Individual and interactive effects of VA mycorrhizal fungi and root-knot nematode, *Meloidogyne incognita*, on cardamom

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Received August 1987; revised March 1988

The effect of inoculation with six species of vesicular-arbuscular (VA) mycorrhizal fungi individually and in different combinations with the root-knot nematode, *Meloidogyne incognita*, simultaneously and sequentially, on cardamom was studied at 6 and 12 months after inoculation. Compared with the control, the growth of cardamom plants was significantly improved by inoculation with VA mycorrhizal fungi, whereas nematode inoculation reduced it. The growth response induced by VA mycorrhizal fungi was similar when inoculation was made simultaneously and after nematode inoculation. VA mycorrhizal fungi significantly reduced the nematode population in roots. Colonization of roots by VA mycorrhizal fungi was also obtained when nematodes were inoculated simultaneously and prior to inoculation by VA mycorrhizal fungi. *Gigaspora margarita* Becker & Hall and *Glomus fasciculatum* (Thaxter *sensu* Gerd.) Gerd. & Trappe were the most effective VA mycorrhizal fungi, able to promote maximum growth response in the absence as well as in presence of root-knot nematodes.

Keywords: Cardamom; Root-knot nematode; VA mycorrhial fungi; Interaction

Cardamom (*Elettaria cardamomum* Maton) is an important export-oriented spice crop grown in India on an area of 94 000 ha with an annual production of 4000 t (Anon., 1984). Decline and poor growth of cardamom seedlings due to heavy incidence of the root-knot nematode, *Meloidogyne incognita*, was reported by Ali and Koshy (1982).

Vesicular-arbuscular (VA) mycorrhizal fungi are reported to be useful as biological control agents against root-invading pathogens such as fungi and nematodes (Roncadori and Hussey, 1977; Bagyaraj *et al.*, 1979; Grandison and Cooper, 1986). The beneficial effects of interaction between VA mycorrhizal fungi and root-knot nematodes were reported to be dependent on a specific mycorrhizal fungus and host involved in the relationship (Schenck *et al.*, 1975). The present study reports the individual and interactive effects of six species of VA mycorrhizal fungi and the root-knot nematode, *Meloidogyne incognita* (Kofoid *et* White) Chitwood, on cardamom in terms of growth response, mycorrhizal colonization and nematode development in the roots, from the experiments at Appangala.

Materials and methods

Cardamom var. Malabar plants, raised from surface-sterilized seeds in pots containing soil fumigated with methyl bromide, were used for the study. The experiment was conducted in earthern pots (35 cm size) containing 8 kg laterite soil (pH 5.8) with available P content of 11.2 ppm. *Glomus fasciculatum* (Thaxter *sensu* Gerd.) Gerd. & Trappe, *Gigaspora margarita* Becker & Hall, *Acaulospora* sp., *Gigaspora calospora* (Nicol. & Gerd.) Gerd. & Trappe, *Glomus versiforme* (Karsten) Berch, *Glomus macrocarpum* (Tul. & Tul.) Gerd. & Trappe were the VA mycorrhizal fungi tested individually and in combination with the nematodes. Mycorrhizal inoculum was prepared in pot cultures by growing sorghum as the host plant for 90 days. There were 26 treatments, replicated six times: T_1, uninoculated control; T_2, inoculated with nematode only at transplanting; T_3–T_8, inoculated with mycorrhizal fungi only (six species of VA mycorrhizal fungi separately) at transplanting; T_9–T_{14}, inoculated simultaneously with nematodes and mycorrhizal fungi at transplanting; T_{15}–T_{20}, inoculated with nematodes at transplanting and with mycorrhizal fungi 21 days after transplanting; T_{21}–T_{26}, inoculated with mycorrhizal fungi at transplanting and with nematodes 21 days later.

Mycorrhizal inoculum containing about 300 spores of each fungus was placed as a thin layer, 2 cm below the soil surface. Three-leaf-stage seedlings were transplanted singly into the pots. The freshly hatched second stage juveniles of *M. incognita* collected from root-knot infested roots were used as nematode inoculum for the several treatments. Inoculum (about 1000 nematodes pot^{-1}) was deposited in four holes at different depths around the base of the seedling. Pots were placed in a completely randomized design under glasshouse conditions. Three replicates were harvested after six months and three after 12 months.

Growth characters, plant height, root length, number of tillers, number of leaves, leaf length and breadth were recorded. Leaf area (P) was calculated using the formula developed by George *et al.* (1984) for the cardamom; $P = 0.813\ B + 0.657\ LB$ where

Contribution No. 630 of Central Plantation Crops Research Institute, Kasaragod, India

0041-3216/89/010021-04 $03.00

L and *B* are the length and breadth of the leaf. Dry weight of the shoot was recorded after drying the samples to constant weight at 70°C. Some hairy roots were fixed in FAA, cleared in 10% KOH and stained in 0.5% trypan blue in lactophenol (Phillips and Hayman, 1970) and mycorrhizal colonization was assessed microscopically. Intensity of infection, expressed as infection grading (IG), was calculated according to Thomas and Ghai (1987). Other root samples (2 g each) were cut into small pieces, stained in boiling acid fuchsin-lactophenol, blended and the nematode population (eggs, different larval stages and adults) assessed using a stereomicroscope.

Results

VA mycorrhizal inoculation resulted in a significant increase in various plant growth characters of cardamom (Table 1). However, the extent of the increase varied with the VA mycorrhizal fungal species inoculated. The growth stimulation was observed both at 6 and 12 months after inoculation. Inoculation with *G. margarita* and *G. fasciculatum* resulted in a maximum of 12- and 10-fold increases in shoot dry weight compared with the control at 6 months. On the other hand, inoculation of the plants with root-knot nematode alone caused a reduction in various growth parameters.

When nematodes and mycorrhizal fungi were inoculated simultaneously (T_9–T_{14}), the growth response induced by VA mycorrhizal fungi was less than in plants inoculated with mycorrhizal fungi alone (T_3–T_8), though they were superior to uninoculated (T_1) and nematode-alone-inoculated (T_2) plants. For example, leaf area was reduced to 90.2 cm^2 $plant^{-1}$ at one year when *G. fasciculatum* and root-knot nematode were simultaneously inoculated, compared with 153.6 cm^2 in *G. fasciculatum* inoculated alone. The detrimental effects of the nematode on the growth of cardamom was more pronounced when nematodes were inoculated prior to inoculations with VA mycorrhizal fungi (T_{15}–T_{20}) than in simultaneous inoculations (T_9–T_{14}) and VA mycorrhizal fungi followed by nematode treatments (T_{21}–T_{26}). The shoot dry weight showed more than 50% reduction when *G. fasciculatum* was inoculated after nematodes compared with its individual inoculation. When VA mycorrhizal fungi were inoculated prior to nematodes (T_{21}–T_{26}), the extent of growth increase was almost similar to that of plants inoculated alone with VA mycorrhizal fungi (T_3–T_8). Growth stimulation was significantly affected in other combined inoculation treatments (T_9–T_{14} and T_{15}–T_{20}), particularly at 12 months. *G. margarita*

Table 1 Growth characteristics of cardamom plants as influenced by inoculation with VA mycorrhizal fungi, root-knot nematode or both

Treatment No.	Inoculation details	Shoot dry weight (g $plant^{-1}$)		Plant height (cm)		Root length (cm)		No. of tillers $plant^{-1}$		No. of leaves $plant^{-1}$		Leaf area (cm^2)	
		6M[a]	12M[a]	6M	12M	6M	12M	6M	12M	6M	12M	6M	12M
T_1	Uninoculated	0.45	2.60	21.4	42.3	14.7	26.0	1.0	3.7	6.0	8.0	25.1	52.0
T_2	Nematode alone	0.11	1.80	12.2	34.0	10.5	17.0	1.0	3.0	4.7	5.0	11.1	28.7
	VA mycorrhizal fungi alone												
T_3	*G. fasciculatum*	4.64	23.73	62.0	87.7	29.3	43.7	4.7	17.7	19.7	34.7	119.7	153.6
T_4	*G. margarita*	5.37	19.87	64.3	85.0	30.3	54.0	5.3	13.0	23.3	31.0	125.4	132.1
T_5	*Acaulospora* sp.	1.62	14.70	32.8	79.0	16.7	40.3	3.0	10.0	12.0	29.3	58.4	121.1
T_6	*G. calospora*	0.81	10.93	16.0	74.0	19.7	33.7	1.7	11.0	5.0	24.0	24.7	119.8
T_7	*G. versiforme*	0.43	9.57	17.7	73.3	15.7	41.3	1.0	10.0	6.0	22.3	23.7	110.7
T_8	*G. macrocarpum*	0.36	8.25	17.8	68.0	15.0	40.3	1.0	9.7	5.7	19.0	24.3	100.2
	VA mycorrhizal fungi and nematode (N) simultaneously												
T_9	*G. fasciculatum* + N	3.44	14.23	55.7	77.7	20.2	34.3	4.3	10.3	18.7	27.0	104.0	90.2
T_{10}	*G. margarita* + N	4.83	18.53	60.3	80.3	20.7	43.7	3.3	11.3	17.0	30.7	102.2	100.4
T_{11}	*Acaulospora* sp. + N	0.27	9.45	9.2	63.0	11.7	32.3	1.0	9.0	3.3	27.0	17.0	81.5
T_{12}	*G. calospora* + N	0.81	7.17	15.5	65.0	11.8	28.0	1.7	6.0	4.0	15.7	18.7	55.5
T_{13}	*G. versiforme* + N	0.49	5.47	18.8	72.3	14.8	34.7	1.0	9.7	5.7	17.7	37.3	93.1
T_{14}	*G. macrocarpum* + N	0.16	5.43	14.7	65.3	9.2	33.7	1.0	4.7	4.3	12.7	18.0	63.8
	Nematode (N) followed by VA mycorrhizal fungi												
T_{15}	N → *G. fasciculatum*	3.02	11.20	46.2	59.3	17.7	32.7	3.7	10.0	15.3	24.0	89.3	79.7
T_{16}	N → *G. margarita*	3.79	11.67	49.2	69.7	19.2	31.3	2.7	9.0	13.3	19.7	99.4	84.7
T_{17}	N → *Acaulospora* sp.	0.27	6.20	9.5	61.0	10.5	26.7	1.0	6.0	3.3	16.0	8.6	68.1
T_{18}	N → *G. calospora*	0.23	4.47	12.7	60.3	9.8	27.7	1.0	4.7	4.0	14.7	10.6	80.2
T_{19}	N → *G. versiforme*	0.33	7.93	17.5	58.0	14.7	31.0	1.0	4.0	5.7	11.7	43.3	75.4
T_{20}	N → *G. macrocarpum*	0.12	5.60	6.3	67.7	10.8	32.0	1.0	4.0	2.3	12.3	13.9	57.7
	VA mycorrhizal fungi followed by nematode (N)												
T_{21}	*G. fasciculatum* → N	4.00	25.83	42.0	91.7	25.3	39.7	3.7	15.3	18.3	38.3	104.1	123.4
T_{22}	*G. margarita* → N	7.55	18.67	64.5	79.3	32.0	45.3	7.0	10.3	26.7	30.0	119.5	109.2
T_{23}	*Acaulospora* sp. → N	1.22	7.77	30.5	72.3	17.3	33.7	2.0	7.3	10.0	18.0	65.0	88.1
T_{24}	*G. calospora* → N	0.83	10.63	20.5	73.0	18.0	30.7	2.0	9.0	6.0	21.3	31.3	88.7
T_{25}	*G. versiforme* → N	0.49	9.83	19.4	67.7	15.8	35.3	1.3	9.3	6.7	15.7	36.5	93.8
T_{26}	*G. macrocarpum* → N	0.35	9.77	20.3	70.7	15.7	36.0	2.0	8.7	6.7	20.7	59.8	91.4
	LSD (P = 0.05)	2.39	7.48	19.2	23.8	9.6	14.2	1.9	5.9	7.6	12.9	54.8	36.4

[a] 6M, 12M: at 6 and 12 months after inoculation

and *G. fasciculatum* were more effective than other VA mycorrhizal fungi tested, even in the presence of nematodes at different inoculation combinations.

Microscopic observations on the cleared and stained roots showed that all six VA mycorrhizal fungi could colonize cardamom roots but to varying levels (Table 2). The extent of mycorrhizal colonization as well as intensity of infection were greater with *G. margarita* and *G. fasciculatum* which were also the most efficient fungi in increasing growth. In the presence of the nematode they colonized cardamom roots better than the other VA mycorrhizal fungi tested.

Nematode counts were very high in plants inoculated with nematodes only. Their numbers in roots decreased in the presence of VA fungi and the extent of reduction was greater when mycorrhizal fungi were inoculated prior to nematode inoculation.

Discussion

The present study revealed that cardamom plants formed symbiotic association with all six species of VA mycorrhizal fungi tested as was evident from the growth stimulation and mycorrhizal colonization in root tissue. The differences in response to inoculation with different VA mycorrhizal fungi could be due to genetic factors as well as to differences in the affinities between the host and microsymbiont under the particular set of soil conditions. The two VA mycorrhizal fungi, *G. margarita* and *G. fasciculatum*, were significantly effective ($P = 0.01$) in improving the vegetative growth of cardamom; inoculation with root-knot nematode resulted in a suppression in the growth of cardamom, indicating the pathogenic relationship between the nematode and the host.

It was also evident from the study that the interaction between VA mycorrhizal fungi and the root-knot nematode was dependent on the sequence in which cardamom plants become colonized by mycorrhizal fungi and infested by the nematode. When VA mycorrhizal fungi were inoculated either simultaneously or after nematodes, the degree of growth improvement was less than in plants inoculated with VA mycorrhizal fungi alone. This indicated that the activities of the nematodes affected the symbiotic relationship, and a less favourable environment was formed for the fungus. The adverse influence on growth was maximum when

Table 2 Percentage mycorrhizal colonization, infection grading and nematode population in roots of cardamom at 6 and 12 months after inoculation with VA mycorrhizal fungi, nematode or both

Treatment No.	Inoculation details	Mycorrhizal[a] colonization (%)		Infection grading (IG)		Nematode[b] popn g^{-1} root	
		6M	12M	6M	12M	6M	12M
T_1	Uninoculated	0	0	0	0	0	0
T_2	Nematode alone	0	0	0	0	20.50	67.94
	VA mycorrhizal fungi alone						
T_3	*G. fasciculatum*	49.30	42.31	26.33	27.46	0	0
T_4	*G. margarita*	54.52	45.44	37.00	32.76	0	0
T_5	*Acaulospora* sp.	33.26	29.68	12.72	11.39	0	0
T_6	*G. calospora*	24.99	27.49	8.37	10.33	0	0
T_7	*G. versiforme*	32.63	23.66	10.33	9.25	0	0
T_8	*G. macrocarpum*	27.01	21.86	7.78	8.11	0	0
	VA mycorrhizal fungi and nematode (N) simultaneously						
T_9	*G. fasciculatum* + N	46.15	40.66	17.33	23.47	6.46	26.63
T_{10}	*G. margarita* + N	45.00	38.08	21.42	23.25	6.28	27.29
T_{11}	*Acaulospora* sp. + N	29.30	24.00	11.37	7.51	6.37	39.46
T_{12}	*G. calospora* + N	25.13	25.25	7.60	8.71	8.54	48.48
T_{13}	*G. versiforme* + N	26.45	23.78	7.33	8.28	10.27	47.48
T_{14}	*G. macrocarpum* + N	24.97	24.00	6.42	7.31	8.87	37.29
	Nematode (N) followed by VA mycorrhizal fungi						
T_{15}	N → *G. fasciculatum*	40.00	32.63	13.67	14.78	6.62	19.58
T_{16}	N → *G. margarita*	44.90	39.23	18.39	21.95	5.99	14.74
T_{17}	N → *Acaulospora* sp.	23.21	21.76	5.72	7.05	11.43	52.99
T_{18}	N → *G. calospora*	24.50	20.15	7.81	6.20	12.27	59.14
T_{19}	N → *G. versiforme*	24.79	20.15	6.56	6.00	9.69	40.69
T_{20}	N → *G. macrocarpum*	23.66	21.62	5.73	6.64	11.41	43.69
	VA mycorrhizal fungi followed by nematode (N)						
T_{21}	*G. fasciculatum* → N	50.02	43.07	21.33	27.21	5.30	37.22
T_{22}	*G. margarita* → N	52.58	42.14	26.67	26.42	5.14	10.80
T_{23}	*Acaulospora* sp. → N	23.47	23.47	8.67	9.00	5.28	9.05
T_{24}	*G. calospora* → N	26.23	22.37	8.42	8.11	4.56	12.97
T_{25}	*G. versiforme* → N	24.42	24.58	7.67	8.67	4.58	20.80
T_{26}	*G. macrocarpum* → N	27.94	22.62	7.33	6.96	5.22	28.6
	LSD ($P = 0.05$)	12.46	13.55	12.14	7.80	4.40	16.05

[a] Values after arc sine transformation
[b] Values after square root transformation
6M, 12M: at 6 and 12 months after inoculation

VA mycorrhizal fungi were inoculated 21 days after nematodes (T_{15}–T_{20}) since it gives competitive advantage for the nematode in allowing its life cycle to be completed in three weeks (Dhawan and Sethi, 1976). When VA mycorrhizal fungi were inoculated before nematode inoculation, the mycorrhiza-induced plant growth response was not affected by inoculation with *M. incognita*. Prior inoculation with VA mycorrhizal fungi resulted in their colonization of roots and later inoculation with the nematodes did not affect the established symbiosis. Many reports are available in the literature to show that mycorrhizal plants are less affected by nematodes compared with non-mycorrhizal plants (Sikora and Schonbeck, 1975; Roncadori and Hussey, 1977; Bagyaraj *et al.* 1979; Kellam and Schenck, 1980; Hussey and Roncadori, 1982). The present study also showed that the interaction between root-knot nematodes and VA mycorrhizal fungi varied with the fungal species inoculated. Similar results were also reported on soybean in interaction studies with different VA mycorrhizal fungi and root-knot nematode (Schenck *et al.*, 1975).

The nematode population was significantly reduced when VA mycorrhizal fungi and nematodes were inoculated simultaneously and one after the other, compared with that in nematode alone inoculated plants, indicating that mycorrhizal fungi present in the root system interfered with the development of nematodes. Sikora and Schonbeck (*ibid.*) also reported a significant reduction in the number of *M. incognita* larvae in a well-established mycorrhizal root system. The exact mechanism of suppression of nematodes by mycorrhizal fungi is not known but it may be due to the physiological changes brought about by mycorrhizae in the root system (Suresh and Bagyaraj, 1984).

Mycorrhizal colonization in cardamom roots was also affected when VAM fungi were inoculated simultaneously with and after nematode inoculation. Kellam and Schenck (1980) reported that *Glomus macrocarpus* and *M. incognita* were mutually inhibitory on soybean and that the presence of nematodes affected mycorrhiza development.

Root-knot nematode is a serious pest in nurseries in various cardamom-growing tracts in India, affecting drastically the growth of seedlings. The present study revealed that prior inoculation with VA mycorrhizal fungi, particularly *G. margarita* and *G. fasciculatum*, was effective in ameliorating the deleterious effects of nematodes and in providing vigorous, healthy seedlings for transplantation in the field.

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