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EFFECTS OF NICKEL AND ROOT-KNOT NEMATODE ON THE GROWTH AND PROTEIN CONTENT OF CHICKPEA

by

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Summary. Single and joint effects of *Meloidogyne javanica* and nickel as $NiCl_2$ (10, 50, 100 and 200 ppm) were studied on the plant growth, flowering, fruit-setting, seed protein and root nodulation on chickpea. Nickel at 100 and 200 ppm caused pigmentation of leaf margins and significantly suppressed plant growth, seed protein and nodulation. The nematode also caused similar negative effects. The infected and treated plants developed more Ni injury (at all concentrations) and root galling (at 50 ppm). Egg mass production and fecundity, however, decreased in the treated plants (except 10 ppm). A synergistic relationship between *M. javanica* and Ni (50 ppm) was apparent resulting in a greater suppression of the variables considered. Ni at 200 ppm reduced the negative effect of the nematode.

Industrial effluents containing nickel, carried to crop production units through irrigation, can adversely affect plant growth (Ajmal and Khan, 1984; Khan *et al.*, 1987) and in general influence the composition of the microflora and microfauna of the soil (Remacle and Houba, 1993). Several reports of the effects of heavy metals on plant pathogens have been published (Nordgren *et al.*, 1985 and 1986) but relatively few have concerned nematodes. In a glasshouse study, heavy metal contaminated soil enhanced the negative effect of *Meloidogyne hapla* by 20% on the growth of lettuce leaves (Temple and Bissessar, 1981). Bissessar (1983) observed significantly more galling caused by *M. hapla*, on celery in soil polluted with heavy metals (primarily nickel).

The present investigation was undertaken to ascertain the effect of nickel chloride on the pathogenicity and reproduction of *Meloidogyne javanica* (Treub) Chitw. and consequent effects on the plant growth and protein contents of chickpea (*Cicer arietinum* L.).

Materials and methods

Four concentrations viz. 10, 50, 100 and 200 ppm of nickel chloride ($NiCl_2 \bullet 6H_2O$) were prepared in distilled water. The solution (550 ml/pot) was added to clay pots filled with 1.5 kg autoclaved soil (field soil and compost 3:1). For each concentration, ten pots were maintained in which *Rhizobium* treated seeds of chickpea were sown (5 seeds/pot). A set of 10 pots which did not receive $NiCl_2$ solution served as a control. A week after sowing, the germinated seedlings were thinned to one per pot. Two days later, five pots of each concentration were inoculated with 2000 freshly hatched juveniles of *M. javanica*. The pots were placed in a screen house and arranged in

a complete randomized block. Watering was done at regular intervals. During the experiment, the plants were regularly observed for the appearance of Ni injury and the number of flower buds formed were recorded. However, pods were counted at harvest.

The plants were harvested three months after sowing and length and fresh and dry weights of shoots and roots were determined. Roots were visually examined to count the functional (pink) and nonfunctional (dark brown) nodules. Roots of nematode inoculated plants were treated with phloxine B (0.15 g/l) to facilitate the counting of galls and egg masses. Fecundity was determined by excising 20 egg masses from each root system (total 100 egg masses) which were blended in 1% NaOCl solution (Khan and Khan, 1994). Protein content (soluble and insoluble) was estimated using the method given by Lowry *et al.* (1951).

The data on plant growth variables, nodulation, flowering, pod formation and seed protein content were subjected to a two-factor analysis of variance (ANOVA) and least significant differences (L.S.D.) were calculated at P=0.05. The data on galling and nematode reproduction were, however, processed for the one-factor ANOVA (Dospekhov, 1984).

Results and discussion

Nickel chloride at 200 ppm caused pigmentation (browning) of the leaf margins of uninoculated plants. However, plants inoculated with *M. javanica* developed pigmentation at 100 and 200 ppm $NiCl_2$, being greater at the higher concentration. Nematode symptoms on roots were considerably higher at 50 ppm of $NiCl_2$ and lower at 200 ppm $NiCl_2$. Nickel chloride (100

TABLE I - *Effect of nickel chloride and* Meloidogyne javanica *on plant growth and dry matter production of chickpea.*

Treatment		Length cm		Fresh Weight g		Dry Weeight g	
$NiCl_2$ ppm	Nematode (J_2) inoculation	Shoot	Root	Shoot	Root	Shoot	Root
0	0	31.4	24.9	11.5	8.6	3.9	1.7
10	0	31.0	24.1	11.4	8.6	3.9	1.7
50	0	28.5*	23.3*	11.3	8.5	3.8	1.7
100	0	26.8*	22.0*	10.6*	8.2	3.5*	1.6*
200	0	25.6*	19.9*	10.1*	7.9*	3.3*	1.5*
0	2000	28.0*	22.8*	10.4*	7.9*	3.5*	1.5*
10	2000	26.5*	22.5*	10.4*	7.9*	3.4*	1.5*
50	2000	23.2*	20.4*	10.2*	7.8*	3.2*	1.4*
100	2000	24.3*	20.1*	9.9*	7.7*	3.2*	1.4*
200	2000	24.2*	18.9*	9.6*	7.2*	3.1*	1.4*
L.S.D. (P=0.05)		1.72	1.28	0.56	0.44	0.18	0.09
F value							
Ni (df=4)		28.3**	24.7**	19.1**	8.3**	23.5**	17.2**
Nematode (df=1)		16.9**	13.5**	14.8**	15.7**	14.9**	15.7**
Ni x Nematode (df=4)		NS	NS	NS	NS	4.1**	4.3**

* = Significantly different from the control at P=0.05, ** = significant at P=0.01; NS=Not significant.

TABLE II - *Effects of nickel chloride and* M. javanica *on root nodulation, flowering, pod formation and seed protein of chickpea.*

$NiCl_2$ ppm	Nematode (J_2) inoculation	Number per root system		Number per root system		Seed protein %
		Functional nodules	Non-functional nodules	Flowers	Pods	
0	0	58	35	37	34	26.7
10	0	58	35	37	33	26.5
50	0	54*	39*	36	33	26.2
100	0	52*	38*	36	31*	24.5*
200	0	49*	31*	34*	28*	24.0*
0	2000	48*	31*	34*	29*	23.4*
10	2000	43*	31*	34*	29*	23.0*
50	2000	42*	30*	33*	27*	21.8*
100	2000	42*	32*	32*	26*	21.3*
200	2000	41*	28*	32*	25*	21.2*
L.S.D. (P=0.05)		2.74	1.90	1.65	1.37	0.98
F value						
Ni (df=4)		25.2**	17.9**	NS	10.2**	9.5**
Nematode (df=1)		28.5**	14.3**	7.2**	10.8**	16.6**
Ni x Nematode (df=4)		4.7**	NS	NS	5.1**	NS

* = Significantly different from the control at P=0.05, ** = significant at P=0.01; NS=Not significant.

and 200 ppm) and *M. javanica* acting alone significantly suppressed the length and fresh and dry weights of shoots and roots of chickpea compared to uninoculated-untreated plants (Table I). The inoculated and Ni treated plants experienced significantly greater suppressions in the variables considered, especially at 50 ppm. At 100 and 200 ppm, a decrease in the negative effect of the nematode occurred, leading to relatively less damaging effect on plants. The interactive effect of Ni and the nematode was significant for dry weights (Table I).

Formation of functional nodules was significantly suppressed at all the concentrations of Ni except 10 ppm (Table II). The nematode alone caused a decline of 17.2% in the number of functional nodules. Nodulation was further suppressed in the inoculated-treated plants. Number of nonfunctional nodules, however, significantly increased in treatments with 50 and 100 ppm $NiCl_2$, whereas in rest of the single or joint treatments, a decline was recorded in the nodule numbers (Table II). Flowering was significantly suppressed in all the treatments except 10, 50 and 100 ppm $NiCl_2$ in uninoculated plants. Pod formation was relatively more affected as a result of treatments with Ni and/or *M. javanica* (Table II). The seeds of the plants treated with Ni (100 and 200 ppm) or inoculated with the nematode contained significantly less protein (Table II). In the joint treatments, especially at 10 and 50 ppm $NiCl_2$, the reduction in seed protein was greater than the sum of the reduction caused by $NiCl_2$ and the nematode separately. The interactive effects of $NiCl_2$ and the nematode were significant for functional nodules and pods (Table II).

The gall formation and egg mass production of *M. javanica* on chickpea roots were significantly higher at 50 ppm $NiCl_2$, whereas at 100 and 200 ppm, significant decreases were observed compared to untreated plants (Table III).

TABLE III - *Effects of Nickel chloride on galling, egg mass production and fecundity of* M. javanica *on chickpea.*

$NiCl_2$ ppm	Number/root system		Number/g dry root		Fecundity
	Galls	Egg masses	Galls	Egg masses	
0	83	78	49	46	271
10	85	78	50	46	276
50	104*	84*	61*	49*	218*
100	87*	59*	54*	37*	160*
200	61*	27*	41*	39*	93*
L.S.D. P=0.05	4.3	3.8	4.1	2.5	11.5
F value	74.9**	77.2**	21.0**	22.4**	96.2**

* = Significantly different from the control at P=0.05, ** = significant at P=0.01.

Number of galls and egg masses per g dry root weight also showed a more or less similar response to nickel. The fecundity, however, significantly decreased at the concentrations of $NiCl_2$ used, except at 10 ppm (Table III).

In conclusion nickel chloride at 100 and 200 ppm significantly suppressed yield and protein contents of chickpea. Higher pigmentation on the foliage of infected plants indicates that nematode infection led to greater uptake of the metal by the roots. The nature of the interaction between *M. javanica* and nickel chloride was concentration dependent. Significantly higher root-knot disease and reproduction of the nematode at 50 ppm $NiCl_2$ led to synergistic interaction, causing yield loss greater than those expected from their individual effects. The interaction at 100 and 200 ppm was antagonistic.

The study demonstrated that nickel at 50 ppm, which is a concentration that may be expected to occur around a nickel source, may result in an increase in the incidence and severity of root-knot disease on chickpea.

Literature cited

AJMAL M. and KHAN A. U., 1984. Effects of breweries effluents on soil and crop plants. *Environ. Pollut., 33*: 341-351.

BISESSAR S., 1983. Effects of heavy metals and *Meloidogyne hapla* on celery grown on organic soil near a nickel refinery. *Pl. Dis., 67*: 11-14.

DOSPEKHOV B. A., 1984. *Field Experimentation.* Mir Publishers, Moscow, Russia, pp. 352.

KHAN M. R. and KHAN M. W., 1994. Effects of simulated acid rain and root-knot nematode on tomato. *Pl. Pathol., 43*: 41-49.

KHAN M. R., SINGH, S. K. and KHAN M. W., 1987. Effect of nickel on growth of lentil plants. *IAWPC Tech. Ann., 14*: 57-60.

LOWRY O. H., ROSEBROUGH N. J., FARR A. L. and RANDALL R. J., 1951. Protein measurement with folin phenol reagent., *J. biol. Chem., 193*: 265-275.

NORDGREN A., BAATH E. and SODERSTROM B., 1985. Soil microfungi in an area polluted by heavy metals. *Can J. Bot., 63*: 448-455.

NORDGREN A., KAURI T., BAATH E. and SODERSTROM B., 1986. Soil microbial activity, mycelial lengths and physiological groups of bacteria in a heavy metal polluted area. *Environ. Pollut., 41*: 89-100.

REMACLE J. and HOUBA C., 1993. Influence of metal pollutants on microbial populations. *In: Frontier in Applied Microbiology.* K. G. Mukerji and V. P. Singh, eds. Rastogi Publishers, India pp. 131-151.

TEMPLE P. J. and BISESSAR S., 1981. Uptake and toxicity of nickel and other metals in crops grown on soil contaminated by a nickel refinery. *J. Pl. Nutr., 3*: 473-482.

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