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RELATIONSHIP BETWEEN INITIAL INOCULUM DENSITY OF *MELOIDOGYNE INCOGNITA* AND GROWTH, PHYSIOLOGY AND OIL YIELD OF *OCIMUM KILIMANDSCHARICUM*

by

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Summary. In pot experiments a negative correlation was observed between various initial inoculum densities of *Meloidogyne incognita* and fresh/dry weight, oil yield, photosynthetic rate, total chlorophyll, sugars, phenols and root-knot development in *Ocimum kilimandscharicum*. The greatest reduction in all growth parameters occurred in plants inoculated with the highest inoculum density of *M. incognita* (16,000 J_2/7.5 kg soil). The highest reproduction factor (45.85) occurred in plants inoculated with 5000 J_2/7.5 kg soil.

Ocimum kilimandscharicum Guerke is an important essential oil bearing plant of the family Labiatae, which is a native of Kenya. Major components of the oil are linalool, camphor and 1,8-cineole (Charles and Simon, 1992), and minor components are ß-sitosterol, oleanolic acid and ursolic acid obtained from the inflorescence (Rastogi and Mehrotra, 1990). The oil is used in confectionary and pharmaceutical industries especially in European countries.

Information on association of plant parasitic nematodes with different species of *Ocimum* is scarse (Goffart, 1931; Buhrer, 1938; Ustinov, 1939). Work carried out at the Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow indicates that the root-knot nematodes, *Meloidogyne incognita* and *M. javanica*, cause significant reduction in oil yield of these crops (Haseeb *et al.*, 1986; Haseeb and Pandey, 1987). Root-knot nematode may also affect photosynthesis and growth of plants (Melakeberhan *et al.*, 1984) by influencing the hosts nutrient content, translocation of photosynthates (Bird and Loveys, 19075) or transpiration (Evans, 1982). The relationship between different initial inoculum levels of *M. incognita* and yield of *O. basilicum*, *O. canum* and *O. sanctum* has recently been evaluated (Haseeb *et al.*, 1988; Haseeb and Butool, 1989; Haseeb *et al.*, 1993), but there is no information on the effect of root-knot infection on growth, physiological and biochemical parameters of *O. kilimandscharicum*.

Materials and methods

Twenty one day old seedlings of *O. kilimandscharicum* of uniform size were transplanted singly into 30 cm clay pots containing an autoclaved soil-compost mixture (7:1). Pots were inoculated with 500, 1,000, 2,000, 4,000, 8,000 and 16,000 freshly hatched second stage juveniles (J_2) of *Meloidogyne incognita* from cultures maintained on brinjal roots (*Solanum melongena* L.) in a glasshouse. Uninoculated pots served as controls. Final data were recorded 75

days after inoculation. Total chlorophyll content was measured according to the method of Arnon (1949). Carbon dioxide gas exchange rate was measured in a closed system using a portable photosynthesis model Li6000 (LiCOR, USA). Estimation of total phenol and total sugars was done according to the methods of Yemm and Willis (1954) and Swain and Hill (1959), respectively. Oil content was determined by hydrodistillation of 100 g fresh herb by Clevenger apparatus (Clevenger, 1928).

Plant growth was determined by measuring shoot/root length, fresh/dry root and shoot weight. Development of galls on roots was rated according to Taylor and Sasser (1978). Post harvest nematode populations in the soil were determined from 250 g samples using Cobb's sieving and decanting technique and Baermann funnel. Nematode populations in 5 g root samples were determined by the maceration technique (Southey, 1986).

The experiment was a completely randomized block design with five replicates of each treatment. Data were subjected to analysis of variance (Cochran and Cox, 1957). Statistically significant differences among the treatments were tested by critical difference (CD) test at 5% and 1% probability levels.

Results and discussion

All inoculum levels of *M. incognita* caused significant reductions in the growth of *O. kilimandscharicum* (Table I). There was a significant ($P \leq 0.01$) reduction in root length, shoot height and shoot fresh weight, compared with the untreated control, which progressively increased with the size of the nematode inoculum although not always significantly. Oil yield also decreased significantly with increasing inoculum, compared with the untreated control except at the level of 500 J_2 per pot (Table I).

Increasing levels of inoculum also caused significant ($P \leq 0.01$) reduction in total sugars, phenol, carbon dioxide exchange rate and chlorophyll (Table II).

The reproduction factor (Pf/Pi) of *M. incognita* was greatest at an inoculum level of 500 J_2 per pot and progressively decreased with increasing inoculum level (Table III). Conversely, the root-knot index increased with an increase in inoculum.

The experimental data clearly show that *M. incognita* is capable of seriously affecting the plant growth, its metabolism and oil yield of *O. kilimandscharicum*. Findings pertaining to the relationship between different inoculum levels

TABLE I - *Effect of initial inoculum densities (Pi) of* Meloidogyne incognita *on the growth and oil yield of* Ocimum kilimandscharicum.

Pi	Plant length (cm)			Plant fresh weight (g)			Plant dry weight (g)			Oil yield (ml)
	Root	Shoot	Total	Root	Shoot	Total	Root	Shoot	Total	
0	41.2	118.2	159.4	43.2	167.2	210.4	8.0	29.6	37.6	0.55
500	38.2	106.8	145.0	38.6	145.2	183.8	7.2	25.7	32.9	0.54
1,000	34.8	99.2	134.0	34.9	132.8	167.7	6.4	23.6	30.0	0.51
2,000	31.5	89.0	120.5	33.2	119.6	152.8	6.10	21.4	27.4	0.45
4,000	28.6	79.5	108.1	32.4	102.8	135.2	5.8	18.4	24.2	0.38
8,000	24.2	66.6	90.8	30.2	75.2	105.4	5.4	13.4	18.8	0.28
16,000	21.6	46.4	68.0	27.4	41.2	68.6	4.9	7.4	12.3	0.20
LSD ($P \leq 0.05$)	1.24	3.33	4.54	1.95	4.46	5.20	0.30	0.68	0.75	0.015
LSD ($P \leq 0.01$)	1.68	4.51	6.14	2.64	6.05	7.05	0.41	0.92	1.02	0.021

TABLE II - *Effect of different initial inoculum densities (Pi) of* M. incognita *on chlorophyll, CO_2 exchange rate, total sugars and phenol content in plants of* O. kilimandscharicum.

Pi	Chlorophyll content (mg/g fresh weight)			CO_2 exchange rate (mg CO_2/ dm^2/hr	Total sugars (mg/g fr. wt.)	Total phenol (mg/g fr. wt.)
	Chl a	Chl b	Total			
0	0.68	0.41	1.11	15.94	9.86	7.82
500	0.59	0.35	0.96	13.80	8.60	6.90
1,000	0.54	0.32	0.88	12.70	7.90	6.20
2,000	0.49	0.30	0.80	11.60	7.30	5.50
4,000	0.43	0.26	0.72	10.40	6.60	5.10
8,000	0.33	0.20	0.55	7.90	5.00	4.00
16,000	0.23	0.14	0.39	5.60	3.60	2.70
LSD (P≤0.05)	0.014	0.024	0.040	0.406	0.306	0.287
LSD (P≤0.01)	0.019	0.033	0.054	0.550	0.415	0.389

TABLE III - *Effect of initial inoculum levels on the reproduction of* M. incognita on *O. kilimandscharicum.*

Pi	Final nematode population (Pf)			Reproduction factor (Rf=Pf/Pi)	Root-knot index (RKI)
	Total root	7.5 kg soil	Total		
0	–	–	–	–	–
500	3,872	14,400	18,272	36.54	0.25
1,000	8,108	18,000	26,108	26.11	1.00
2,000	19,342	23,400	42,742	21.37	1.75
4,000	31,658	32,600	64,258	16.06	3.00
8,000	44,320	39,600	83,920	10.49	3.75
16,000	45,600	42,600	88,200	5.51	4.00
LSD (P≤0.05)	1,378	1,142	3,008	0.881	0.195
LSD (P≤0.01)	1,880	1,558	4,102	1.2	0.267

of *M. incognita* and fresh/dry weight of plant, total chlorophyll content, total sugars, phenol, CO_2 exchange rate and oil yield are in general agreement with previous reports (Melakeberhan *et al.*, 1986; Hasseb *et al.*, 1990; Haseeb *et al.*, 1993).

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Literature cited

ARNON D. I., 1949. Copper enzymes in isolated chloroplasts: Polyphenol oxidase in *Beta vulgaris*. *Plant Physiol., 24*: 1-5.

BIRD A. F. and LOVEYS B. R., 1975. The incorporation of photosynthates by *Meloidogyne javanica*. *J. Nematol., 7*: 111-113.

BUHRER E. M., 1938. Additions to the list of plants attacked by the root-knot nematode (*Heterodera marioni*). *Plant Dis. Rept., 22*: 216-234.

CHARLES D. J. and SIMONS J. E., 1992. Essential oil constitutents of *Ocimum kilimandscharicum* Guerke. *J. Ess. Oil Res., 4*: 125-128.

CLEVENGER J. F., 1928. Apparatus for the determination of volatile oils. *J. Am. Pharmaceut., Assoc., 17*: 346.

COCHRAN W. G. and COX G. M., 1957. *Experimental Designs*, Vol. II, John Wiley and Sons Inc., New York, 611 pp.

EVANS K., 1982. Water use, calcium uptake and tolerance of cyst nematode attack in potato. *Potato Res., 25*: 71-88.

GOFFART H., 1931. Beobachtungen uber Pflanzenschadliche Nematoden Gartenpflanzen. *Garterobauwiss, 5*: 353-359.

HASEEB A. and BUTOOL F., 1989. Pathogenicity and reproduction of *Meloidogyne incognita* on *Ocimum sanctum. Int. Nematol. Network Newsl., 6*: 27-30.

HASEEB A., BUTOOL F. and PANDEY R., 1988. Influence of different initial population densities of *Meloidogyne incognita* on the growth and oil yield of *Ocimum basilicum* cv. French. *Indian J. Plant. Pathol., 6*: 176-179.

HASEEB A. and PANDEY R., 1987. Incidence of root-knot nematodes in medicinal and aromatic plants- New host records. *Nematropica, 17*: 209-212.

HASEEB A., PANDEY R. and HUSAIN A., 1986. Studies on the nematode diseases of *Ocimum* species. Proc. 58th Ann. Sess. Nat. Acad. Sci., India, 76 pp.

HASEEB A., SHUKLA P. K. and BUTOOL F., 1993. Relationship between initial inoculum densities of *Meloidogyne incognita* and growth/oil yield of Indian basil. *Indian J. Plant Pathol., 11*: 55-58.

HASEEB A., SHRIVASTAVA N. K. and PANDEY R., 1990. The influence of *Meloidogyne incognita* on the growth, physiology, nutrient concentrations and alkaloid yield of *Hyoscyamus niger. Nematol. medit., 18*: 127-129.

MELAKEBERHAN H., BROOKE R. C. and WEBSTER J. M., 1986. Relationship between physiological response of french beans of different ages to *Meloidogyne incognita* and subsequent yield loss. *Plant. Pathol., 35*: 203-213.

MELAKEBERHAN H., WEBSTER J. M. and BROOKE R. C., 1984. Improved techniques for measuring the CO_2 exchange rate of *Meloidogyne* infected bean plants. *Nematologica: 30*: 213-221.

RASTOGI R. P. and MEHROTRA B. N. (Eds), 1990. *Compendium of Indian Medicinal Plants*, Vol. II: 496, P.I.D.-C.D.R.I. (Lucknow - New Delhi) Publication, India, 859 pp.

SOUTHEY J. F., 1986. *Laboratory Methods for Work with Plant and Soil Nematodes*, M.A.F.F., H.M.S.O., London, 202 pp.

SWAIN T. and HILL W. E., 1959. Phenolic contents of *Prunus domestica I.* The quantitative analysis of phenolic constituents. *J. Soc. Food Agric., 10*: 63-68.

TAYLOR A. L. and SASSER J. N., 1978. *Biology, Identification and Control of Root-Knot Nematodes* (*Meloidogyne* species). Coop. Publ. Dept. Plant. Pathol., North Carolina State Univ. and U.S. Agency Int. Dev., Raleigh., N.C. 111 pp.

USTINOV A. A.,1939. *The Root-Knot Nematodes, Heterodera marioni (Cornu), in Nematodes of Agricultural Crops* (E. S. Kirjanova ed), Leningrad Acad. Sci., Moscoz and Leningrad, 274 pp.

YEMM E. W. and WILLIS A. J., 1954. The estimation of carbohydrate in plant extract by anthrone. *J. Biochem., 57*: 508-514.

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