

EFFECT OF CADMIUM ON MULTIPLICATION OF THE ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA*, AND ITS ACCUMULATION IN TOMATO PLANTS

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Summary. Heavy metal pollution is posing a problem as it is continuously being added to agricultural soils through industrial effluents in wastewater. In the present study, tomato (*Solanum lycopersicum* cv. Pusa Ruby) plants were treated with the soil pollutant cadmium in the presence of the root-knot nematode, *Meloidogyne incognita*. The soil was treated with 7.5, 15.0, 30.0 and 60.0 ppm concentration of Cd and subsequently inoculated with 5,000 second stage juveniles of *M. incognita*/pot. There was an antagonistic association between Cd and the nematode. Nematode disease severity (i.e. degree of galling) decreased with the increase in concentration of the heavy metal. A decrease was also observed in nematode reproduction (eggs/egg mass and egg masses/root system) and size of soil population at all concentrations of Cd. Accumulation of the heavy metal was greater in nematode infected plants than in uninfected plants and in roots as compared to shoots in both the infected and uninfected tomato plants. Cadmium not only caused retardation in the growth of females but also caused aberration in their shapes. The degree of retardation and aberration was directly proportional to the concentration of the heavy metal. The antagonistic interaction between Cd and *M. incognita* multiplication was also found to be concentration dependent.

Key words: Nematode reproduction, root-knot nematode, *Solanum lycopersicum*.

Heavy metals are common environmental contaminants arising from metal mining and numerous other industrial, urban and agricultural activities (Foy *et al.*, 1978) and are being continuously added to the soil. Soil, water and plant pollution is rising due to the increases in urbanisation and industrialisation, which have resulted in increased generation of municipal waste water (Emongor, 2007).

Sewage sludge, industrial waste, rock phosphate contaminated with many toxic metals (e.g. Pb, Cd, Ni and Cr), ultimate disposal of treated and untreated waste effluents and indiscriminate use of fertilizers and pesticides in agriculture greatly influence soil health (Kar *et al.*, 2007). Soil health ultimately has its impact on soil biota, which in turn affects plant health (Langat *et al.*, 2008). Among the inorganic contaminants of waste water, heavy metals are gaining importance due to their tendency to be adsorbed by soil colloids and thereafter to be released in soil solutions.

The importance of cadmium (Cd) as a pollutant of the marine environment has been recognized for some time (Popham and Webster, 1979). It is a highly toxic heavy metal and is introduced into the soil-system as a by-product of industrial processes. It accumulates and may reach concentrations well in excess of those naturally occurring in the environment (Douglas-Wilson, 1972; Eisler, 1981; Wright, 1978). The toxicity of several Cd compounds found to be toxic to saprophytic and plant parasitic nematodes depends mainly upon their water solubility and method of application (Feldmesser and Rebois, 1966). The disposal of domestic waste water and industrial effluents have been matters of great concern in recent years (Reed and Matsumoto, 1988).

Pollutants also change the physical and chemical

characteristics of the soil, which in turn may have an impact on the population characteristics of soil-borne pathogens. They may also affect the pathogenesis of these organisms, in different ways; it may be increased or decreased through a direct effect of the pollutants on the organisms or indirectly through pollutant-induced changes in the host plant or through changes in other aspects of the environment (Bissesar *et al.*, 1983).

“Pollution Pathology” has emerged as a distinct discipline studying the interactive effects of pollutants and pathogenic agents and their impact on crops (Rich, 1964; Darley and Middleton, 1966; Heck, 1966, 1968; Kosuge, 1969; Heagle, 1973).

In the present study, an effort has been made to assess the toxic effects of Cd on the development, multiplication, fecundity and number of galls of the root-knot nematode, *Meloidogyne incognita* (Kofoid *et al.* White) Chitw. and its accumulation in tomato (*Solanum lycopersicum* L.) cv. Pusa Ruby.

MATERIALS AND METHODS

The experiment was conducted in the presence and the absence of Cd used in the form of the nitrate salt [Cd(NO₃)₂·4H₂O].

The population of *M. incognita* was raised and maintained on tomato plants in concrete culture beds, using single egg masses collected from severely infected roots of tomato growing in the Aligarh Muslim University campus. The species of root-knot nematode was identified by close examination of the perineal patterns of the females and morphometrics of the juveniles (Eisenback *et al.*, 1981). The second stage juveniles (J2) of *M. incog-*

nita were obtained by incubating nematode egg masses collected from the infected tomato plant roots. The egg masses were placed in tap water in Petri dishes at room temperature for five days to allow the eggs to hatch. The J2's in the water suspension were counted with the aid of a counting dish under a stereo microscope.

The tomato seedlings were grown in clay pots (25-cm-diameter) containing an autoclaved mixture of soil, sand and compost in the ratio of 3:1:1. After three weeks, a tomato seedling was transplanted into each of the fifteen 5-cm-diameter clay pots (three replicates \times five treatments) containing 1 kg autoclaved soil (sand 72%, silt 17%, clay 8%, organic matter 3.4-3.8%, pH ranging from 7.1 to 7.8). The pots were lined with polythene to prevent the absorption by and movement of the heavy metal through the pots.

A stock solution of cadmium was prepared considering the molecular weight of the salt (308.47). Dilutions were prepared so that 100 ml of each concentration added to the autoclaved pot soil produced concentrations of 7.5, 15.0, 30.0 and 60.0 ppm (on a dry weight basis) of the metal in relation to the known quantity of soil used per pot. In order to maintain an equal level of nitrogen in the different pots, appropriate quantities of NaNO_3 were added, taking into account the amount of NO_3 present in the different doses of $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$. Cadmium was applied to the soil just after transplantation of the seedlings in the pots and one week after the addition of Cd, the soil in each pot was inoculated with 5,000 3-days-old second stage juveniles of the root-knot nematode. Untreated and inoculated pots served as a control. Treatments were replicated three times in a completely randomized design on benches in a greenhouse. Tomato seedlings were watered regularly as and when required.

The experiment was terminated 3 months after inoculation and the plants were carefully uprooted and the roots washed free of soil. The galled roots were fixed in FAA and stained in 0.01% cotton blue in lactophenol to differentiate nematode females within the roots. The females were excised from the roots using dissecting needles under a stereoscopic microscope and mounted in lactophenol on cavity slides. Cross-sectional area of the females was determined following the method of Bird (1959). Five females per replicate were measured. Outlines of the females were drawn on a paper of uniform thickness using a Camera Lucida. The paper was cut along these outlines and the cut out areas were weighed (W1). Similarly, a 10^6 square micron ($\text{sq}\mu$) of the same paper, obtained by drawing this area by the above method using a micrometre slide, was also weighed (W2). The cross-sectional area of the females from different treatments was calculated using the formula:

$$\text{Cross-sectional area of the females} = \text{W1}/\text{W2} \times 10^6 \text{sq}\mu$$

Linear regression analysis of cross-sectional area of females of *M. incognita* at different concentrations of Cd was performed.

Determination of nematode population in soil and roots. The soil in each pot was thoroughly mixed and a 200 g sub-sample was processed according to Cobb's sieving and decanting method followed by the Baermann's funnel technique. The nematode suspension from each sub-sample was collected separately in a beaker and the volume adjusted to 100 ml. The nematode suspension was then agitated with the help of a pipette and three 10-ml aliquots were removed and placed into counting dishes and the nematodes counted. The mean number of J2s from the three individual counts was used to calculate the total nematode population density in 1 kg soil.

For estimation of the nematode population in the roots, a 1.0-g root sub-sample from each replicate was blended with enough water in a Waring blender for approximately 30-40 seconds. The homogenate was collected in a beaker and the volume adjusted to 100 ml. The nematode population was counted as described for the soil samples (Southey, 1986). The entire root system from each replicate pot was used for counting root galls and egg masses under a stereoscopic microscope. To differentiate egg masses from root tissue, the roots were stained in a Phloxine B solution (0.15 g/l water) for about 20 minutes. The mean number of eggs/egg mass was determined by counting the eggs extracted from 100 egg masses from each of the five replicates per treatment, obtained by agitating the egg masses in a solution of 1% NaOCl in a Waring blender (Khan and Khan, 1994). The nematode reproduction factor (*Rf*) was calculated by the formula $Rf = Pf/Pi$ (where, *Pi* is the initial population and *Pf* the final population of the nematode).

Determination of the heavy metal accumulated in plant parts. The plant samples for analysis of the heavy metal were prepared by washing them several times with double distilled water and then dried at 105 °C for 24 h. The dried plant material was ground with a pestle and mortar and 5 g of the powdered material were digested in 20 ml of boiling Analar HNO_3 in a 50 ml Kjeldhal flask. The digestion was usually completed within about half an hour. The digests were adjusted to 25 ml by adding the required quantity of HNO_3 and the quantity of Cd was determined using an Atomic Absorption Spectrophotometer (Harding and Whitton, 1981). Also, a stock solution of 100 ppm of Cd was prepared using cadmium oxide and different concentrations obtained by dilution. The wavelength was set at 228.8 nm and readings of the standard solutions of known concentrations were noted so that the concentrations in plant samples could be calculated by interpolation.

The study was conducted for two consecutive years. However, as the data were similar in both years, only those of the second year are presented.

Statistical analysis. The data were subjected to analysis of variance and the means were compared using Fisher's protected LSD test at $P = 0.05$.

RESULTS AND DISCUSSION

There was a significant decrease in nematode population, fecundity and number of galls at all doses of cadmium as compared to the control (untreated-inoculated) (Table I). Also, a direct correlation was observed between the reduction in nematode population density and the concentration of Cd. In the pots that received Cd, maximum Rf (2.59), number of egg masses/g root (19), eggs/egg mass (125) and galls/root system of tomato (28) occurred at 7.5 ppm concentration. However, in the control these variables were greater (Rf 3.7, egg mass/g root 95, eggs/egg mass 310 and galls/root system 85) than in all Cd treated-inoculated plants. A non-significant effect of Cd was observed on Rf when comparing 15.0 and 30.0 ppm treatments and in fecundity and number of galls when comparing 15.0 and 30.0 and 60.0 ppm treatments.

The toxic effect of Cd also altered the normal growth of *M. incognita* females (Table II). Cadmium exhibited toxicity at all concentrations (7.5-60.0 ppm). The retardation and deformation in size and shape of the mature females was proportional to the increase in concentra-

tions of Cd. It was also evident, from the regression line, that there was a negative linear relationship between the concentration of Cd and the cross-sectional area of females of *M. incognita* (Fig. 1). The significant percentage reductions in the growth of females were 21.9, 37.0, 60.1 and 74.7 at 7.5, 15.0, 30.0 and 60.0 ppm, respectively (Table II, Fig. 2).

The amount of Cd accumulated was generally greater in the plants inoculated with *M. incognita* than in the uninoculated plants. Moreover, the metal accumulation was more in roots than in shoots irrespective of the nematode inoculation. In the uninoculated treated plants it was 1.5, 4.0, 12.0 and 15.0 µg/g in the shoots and 4.0, 11.0, 12.5 and 12.5 µg/g in the roots at 7.5, 15.0, 30.0 and 60.0 ppm of Cd in the soil, respectively. The accumulation was greater in the inoculated set of plants, being 5.0, 10.0, 12.0 and 12.5 µg/g in shoots and 6.0, 13.0, 14.5 and 15.0 µg/g in roots, respectively (Table III).

All concentrations of Cd applied to the soil were toxic to the nematode and reduced its development and multiplication. Results indicating a reduction in Rf, egg masses, eggs/egg mass and of galls/root system following an increase in the concentration of Cd agree with earlier

Table I. Effect of cadmium (Cd) on reproduction of *Meloidogyne incognita* (Mi) on tomato in a greenhouse 3 months after soil infestation.

Treatment	Females/root	Juveniles/kg soil	Total	Rf ¹ = Pf/Pi	Egg masses/g root	Eggs/egg mass	Galls/root
Control (untreated + inoculated)	265	18,514	18,779	3.75	95	310	85
Cd (7.5 ppm) + Mi	145	12,834	12,979	2.59	19	125	28
Cd (15.0 ppm) + Mi	127	1,849	1,876	0.39	11	64	9
Cd (30.0 ppm) + Mi	40	713	753	0.15	8	38	6
Cd (60.0 ppm) + Mi	24	316	340	0.075	3	23	4
LSD (P = 0.05)	97.45	1177.63	-	0.97	6.42	42.67	9.89
LSD (P = 0.01)	141.78	1713.33	-	1.40	9.34	62.0	14.

Each value is the average of three replicates.

¹Rf = Pf/Pi, where Rf = Reproduction factor, Pf = Final population, Pi = Initial population.

Table II. Effect of cadmium (Cd) on the growth of females of *M. incognita* in tomato cv. Pusa Ruby in a greenhouse 3 months after soil infestation.

Treatment	Cross-sectional area of nematode females (sqµ)	% reduction over control
Untreated	203,611.7 (201,115.2)	-
7.5	158,942.4 (162,938.4)	21.94
15.0	128,442.2 (124,761.6)	36.92
30.0	81,225.4 (86,584.7)	60.11
60.0	51,586.1 (48,407.9)	74.67
LSD (P = 0.05)	23,617.39	
LSD (P = 0.01)	34,360.95	

Each value is the average of three replicates.

Regression equation $y = 124761.56 - 38176.82(x - 2.00)$

In parentheses are given the values calculated using the regression equation.

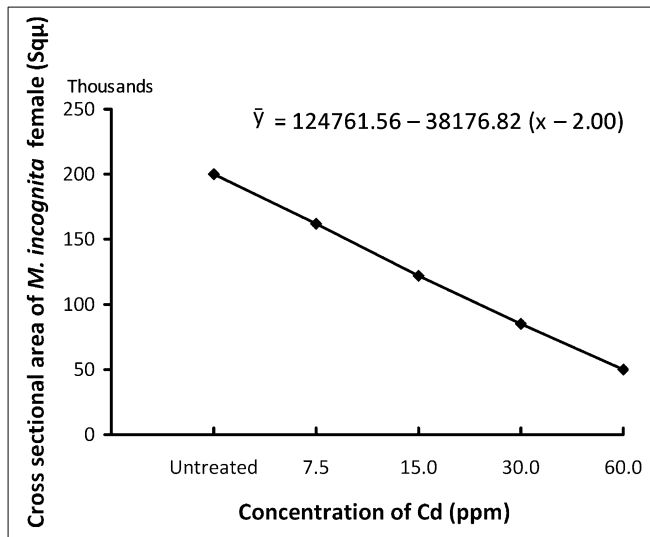


Fig. 1. Regression line showing the negative linear relationship between different concentrations of cadmium (Cd) and cross-sectional areas of females of the root-knot nematode, *Meloidogyne incognita*.

studies (Parveen, 2004), where decrease in hatching and increase in mortality of juveniles of *M. incognita* had a direct correlation with the concentration of Cd, which, in turn, had an impact on nematode development, multiplication and disease development. The results of this investigation also agree with those of Khan *et al.* (2006), who reported an antagonistic association between *M. incognita* and a higher dose of Ni (400 mg/l) in soil.

Van Kessel *et al.* (1989) reported retardation in growth, at the early juvenile stage of *Caenorhabditis elegans* (Maupas, 1900) Dougherty, 1955 (a marine nematode), at 160 and 320 μM concentration of CdCl_2 . This nematode did not reach the adult stage and, therefore, did not reproduce.

In the presence of Cd, the females of *M. incognita* showed significant retardation of growth and deformity in their shape and size as compared to the control. Various workers have shown that the application of Cd to the soil alters the nutrient concentration and growth of plants (Walker *et al.*, 1977).

The presence of heavy metals in the soil changes its nutrient status and the availability of nutrients to plants

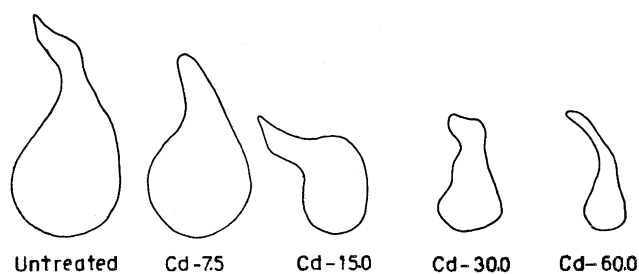


Fig. 2. Representative females from different treatments of Cd three months after soil infestation with *M. incognita*.

due to the nutrient-heavy metal interaction. It has also a great impact on the population, growth and development of soil biota (Samiullah *et al.*, 1996). Owino *et al.* (1992) found that egg development and parasitism of *M. incognita* and *Heterodera schachtii* Schmidt in the presence of sodium hypochlorite decreased with an increase in the duration of the treatment and the concentration of sodium hypochlorite.

Toxic heavy metals also interfere with the uptake and metabolism of nutrients and this may result in physiological starvation of females and juveniles of the nematode (Popham and Webster, 1979). The growth of females of *M. incognita* may be affected directly due to the toxic effects of the heavy metal or indirectly by feeding of females on the host plants growing under stress conditions.

The results clearly indicated that the heavy metal accumulated in both infected and uninfected plants but that the accumulation was more in the nematode inoculated plants. Moreover, the amount of Cd was greater in roots than in shoots in both infected and uninfected plants. This could be attributed to disruption in the translocation of the nutrients caused by the nematode and therefore greater accumulation of cadmium in the roots (Willcox-Lee and Loria, 1987). Due to the disruption of the root physiology, the translocation of the heavy metal to the shoots of infected plants was less controlled than to the shoots of the uninfected plants and therefore resulted in greater accumulation of Cd in the shoots of the infected plants. The increase in accumulation due to the presence of the nematode was greater at low concentrations of the metal (i.e., 7.5 and 15.0 ppm). It is quite apparent from the results that in the absence of the nematode the movement of Cd was more restricted than in inoculated plants. In the inoculated plants, it appears that the damage caused by the nematode predisposed the plants to increased uptake of Cd, thus causing more damage to the plants.

These findings agree with Bisessar *et al.* (1983), who found that the nickel content of stalks and leaves of plants grown in soil contaminated with the heavy metal and infested with the root knot nematode *Meloidogyne hapla* Chitw. were significantly higher than that in stalks and leaves of plants grown in the same soil without the nematodes. They reported the heavy metal concentrations in celery as greatest in roots, followed by leaves and then stalks. This had also been observed by other researchers (Agarwala *et al.*, 1977; Cataldo *et al.*, 1978).

However, Nyczepir *et al.* (2006) reported contradictory results. These authors found that Ni deficiency was greater in pecan seedlings (in the young leaflets) growing in *Meloidogyne paritityla* Kleyhans infested soil vs uninoculated soil. It may be concluded that it is not always true that metal accumulation will be greater in plants infected with root knot nematodes as it is highly variable and depends upon the metal concentration in the soil.

Relatively little is known about the factors that control

Table III. Concentration of cadmium (Cd) in tomato cv. Pusa Ruby infected with *M. incognita* in a greenhouse 3 months after soil infestation.

Treatment (rates of Cd in ppm)	Amount of cadmium in plant material on a dry weight basis ($\mu\text{g/g}$)					
	Uninoculated			Inoculated		
	Shoot	Root	Average	Shoot	Root	Average
0	-	-	-	-	-	-
7.5	1.5	4.0	2.75	5.0	6.0	5.50
15.0	4.0	11.0	7.50	10.0	13.0	11.50
30.0	12.0	12.5	12.25	12.0	14.5	13.25
60.0	15.0	12.5	13.75	12.5	15.0	13.75
LSD (P = 0.05)	1.36	1.49	-	1.28	1.51	-
LSD (P = 0.01)	1.98	2.17	-	1.86	2.20	-

Each value is the average of three replicates.

Inoculum level = 5000 J_2 of *M. incognita*/kg soil.

the availability of the metals at the root/soil interface. Koepp (1977) reviewed the uptake and translocation of lead (Pb). He found that translocation of heavy metals is highly dependent on the physiological status of the plant.

The present study clearly indicates that there was direct correlation between the concentration of the heavy metal and development and pathogenesis of the nematode. All concentrations tested were nematicidal and the highest concentration of cadmium was found to be highly toxic for the survival of the nematode. Inference can also be drawn from the present study that soils contaminated with heavy metals, particularly Cd, at high concentrations affect the growth of tomatoes either directly or indirectly by affecting the nematode population in the soil. However, field trials are required to determine the losses in yield of tomato crops grown in soils contaminated with heavy metals and nematodes.

This study may help in understanding the behaviour of pathogens under stressed conditions, to trace out the impact of the stress on the pathogenesis and pathogenicity of the organism concerned, and to understand and evaluate the effects of heavy metal pollution in the soil eco-system and its effects on plant growth, which are suitable indicators for assessing soil health. This role for nematodes as bio-indicators of soil health has already been demonstrated (Bongers and Ferris, 1999; Chen *et al.*, 2003).

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