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Damage potential and reproductive fitness of root-knot nematode on some medicinal herbs in Korea

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Summary. The damage potential and reproduction of root-knot nematodes, *Meloidogyne hapla* on medicinal herbs, *Angelica dahurica*, *Codonopsis pilosula* and *Glycyrrhiza uralensis*, was tested in potted soil under glasshouse conditions. Three-week-old seedlings were inoculated with initial population densities (Pi) of 1000, 2000, 3000, 4000 and 5000 second-stage juveniles (J2)/plant. Significant damage was observed on shoot and root lengths, fresh root weight and root diameter of *A. dahurica*, *C. pilosula* and *G. uralensis* by all Pi levels 90 day post-inoculation. Damage increased with increase in Pi. 5000 Pi caused 31.7, 34.0 and 25.2% reduction in root lengths of *A. dahurica*, *C. pilosula* and *G. uralensis*, respectively. Higher root galling index was observed on *A. dahurica* than on *C. pilosula* and *G. uralensis* at all Pi levels. At the highest Pi, root gall index was 5.0, 4.2 and 3.8 on *A. dahurica*, *C. pilosula* and *G. uralensis*, respectively. Increasing rate of Pi exponentially reduced reproduction factor (Rf) of *M. hapla* on all the three species of medicinal herbs. However, the Rf was higher on *A. dahurica* than on *C. pilosula* and *G. uralensis* at all Pi levels.

Key words: *Angelica dahurica*, *Codonopsis pilosula*, *Glycyrrhiza uralensis,* Korea, medicinal plants, *Meloidogyne hapla*, pathogenicity.

Medicinal herbs have always been important and are in use directly or indirectly for the benefit of mankind. In the past they were only available from mountains and other inaccessible areas, thus making them rare commodities. However, with the advance in agriculture and the increased availability of markets, cultivation of medicinal herbs started as cash crop and has increased many folds to meet the requirement of pharmaceutical industries. However, due to limited arable land in Korea, the same fields have been continuously used to cultivate the same species of medicinal herbs for several years. In such an intensive and continuous cultivation system, soil borne diseases, especially nematodes, often become an important constraint for susceptible hosts. Comprehensive lists of nematode pests of medicinal herbs and their distribution have been compiled (Kim *et al.*, 1987; Choi & Park, 1991; Park *et al.*, 1992; 1993) but the study of nematodes as pests of medicinal herbs has received little attention so far.

Root-knot nematodes (*Meloidogyne* spp.) are found throughout the world and can infect many plant families. Northern root-knot nematode, *Meloidogyne hapla* Chitwood, is commonly found in fields cultivated with medicinal herbs in Korea (Kim *et al.*, 1987; Park *et al.*, 1992, 1993, 1998 a,b,c; Chung *et al.*, 2004). Although the pathogenicity of *M. hapla* on various crops in Korea has been reported (Cho *et al.*, 1986; Cho *et al.*, 1994), the damage potential of *M. hapla* on medicinal herbs remained mostly unexplored. A few reports exist on the susceptibility of *Angelica gigas*, *Paeonia lactiflora* and *Discorea polystachya* to *M. hapla* (Han, 1994; Park *et al.*, 1999). Information on host suitability of medicinal herbs for *M. hapla* is important for continued sustainable production and to determine sustainable management practices. The objective of the present work was to evaluate the damage potential and reproduction fitness of *M. hapla* on *Angelica dahurica*, *Codonopsis pilosula* and *Glycyrrhiza uralensis*, which are commonly, cultivated medicinal herbs in Korea.

MATERIALS AND METHODS

The effect of initial population density (Pi) of *M. hapla* on plant growth, root galling and nematode reproduction was studied on *A. dahurica, C. pilosula* and *G. uralensis* in potted soil under glasshouse conditions. A single population of *M. hapla* was maintained on tomato plants (*Lycopersicon esculentum* Mill) *cv.* Rutgers starting from a single nematode egg mass. To obtain inocula for experiments, *M. hapla* eggs were extracted from galled tomato roots by shaking in 1% NaOCL (Hussy & Baker, 1973). Extracted eggs were gently washed in a stream of tap water three times to remove NaOCl. Eggs were separated from root residues by centrifugation (Coolen, 1979) and incubated for 4 days using modified Baermann trays to obtain second-stage juveniles (J2) for inoculation (Rodriguez-Kabana & Pope, 1981). The population of J2 released was determined from 10 replications of 1-ml aliquots of the inoculum suspension.

Three seeds of each species of the medicinal herbs, *A. dahurica, C. pilosula* and *G. uralensis* were sown into 15-cm diameter clay pots, containing 1000 cm^3 of steam-sterilized soil-compost mixture (3:1). After three weeks of germination, plants were thinned to one/pot and inoculated separately with 1000, 2000, 3000, 4000 and 5000 freshly hatched J2 of *M. hapla* suspended in 30 ml water/pot. J2 were added to soil around the roots in each pot by removing topsoil to a depth of 2-3 cm and pipetting nematode suspension on to the exposed roots. Roots were then covered with soil. Control pots received only water. Each treatment was replicated five times. Pots were arranged in complete randomized block design on benches in a glasshouse maintained at 23-26°C. Plants in pots were watered as needed to maintain the soil at field capacity and fertilized once with urea (0.20g/pot) 40 days after nematode inoculation. The experiment was terminated at 90 day post-inoculation. Plants were carefully uprooted from pots and the roots were washed gently with tap water to free them from adhering soil particles and stained with Phloxine B (0.15 g l^{-1} tap water). Plant shoot and root lengths, fresh root-weight, and root-diameter were recorded. Root galling index in nematode infected plants was assessed on a 0-5 scale according to the percentage of galled tissue, as follows (0 = 0-10%, 1 = 11-20%, 2 = 21-50%, 3= 51-80%, 4 = 81-90%, and 5= 91-100% (Barker, 1985). Nematodes from 100 cm^3 samples of infested potting soil and eggs from 5 g roots of each plant were extracted by centrifugation (Coolen, 1979), as described for inoculum preparation. Extracted eggs and nematodes were used to estimate final nematode population densities. The reproduction factor, Rf = Pf/Pi, was calculated (Pf is the average final population count of eggs and nematodes and Pi, the original inoculum of J2). All data were subjected to statistical analysis. Analyses of variance were carried out using Statistix 7.0 (NH Analysis software, Roseville, MN). Regression analyses were conducted by the least-squares program for nonlinear models of Statistical Analysis System 6.08 (SAS Institute Inc., Cary, NC). Duncan's multiple range tests was employed to test for significant difference between treatments at $P \geq 0.05$.

RESULTS AND DISCUSSION

The data obtained at 90 day post-inoculation, showed that plant shoot and root lengths, fresh root weight and root diameter of *A. dahurica, C. pilosula* and *G. uralensis* were reduced at all Pi compared with the non-inoculated (control) (Tables 1-3). However, the suppression of plant growth increased with the increased Pi. Inoculation with 5000 J2 of *M. hapla* reduced shoot length by 27.5, 33.0 and 23.9% in *A. dahurica, C. pilosula* and *G. uralensis*, respectively compared with the control. Fresh-weight, length and diameter of roots showed similar decreasing responses to increasing Pi. At the highest Pi (5000 J2) root length decreased by 17.9, 33.0 and 17.8%; fresh-root weight decreased by 43.8, 38.9 and 44.0%, and root-diameter decreased by 22.2, 44.1 and 23.1% in *A. dahurica, C. pilosula* and *G. uralensis,* respectively compared with the control. Similarly root gall index increased with the increase in Pi (Fig. 1). However, a greater root gall index was observed on *A. dahurica* than on *C. pilosula* and *G. uralensis* at all Pi levels ($P > 0.05$). At the highest Pi, root gall index was 5.0, 4.2 and 3.8 on *A. dahurica, C. pilosula* and *G. uralensis,* respectively.

These results on *A. dahurica*, *C. pilosula* and *G. uralensis* clearly indicated that *M. hapla* caused substantial damage in terms of both quality and quantity of roots, which are used for pharmaceutical purposes. Han (1994) has also reported that 20% and 30% decrease in root-growth of a medicinal herb, *Angelica gigas* in Korea when inoculated with 1000 and 5000 J2 of *M. hapla*, Pandey & Haseeb (1997) reported that *M. incognita* significantly reduced plant growth and suppressed

Table 1. Effect of population densities of *Meloidogyne hapla* on growth of *Angelica dahurica* 90 days post-inoculation.

Inoculum density (Pi)	Shoot		Root					
	Length (cm)	Reduction (%)	Length (cm)	Reduction (%)	Fresh Wt (g)	Reduction (%)	Diameter (mm)	Reduction (%)
0 (Control)	21.5 a	–	23.0 a	–	13.7 a	–	12.2 a	–
1000	20.0 b	7.0	21.5 b	6.5	12.6 b	8.0	11.0 b	9.8
2000	18.7 c	13.0	19.0 c	18.6	11.4 c	16.8	10.4 b	14.8
3000	17.5 d	18.6	17.6 d	23.5	10.5 d	23.4	9.5 c	22.1
4000	16.7 de	22.3	16.2 e	29.6	9.2 e	32.8	9.0 c	26.2
5000	16.2 e	24.7	15.7 e	31.7	8.9 e	35.0	8.8 c	27.9

Table 2. Effect of population densities of *Meloidogyne hapla* on growth of *Codonopsis pilosula* 90 days post-inoculation.

Inoculum density (Pi)	Shoot		Root					
	Length (cm)	Reduction (%)	Length (cm)	Reduction (%)	Fresh Wt (g)	Reduction (%)	Diameter (mm)	Reduction (%)
0 (Control)	22.9 a	–	10.6 a	–	0.90 a	–	3.4 a	–
1000	21.0 b	8.3	10.1 a	4.7	0.80 ab	11.1	3.1 b	8.8
2000	18.0 c	21.4	9.3 b	12.3	0.72 bc	20.0	2.8 c	17.6
3000	16.0 d	30.1	8.5 c	19.8	0.66 cd	26.7	2.5 cd	26.5
4000	15.0 e	34.5	7.5 d	29.2	0.58 d	35.6	2.2 d	35.3
5000	13.6 f	40.6	7.0 d	34.0	0.56 d	37.8	2.1 d	38.2

Table 3. Effect of population densities of *Meloidogyne hapla* on growth of *Glycyrrhiza uralensis* 90 days post-inoculation.

Inoculum density (Pi)	Shoot		Root					
	Length (cm)	Reduction (%)	Length (cm)	Reduction (%)	Fresh Wt (g)	Reduction (%)	Diameter (mm)	Reduction (%)
0 (Control)	14.2 a	–	13.5 a	–	1.5 a	–	5.2 a	–
1000	13.4 ab	5.6	12.8 ab	5.2	1.3 b	13.3	4.8 ab	7.7
2000	12.8 ab	9.9	11.9 bc	11.9	1.1 c	26.7	4.4 abc	15.4
3000	11.7 c	17.6	10.8 cd	20.0	1.0 cd	33.3	4.1 bc	21.2
4000	10.7 cd	24.6	10.3 d	23.7	0.96 cd	36.0	3.9 c	25.0
5000	10.0 d	29.6	10.1 d	25.2	0.88 d	41.3	3.8 b	26.9

Wt: weight. Columns means followed by the same letter do not differ significantly, according to Duncan's Multiple Range Test (P = 0.05)

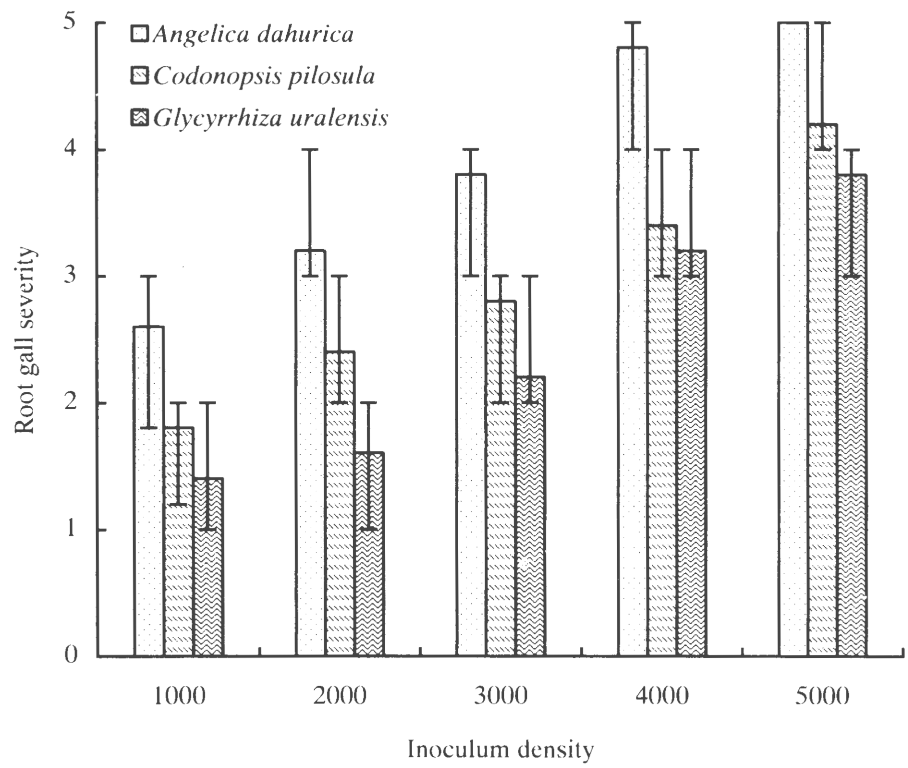

Fig. 1. Relationship between inoculum densities (Pi) of *Meloidogyne hapla* and root galling index on *Angelica dahurica, Codonopsis pilosula* and *Glycyrrhiza uralensis* 90 days post-inoculation.

root development in medicinal herbs *Ammi visnaga*, *Costus speciosus* and *Solanum indicum* and the damage varied proportionally to nematode Pi. Park *et al.* (1999) studied the pathogenicity of *M. hapla* on *Paeonia lactiflora* (a medicinal herb), in pots under glasshouse conditions in Korea. Similarly, they found that the weight, length and diameter of the main root decreased significantly with the increase in Pi up to 10000 eggs/plant.

The reproductive factors (Rf) of *M. hapla* were greater on *A. dahurica* than on *C. pilosula* and *G. uralensis* at all inoculum levels (Fig. 2). This difference may be linked to root biomass or degree of susceptibility. However, higher Pi resulted in lower Rf values in these glasshouse experiments. The Rf of *M. hapla* on *A. dahurica*, *C. pilosula* and *G. uralensis* roots decreased exponentially with increase in Pi (Fig. 2). Rf values of 36.2, 9.0 and 6.5 were recorded at the lowest Pi, whereas the minimum Rf values were 10.7, 3.5 and 2.7 at the highest Pi, on *A. dahurica*, *C. pilosula* and *G. uralensis*, respectively. This may be explained by the destruction of root systems by high nematode populations, which fostered competition for nutrition among the developing nematodes within a root system. Consequently, many juveniles would not have been able to enter the viable roots and, thus, they died of starvation. Similar observations have been made by Pandey & Haseeb (1997) and Park *et al.* (1999) on the pathogenicity of root-knot nematodes on some medicinal herbs. *A. dahurica*, *C. pilosula* and *G. uralensis* are cultivated for more than 180 days for pharmaceutical purposes, so that even a relatively low density of *M. hapla* would be likely to build up to damaging levels before harvest.

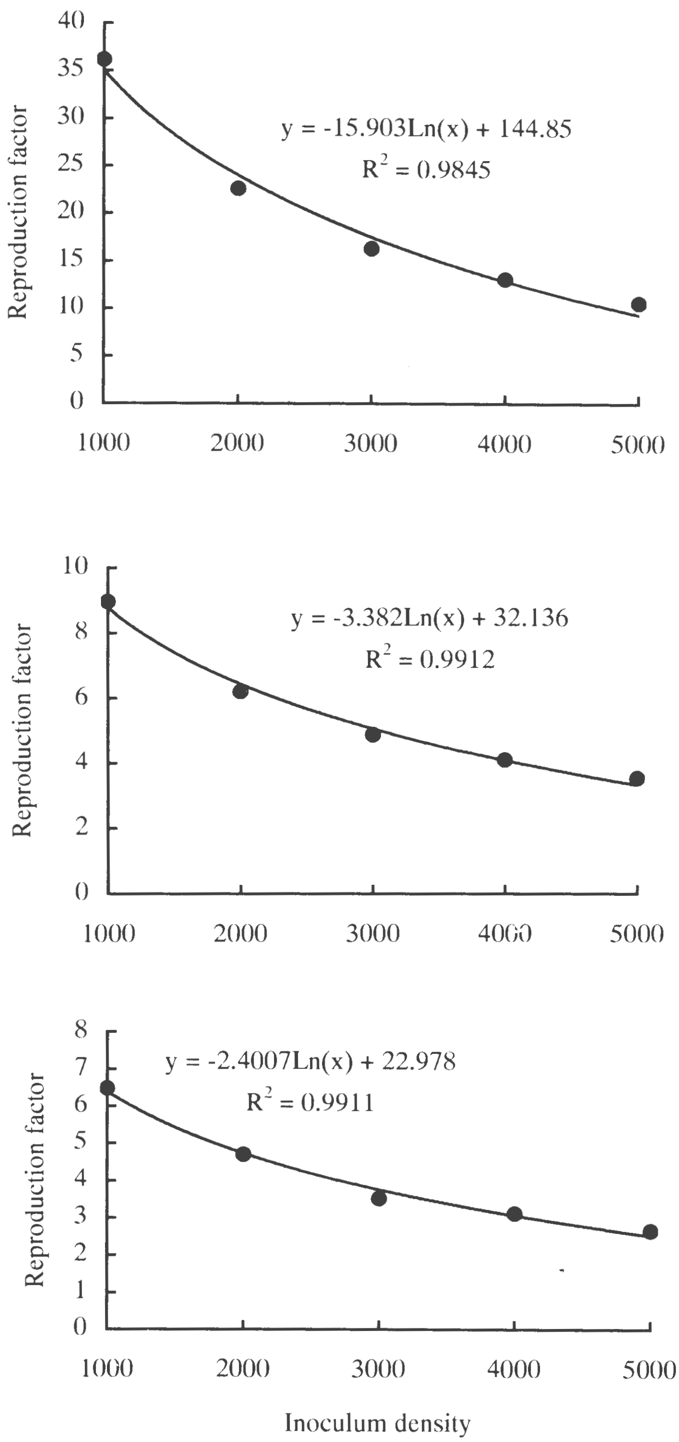

Fig.2. Relationships between inoculum densities (Pi) of *Meloidogyne hapla* and its reproduction (Rf) on *Angelica dahurica*, *Codonopsis uralensis* and *Glycyrrhiza uralensis* 90 days post-inoculation. Each point represents the average of five replicated plants. Lines represent the predicted function calculated by fitting the expanded negative exponential model to the data.

The present results indicated that *M. hapla* was more pathogenic to *A. dahurica* than to *C. pilosula* and *G. uralensis*. The higher level of pathogenicity was the most apparent in root galling index.

Since *M. hapla* has high damage potential and reproductive capacity on *A. dahurica, C. pilosula* and *G. uralensis*, more research is needed for the development of resistant or tolerant cultivars of these herbs. Currently, chemical control methods may be required in nematode-infested soil.

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So Deuk Park, Zakaullah Khan и Young Ho Kim. Потенциал вредоносности и особенности размножения *Meloidogyne hapla* на некоторых медицинских растениях Кореи.

Резюме. Потенциал вредоносности и особенности размножения были исследованы в условиях эксперимента в теплице на трех видах медицинских растений: *Angelica dahurica*, *Codonopsis pilosula* and *Glycyrrhiza uralensis*. В горшки с растениями возрастом три недели были внесены *M. hapla* с начальной плотностью популяции (Pi) в 1,000, 2,000, 3,000, 4,000 и 5,000 личинок второй стадии на растение. Угнетение роста растений, которое оценивали по длине побегов и корней, весу и диаметру корней, наблюдали у *A. dahurica*, *C. pilosula* и *G. uralensis* при всех уровнях Pi через 90 дней после внесения *M. hapla*. При этом степень вреда возрастала с увеличением Pi. Так, Pi = 5000 вызывало соответственно 31.7; 34.0 и 25.2%-ное сокращение длины корней у *A. dahurica*, *C. pilosula* и *G. uralensis*. У *A. dahurica* было отмечено более выраженное развитие галлов, чем у *C. pilosula* и *G. uralensis* при всех значениях Pi. При максимальном значении Pi, степень развития галлов оценивалась в 5.0; 4.2, и 3.6 у *A. dahurica*, *C. pilosula* и *G. uralensis*, соответственно. С повышением Pi экспоненциально снижался фактор размножения (Rf) M. hapla на всех трех видах медицинских растений. Однако, Rf был выше на *A. dahurica,* чем на *C. pilosula* и *G. uralensis* при всех значениях Pi. Такие особенности *A. dahurica*, *C. pilosula* и *G. uralensis* не позволяют использовать эти растения в севообороте для смены других восприимчивых растений.
