

RELATIONSHIP BETWEEN INITIAL POPULATION DENSITY OF *MELOIDOGYNE INCOGNITA* AND GROWTH, OIL YIELD, AND BIOCHEMICAL CHANGES IN PLANTS OF *OCIMUM CANUM*

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RESUMEN

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Los efectos de varias densidades iniciales de inóculo (Pi) del nematodo de las agallas de la raíz, *Meloidogyne incognita*, en el crecimiento de la planta, los cambios bioquímicos y el rendimiento de aceite de *Ocimum canum* fueron examinados en un experimento de invernadero. El largo de las raíces y de los brotes, el peso de las plantas frescas y secas, el contenido de clorofila de las hojas, el contenido total de azúcar y fenol de las hojas y el rendimiento de aceite decrecieron con un incremento en Pi. Las agallas de las raíces fueron directamente proporcionales al Pi y la reproducción del nematodo fue directamente dependiente. La proporción de reproducción fue la más alta a un Pi de 500 juveniles de segundo estadio/2.5 kg de suelo y la más baja a un Pi de 18 000 juveniles.

Palabras clave: *Meloidogyne incognita*, nematodo agallador de la raíz, *Ocimum canum*, patogenicidad.

Several species of the genus *Ocimum*, such as *O. basilicum* Benth, *O. canum* Sims, *O. gratissimum* L., *O. kilimandscharicum* Guerke, and *O. sanctum* L., are highly priced medicinal and aromatic plants used for essential oils. *Ocimum canum* is a high oil-yielding species which has extensive application in perfumery and pharmaceutical products, The major constituents of its oil are methyl chavicol, camphor, citral, linalool, and limonene (Rastogi and Mehrotra, 1993).

The root-knot nematodes, *Meloidogyne incognita* (Kofoid and White) Chitwood and *M. javanica* (Treub) Chitwood, are major constraints to cultivation of medicinal and aromatic plants (Haseeb, 1994; Haseeb and Pandey, 1987). Although the severity of plant damage resulting from nematode infection depends on the species and population density of the nematode (Seinhorst, 1961; Webster, 1969), information on the effect of various pre-plant population densities of *M. incognita*

on the yield of *O. cimum* is still lacking. The objective of the present investigation was to determine the effect of different initial inoculum densities of *M. incognita* on the development of disease, nematode multiplication, plant growth, oil yield, chlorophyll content, CO₂ exchange rate, and total sugar and phenol content of *O. canum*.

Seed of *O. canum* (local name, Kali Tulsi) were sown in earthen pots containing a steam sterilized soil-compost (8:2) mixture. Pots were irrigated with autoclaved water as required. Twenty-one-day-old seedlings of uniform size were transplanted singly into 15-cm-diam clay pots containing 2.5 kg of a 9:1 mixture of autoclaved soil (73.2% sand, 8.6% silt, 18.2% clay; pH 7.8) and compost. Two days after transplanting, plants were inoculated with 0, 500, 1 000, 2 000, 4 000, 8 000, or 16 000 freshly hatched second-stage juveniles (J2) of *M. incognita* obtained by hand picking of egg masses from infected brinjal roots

(*Solanum melongena* L.) maintained in a glasshouse.

Physiological and biochemical data were collected from *O. canum* plants at 50 days after nematode inoculation. Carbon dioxide gas exchange rate of the third leaf (from apex) was measured in a closed polycarbonate chamber with the infra-red gas analyzer of a portable photosynthesis model LI 6000 (LiCOR, U.S.A.). Chlorophyll content of the third leaf was measured by grinding a 0.2 g leaf sample in 80% acetone with calcium carbonate and recording the absorbances of extract (25 ml final volume) at 645 and 663 nm using a Baush and Lomb Spectronic-21 (Arnon, 1949). Total chlorophyll content was calculated from the absorbances.

Estimation of total sugar was by extraction of a leaf sample (0.5 g) in ethanol and addition of anthrone reagent (Yemm and Willis, 1954). The total phenol content in leaves was estimated by extraction of a leaf sample (0.5 g) in methanol and addition of folincio calteu reagent and Na_2CO_3

(Swain and Hill, 1959). The absorbances for the estimation of sugar and phenol were 620 and 600 nm, respectively, and the contents were estimated by the glucose and gallic acid standard curves, respectively. Oil yield was determined by hydrodistillation of fresh herb using the Clevenger apparatus (Clevenger, 1928).

Taproot length, shoot height, fresh and dry weights of roots and shoots, and nematode infection were measured at 75 days after inoculation. Root gall indices were rated on a scale of 0-4, where 0=no root galling, 1=slight galling (1-25%), 2=moderate galling (26-50%), 3=severe galling (51-75%), and 4=very severe galling (76-100%). Final nematode population density from 250 g soil was determined by using Cobb's sieving and decanting technique followed by Baermann funnel (Southey, 1986). The J2 population obtained in 250 g soil was multiplied by 10 to transform it to total (2.5 kg) soil per pot. Nematode population in roots was determined by maceration of 5 g root in a Waring blender

Table 1. Effect of initial inoculum densities (Pi) of *Meloidogyne incognita* on the growth and oil yield of *Ocimum canum*.²

Pi	Length (cm)		Dry weight (g)		Oil yield (ml/ 100 g fresh wt.)
	Root	Shoot	Root	Shoot	
0	36.4	52.6	2.5	23.2	0.72
500	28.2	46.8	2.2	20.2	0.62
1 000	24.2	40.4	2.0	17.7	0.54
2 000	21.6	34.6	2.4	16.1	0.47
4 000	19.2	31.0	2.8	14.3	0.42
8 000	17.0	28.4	3.1	13.0	0.38
16 000	14.2	27.2	3.6	11.7	0.33
L.S.D. (P ≤ 0.05)	1.52	2.17	0.18	0.93	0.039
L.S.D. (P ≤ 0.01)	2.06	2.94	0.24	1.27	0.053

²Each value is an average of 5 replicates.

Table 2. Effect of initial inoculum densities (Pi) of *Meloidogyne incognita* on chlorophyll content, CO₂ exchange rate, total sugar, and total phenol content in plants of *Ocimum canum*.^a

Pi	Chlorophyll content (mg/g fresh wt.)	CO ₂ exchange rate (mg CO ₂ /dm ² /hr)	Total sugar (mg/g fresh wt.)	Total phenol (mg/g fresh wt.)
0	1.09	15.04	5.00	6.20
500	0.95	13.06	4.30	5.40
1 000	0.84	11.31	3.80	4.80
2 000	0.78	10.83	3.50	4.50
4 000	0.72	9.19	3.10	4.10
8 000	0.69	8.26	2.90	3.70
16 000	0.65	7.35	2.60	3.10
L.S.D. (P ≤ 0.05)	0.008	0.033	0.058	0.055
L.S.D. (P ≤ 0.01)	0.012	0.045	0.079	0.075

^aEach value is an average of 5 replicates.

(Pinochet *et al.*, 1995). The population obtained in 5 g root was divided by 5 and then multiplied by root fresh weight (g) to determine total final population in roots.

The experimental design was a randomized complete block with five replications. Data were analyzed by analysis of variance as described by Cochran and Cox (1957). Significant differences among the treatments were tested by least significant difference (L.S.D.) at $P \leq 0.05$ or $P \leq 0.01$.

Several plant growth parameters decreased as initial inoculum densities of *M. incognita* increased (Table 1). Root length, shoot height, shoot dry weight, and oil yield were significantly ($P \leq 0.01$) reduced at the inoculum density of 500 J2 per pot, and with the further increases in Pi, reductions were generally more severe.

Effects of *M. incognita* inoculation on root biomass were inconsistent. Significant ($P \leq 0.01$) reduction of root dry weight was observed at 500 and 1 000 J2/pot, as compared to the uninoculated control (Table 1). Root dry weights at the 0 and 2 000 J2/pot inoculum levels did not differ, but at

higher inoculum densities, root weight was significantly ($P \leq 0.01$) increased as compared to the uninoculated control. This was due to increased size and severity of gall formation on the roots. Results with root and shoot fresh weight were similar to those observed with dry weight (data not shown).

Reduction ($P \leq 0.01$) in chlorophyll content in leaves of *O. canum* was found at Pi of 500 or greater J2 as compared with uninoculated plants (Table 2). Carbon dioxide exchange rate, total sugar content, and phenol content in leaves were reduced ($P \leq 0.01$) at Pi of 500 J2 per pot. These parameters showed further decreases ($P \leq 0.05$) as the inoculum level increased.

Data on nematode populations in soil and roots, reproductive rate, and root gall index indicated significant ($P \leq 0.01$) differences among most of the treatments (Table 3). The lack of significant differences in these parameters between Pi of 8 000 and 16 000 J2 per pot indicate that the nematode reproductive rate was decreased at Pi of 8 000 J2 per pot and

Table 3. Effect of initial inoculum densities (Pi) on final population level and reproduction of *Meloidogyne incognita* in *Ocimum canum*.^y

Pi	Final nematode population			Reproduction factor (Pf/Pi)	Root-gall index (0-4)
	Total root	2.5 kg soil	Total (Pf) ^x		
0	0	0	0	0.0	0.00
500	5 700	17 200	22 900	45.9	1.00
1 000	14 500	20 600	35 100	35.1	1.25
2 000	27 600	24 800	52 400	26.2	2.75
4 000	40 000	31 600	71 600	17.9	3.50
8 000	52 700	35 400	88 100	11.0	4.00
16 000	59 000	37 000	96 000	6.0	4.00
L.S.D. (P ≤ 0.05)	6 256	2 168	8 270	4.08	0.12
L.S.D. (P ≤ 0.01)	8 533	2 956	11 279	5.54	0.16

^yEach value is an average of 5 replicates.

^xAverage value per pot (total root system and all soil).

above. Results indicate that *O. canum* is highly susceptible to *M. incognita* and that severe damage may occur at high initial population densities.

Losses in crop yield can be better understood by determining the influence of nematodes on the morphological and physiological process of yield formation (Loomis and Adams, 1980; Melakeberhan *et al.*, 1985). The decrease in chlorophyll content with the increase in nematode population density may in turn result in a decrease in the photosynthetic rate. The rate of photosynthesis is a crucial parameter influencing crop yield (Loveys and Bird, 1973; Melakeberhan *et al.*, 1985; Wallace, 1974). The results of the present investigation, in which increasing Pi of *M. incognita* reduced shoot height and weight, oil yield, total chlorophyll content, photosynthetic rate, and total sugar and phenol content, are in general agreement with previous reports (Bird, 1974; Haseeb *et al.*,

1993; Melakeberhan *et al.*, 1985; Meon *et al.*, 1978).

In general, *M. incognita* disrupts the vascular tissue of roots, which hinders the translocation of elements in roots and shoots, and decreases chlorophyll content and photosynthetic rates (Loveys and Bird, 1978; Evans, 1982; Melakeberhan *et al.*, 1985). This sequence of events apparently leads to the suppression of plant growth and oil yield of *O. canum*.

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