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Damage Threshold of Root-Knot Nematode, *Meloidogyne incognita* on Common Bean Influenced by Planting Dates, and Inoculum Levels under Greenhouse Conditions

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ABSTRACT

Two experiments were conducted to study the effect of root-knot nematode, *Meloidogyne incognita* at three inoculum levels on the growth characteristics of common bean (*Phaseolus vulgaris* L.) cv. Xera and the response of plants towards the nematode on two different planting dates (Autumn, and Early spring seasons). Data indicated that different *M. incognita* inoculum levels at the two growing seasons had distinct effects on the growth of common bean. The results revealed that the root galling severity, and damage index (DI) increased significantly ($P \leq 0.05$) with increasing the inoculum levels of *M. incognita* on common bean. However, root galling was more severe on common bean plants as the maximum index rate was 8.6 and 9.0, seventy days after inoculation (Pi = 3000 J_2s) in autumn and spring seasons, respectively. Similarly, the final nematode population and egg masses/root increased with increasing the inoculum levels of *M. incognita* during the early spring season compared with the autumn season. All the growth parameters were significantly ($P \leq 0.05$) reduced in all infected plants as compared with control (uninfected) plants. At lower initial inoculum levels the extent of reduction in plant growth was low, but at higher inoculum levels, reduction in plant growth parameters was remarkable, in comparison to control plants in the two growing seasons, but more reduction was observed in the early spring season. The highest percent reduction was achieved by inoculation levels 2000 and 3000 J_2s in the whole plant fresh weight, plant lengths, root, fresh weight, pods weight~~s~~, and number of nodules per root system. With an increase in initial inoculum levels of *M. incognita*, physiological parameters of common bean were decreased. In response to nematode infection, the data clearly clarified that root-knot nematode adversely affected the plant growth, nitrogen, phosphorus, potassium percentages, total protein and chlorophyll contents in nematode-infected common bean plants.

***Keywords*: *Meloidogyne incognita*, *Phaseolus vulgaris*,** planting dates**,** inoculum levels, physiological parameters.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is the most important legume for human consumption worldwide, and an important source of vegetable protein, minerals, antioxidants, and bioactive compounds. The N_2-fixation capacity of this crop reduces its demand for synthetic N fertilizer application to increase yield and quality (Karavidas et al., 2022). It is mostly grown in all districts of Egypt, during two seasons, spring and autumn, when the temperature is most suitable for its growth and yield. The cultivated area of bean in Egypt has been recorded to be more than 26028 hectares annually in 2020 according to the official data from the Egyptian Ministry of Agriculture. The common bean is often damaged by root-knot nematodes under both field and greenhouse conditions. Plant parasitic nematodes are the major pathogens of both temperate and tropical agriculture crops, which have a global economic effect of more than US$ 100 billion each year (Abdel-Baset et al., 2020). Among plant parasitic diseases, root-knot disease is caused by root-knot nematodes, *Meloidogyne* spp., of which *M. incognita* is the most destructing species that results in huge economic losses. These nematodes invade and colonize host plant roots subvert the host machinery to their own benefit and overcome host defenses (Haegeman et al., 2012). Feeding site formation enables the parasite to withdraw large amounts of nutrients from the plant vascular system. Many morphological and physiological changes occur during the formation of feeding sites in the host (Sharf and Hisamuddin, 2019). An adequate supply, uptake, and a balanced distribution of nutrient elements within a plant are necessary for normal plant growth. When nematodes infect plants, the nutrient status changes and alter the host physiology. Many investigators reported that the effect of plant parasitic nematodes on the uptake of nutrient elements and distribution within the plant varies with nematode species, host type, and stage of infection, whether measurements were taken in different plant parts or infected and non-infected parts (Melakeberhan et al., 1987). Although these reports show that plant-parasitic nematodes change the level and distribution of nutrients within the plant, the experimental data were taken only once during the period of nematode infection and do not establish a relationship with other physiological processes such as photosynthesis. In a series of studies where common bean, *P. vulgaris* plants of different ages were infected with *M. incognita*, reduced rates of photosynthesis and crop yield with increasing inoculum levels (Sharf and Hisamuddin, 2019). Root-knot nematodes, affect the water and nutrients absorption and translocation in host plants; photosynthesis rate decreases in infected plants which are negatively correlated with inoculum levels; the photosynthetic products move toward the roots specifically into giant cells that was developed by the nematode infections and support nematode development and reproduction (Maleita et al., 2012).

The aim of this work was to study I- the effects of root-knot nematode, *M. incognita* on common bean growth parameters. II- estimating damage threshold at two planting dates.

MATERIALS AND METHODS

Two greenhouse experiments were conducted at Ismailia Agricultural Research Station, Egypt, to study the effect of root-knot nematode *Meloidogyne incognita* on common bean growth. The first experiment was carried out during September – December 2021, while the second one was carried out during February – May 2022.

Source of seeds and greenhouse preparation

Seeds of the common bean (*Phaseolus vulgaris* L.) cv. Xera was obtained from the Vegetable Crop Research Department, Horticulture Research Institute, Agriculture Research Center, Giza, Egypt. The seeds were surface-sterilized before sowing them into 25cm-diameter pots filled with steam-sterilized soil sandy clay in the ratio of 1:4 (3kg). Thinning was done one week after sowing so as one seedling per pot.

Inoculum preparation

The nematode was obtained from common bean farm had a natural infestation of only *M. incognita*, as a serious pathogen of bean plant. The adult females were removed to identify the nematode species, according to the female perennial pattern (Taylor and Sasser, 1978). The nematode *M. incognita* population was maintained as a pure culture, population in the roots of eggplant (*Solanum melongena* L.) cv. Travita in the greenhouse. *M. incognita* eggs were extracted from the roots using a 0.5% NaOCL solution for 3 min., according to the method of Hussey and Barker (1973). The eggs were incubated in egg hatching cups to provide second-stage juveniles (J_2s). Two - weeks old seedlings were inoculated with 1000, 2000, and 3000 nematodes (0.3, 0.6, and 1 nematode/1g soil). The inoculums consisted of a 20ml suspension of second-stage juveniles (J_2s) and eggs.

Inoculation procedure

The inoculum was pipetted into a depression made around the common bean roots and covered with soil. Non-inoculated plants served as control. The treatments were arranged in a randomized complete block design and replicated three times.

Data collection

The experiment was terminated 70 days after inoculation and plant growth data were collected. While, nematode assessment data were collected 21, 45, and 70 days after inoculation.

Nematode Assessment

The common bean roots were carefully, washed and assessed for galling according to the method described by Sharma et al. (1994). Gall index (GI): 1 = no galls; 2 = 1 to 5 galls; 3 = 6 to 10 galls; 4 = 11 to 20 galls; 5 = 21 to 30 galls; 6=31 to 50 galls; 7 = 51 to 70 galls; 8 = 71 to 100 galls; and 9 = >100 galls per root system. Gall size (GS) and percent galls area (GA) are also, rated on a 1-9 scale. For GS: 1 =no galls; 3 = very small galls (about 10 % increase in the root area at the galled region over non-galled normal root area); 5 = small galls (about30% increase); 7 = medium galls (about 31 to 50% increase) and 9 = big galls (about 51 to 100% increase). For GA: 1 = no galls; 3 = 1 to 10% root area galled; 5 = 11 to 30 % root area galled; 7 = 31 to 50% root area galled, and 9 = >50% root area galled (Sharma et al., 1994). Damage index (DI) is calculated by dividing the sum of GI, GS, and GA by three for each replicate (Sharma et al., 1994). The number of egg masses/ root system and egg mass index (EI) as well as the number of second stage juveniles in each pot were recorded (Goodey, 1957).

Plant growth assessment

Plants were gently uprooted and the root system separated from the shoot system at the first basal node. The root systems were carefully and thoroughly washed before taking their fresh weights (g). Fresh shoot weights (g), length (cm), pods weights (g), and numbers of nodules per root system were obtained. The percentage of reduction in plant (R %) was calculated using the formula.

$$R\% = \frac{\text{Control plants} - \text{Infected plants}}{\text{Control plants}} \times 100$$

Plant physiology parameters:

Leaf mineral contents

Five grams of mature leaves were randomly collected after harvesting. The samples of leaves were washed with tap water, rinsed twice in distilled water and air dried in an oven at 70°C. The dried leaves were ground and digested by H_2O_2 and H_2SO_4 according to Evenhuis and Dewaard (1980). Suitable aliquots were taken for the determination of the mineral content. Nitrogen was determined by the Kjeldahl method (Anonymous, 1995). Phosphorus was determined according to Murphy and Riley (1962). Potassium was determined with a flame photometer. The concentrations of N, P, and K were expressed as percentages.

Total protein (mg/g FW)

It was estimated by the Bradford method (Bradford, 1976) at 595 nm. 0.2g fresh leaves were homogenized in a prechilled mortar with 1ml of 0.1M phosphate buffer (pH= 7). Then, the suspension obtained was filtered through one layer of muslin cloth and then centrifuged at 10000 rpm for 15 min., 4ºC (Urbanek et al., 1991). Two ml of Bradford reagent was added to 200 µl leaf extract.

Chlorophyll contents

Leaf chlorophyll contents were estimated using SPAD-502 apparatus (Castelli et al., 1996). Five readings were taken on the middle third of the surface lamina, the instrument itself, providing the average value given the instrument's limited reading area ($6mm^2$) in order to monitor any variations due to uneven pigment distribution.
Temperature data were obtained from Central Laboratory of Agricultural Climate, Ministry of Agriculture, Giza, Egypt (Table 1).

Table 1: Maximum and minimum temperature (°C) during growing seasons 2021-2022

Date	**Temperature °C**		
	Maximum	**Minimum**	**Average**
September, 2021	28	21	24.5
October	26	20	23
November	23	17	20
December	21	11	16
February, 2022	18	9	13.5
March	23	16	19.5
April	27	19	23
May	30	20	25

Statistical analysis

All experiments were performed twice. Analyses of variance were carried-out using MSTAT-C program version 2.10 (Anonymous, 1991). Means were separated using the least significant differences (LSD) method at $P \leq 0.05$ (Gomez &Gomez, 1984).

RESULTS

Nematode severity and damage on common bean cv. Xera in autumn, and early spring seasons.

During the two growing seasons, results in Table (2) revealed the root galling severity, and damage index (DI) increased with increasing the inoculum levels of *M. incognita* on common bean cv. Xera. However, root galling was more severe on common bean plants as the maximum number of root galls/root system was 102, and 126, (70) days after inoculation at Pi 3000 J_2s in autumn and spring seasons, respectively. The damage index was 8.6 in the autumn season, and 9.0 in early spring season. When *M. incognita* population increased with time, the penetration of second stage juveniles increased and the number of galls also increased. Data also, revealed that no egg masses were observed 21 days after nematode inoculation due to root-knot nematode completing their entire life cycle within 25 to 30 days. Data recorded 28.0 egg masses/ root system 70 days after nematode inoculation at Pi 3000 J_2s in the autumn season. Moreover, the highest number of the egg masses/root system was 46 after70 days of nematode inoculation at Pi 3000 J_2s in early spring season. Moreover, the results showed that the final nematode population was 286 juveniles per 250 g of soil, 70 days after nematode inoculation at Pi 3000 J_2s.

Table 2: Reaction of common bean cv. Xera in relation with three initial population densities of *Meloidogyne incognita* in autumn and early spring seasons.

Seasons/year	Nematode parameters								
	Days after inoculation	No. of root galls/ root	G.I	Gall size (GS)	Gall area (GA)	Damage index (DI)	No. of J_2/250 g soil	No. of egg masses/root system	E.I
	1000 J_2s								
Autumn, 2021	21	7.0	2.0	1.6	1.6	1.7	-	-	1.0
	45	21.0	4.6	3.0	3.0	3.5	20.0	3.3	2.0
	70	39.0	5.3	3.6	3.6	4.2	86.0	10.0	3.3
Early Spring, 2022	21	11.0	3.6	3.0	3.6	3.4	-	-	1.0
	45	31.0	5.6	5.6	5.6	5.6	53.0	7.0	3.0
	70	66.0	7.0	6.3	6.3	6.5	106.0	17.7	4.0
Mean		29.2	4.7	3.8	4.0	4.2	44.4	6.3	2.3
2000 J_2s									
Autumn, 2021	21	27.0	5.6	5.6	3.0	4.7	-	-	1.0
	45	54.0	7.0	7.0	5.0	6.3	40.0	7.0	3.0
	70	71.0	7.3	7.0	6.3	6.8	153.0	19.0	4.3
Early Spring, 2022	21	36.0	5.3	6.3	6.3	6.0	-	-	4.0
	45	66.0	6.6	7.6	7.6	7.3	86.0	13.0	4.0
	70	93.0	8.0	8.3	7.6	8.0	213.0	29.0	5.3
Mean		57.8	6.6	7.0	6.0	6.5	82.2	11.6	3.1
3000 J_2s									
Autumn, 2021	21	38.6	6.0	6.3	5.0	5.7	-	-	1
	45	73	.8.0	7.0	6.3	7.1	120	14.3	4
	70	102	8.6	8.3	7.6	8.2	286	28.0	5
Early Spring, 2022	21	48	7.0	6.6	7.0	6.8	-	-	1
	45	91	8.3	8.4	9.0	8.5	220	22.0	5
	70	126	9.0	9.0	9.0	9.0	433	46.0	6
Mean		79.9	7.6	7.6	7.3	7.5	176.6	18.4	3.4
$LSD_{0.05}$									
Population(A)		1.48	0.2	0.44	0.47	0.25	9.8	0.68	0.47
Season (B)		1.2	0.7	0.36	0.38	0.21	8.0	0.56	0.38
Time ©		1.48	0.2	0.44	0.47	0.25	9.8	0.68	0.47
Interaction A*B* C		3.6	0.5	1.08	1.15	0.63	24.0	168	1.1
Correlation									
Population(A)		+	+	+	+	+	+	+	+
Season (B)		+	+	+	+	+	+	+	+
Time (C)		+	+	+	+	+	+	+	+
Correlation A*B		+	+	+	-	+	+	+	+
Correlation A*C		+	-	-	-	-	+	+	+
Correlation B*C		+	-	-	-	-	+	+	+
Correlation A*B*C		+	-	-	-	-	+	+	+

Data are averages of 3 replicates. (+) significantly, (-) non significantly*. Gall index (GI) or egg mass index (EI) was determined according to Sharma et al. (1994), gall (GI) and egg mass indices (EI) 1 = no galls or egg masses, 2 = 1-5 galls, 3 = 6-10 galls, 4 = 11-20 galls, 5 = 21-30 galls, 6 = 31-50 galls, 7 = 51-70 galls, 8 = 71-100 galls, and 9 = > 100 galls or egg masses per plant.

in the autumn season. Meanwhile, data recorded 433 juveniles per 250 g in the early spring season. Recorded data clarified that there was a significant correlation ($P \leq 0.05$) with the inoculum densities of root-knot nematodes, *M. incognita* 1000, 2000, and

3000, time, and seasons (autumn, and early spring) on root galls/ root system, No. of egg masses/root system, and No. of J_{2s}/250 g soil (Table 2).

The relationship between nematode inoculum, and common bean growth at two growing seasons, autumn, and early spring seasons.

Data in Table (3), revealed that different *M. incognita* inoculum levels at the two growing seasons had distinct effects on the growth of common bean cv. Xera. All the growth parameters were significant ($P \leq 0.05$) reduced in all nematode inoculated plants as compared with control plants. At lower initial inoculum levels 1000 J_2s the extent of reduction in plant growth was low, but at higher inoculum levels 3000J_2s, reduction in plant growth parameters was remarkable, in comparison to control plants in two growing seasons. Shoot weight was decreased at lower and higher inoculum levels of *M. incognita*. At the lowest inoculum level 1000J_2s, the reduction was low as compared to the control, it was noted that shoot weight was more affected in the early spring season, it recorded 19g compared with 22.3g in the autumn season.

However, at the inoculum level 2000 J_2s, the reduction in plant growth, over the control was significant. The greatest reduction in shoot length was observed with the higher number of second-stage juveniles 3000J_2s, it recorded 15.6cm in spring season compared with 22.6 cm in the autumn season. From the data, it is clear that at lower as well as a higher inoculum levels, reduction in yield occurred when compared with the control. The yield in terms of pods weight was reduced when the plants were infected with the highly nematode inoculum 3000J_2s, but more affected in the early spring season, 8g compared with 10g in the autumn season. The same trend regarding number of nodules per root system was recorded.

Also, the study indicated that there was a significant correlation ($P \leq 0.05$) with the inoculum densities of root-knot nematodes, *M. incognita* 1000, 2000, and 3000, and seasons (autumn season, and early spring) on all growth parameters i.e. shoot fresh weight(g) shoot length (cm) root, fresh weight(g), number of nodules /root system and pod weights (g) of common bean cv. Xera (Table 3).

The percentage reduction in the growth parameters was recorded in Table (4). Results revealed that at higher inoculum levels, reduction in plant growth parameters was noticeable in two growing seasons. The highest percent reduction was achieved by the inoculation levels 2000 and 3000 J_2s on the whole plant fresh weight, plant lengths, root, fresh weight, and a number of nodules per root system. Meanwhile, the highest reduction of both above-mentioned criteria was conducted in the early spring season compared with the autumn season. With the highly nematode inoculum 3000J_2s. The percent of reduction in plant fresh weight, plant length, root, fresh weight, and number of nodules per root system were 45.0, 36.5, 37.0, 40.0, and 62.5% in the autumn season, respectively, while it was 42.0, 53.5, 55.0, 53.0, and 73.5% in the early spring season, respectively.

Effect of root-knot nematode on the plant physiology at two growing seasons, autumn and early spring.

The root-knot nematode infection caused changes in the metabolic reaction of common bean plants. Nitrogen, phosphorus, potassium, protein contents, and chlorophyll contents were analyzed in the nematode infected plant. Data in Table (5) cleared that the highest reduction was recorded in plants inoculated with the highest inoculum levels 3000J_2s at two growing seasons. The changes in nitrogen concentration, after root-knot nematode infection, altered host metabolic pathways. The shoot nitrogen content decreased with increasing the inoculum levels in treatments 1000

Table 3: Growth parameters of common bean cv. Xera at three initial population densities of *Meloidogyne incognita* in autumn, and early spring seasons

Seasons/Year	Plant parameters																			
	1000 J_2s					2000 J_2s					3000 J_2s					Non-inoculated				
	Shoot fresh weight (g)	Shoot length (cm)	Root, fresh weight(g)	Pods weights (g)	Nodules /root system	Shoot fresh weight (g)	Shoot length (cm)	Root fresh weight (g)	pods weights (g)	Nodules /root system	Shoot fresh weight(g)	Shoot length (cm)	Root, fresh weight(g)	Pods weights (g)	Nodules /root system	Shoot fresh weight(g)	Shoot length (cm)	Root, fresh weight(g)	Pods weights (g)	Nodules /root system
Autumn, 2021	22.3	30	4.8	13	84	18.3	24.3	3.7	11.7	66	15	22.6	3.4	10	39.6	27.3	35.6	5.4	16.6	105.6
Early Spring, 2022	19.0	29	4.0	13	87	17.0	24.3	3.1	11.3	67	15	15.6	2.5	8	32.6	26.0	33.6	5.7	17.0	124.6
Mean	20.6	29.5	4.4	13	85.5	17.6	24.3	3.4	11.5	66.5	15	19.1	2.9	9	36.1	26.6	34.6	5. 6	16.8	115.2
$LSD_{0.05}$																				
Population(A)	1.51	1.35	0.35	0.64	4.7	1.51	1.35	0.35	0.64	4.7	1.51	1.35	0.35	0.64	4.7	00	00	00	00	00
Season (B)	1.07	0.95	0.24	0.45	3.3	1.07	0.95	0.24	0.45	3.3	1.07	0.95	0.24	0.45	3.3	00	00	00	00	00
Interaction A*B	2.14	1.91	0.49	0.9	6.7	2.14	1.91	0.49	0.9	6.7	2.14	1.91	0.49	0.9	6.7	00	00	00	00	00
Correlation																				
Population(A)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	00	00	00	00	00
Season (B)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	00	00	00	00	00
Correlation A*B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	00	00	00	00	00

Data are averages of 3 replicates, (+) significantly, (-) non significantly

Table 4: Percent reduction in common bean cv.Xera growth parameters at three inoculum densities of *Meloidogyne incognita* in autumn and early spring seasons.

Seasons/Year	Plant parameters (R%)														
	1000 J_2s					2000 J_2s					3000 J_2s				
	R% Shoot fresh weight	R% Shoot length	R% Root fresh weight	R% pods weights	R% Nodules /root system	R% Shoot fresh weight	R% Shoot length	R% Root, fresh weight	R% pods weights	R% Nodules /root system	R% Shoot fresh weight	R% Shoot length	R% Root, fresh weight	R% pods weights	R% Nodules /root system
Autumn, 2021	18.3	15.7	11	21.6	20	33	31.7	31	29.5	37.5	45	36.5	37	40	62.5
Early Spring, 2022	26.9	13.6	29.8	23.5	30	34.6	27.6	45.6	33.5	46	42	53.5	56	53	73.8

R%=Control plants – infected plants /Control plants X100

Table 5: Nitrogen, phosphorus, and potassium percentages, protein and chlorophyll contents of common bean cv. Xera at three inoculum densities of *Meloidogyne incognita* in autumn and early spring seasons.

Seasons/ Year	Chemical components																			
	1000 J_2s					2000 J_2s					3000 J_2s					Non-inoculated				
	Nitrogen	Phosphorus	Potassium	Total Protein	Chlorophyll content	Nitrogen	Phosphorus	Potassium	Total Protein	Chlorophyll content	Nitrogen	Phosphorus	Potassium	Total Protein	Chlorophyll content	Nitrogen	Phosphorus	Potassium	Total Protein	Chlorophyll content
Autumn, 2021	3.1	1.9	0.26	22.0	37.0	2.2	1.9	0.20	20.7	27.3	2.1	1.5	0.17	19.1	23.0	3.6	2.2	0.39	27.2	51.6
Early Spring, 2022	3.3	1.8	0.25	22.6	40.0	2.8	1.6	0.197	21.0	34.6	2.3	1.3	0.13	19.3	26.6	3.8	2.0	0.40	25.3	47.0
Mean	3.2	1.9	0.25	22.3	38.6	2.5	1.8	0.198	20.8	31.0	2.2	1.4	0.15	19.2	24.8	3.7	2.1	0.39	26.2	49.3
LSD 0.05																				
Population(A)	0.54	0.26	0.14	0.7	2.5	0.54	0.26	0.14	0.7	2.5	0.54	0.26	0.14	0.7	2.5	00	00	00	00	00
Season (B)	0.38	0.19	0.1	0.51	1.78	0.38	0.19	0.1	0.51	1.78	0.38	0.19	0.1	0.51	1.78	00	00	00	00	00
Interaction A*B	0.77	0.38	0.2	0.2	3.5	0.77	0.38	0.2	0.2	3.5	0.77	0.38	0.2	0.2	3.5	00	00	00	00	00
Correlation																				
Population(A)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	00	00	00	00	00
Season (B)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	00	00	00	00	00
Correlation A*B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	00	00	00	00	00

Data are averages of 3 replicates, (+) significantly, (-) non significantly

Table 6: Percent reduction in nitrogen, phosphorus, potassium concentrations, protein contents and chlorophyll content of common bean cv. Xera at three inoculum densities of *Meloidogyne incognita* in autumn and early spring seasons.

Seasons/Year	Chemical components (R%)														
	1000 J_2s					**2000 J_2s**					**3000 J_2s**				
	R% Nitrogen	R% Phosphorus	R% Potassium	R% Total Protein	R% Chlorophyll content	R% Nitrogen	R% Phosphorus	R% Potassium	R% Total Protein	R% Chlorophyll content	R% Nitrogen	R% Phosphorus	R% Potassium	R% Total Protein	R% Chlorophyll content
Autumn, 2021	13.8	13.6	33.0	19.0	28.0	39	13.6	48.7	23.8	47.0	41.6	31.8	56.0	29.7	55.0
Early Spring, 2022	13.0	10.0	37.5	10.6	14.8	26	20.0	50.7	17.0	26.0	39.0	35.0	67.5	24.0	43.4

R%=Control plants – Infected plants /Control plants X100 and 3000 J_2s over the control plants. The root-knot nematode, *M. incognita* caused a reduction in the leaf protein content with the high inoculum levels 3000J_2s compared with the control in the two growing seasons. The same trend with phosphorus, and potassium contents was recorded. Also, the study found that there was a significant correlation ($P\leq0.05$) with the inoculum densities of root-knot nematodes, *M. incognita* (1000, 2000, and 3000), and nitrogen, phosphorus, potassium percentages, protein and chlorophyll contents Table (5).

Data in Table (6) cleared that the highest percent reduction was recorded in plants inoculated with the highest inoculum levels 3000J_2s by 41.6, 31.8 and 56.0% in nitrogen, phosphorus and potassium concentrations, respectively in autumn season. Moreover, they were recorded percentage reduction by 39.0, 35.0 and 67.5%, respectively in early spring season. The total chlorophyll contents were decreased with an increase in the inoculum levels of *M. incognita*. At the lower inoculum level the percent reduction in the chlorophyll content was decreased by 28.0%, however, at the higher inoculum level the percent reduction in the chlorophyll content was decreased by 55.0% in the autumn season. Meanwhile, the percent reduction in chlorophyll content was 14.0, and 43.4% in early spring season, respectively.

DISCUSSION

The main symptom of root-knot nematode infection is the presence of root galls, derived from complex physiological and biochemical changes caused by parasites. Such changes lead to cell hypertrophy and hyperplasia, compromising water and nutrient absorption by roots and consequently impairing plant growth and yield (Hussain et al., 2016). The results of our study demonstrated that common bean (*Phaseolus vulgaris* L) cv. Xera plants had severely damaged threshold level (DT) by the root-knot nematode, *Meloidogyne incognita* under two planting dates (autumn, 2021, and early spring, 2022). From our results, it is evident that damage index (DI) was retarded at different inoculum levels (1000, 2000, and 3000J_2s) of the root-knot nematode, *M. incognita.* This damage was greatly influenced by the season of planting (temperature degrees prevalent during common bean growing), and an increase in inoculum levels of *M. incognita*, with maximum reduction in the higher nematode levels. Damage index (DI) increased with the increasing of the inoculum levels of *M. incognita* on common bean cv. Xera with the maximum index rate 8.2, 70 days after inoculation at Pi 3000 J_2s in autumn, 2021 while, it was 8.6 in early spring, 2022.

In the present study, all the inoculum densities of *M. incognita* resulted in significant reductions in growth, yield and increases in nematode infestations in two growing seasons. The reduction in growth parameters may be due to the poor development of lateral roots as a result of high nematode infection rate. The effect of different initial inoculum levels on the growth and yield of diverse plants suffering from these pests has been investigated (Haider et al., 2003; Hussain et al., 2011; Kayani et al., 2017). According to Mukhtar and Kayani (2020) the initial density of nematodes is responsible for a subsequent reduction in the yield of crops and an increase in nematode populations.

In the present study, final nematode populations and gall formations proportionally affected plant growth. Also, our study indicated that the interactions of the inoculum densities of RKN on the growth, yield, and nodulation of common bean cv. Xera, and the interaction of seasons of these parameters were significant.The growth and physiological parameters of infected common bean were significantly decreased when plants were grown in early spring, more than plants grown in autumn season.

The effect of nematodes on growth and physiological parameters of common bean grown in early spring may be due to the high relative temperature prevalent during different stages of common bean grown season (March- April) as temperature averages were 23 and 25 °C, comparing with 16, and 20°C in autumn season. The low temperature (less than 20°C) reduced nematode activity and their ability to attack plants and reproduce (Evans and Perry, 2009). In addition–nematode life cycle is delayed and it may take more than two months (Korayem et al., 2015) hence bean plants reach maturity before the emergence of the first nematode generation which causes dramatic damage to the hosts (Korayem et al., 2012). High temperature is an important factor affecting the expression of resistance to root-knot nematodes in several crop plants. Generally, plant resistance to plant-parasitic nematodes reduced as temperature increases beyond an upper threshold for heat stability. This threshold is determined by temperature effects on the nematode and (or) the crop plant. In common bean (*P. vulgaris*) the level of resistance to *M. incognita* was reduced at 28°C, relative to 16 or 21 °C (Omwega et al., 1990).

Data also showed that nitrogen, potassium, phosphorous, and protein contents decreased in the infected plants compared with the uninfected plants either grown in autumn, or in early spring. Similar results were obtained by Abdel-Monaim et al. (2018), who found that N, P, K, and protein in cowpea were reduced by *Meloidogyne* spp., infection under field conditions. Also, Abdel-Baset et al. (2020) reported that the protein contents in eggplant were reduced by *M. arenaria*. This was likely due to damage caused by the increasing numbers of nematodes that invaded plant roots, and probably ceased the nutrient and water uptake (Karssen and Moens, 2006). Nitrogen is required for the cellular synthesis of enzymes, proteins, chlorophyll, DNA, and RNA, which are important in plant growth and the production of food (Banerjee et al., 2006).

In this study, a significant reduction in shoot nitrogen content was also noticed with an increase in the initial inoculum level. With the increase in nematode population, there was a corresponding decrease in the number of nodules, the nitrogen content of shoot, and the protein contents of grain of *Phaseolus aureus* (Sharf and Hisamuddin, 2019). In the soil, plant parasitic nematodes are attracted to their hosts by the concentration gradients formed by root exudates, which provide a recognition signal, but can also repel nematodes. However, it is not clear whether mineral nutrients play an important role in this process. Some studies show that nematodes cause a drop in root system activity and growth, for example, nematodes are cited as the main agents responsible for potassium deficiency in apples (Simone et al., 2013). In cotton, an attack by *Rotylenchulus reniformis* Linford and Oliveira can cause significant losses but does not affect cotton plant aerial part growth in the presence of high levels of available potassium (Simone et al., 2013). Similar responses have also been observed for micronutrients (Huber and Wilhelm, 1988). The leaf protein content, in common bean, was decreased with an increase in *M.incognita* inoculum levels, with a maximum reduction in Pi (3000 J_2s) plants. A reduction in protein contents with an increase in inoculum levels indicated that the developing nematode continuously withdrew a large amount of nutrients from the plant through the giant cells (Sharf and Hisamuddin, 2019). In nematode-infected plants, the total chlorophyll contents were decreased with increasing the inoculum levels of *M. incognita* in the two growing seasons, due to insufficient supply of photosynthesis, in turn the pods weights were decreased (Sharf and Hisamuddin, 2019).

Conclusively, the current study suggested that root-knot nematode, *M. incognita* applied at three different inoculum levels adversely affected plant growth and chemical constituents of common bean plants. A study that could help in estimating damaging threshold. However, further studies are needed to determine damaging threshold of *M. incognita* on different plant species.

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الملخص العربى

تاثيرات مواعيد زراعه الفاصوليا الخضراء ومستويات العدوى على حد الضرر لنيماتودا تعقد الجذور *Meloidogyne incognita* تحت ظروف الصوبة

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اجريت تجربتان لدراسة تاثير ثلاث مستويات عدوى من نيماتودا تعقد الجذور *Meloidogyne incognita* على خصائص وصفات نمو نباتات الفاصوليا الخضراء صنف "اكزيرا" ومدى استجابة النباتت للاصابة تحت موعدان للزراعة (خريف – ربيعى مبكر) و أوضحت النتائج أن مستويات العدوى *M. incognita* المختلفة كان لها تأثيرات واضحة على نمو نباتات الفاصوليا خلال موسمي النمو. أظهرت النتائج ان زيادة مستويات العدوى من *M. incognita* ادت الى زيادة معنوية فى مؤشر اعداد العقد الجذرية النيماتودية وكذلك مؤشر الضرر على جذور نباتات الفاصوليا. حيث كان معدل مؤشر اعداد العقد الجذرية النيماتودية بعد ٧٠ يوم من العدوى بلقاح ٣٠٠٠ يرقة / نبات (٨,٦ و ٩,٠) خلال موسمى الخريف والربيعى المبكر على التوالي. وبالمثل لوحظ انه بزيادة مستويات العدوى من *M. incognita* كان هناك زيادة فى اعداد كل من الطور اليرقى المعدى فى التربة وكذلك كتل البيض على جذور النباتات خلال موسم الربيعى المبكر مقارنة بموسم الخريف. حيث ادت جميع مستويات العدوى من نيماتودا تعقد الجذور الى انخفاض معنوى فى معايير نمو نباتات الفاصوليا مقارنة بالكنترول (المقارنة) حيث زاد معدل انخفاض نمو النباتات زيادة ملحوظة بزيادة مستويات العدوى خلال موسيمى النمو وكان اكثر تاثرا فى موسم الربيعى المبكر. جدير بالذكر انه بزيادة مستويات اللقاح من نيماتودا تعقد الجذور (٢٠٠٠- ٣٠٠٠) يرقة لكل نبات ادى ذلك الى انخفاض معنوى فى الاوزان الرطبة لكل من المجموع الخضرى والجذرى لنباتات الفاصوليا بالاضافة الى انخفاض فى اطوال النباتات واوزان القرون وكذلك اعداد العقد البكتيرية الجذرية على الجذور خلال موسمى النمو. اشارت النتائج ايضا ان بزيادة مستويات العدوى لنيماتودا تعقد الجذور *M. incognita* انخفضت بعض المعاييرالفسيولوجية لنباتات الفاصوليا، حيث كان لنيماتودا تعقد الجذور تاثيرا سلبيا على محتويات النباتات من النيتروجين، والفوسفور، والبوتاسيوم وكذلك محتوى النباتتات من البروتينات والكلوروفيل.