

## Research Article

## Relationship between initial population density of *Meloidogyne javanica* and susceptibility of three cucumber cultivars

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**ABSTRACT-** Root-knot nematodes (*Meloidogyne* spp.) cause approximately 5% yield losses in global crop production and have a wide host range in tropical and subtropical regions. This study investigated the relationship between initial *Meloidogyne javanica* population density and resistance in three cucumber cultivars under greenhouse conditions. Seeds of three cucumber cultivars ('Super Dominus', 'Bate-Alpha', and 'Sina') were sown in plastic pots containing 1 kg steam-sterilized sandy-loam soil and maintained under greenhouse conditions at  $27 \pm 4$  °C. Seedlings at the four-leaf stage were inoculated with different inoculum densities of *M. javanica* (0, 0.5, 1, 2, 3, 4, and 8 eggs/g soil). Plant growth and nematode infestation indices were evaluated two months after inoculation. The gall index (GI) and reproduction factor (Rf) enabled cultivar resistance evaluation. Results showed that *M. javanica* was pathogenic for all tested cultivars at any initial population density, causing severe (> 50%) growth reductions at higher densities. Based on Canto-Saenz's host suitability criteria (GI > 2 and Rf > 1), all cultivars were classified as susceptible.

**INTRODUCTION**

Root-knot nematodes (*Meloidogyne* spp.) are among the most economically significant plant pathogens responsible for approximately 5% of global crop losses (Mitiku 2018). Among them, *Meloidogyne javanica* is particularly damaging to cucumber (*Cucumis sativus*), causing substantial yield reductions under both greenhouse and field conditions (Viggiano et al., 2014).

Several studies have shown an inverse relationship between the initial population density ( $P_i$ ) of root-knot nematodes and plant growth parameters (Barillas-Argueta, 1993; Charegani et al., 2012; Dammini Premachandra et al. 2015; Verdejo-Lucas and Talavera, 2019; Shekoochi et al., 2021). For instance, Barillas-Argueta et al. (1993), found that *M. incognita* significantly reduced shoot dry weight in kenaf (*Hibiscus cannabinus*), with the highest  $P_i$  (5000 eggs + second stage juveniles [J2s] per 500 cm<sup>3</sup> soil) resulting in a 46% reduction compared to untreated plants. They also reported that the nematode reproduction factor (Rf) increased by 84% at the highest  $P_i$  compared to the lowest  $P_i$  (100 eggs + J2s), with the final population ( $P_f$ ) rising proportionally with  $P_i$ . A similar trend was observed in tomato, where increasing  $P_i$  levels of *M. incognita* correlated with reduced plant growth, higher gall index (GI), and greater nematode reproduction. In spinach, inoculation with 10 J2s per cm<sup>3</sup> soil resulted in 43%, 46%, and 45%

reductions in shoot length, shoot weight, and root length, respectively (Dammini Premachandra et al., 2015).

Resistance also plays a crucial role in moderating the nematode impact. In tobacco, Hanounik et al. (1975) inoculated resistant and susceptible cultivars with  $P_i$  levels ranging from 0 to 64 *M. incognita* eggs per 1.5 cm<sup>3</sup> of soil. Even at the highest  $P_i$ , the resistant cultivar exhibited greater leaf weight and shoot height than those of the susceptible cultivar. Similarly, Fourie et al. (2010) reported that *M. incognita* Rf values remained significantly lower in resistant soybean cultivars than in susceptible ones across increasing  $P_i$  levels. In chickpea, varying  $P_i$  levels of *M. javanica* revealed cultivar-dependent differences in susceptibility in two cultivars ('Haberu' and 'Geletu') that showed high Rf values. Those two cultivars were classified as susceptible, while others were moderate hosts (Kefelegn et al., 2024).

Comparable cultivar-dependent differences have been reported in cucumber. Aboulipour et al. (2011) found that 13 greenhouse cucumber cultivars were susceptible to *M. javanica*, while two local cultivars from Isfahan ('Chambar' and 'Dastgerdi') were considered as tolerant. Likewise, Charegani et al. (2012) showed that increasing initial densities of *M. javanica* and *M. incognita* intensified yield losses in both cucumber and tomato plants under greenhouse conditions. More recently, Ghanbari et al. (2024) evaluated 11 cucumber hybrids against *M. incognita*

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under greenhouse conditions and reported that none of them displayed significant resistance indices.

Given these findings, the present study investigated the relationship between *M. javanica* initial population density and susceptibility of three cucumber cultivars ('Super Dominus', 'Bate-Alpha', and 'Sina') under controlled greenhouse conditions.

## MATERIALS AND METHODS

### *Nematode inoculum*

Root-knot nematode-infected cucumber roots with gall symptoms were collected from a greenhouse in the Boyer-Ahmad region of Iran. The roots were gently washed with tap water and a single egg mass was used to inoculate the four-leaf-stage cucumber seedlings (cv. 'Super Amelia'). The nematode species was identified as *M. javanica* based on the female perineal pattern, following a method described by Taylor and Netscher (1974).

Eggs of *M. javanica* were extracted from galled cucumber roots using the method of Hussey and Barker (1973). Briefly, thoroughly washed roots were cut into 1-2 cm pieces and blended in 0.5% sodium hypochlorite (NaOCl) solution for 40 s using an electric blender. The suspension was poured through a 200-mesh sieve placed over a 500-mesh sieve and immediately rinsed with tap water to remove residual NaOCl. Eggs retained on the 500-mesh sieve were collected by washing in a clean beaker for subsequent use.

### *Greenhouse study*

Seeds of three cucumber cultivars ('Sina', 'Bate-Alpha', and 'Super Dominus') were sown in plastic pots containing 1 kg steam-sterilized sandy-loam soil and maintained under greenhouse conditions at  $27 \pm 4$  °C. Three-week-old seedlings at the four-leaf stage were inoculated with varying densities of *M. javanica* eggs (0, 500, 1000, 2000, 3000, 4000, and 8000 eggs per pot). The experiment was arranged in a completely randomized design (CRD) with four replicates per treatment.

Two months after inoculation, plant growth parameters, including shoot length, shoot fresh and dry weights, and root fresh weight, were recorded. Roots were gently washed with tap water, and fresh weight was measured. To assess nematode infestation, roots were stained with fuchsine acid (Byrd et al. 1983), and the number of galls and egg masses per root system was counted using the method of Taylor and Sasser (1978). The GI was determined based on Taylor and Sasser (1978) scale, as shown in Table 1.

**Table 1.** Root gall index according to Taylor and Sasser (1978)

Number of galls per root system	Gall index (GI)
0	0
1-2	1
3-10	2
11-30	3
31-100	4
More than 100	5

The total number of eggs per root system was determined using the NaOCl method (Hussey and Barker, 1973). The

Rf was calculated as the ratio of the *Pf* (total number of nematodes per pot) to the *Pi* (initial population of nematode). Resistance levels of the cucumber cultivars were assessed using the modified Canto-Saenz host suitability designations (Sasser et al., 1984) (Table 2).

### *Statistical analysis*

Data were subjected to two-way analysis of variance (ANOVA), and treatment means were compared using the least significant difference (LSD) test. Differences were considered statistically significant at  $P < 0.01$ . All statistical analyses were performed using SAS software (version 9.4, SAS Institute Inc., Cary, NC).

**Table 2.** Resistance classification based on gall index and the reproduction factor according to Canto-Saenz's scheme (Sasser et al., 1984)

Gall index (GI)	Reproduction factor (RF)	Degree of resistance
$\leq 2$	$\leq 1$	R = Resistant
$\leq 2$	$> 1$	T = Tolerant
$> 2$	$\leq 1$	H = Hypersusceptible
$> 2$	$> 1$	S = Susceptible

## RESULTS AND DISCUSSION

Crop damage and yield reduction were influenced by several factors, including plant-parasitic nematodes, environmental conditions, soil type, and cultivar susceptibility (Ravichandra, 2014). This study evaluated the effect of different initial population densities (*Pi*) of *M. javanica* on three cucumber cultivars ('Bate-Alpha', 'Super Dominus', and 'Sina') under greenhouse conditions. Based on Canto-Saenz host suitability designations, all three cultivars were classified as susceptible, even at the lowest population density (0.5 eggs/g soil). At higher population densities (*Pi* = 4 and 8 eggs/g soil), plant growth was severely suppressed.

### *Effect of nematode populations on plant growth*

Plant growth parameters declined in all three cultivars as nematode *Pi* increased. Significant reductions ( $P < 0.01$ ) were observed in shoot dry weight (particularly in 'Sina'), shoot length and root fresh weight (in 'Bate-Alpha' and 'Sina'), and shoot fresh weight in all inoculated plants compared to the non-inoculated controls. Also, 'Bate-Alpha' exhibited a significant shoot dry weight reduction at *Pi* = 2, 3, 4, and 8 eggs/g soil. 'Super Dominus' showed a significant shoot dry weight reduction at *Pi* = 4 and 8 eggs/g soil, and shoot length in 'Super Dominus' was significantly reduced at *Pi* = 1, 2, 3, 4, and 8 eggs/g soil ( $P < 0.01$ , Table 3).

These results indicated that all tested cultivars were highly sensitive to nematode pressure, with measurable growth suppression even at relatively low inoculum levels.

### *Nematode reproduction and host susceptibility*

In 'Bate-Alpha', the Rf was highest at *Pi* = 4 eggs/g soil, while both egg counts and egg mass numbers per root system peaked at *Pi* = 8 eggs/g soil. Gall numbers per root system were significantly greater at *Pi* = 4 eggs/g soil compared to *Pi* = 0.5, 1, 2, and 3 eggs/g soil.

In 'Super Dominus', egg counts per root system were significantly higher at  $Pi = 8$  eggs/g soil than at  $Pi = 0.5, 1, 2,$  and  $3$  eggs/g soil. Gall numbers per root system were highest at  $Pi = 4$  eggs/g soil, significantly exceeding those at  $Pi = 0.5, 1, 2,$  and  $8$  eggs/g soil. Similarly, the number of egg

masses was significantly higher at  $Pi = 8$  eggs/g soil compared to  $Pi = 0.5, 1,$  and  $2$  eggs/g soil. The highest Rf was observed at  $Pi = 2$  eggs/g soil, which was significantly greater than those at  $Pi = 0.5, 1, 4,$  and  $8$  eggs/g soil.

**Table 3.** Mean plant growth parameters of three cucumber cultivars ('Bate-Alpha', 'Super Dominus', and 'Sina') inoculated with different initial population densities ( $Pi$ , eggs/g soil) of *Meloidogyne javanica*, 60 days after inoculation

Cultivar	$Pi$	Root fresh weight (g)	Shoot fresh weight (g)	Shoot dry weight (g)	Shoot height (cm)
Bate-Alpha	0	3.84 <sup>a</sup> ± 0.49	10.17 <sup>a</sup> ± 0.09	1.48 <sup>a</sup> ± 0.14	35 <sup>a</sup> ± 1.53
	0.5	2 <sup>bc</sup> ± 0.29	7.93 <sup>b</sup> ± 0.28	0.96 <sup>ab</sup> ± 0.11	28.27 <sup>b</sup> ± 1.51
	1	2.67 <sup>bc</sup> ± 0.21	6.66 <sup>c</sup> ± 0.15	0.86 <sup>ab</sup> ± 0.36	23.2 <sup>c</sup> ± 0.15
	2	2.8 <sup>b</sup> ± 0.09	5.54 <sup>d</sup> ± 0.26	0.67 <sup>b</sup> ± 0.04	26.6 <sup>bc</sup> ± 1.29
	3	2.66 <sup>bc</sup> ± 0.08	4.25 <sup>e</sup> ± 0.07	0.66 <sup>b</sup> ± 0.01	22.17 <sup>c</sup> ± 0.94
	4	1.61 <sup>cd</sup> ± 0.11	3.59 <sup>e</sup> ± 0.22	0.47 <sup>b</sup> ± 0.04	16.5 <sup>d</sup> ± 1
	8	0.85 <sup>d</sup> ± 0.12	2.18 <sup>f</sup> ± 0.18	0.39 <sup>b</sup> ± 0.05	14.17 <sup>d</sup> ± 0.5
	Super Dominus	0	2.67 <sup>a</sup> ± 0.11	9.21 <sup>a</sup> ± 0.15	0.95 <sup>a</sup> ± 0.05
0.5		1.94 <sup>ab</sup> ± 0.12	7.21 <sup>b</sup> ± 0.34	0.84 <sup>a</sup> ± 0.11	27 <sup>ab</sup> ± 0.76
1		1.58 <sup>b</sup> ± 0.09	6.86 <sup>b</sup> ± 0.14	0.77 <sup>a</sup> ± 0.03	25.43 <sup>b</sup> ± 0.81
2		1.88 <sup>ab</sup> ± 0.13	6.59 <sup>b</sup> ± 0.36	0.75 <sup>a</sup> ± 0.07	19.96 <sup>c</sup> ± 0.23
3		1.74 <sup>ab</sup> ± 0.39	4.87 <sup>c</sup> ± 0.44	0.74 <sup>a</sup> ± 0.08	19.83 <sup>c</sup> ± 0.18
4		1.52 <sup>b</sup> ± 0.34	4.83 <sup>c</sup> ± 0.07	0.45 <sup>b</sup> ± 0.05	19.93 <sup>c</sup> ± 0.54
8		0.53 <sup>c</sup> ± 0.09	2.66 <sup>d</sup> ± 0.16	0.37 <sup>b</sup> ± 0.03	16.9 <sup>d</sup> ± 0.49
Sina		0	3.06 <sup>a</sup> ± 0.32	18.34 <sup>a</sup> ± 0.11	2.05 <sup>a</sup> ± 0.25
	0.5	1.59 <sup>b</sup> ± 0.07	13.56 <sup>b</sup> ± 0.5	1.44 <sup>b</sup> ± 0.01	44.37 <sup>b</sup> ± 2.52
	1	1.27 <sup>b</sup> ± 0.15	11.36 <sup>b</sup> ± 0.45	1.27 <sup>b</sup> ± 0.05	28.67 <sup>c</sup> ± 0.83
	2	1.32 <sup>b</sup> ± 0.17	6.50 <sup>c</sup> ± 0.57	0.65 <sup>c</sup> ± 0.04	26.00 <sup>cd</sup> ± 1.15
	3	1.88 <sup>b</sup> ± 0.35	6.59 <sup>c</sup> ± 0.73	0.50 <sup>c</sup> ± 0.03	26.47 <sup>cd</sup> ± 1.3
	4	1.06 <sup>b</sup> ± 0.03	4.39 <sup>c</sup> ± 0.75	0.41 <sup>c</sup> ± 0.08	22.10 <sup>de</sup> ± 0.21
	8	0.99 <sup>b</sup> ± 0.12	2.06 <sup>d</sup> ± 0.21	0.23 <sup>c</sup> ± 0.03	19.50 <sup>e</sup> ± 0.5

Data for each cultivar were analyzed separately. Values represent mean ± standard error ( $n = 4$ ). Means within the same column for each cultivar followed by different letters differ significantly according to the least significant differences (LSD) test at  $P < 0.01$ .

**Table 4.** Mean nematode indices of *Meloidogyne javanica* on three cucumber cultivars ('Bate-Alpha', 'Super Dominus', and 'Sina') inoculated with different inoculum densities ( $Pi$ , eggs/g soil), 60 days after nematode inoculation

Cucumber cultivar	$Pi$	Eggs/root system	Galls/root system	Egg masses/root system	Reproduction factor	Gall index
Bate-Alpha	0.5	1313 <sup>c</sup> ± 59.28	18 <sup>d</sup> ± 1.15	6.7 <sup>e</sup> ± 0.88	2.64 <sup>d</sup> ± 0.12	3
	1	2300 <sup>e</sup> ± 208	22 <sup>d</sup> ± 2	6 <sup>e</sup> ± 0.58	2.31 <sup>d</sup> ± 0.21	3
	2	9167 <sup>d</sup> ± 338	60.7 <sup>c</sup> ± 5.33	36.7 <sup>d</sup> ± 4.67	4.6 <sup>b</sup> ± 0.17	4
	3	14933 <sup>c</sup> ± 176	95 <sup>b</sup> ± 3	60.7 <sup>c</sup> ± 3.84	5 <sup>b</sup> ± 0.06	4
	4	25467 <sup>b</sup> ± 375	173 <sup>a</sup> ± 8.19	122 <sup>b</sup> ± 6.12	6.4 <sup>a</sup> ± 0.1	5
	8	31100 <sup>a</sup> ± 550	163 <sup>a</sup> ± 3.53	155 <sup>a</sup> ± 4.36	3.91 <sup>c</sup> ± 0.07	5
Super Dominus	0.5	1633 <sup>d</sup> ± 46.67	11.67 <sup>d</sup> ± 0.33	7.67 <sup>c</sup> ± 0.33	3.3 <sup>d</sup> ± 0.1	3
	1	5740 <sup>d</sup> ± 80.83	18 <sup>d</sup> ± 3.06	14.67 <sup>c</sup> ± 0.67	5.77 <sup>cd</sup> ± 0.09	3
	2	28933 <sup>c</sup> ± 2469	132 <sup>c</sup> ± 5.29	119 <sup>b</sup> ± 3.71	14.57 <sup>a</sup> ± 1.23	5
	3	40600 <sup>b</sup> ± 2910	165 <sup>ab</sup> ± 8.11	129 <sup>ab</sup> ± 5.7	13.56 <sup>ab</sup> ± 0.97	5
	4	44600 <sup>ab</sup> ± 1928	178 <sup>a</sup> ± 6.11	131 <sup>ab</sup> ± 5.46	11.16 <sup>b</sup> ± 0.47	5
	8	51267 <sup>a</sup> ± 1443	152 <sup>bc</sup> ± 5.03	146 <sup>a</sup> ± 3.79	6.40 <sup>c</sup> ± 0.17	5
Sina	0.5	1287 <sup>e</sup> ± 94.04	20 <sup>e</sup> ± 2	4.67 <sup>e</sup> ± 1.33	2.58 <sup>d</sup> ± 0.19	3
	1	2933 <sup>e</sup> ± 66.7	51.34 <sup>d</sup> ± 6.67	35.33 <sup>d</sup> ± 7.42	2.97 <sup>d</sup> ± 0.06	4
	2	13333 <sup>d</sup> ± 751	92.67 <sup>c</sup> ± 4.06	59.33 <sup>c</sup> ± 4.06	6.69 <sup>b</sup> ± 0.38	4
	3	17683 <sup>c</sup> ± 524	103 <sup>c</sup> ± 8.51b	74.67 <sup>c</sup> ± 1.76	5.92 <sup>bc</sup> ± 0.17	5
	4	34567 <sup>b</sup> ± 1189	127 <sup>b</sup> ± 6.36	114 <sup>b</sup> ± 3.06	8.67 <sup>a</sup> ± 0.30	5
	8	43433 <sup>a</sup> ± 698	171 <sup>a</sup> ± 9.61	141 <sup>a</sup> ± 5.57	5.45 <sup>c</sup> ± 0.09	5

Data for each cultivar were analyzed separately. Values represent mean ± standard error ( $n = 4$ ). Means within the same column for each cultivar followed by different letters differ significantly according to the least significant differences (LSDs) test at  $P < 0.01$ .

In 'Sina', the number of eggs, gall, and the value of egg mass per root system at  $Pi = 8$  eggs/g soil and Rf at  $Pi = 4$  eggs/g soil were significantly higher than at other nematode population levels. According to the Canto-Saenz classification system ( $GI > 2$  and  $Rf > 1$ ; Table 4), all three cultivars were classified as susceptible to *M. javanica*.

These findings are consistent with previous research showing that host susceptibility, nematode reproduction, and crop damage are strongly influenced by the initial population density ( $Pi$ ) of root-knot nematodes (*Meloidogyne* spp.) (Seinhorst, 1965; Seinhorst, 1970; Greco and Di Vito, 2009; Maleita et al. 2012; Charegani et al., 2012; Moosavi, 2014; Mosahaneh et al., 2020; Padilla-Hurtado et al., 2022). For instance, Maleita et al. (2012) studied the effects of *M. hispanica* and *M. javanica* at 5, 10, and 20 eggs/g soil on both susceptible (Easypeel and Moneymaker) and resistant (Motelle and VFNT-Cherr) tomato genotypes. They found high GI ( $GI \geq 4$ ) and RF ( $Rf \geq 3.44$ ) even in resistant cultivars carrying the *Mi*-gene. Increasing  $Pi$  disrupted *Mi*-gene-mediated resistance, suggesting a density-dependent breakdown of host defense mechanisms.

A similar trend was observed by Dammini Premachandra and Gowen (2015), who reported that *M. incognita* caused over 40% reduction in spinach growth at high densities (up to 10,000 J2s per 1000 cm<sup>3</sup> soil). Similarly, Fourie et al. (2010) found that Rf values declined as  $Pi$  increased in soybean cultivars (Prima 2000: susceptible; LS5995: resistant) infected with *M. incognita* race 2.

In our study, while the highest Rf values were observed at moderate  $Pi$  levels (4 eggs/g soil in 'Bate-Alpha' and 'Sina', while having 2 eggs/g soil in 'Super Dominus'), Rf values significantly decreased at  $Pi = 8$  eggs/g soil. Plants at this density were severely stunted and exhibited smaller root systems, likely limiting the food supply for nematodes and constraining reproduction. Seinhorst (1966) and similar studies (Jones & Perry, 2011) showed that intraspecific competition for limited feeding sites and host resources intensifies among root-knot nematodes at high  $Pi$ . This density-dependent regulation reduces individual fecundity, increases mortality, and ultimately suppresses  $Pf$  compared to that observed at moderate  $Pi$  levels. These results support previous reports that excessively high  $Pi$  values can reduce Rf due to the insufficient host tissue (Charegani et al., 2012; Moosavi, 2014; Mosahaneh et al., 2020).

Prakash Sharma and Sharma (2015) also reported that increasing the  $Pi$  of *M. incognita* reduced water and nutrient absorption, nutrient translocation, and photosynthesis in tomato (cv. PT-3), resulting in impaired plant growth and less biomass accumulation.

Consistent with these findings, we observed reductions in shoot biomass at  $Pi = 8$  eggs/g soil. Specifically, shoot dry weight declined by 61% (in 'Bate-Alpha'), 73% (in 'Super Dominus'), and 89% (in 'Sina') compared to non-inoculated controls. Shoot length decreased by 60%, 40%, and 65%, and shoot fresh weight decreased by 79%, 71%, and 89%, respectively. These findings confirmed that damage caused by *M. javanica* is directly proportional to the initial nematode population density.

Our findings are also consistent with those of Kefelegn et al. (2024) who reported that multiple chickpea cultivars were susceptible or moderate hosts for *M. javanica* based on both Rf and GI values.

## CONCLUSION

This study confirmed that *M. javanica* is pathogenic to all three tested cucumber cultivars ('Bate-Alpha', 'Super Dominus', and 'Sina') at all initial population densities. Severe damage (> 50% reduction in plant growth parameters) was observed at high nematode densities. According to the Canto-Saenz host suitability criteria, all cultivars were classified as susceptible within the tested  $Pi$  range.

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## CRediT AUTHORSHIP CONTRIBUTION STATEMENT

Conceptualization: Habiballah Chadegani; Methodology: Habiballah Chadegani and Sedigheh Gholampour; Software: Habiballah Chadegani; Validation: Habiballah Chadegani; Formal analysis: Habiballah Chadegani; Investigation: Sedigheh Gholampour; Resources: Sedigheh Gholampour; Data curation: Habiballah Chadegani; Writing: Habiballah Chadegani; Visualization: Habiballah Chadegani; Supervision: Habiballah Chadegani.

## DECLARATION OF COMPETING INTEREST

The authors declare no conflicts of interest.

## ETHICAL STATEMENT

This study did not involve any experiments on humans or animals. All experimental procedures complied with institutional, national, and international guidelines for research ethics.

## DATA AVAILABILITY

The raw data supporting this research will be made available by the authors upon request.

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