	1000 spores/kg soil + 0.5%	4550 b	-
GF+NC	1000 spores/kg soil + 1.0%	1040 c	-
GF+NC	1000 spores/kg soil + 2.0%	440 d	-
MI+GF+NC	(1000 J_2 +1000 spores)/kg soil + 0.25%	8995 e	730 d
MI+GF+NC	(1000 J_2 + 1000 spores) /kg soil + 0.5%	4280 b	540 e
MI+GF+NC	(1000 J_2 + 1000 spores) /kg soil + 1.0%	1210 c	280 f
MI+GF+NC	(1000 J_2 + 1000 spores) /kg soil + 2.0%	590 d	195 f

Figures followed by similar letters are statistically not significantly different ($P \leq 0.05$).

Table III. Effect of decomposition period of neem cake on viability of chlamydospores of *G. fasciculatum* and mortality of *M. incognita* grown in pots containing 1 kg soil.

Neem cake treatment		Spore viability (%)	Nematode mortality (%)
Dose	Decomposition period (week)		
0.25%	1	100 a	22 a
	2	98 a	54 b
	4	95 ab	80 c
	6	90 bc	100 d
0.5%	1	88 c	30 e
	2	76 d	62 f
	4	65 e	80 c
	6	52 f	100 d
1.0%	1	55 f	50 b
	2	50 fi	70 g
	4	40 g	90 hj
	6	30 h	100 d
2.0%	1	45 ig	80 c
	2	32 j	95 dh
	4	30 j	100 b
	6	20 k	100 d
VAM + MI	1	100 a	10 i
	2	100 a	30 e
	4	98 a	55 b
	6	98 a	85 cj

Figures followed by similar letters are statistically not significantly different ($P \leq 0.05$).

creased and nematode mortality increased with increase in dose and exposure period to the cake in amended soil (Table III).

DISCUSSION

The nematicidal principles (azadirachtin, nimbidin, phenolics, aldehydes, ketones etc.) and manurial properties (43.2% C, 3.4% N and 0.4% P) of this cake have been reported (Khan, 1976; Mojumder, 1995). VAM fungi improve plant health through nutrient mobilization from soil and suppress various diseases in plants caused by nematodes and other pathogens (Gerdemann, 1968; Hussey and Roncadori, 1982; Linderman, 1994). Although the effect of neem cake was more pronounced at higher doses, similar effects could be obtained at lower doses if used in combination with *G. fasciculatum*. It is evident that mycorrhizal colonization was greatest at lower doses of the cake than at higher doses. Suppression could be attributed to lower production of chlamydospores as well as loss of their viability. These chlamydospores are the primary propagules involved in root colonization on germination. The adverse effects of neem cake on growth and sporulation of certain *Fusarium* wilt and *Rhizoctonia* or *Sclerotium* root-rot fungi in amended soil has been reported (Locke, 1995). Ornamental plants are often grown in soil amended with large quantities of composted organic matter and most of the amendments are known to enhance sporulation and root colonization by VAM (Singh, 2000). Moreover, neem cake, besides its manurial properties, not only suppressed the nematode but at higher doses adversely affected also the mycorrhizal root colonization and sporulation of *G. fasciculatum* on this test plant. The VAM fungus, *G. fasciculatum* besides favouring growth of this ornamental also adversely affected the development of *M. incognita*. And thus, if using neem cake as an organic source of soil amendment in gladiolus, in combination with VAM, it would be advisable to select lower doses of this cake to protect the crop from root-knot damage.

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