


**Original Research**

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## Evaluation for resistance of some spinach (*Spinacia oleracea* L.) cultivars against root-knot nematode, *Meloidogyne javanica*

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**Abstract**

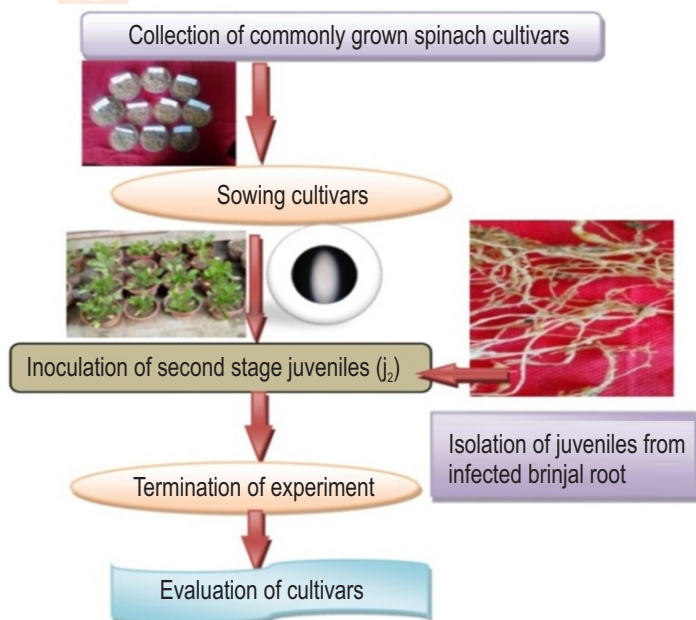
**Aim:** The present study was carried out to screen ten cultivars of spinach (*Spinacia oleracea* L.) for their response to *Meloidogyne javanica* under greenhouse conditions with the aim to exploit them for nematode management programme.

**Methodology:** Ten commonly grown spinach cultivars were tested for their reaction to *Meloidogyne javanica* by inoculating 2000 second-stage juveniles in pots. Five seeds of each cultivar were surface sterilized with 1.0% NaOCl and sown in sterilized clay pots, placed in a completely randomized design. After three months the experiment was terminated and the plant growth, physiological and nematode infested parameters were evaluated.

**Results:** None of the tested cultivar was immune, highly resistant or resistant to nematode infestation. Cultivars Pusa Bharti and Pahari were moderately resistant whereas Giant, All green and Kaveri were highly susceptible to nematode infestation. Five cultivars Pure green, Solan Harit, Green flavor, Super green and Kantadar were found to be susceptible.

**Interpretation:** Growing moderately resistant cultivar in severely infested field with *M. incognita* may assist to minimize the root-knot nematode multiplication and thereby, reduce the crop damage below the threshold level. Thus using resistant cultivars against *M. incognita* can prove to be an environmentally friendly and cost-effective management approach.

**Key words:** *Meloidogyne javanica*, Root-knot nematode, Spinach cultivars



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## Introduction

*Spinacea oleracea* L. is an economically important vegetable crop that belongs to family Amaranthaceae. The miraculous nutritive and medicinal value makes its usable for various macro elements deficiencies and in therapeutic purposes. It is a rich source of dietary potassium, magnesium, iron as well as several vitamins and has an added benefit of low calorie content. Spinach is a proven source of essential nutrients such as carotene (a precursor of vitamin A), ascorbic acid, and their role in antioxidant activities (Lomnitski et al., 2000; Edenharder et al., 2001). It is consumed raw, canned, boiled, pureed, frozen, dehydrated, cooked and baked form (Slavin and Lloyd, 2012). Spinach-generated phytoconstituents furnishes to the anti-cancer, anti-obesity, hypolipidemic and hypoglycemic, properties (Roberts et al., 2016). It grows all over the world as a cool-season annual green leafy vegetable in the tropical and sub tropical regions (Vazquez et al., 2013).

The world's total production of spinach was 30 M tonnes covering an area of 920,804 ha (FAO, 2020). China is the largest producer in the world with 28,520,698 tonnes production volume per year. (FAO, 2020) and in India it cultivated in Uttar Pradesh, West Bengal, Maharashtra, Rajasthan, Punjab, Haryana and Gujarat. Unfortunately, it is prone to attack by various phytopathogens including fungi, bacteria, viruses and nematodes. A lot of research work has been done on different fungal, viral and bacterial diseases of spinach (Larsson and Gerhardson, 1992; Aslam et al., 2019). But no detailed study for the nematode infestation has been reported except some sporadic findings. Vegetable fields that are heavily infested with plant-parasitic nematodes have been reported with 80% loss (Anamika et al., 2011). Plant-parasitic nematodes (PPNs) are one of the major limiting factors in the crop production and cause severe yield losses worldwide (Asif et al., 2018; Khan et al., 2019; Ghareeb et al., 2020).

At global scale, the estimated average annual yield loss of various crops by plant parasitic nematodes is 21.3% of total crop production and 1.58 billion USD (Kumar et al., 2020). Among PPNs, root-knot nematodes (*Meloidogyne* spp.) are the virulent and devastating pest for the spinach (Murungi et al., 2018) causes huge damage to the agricultural sector (Asif et al., 2018; Khan et al., 2019). The yield loss due to *Meloidogyne* spp. in spinach was estimated to be 50-100% (Onkendi et al., 2014). This economic loss caused by the nematode could be curtailed by the involvement of different approaches in the form of chemical treatments, biocontrol agents, organic amendments and cultural practices such as crop rotation, soil solarization and antagonistic plants (Noureldeen et al., 2021; Khan et al., 2021; Hasan et al., 2021) However, for the nematode management programme these strategies should not be the sole prime option. The application of chemicals is an ordinary and most widely used tactic for the nematode management but their exploration is not feasible due to high cost benefit ratio and environmental pollution (Mukhtar et al., 2013). Among the most efficient alternatives to pesticides, natural

plant resistance is one of the economically feasible and environmentally benign methods for the nematode management (Khan et al., 2018; Ansari, et al., 2018). Additionally, the use of resistant cultivar is the key approaches having resistant genes which prevent nematodes infestation on the plant (Williamson et al., 2009). The cultivars referred as resistant to root-knot nematodes have comparatively better crop yield as compared to susceptible cultivars (Mukhtar et al., 2014). This variability in tolerance might be related to their genetic makeup, coding for a resistance mechanism possessed by a particular cultivar (Anwar and McKenry, 2007). In this concern deployment of rotation strategies of susceptible and resistant cultivars can raise the longevity of resistance genes (Nilusmas et al., 2020). Keeping this view in mind, the current study was conducted to analyze the magnitude of disease resistance in ten spinach cultivars against root-knot nematode, *M. javanica* under greenhouse conditions.

## Materials and Methods

**Collection of spinach cultivars:** Seeds of ten spinach cultivars of spinach namely, Giant, All green, Green flavor, Kantedar, Pure green, Super green, Kaveri, Pahari, Solan Harit and Pusa Bharti were collected from distinct places of Uttar Pradesh and Delhi (India). The cultivar Giant was obtained from the market of Akbarabad, Badaun. Seeds of cultivars viz., All green, Green flavor, Kantedar, Pure green and Super green were acquired from the local market of Aligarh and the remaining cultivars namely; Pahari, Pusa Bharti, Solan Harit, and Kaveri were procured from IARI, New Delhi (India).

**Source of nematode inoculum:** Inoculum of root-knot nematodes were collected from infested brinjal fields of small village Plakna near Ozone city, Aligarh. The root-knot nematode, *Meloidogyne javanica* was selected as test pest in the current experiment which was identified through perineal pattern technique (Netscher and Taylor, 1974).

**Maintenance of inoculum:** Inoculum was maintained by multiplying the juveniles of *M. javanica* on brinjal in greenhouse conditions. Plants were gently uprooted at an interval of 90 days and washed to remove adhering soil. Extraction and collection of second stage of juveniles (J2) were carried out from hatched eggs by incubating (27±2°C) handpicked egg masses following the method of Hussey and Barker (1973).

**Screening assay:** Screening of spinach cultivars for resistance to *M. javanica* was conducted under green house condition in the Department of Botany, Aligarh Muslim University, Aligarh, India. Five seeds of each cultivar were surface sterilized with 1.0% NaOCl and sown (5 seeds per pot) in the sterilized clay pots filled with 1 kg per pot (6 inches) sandy loam soil and manure (3:1 ratio). Thinning of seedlings was performed after three weeks leaving one healthy seedling per pot.

**Inoculation technique:** After two days, each plant was inoculated with freshly hatched juveniles (2000 per pot) of *M.*

*javanica* by making three holes around the roots of each cultivar. Thereafter, 2.5 ml of nematode suspension containing 2000 number of juveniles of *M. javanica* was poured into the holes. On the other hand, uninoculated plants served as control. Each cultivar was replicated five times and placed in a completely randomized design. The plants were watered when needed and handled with care to avoid any kind of experimental error.

**Data collection:** After 90 days, all the cultivars were uprooted carefully and roots were washed to remove adhering soil particles. Morphological parameters (shoot and root length; the fresh and dry weight of shoot and root; the number of leaves/plant and surface area index) of each cultivar were measured separately. Physiological parameters namely chlorophyll and carotenoid content were estimated following the method of Mackinney (1941) and Maclachlan and Zalik (1963), respectively.

The percent reduction in the growth parameters over their control was calculated by the following formula: (Hooper, 1986).

$$\% \text{Reduction} = \frac{\text{Value in uninoculated} - \text{Value of inoculated}}{\text{Value in uninoculated}} \times 100$$

Root-knot nematode infestation caused by *M. javanica* was assessed by pathological parameters such as number of galls per plant, number of egg masses per plant, number of eggs per egg masses and nematode population density in 250 g soil of each infected plant. Host rating status towards the nematode was assessed by estimating the number of galls per root system following the method of Taylor and Sasser (1978).

**Statistical analyses:** The experimental data were analyzed statistically by One-way ANOVA using SPSS-17 statistical software (SPSS Inc., Chicago, IL, USA). The mean values were statistically compared by Duncan's Multiple Range Test at  $p \leq 0.05$ .

## Results and Discussion

The use of resistant cultivars is considered as an eco-friendly and sustainable strategy to manage root-knot nematode. In this study, the response of ten spinach cultivars towards *M. javanica* was evaluated based on the number of galls and reduction in plant growth characters. In this study, all the tested cultivars exhibited significant variations in response to root-knot nematode infestation. Among all cultivars, none of them was immune or highly resistant against *M. javanica*. The cultivars Giant, All green and Kaveri were highly susceptible to the root-knot nematodes infestation. The cultivars Pure green, Solana Harit, Green flavor, Super green and Kantedar displayed susceptible behavior in terms of nematode reproduction and suppression in the growth characters and were less destructive than highly susceptible cultivars. However, cultivars namely, Pusa Bharti and Pahari showed moderately resistant responses. All the cultivars exhibited significant variations in relation to the formation of number of galls, number of egg masses, number of

eggs per egg masses and nematode population per 250 g of soil. The results revealed that highly susceptible cultivars (Giant, All green and Kaveri) showed maximum galling in roots, egg masses per root system, eggs per egg mass and nematode population. Highly susceptible cultivars namely, Giant (204), All green (138) and Kaveri (112) displayed highest number of galls followed by susceptible cultivars namely, Pure green (97), Solan Harit (91), Green flavor (85), Super green (71) and Kantedar (64) in terms of number of gall per root. Similarly, the highest number of egg masses were recorded in highly susceptible, i.e., Giant (109), All green (101) and Kaveri (94), respectively.

Similar trend was observed for the egg production and nematode population per root system. The maximum number of eggs (121) and nematode population (2174) were found in highly susceptible cultivars namely Giant (121 and 2174), All green (110 and 2031) and Kaveri (105 and 1946) where as minimum was found in Pahari (19 and 1012) and Pusa Bharti (27 and 1123). This higher invasion rate of juveniles and development of root gall usually depends on the compatible interactions of nematode and susceptible host plants (Zhou et al., 2000). On the other hand, the minimum numbers of galls were observed in moderately resistant cultivars, Pusa Bharti (8) and Pahari (5). Similarly, the lowest number of egg masses were found in cultivar Pusa Bharti (13) and Pahari (8). In addition to this, similar trend was followed by the egg production and nematode population. Resistance or susceptibility and genetic differences between nematode populations are determined by the host genetic factor (Petitot et al., 2020; Haq et al., 2021). The resistant plants consists specific resistance genes against pathogens (Cousins and Walker, 2000) and some defense mechanism participate in suppression of nematode invasion on host plants (Chaudhary et al., 2019; Hada et al., 2021).

The degrees of resistance and tolerance are not always same due to separate genetic control in some plant nematode interactions (Wille et al., 2019; Gartner et al., 2021). Resistance and susceptibility to plant parasitic nematodes are determined by the effect of the plant on the nematode's ability to reproduce (Cook and Evans, 1987) where resistant plants contain fewer developed nematodes than susceptible plants (Kayani and Mukhtar, 2018). Likewise, susceptible cultivars viz., Pure green, Solan Harit, Green flavor, Super green and Kantedar follow similar trend in terms of galls, number of egg masses, egg per egg masses and nematode population (Table 2). Similar, observation was reported by Vovlas et al. (2005), who stated that the number of root galls has a direct relationship with the nematode population density where nematode population in the susceptible plant accomplish their full development while lesser development was reported in resistant cultivar. Besides the number of galls nematode also caused significant increase in the inadmissible characters in the form of abnormalities of infected root of spinach. The root-knot nematode, *M. javanica* showed variable effects on the plant growth characters of all the tested cultivars. These cultivars displayed significant variation in reduction in terms of shoot and root length, fresh and dry weight of shoot and root and

**Table 1:** Effect of infestation by the root-knot nematode *M. javanica* on growth parameters of different spinach cultivars

Cultivars		Shoot length	Percent reduction	Root length	Percent reduction	Fresh shoot weight	Percent reduction	Fresh root weight	Percent reduction	Dry shoot weight	Percent reduction	Dry root weight	Percent reduction
All green	C	40.9 <sup>a</sup>	46.4 <sup>ab</sup>	64.2 <sup>a</sup>	43.6 <sup>a</sup>	62.83 <sup>a</sup>	45.3 <sup>a</sup>	29.63 <sup>abc</sup>	41.5 <sup>a</sup>	5.40 <sup>a</sup>	43.7 <sup>a</sup>	2.15 <sup>ef</sup>	43.0 <sup>ab</sup>
	IC	21.9 <sup>ghi</sup>		36.2 <sup>hi</sup>		34.36 <sup>gh</sup>		17.33 <sup>fg</sup>		3.04 <sup>fgh</sup>		1.22 <sup>i</sup>	
Giant	C	39.6 <sup>ab</sup>	48.7 <sup>a</sup>	65.0 <sup>a</sup>	45.0 <sup>a</sup>	59.81 <sup>a</sup>	47.0 <sup>a</sup>	28.02 <sup>bcd</sup>	43.0 <sup>a</sup>	5.25 <sup>a</sup>	44.6 <sup>a</sup>	2.54 <sup>a</sup>	45.8 <sup>a</sup>
	In	20.3 <sup>hij</sup>		35.7 <sup>hi</sup>		31.69 <sup>h</sup>		15.97 <sup>g</sup>		2.90 <sup>h</sup>		1.37 <sup>k</sup>	
Green flavor	C	34.6 <sup>cd</sup>	36.4 <sup>cd</sup>	58.5 <sup>c</sup>	34.7 <sup>bcd</sup>	61.43 <sup>a</sup>	35.5 <sup>cd</sup>	27.24 <sup>cd</sup>	32.0 <sup>cd</sup>	4.57 <sup>bc</sup>	31.2 <sup>de</sup>	1.88 <sup>g</sup>	32.7 <sup>de</sup>
	In	22.0 <sup>ghi</sup>		38.2 <sup>hi</sup>		39.62 <sup>de</sup>		18.52 <sup>fg</sup>		3.14 <sup>fgh</sup>		1.26 <sup>ki</sup>	
Kantedar	C	36.6 <sup>bc</sup>	31.8 <sup>e</sup>	63.0 <sup>a</sup>	30.9 <sup>d</sup>	60.87 <sup>a</sup>	32.0 <sup>d</sup>	27.31 <sup>cd</sup>	28.0 <sup>ef</sup>	4.50 <sup>bc</sup>	27.0 <sup>f</sup>	2.18 <sup>ef</sup>	26.2 <sup>f</sup>
	In	24.9 <sup>g</sup>		43.5 <sup>g</sup>		41.39 <sup>d</sup>		19.66 <sup>f</sup>		3.28 <sup>fgh</sup>		1.60 <sup>h</sup>	
Kaveri	C	41.5 <sup>a</sup>	43.6 <sup>b</sup>	62.4 <sup>ab</sup>	42.9 <sup>a</sup>	60.43 <sup>a</sup>	41.5 <sup>b</sup>	31.00 <sup>a</sup>	40.0 <sup>a</sup>	4.63 <sup>bc</sup>	38.5 <sup>b</sup>	2.27 <sup>de</sup>	42.0 <sup>b</sup>
	In	23.4 <sup>ghi</sup>		35.6 <sup>hi</sup>		35.35 <sup>fgh</sup>		18.60 <sup>fg</sup>		2.84 <sup>h</sup>		1.31 <sup>kl</sup>	
Pahari	C	39.6 <sup>ab</sup>	25.7 <sup>f</sup>	59.1 <sup>bc</sup>	23.3 <sup>e</sup>	62.38 <sup>a</sup>	24.8 <sup>e</sup>	25.45 <sup>de</sup>	22.7 <sup>g</sup>	4.42 <sup>c</sup>	20.1 <sup>h</sup>	2.09 <sup>f</sup>	24.6 <sup>fg</sup>
	In	29.4 <sup>ef</sup>		45.3 <sup>fg</sup>		46.90 <sup>c</sup>		19.67 <sup>f</sup>		3.53 <sup>efg</sup>		1.57 <sup>hi</sup>	
Pure green	C	31.9 <sup>de</sup>	39.2 <sup>c</sup>	58.5 <sup>c</sup>	37.7 <sup>b</sup>	58.47 <sup>a</sup>	37.9 <sup>c</sup>	30.78 <sup>ab</sup>	36.7 <sup>b</sup>	4.68 <sup>bc</sup>	35.6 <sup>c</sup>	2.39 <sup>bc</sup>	38.3 <sup>c</sup>
	In	19.3 <sup>i</sup>		36.4 <sup>hi</sup>		36.30 <sup>efg</sup>		19.48 <sup>f</sup>		3.01 <sup>gh</sup>		1.47 <sup>ji</sup>	
Pusabharti	C	39.2 <sup>ab</sup>	27.6 <sup>f</sup>	53.6 <sup>d</sup>	26.1 <sup>e</sup>	53.8 <sup>b</sup>	28.0 <sup>b</sup>	26.02 <sup>de</sup>	25.5 <sup>g</sup>	5.07 <sup>ab</sup>	23.9 <sup>g</sup>	2.31 <sup>cd</sup>	23.1 <sup>g</sup>
	In	28.3 <sup>f</sup>		39.6 <sup>h</sup>		38.76 <sup>def</sup>		19.38 <sup>f</sup>		3.8 <sup>5de</sup>		1.77 <sup>g</sup>	
Solanharit	C	39.0 <sup>ab</sup>	38.5 <sup>c</sup>	48.8 <sup>ef</sup>	36.2 <sup>bc</sup>	53.19 <sup>b</sup>	37.3 <sup>c</sup>	25.42 <sup>d</sup>	34.5 <sup>bc</sup>	5.34 <sup>a</sup>	32.4 <sup>d</sup>	2.48 <sup>ab</sup>	35.3 <sup>d</sup>
	In	23.9 <sup>gh</sup>		31.1 <sup>j</sup>		33.35 <sup>gh</sup>		16.65 <sup>fg</sup>		3.60 <sup>ef</sup>		1.60 <sup>h</sup>	
Super green	C	30.5 <sup>ef</sup>	34.2 <sup>de</sup>	51.6 <sup>de</sup>	32.4 <sup>cd</sup>	59.68 <sup>a</sup>	33.3 <sup>d</sup>	23.12 <sup>e</sup>	30.9 <sup>de</sup>	4.23 <sup>cd</sup>	30.0 <sup>e</sup>	2.16 <sup>ef</sup>	29.9 <sup>e</sup>
	In	20.0 <sup>i</sup>		34.8 <sup>i</sup>		39.80 <sup>de</sup>		15.97 <sup>g</sup>		2.96 <sup>gh</sup>		1.51 <sup>hi</sup>	

C-Control, IC-Inoculated; Values are mean of four replicates; Initial inoculum level = 2000 second stage juveniles of *Meloidogyne javanica* per pot; Means on each column followed by the same letters are not significantly different according to Duncan's Multiple Range Test at  $P \leq 0.05$

foliage growth over their respective controls. The maximum reduction in terms of shoot and root lengths were observed in cultivars Giant, All green and Kaveri; the percent reduction noted in shoot length was 48.7, 46.6 and 43.6 and root length was 45.0, 43.6 and 42.9 percent, respectively. Alternatively, the minimum reduction was recorded in moderately resistant cultivars viz., Pusa Bharti and Pahari; the percent reduction recorded in shoot length were 27.6 and 25.7 and root length were 26.1 and 23.3, respectively. The growth reduction in susceptible cultivars namely, Pure green, Solan Harit, Green flavor, Super green and Kantedar found to be greater than moderately resistant and lesser than highly susceptible cultivar. Lesser reduction in the susceptible cultivars than highly susceptible cultivar may be due to lower invasion rate of juveniles into the plants, which leads to a lesser population of nematodes in host plants. Likewise, all cultivars responded differently in terms of fresh and dry weight of shoot and root. However, the highest reduction in these parameters was found in cultivars Giant, All green and Kaveri.

The values obtained for fresh shoot weight were 47.0%, 45.3% and 41.5% and fresh root weight was 43.0%, 41.5% and 40.0% for cultivars Giant, All green and Kaveri respectively. The lowest reduction in these parameters were observed in cultivars Pusa Bharti and Pahari. Similarly, the maximum reduction in dry weight of shoot and root was recorded in Giant, All green and Kaveri while minimum in Pusa Bharti and Pahari. A similar trend was observed in foliage growth (Number of leaves and leaf area

index) of the plants of all cultivars (Table-1 and 2). The significant reduction in growth attributes are the main cause of damaged roots promoted by the penetration or feeding of juveniles that eventually decrease the efficiency of water absorption by the root system. The results are in accordance with Wyss (2002); Di Vito *et al.* (2004), they observed that due to heavy formation of galls and egg masses in the root system, the absorption and translocation of water and minerals in the plant is halted as a result of which there is a reduction in photosynthesis and plant growth.

Similar observations on the reduction of growth characters and their role in suppression in the absorption of water and nutrients from the soil and translocation of photosynthates have also been reported by Siddique and Grundler (2018) and El-Sagheer (2019). Nematode infestation reduced photosynthetic pigment (chlorophyll and carotenoid contents) in inoculated plants as compared to uninoculated plants. Chlorophyll and carotenoid contents showed a significant reduction in the inoculated plants due to nematode attack. The maximum reduction was observed in cultivars, Giant (47.5%), All green (44.6%) and Kaveri (41.1%). On the other hand, the minimum reduction was noticed in Pusa Bharti (24.2%) and Pahari (21.6%). Lesser reduction in the chlorophyll content in the remaining five cultivars, Puregreen (39.0%), solan Harit (35.0%), Green flavor (33.1%), Super green (30.7%) and Kantedar (28.6%) was noted over their respective control. A similar trend was also found in the carotenoids content; the greatest reduction was recorded in

Table 2: Effect of root-knot nematode *M. javanica* on different cultivars of spinach in relation to the physiological and nematode infestation parameters

Cultivars	No. of leaves/ plant	% reduction over control	Leaf area index/ plant (cm <sup>2</sup> )	% reduction over control	Chlorophyll content (mg g <sup>-1</sup> )	% reduction over control	Carotenoids content (mg g <sup>-1</sup> )	% reduction over control	Galls/ plant	Egg masses/ plant	Egg masses/ masses	Nematode population (250 g soil)	Reaction
All green	C 20 <sup>cd</sup> 11 <sup>hi</sup>	45.0 <sup>ab</sup>	44.89 <sup>a</sup> 25.09 <sup>cd</sup>	44.1 <sup>a</sup>	1.83 <sup>b</sup> 1.01 <sup>lm</sup>	44.6 <sup>a</sup>	0.294 <sup>a</sup> 0.168 <sup>k</sup>	42.6 <sup>a</sup>	- 138 <sup>b</sup>	- 101 <sup>b</sup>	- 110 <sup>b</sup>	- 2031 <sup>b</sup>	Highly Susceptible
Giant	C 27 <sup>a</sup>	48.1 <sup>a</sup>	45.31 <sup>a</sup>	46.0 <sup>a</sup>	1.99 <sup>a</sup>	47.5 <sup>a</sup>	0.276 <sup>b</sup>	44.0 <sup>a</sup>	-	-	-	-	Highly Susceptible
Green flavor	C 17 <sup>def</sup> 11 <sup>hi</sup>	34.0 <sup>de</sup>	24.46 <sup>cd</sup> 6.65 <sup>hi</sup>	32.1 <sup>de</sup>	1.04 <sup>i</sup> 1.46 <sup>h</sup>	33.1 <sup>cd</sup>	0.154 <sup>lm</sup> 0.212 <sup>i</sup>	32.5 <sup>bc</sup>	204 <sup>a</sup>	109 <sup>a</sup>	121 <sup>a</sup>	-	Highly Susceptible
Kantedar	C 23 <sup>bc</sup> 16 <sup>efg</sup>	30.0 <sup>f</sup>	22.90 <sup>d</sup> 16.69 <sup>f</sup>	27.1 <sup>f</sup>	0.97 <sup>m</sup> 1.54 <sup>ef</sup>	28.6 <sup>e</sup>	0.143 <sup>n</sup> 0.206 <sup>g</sup>	26.4 <sup>de</sup>	85 <sup>f</sup>	59 <sup>f</sup>	71 <sup>f</sup>	1463 <sup>f</sup>	Susceptible
Kaveri	C 17 <sup>def</sup> 9	42.8 <sup>b</sup>	42.25 <sup>a</sup> 24.54 <sup>cd</sup>	41.9 <sup>ab</sup>	1.09 <sup>k</sup> 1.72 <sup>c</sup>	41.1 <sup>b</sup>	0.151 <sup>m</sup> 0.229 <sup>e</sup>	40.9 <sup>a</sup>	64 <sup>n</sup>	37 <sup>n</sup>	53 <sup>h</sup>	1287 <sup>h</sup>	Susceptible
Pahari	C 19 <sup>de</sup> 14 <sup>gh</sup>	23.8 <sup>g</sup>	7.05 <sup>h</sup> 5.64 <sup>i</sup>	19.9 <sup>g</sup>	1.52 <sup>g</sup> 1.19 <sup>j</sup>	21.6 <sup>f</sup>	0.135 <sup>o</sup> 0.197 <sup>h</sup>	20.9 <sup>f</sup>	112 <sup>c</sup>	-	-	-	Highly Susceptible
Pure green	C 23 <sup>bc</sup> 13 <sup>gh</sup>	39.5 <sup>c</sup>	29.04 <sup>bc</sup> 17.77 <sup>ef</sup>	38.8 <sup>bc</sup>	1.49 <sup>gh</sup> 0.90 <sup>n</sup>	39.0 <sup>b</sup>	0.157 <sup>l</sup> 0.101 <sup>q</sup>	35.2 <sup>b</sup>	051	08	19	-	Moderately Resistant
Pusabharti	C 20 <sup>cd</sup> 14 <sup>gh</sup>	26.1 <sup>g</sup>	14.43 <sup>g</sup> 11.16 <sup>gh</sup>	22.6 <sup>g</sup>	1.67 <sup>d</sup> 1.26 <sup>j</sup>	24.2 <sup>f</sup>	0.248 <sup>d</sup> 0.185 <sup>i</sup>	25.3 <sup>e</sup>	97 <sup>d</sup>	81 <sup>d</sup>	87 <sup>d</sup>	-	Susceptible
Solanharit	C 19 <sup>de</sup> 11 <sup>hi</sup>	36.9 <sup>cd</sup>	33.5 <sup>e</sup> 21.8 <sup>de</sup>	34.7 <sup>cd</sup>	1.80 <sup>b</sup> 1.17 <sup>k</sup>	35.0 <sup>c</sup>	0.262 <sup>c</sup> 0.173 <sup>j</sup>	33.7 <sup>bc</sup>	08 <sup>f</sup>	13 <sup>f</sup>	27 <sup>f</sup>	1123 <sup>i</sup>	Susceptible
Super green	C 25 <sup>ab</sup> 17 <sup>def</sup>	31.9 <sup>ef</sup>	45.20 <sup>a</sup> 31.77 <sup>b</sup>	44.1 <sup>a</sup>	1.58 <sup>e</sup> 1.09 <sup>k</sup>	30.7 <sup>de</sup>	0.181 <sup>i</sup> 0.127 <sup>p</sup>	29.8 <sup>cd</sup>	91 <sup>e</sup>	68 <sup>e</sup>	76 <sup>e</sup>	1626 <sup>e</sup>	Moderately Susceptible

C-Control; IC-Inoculated. Values are mean of four replicates; Initial inoculum level = 2000 second stage juveniles of *Meloidogyne javanica* per pot; Means on each column followed by same letters are not significantly different according to Duncan's Multiple Range Test at P≤0.05

Table 3: A matrix of Pearson's correlation coefficient between measured variables of spinach cultivars

Variables	SL	RL	SFW	RFW	SDW	RDW	NO.L	LAI	CHL	CTND	NO.G	NO.EM	NO.EGGS	NP
SL	1													
RL	0.644*	1												
SFW	0.605	0.828**	1											
RFW	0.561	0.731*	0.596	1										
SDW	0.800**	0.317	0.372	0.367	1									
RDW	0.589	0.288	0.373	0.296	0.785**	1								
NO.L	-0.194	0.045	0.322	-0.338	-0.099	0.324	1							
LAI	-0.601	-0.658*	-0.601	-0.714*	-0.550	-0.215	0.376	1						
CHL	0.798**	0.270	0.351	0.052	0.829**	0.774**	0.192 <sup>ns</sup>	-0.202	1					
CRTN	0.621	0.088	-0.056	-0.092	0.678*	0.317	-0.251 <sup>ns</sup>	-0.188 <sup>ns</sup>	0.745*	1				
NO.G	-0.743*	-0.570	-0.815**	-0.628	-0.721*	-0.682*	-0.208 <sup>ns</sup>	0.569 <sup>ns</sup>	-0.603	-0.172	1			
NO.EM	-0.770**	-0.671*	-0.850**	-0.537	-0.756*	-0.752*	-0.321 <sup>ns</sup>	0.603 <sup>ns</sup>	-0.703*	-0.271	0.945**	1		
NO.EGGS	-0.764*	-0.650*	-0.847**	-0.543	-0.767**	-0.753*	-0.306 <sup>ns</sup>	0.609 <sup>ns</sup>	-0.693*	-0.251	0.956**	0.997**	1	
NP	-0.681*	-0.619	-0.868**	-0.482	-0.687*	-0.701*	-0.402 <sup>ns</sup>	0.559 <sup>ns</sup>	-0.638*	-0.185	0.941**	0.986**	0.984**	1

SL: Shoot length, RL: Root length, SFW: Shoot fresh weight, RFW: Root fresh weight, SDW: Shoot dry weight, RDW: Root dry weight, NO.L: Number of leaves, LAI: Leaf area index, CHL: Chlorophyll, CRTN: Carotenoids, NO.G: Number of galls, NO.EM: Number of egg masses, NP: Nematode population, \*Correlation is significant at the 0.05 level (2-tailed), \*\*Correlation is significant at the 0.01 level (2-tailed), nsCorrelation is not significant

cultivars, Giant (44.0%), All green (42.6%) and Kaveri (40.9%). Whereas, cultivars Pusa Bharti (25.3%) and Pahari (20.9%) showed the lowest reduction in carotenoid content. Other cultivars also showed variability in carotenoid content due to nematode infestation as shown in Table 2. Similar results of reduction in total chlorophyll and carotenoid content has also been noted in lentil and tomato infected by *Meloidogyne incognita* (Ansari et al., 2020; Asif et al., 2017). According to Stahl and Sies. (2003); Dall'Osto et al. (2006) reported that in plants, carotenoids provide photo-oxidative protection against harmful effects of singlet oxygen and lipid radicals and from chlorophyll triplet. In nematode infected plant, the accumulation of carotenoid precursors and effectively chlorophyll, at the feeding sites could be beneficial to root-knot nematode (Kyndt et al., 2017). The reduction of chlorophyll and carotenoid controls in nematode infected plant may be due to the accumulation inside feeding site of the nematode.

Regression equations and trend lines showed positive and significant relationships between number of galls and the percent reduction in shoot length ( $R^2 = 0.9190$ ), root length ( $R^2 = 0.8870$ ), shoot fresh weight ( $R^2 = 0.9289$ ), root fresh weight ( $R^2 = 0.8817$ ), shoot dry weight ( $R^2 = 0.9058$ ), root dry weight ( $R^2 = 0.8652$ ), number of leaves ( $R^2 = 0.9140$ ) and leaf area index ( $R^2 = 0.8847$ ). Pearson's correlation coefficients also showed a strong and negative correlations between nematode infestation parameters and all plant growth attributes of tested cultivars. A strong, negative and significant correlation was noted between number of galls and shoot fresh weight ( $r = -0.815$  at  $P = 0.01$ ) followed by shoot length ( $r = -0.743$  at  $P = 0.05$ ), shoot dry weight ( $r = -0.721$  at  $P = 0.05$ ) and root dry weight ( $r = -0.682$  at  $P = 0.05$ ). Whereas the weak, negative and non-significant correlation was displayed by number of leaves, and nematode induced parameters such as number of galls, egg masses, eggs and nematode population. In addition, positive and non-significant correction was observed in between the leaf area index and nematode induced parameters (Table 3). The findings of the present study revealed significant difference among all the screened cultivars against *M. javanica* in reference to plant growth and nematode induced parameters. The minimum multiplication and infestation of nematode were examined on moderately resistant cultivars.

It can be concluded from the current study that the best way to reduce nematode reproduction is using resistant or moderately resistant cultivars in fields infested with the root-knot nematode, *M. javanica*. Furthermore, the exploration of moderately resistant cultivars viz., Pusa bharti and Pahari could be used in breeding programs to develop new cultivars resistant to nematode.

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### Add-on Information

**Authors' contribution:** S. Fatima: Conceptualization, writing, visualization, writing-original draft; M. Asif: Conceptualization, investigation, writing-original draft; T. Ansari, F. Khan: Analysis, review and editing; M. Shariq, A. Khan: Review and editing; M. Ikram: Cross checking and editing; M.A. Siddiqui: Supervision, review and editing; All authors have read and agreed to published version of the manuscript.

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