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Evaluation of Cabbage Cultivars through Screening against Root-knot Nematode, *Meloidogyne incognita*

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ABSTRACT

The root-knot nematode, *Meloidogyne incognita* is a soil-borne and sedentary endoparasitic nematode, reduces the growth and yield of almost all vegetable crops. This experiment was performed to found the level of susceptibility/resistance of five cabbage cultivars against root-knot nematode, *M. incognita* under greenhouse conditions. All the cultivars showed a different level of susceptibility against *M. incognita*, none of the cultivars was found completely resistant or immune. The cultivar 'Mahi' was found susceptible as 41 galls were observed on the roots and showed maximum reductions in growth, yield and photosynthetic pigments among all five cultivars evaluated. Other cultivars viz., Early-55, Quick ball and Golden acre were moderately susceptible with 28, 23 and 19 galls respectively and comparatively less reduction in growth, yield and photosynthetic pigments. Cultivar 'Parvati super cross' with 6 galls considered resistant because it showed a significantly less or negligible reduction in both growth and yield than other susceptible and moderately susceptible cultivars. A negative correlation was occurred between number of galls and parameters of plant growth and yield.

***Key words*:** Root-knot nematode, *M. incognita*, Screening, Resistance, Susceptibility

Cabbage (*Brassica oleracea* L.) belonging to the mustard family Brassicaceae, is an important vegetable crop that mostly grown during winter season. It is a green leafy, biennial plant which grow as an annual vegetable crop containing condensed leaved heads (edible part). It is one of the richest sources of energy, Vitamin C and health benefits and consume as both cooked and salad as well. India ranks second (8,755,000 tons annually) after China (33,881,515 tons annually) in cabbage production [1]. In India, West Bengal (29.55 million tons) is the leading vegetables producing state followed by Uttar Pradesh (27.70 million tons) during the year 2018-19 [2].

Plant-parasitic nematodes (PPNs) are more devasting soil-borne pathogens which severely reduces the growth and yield of agriculture crops. At present, about 4100 species of PPNs have been described, which substantially burden the agriculture industry [3-4]. Among PPNs, root-knot nematodes (genus *Meloidogyne* spp.) are more harmful pests worldwide. Globally >100 species of root-knot nematodes (genus *Meloidogyne* spp.) have been identified. Among them four species, *M. incognita*, *M. arenaria*, *M. javanica* and *M. hapla* are major and more pathogenic to cause significant losses to almost all agricultural crops up to 90% as well as predispose the crops to other soil-borne pathogens [5-6]. According to Rawal [7], *M. incognita* is the single most and more damaging species in all species of genus *Meloidogyne* around the world. More than 3000 plants, including vegetables, fruits, and 226 weeds, are infected by root-knot nematodes [8]. Global losses due to root-knot nematodes reach up to 78 billion $ in agriculture crops [9]. Jain *et al*. [10] find out 14.1% national loss in India to different agriculture crops due to root-knot nematodes (*Meloidogyne* spp.). Sing and Kumar [11], and Sing *et al*. [12] reported 6% yield losses due to PPNs on cole crops.

Although several approaches viz. bio control agents [13], chemical nematicides, soil amendments [14], use of antagonistic plants and crop rotation [15] apply in nematode infested fields to manage nematodes and reduce their development. It has been found that uses of chemical nematicides for nematode management, potent and effective but detrimental to human health, soil and the environment where it applies [16]. However, uses of resistant cultivars are considered as one of the primaries, economically suitable and ecofriendly method to manage nematodes [17-18]. The aim of this work was to found resistance or susceptibility in 5 cabbage cultivars against root-knot nematode, *M. incognita* under greenhouse condition.

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MATERIALS AND METHODS

Arrangement of seeds of cabbage cultivars and nematode inoculum

Five cabbage cultivars seeds (Mahi, Early-55, Quick ball, Golden acre and Parvati super cross) purchased from seed shop, Aligarh, Uttar Pradesh, India. For nematode inoculum, infected roots of eggplant with root-knot nematodes (Fig 1A) collected from eggplant field which situated about 20-25 km from experimented greenhouse, Aligarh Muslim University, Aligarh, Uttar Pradesh, India. Root-knot nematode species was recognized on the basis of perineal patterns [19] (Fig 1B). Recognized species, *M. incognita* was maintained on susceptible variety of eggplant for further experiment. Egg masses were handpicked from the eggplant roots in a petri plate containing distilled water (DW). All the eggs were assembled and poured into petri plate having 25 µm pore size sieves with distilled water and incubated at 27 ± 2 °C for egg hatching. After 24 h all the hatched second stage of juveniles (J2s) of *M. incognita* (Fig 1C) were collected, and distilled water added for further hatching.

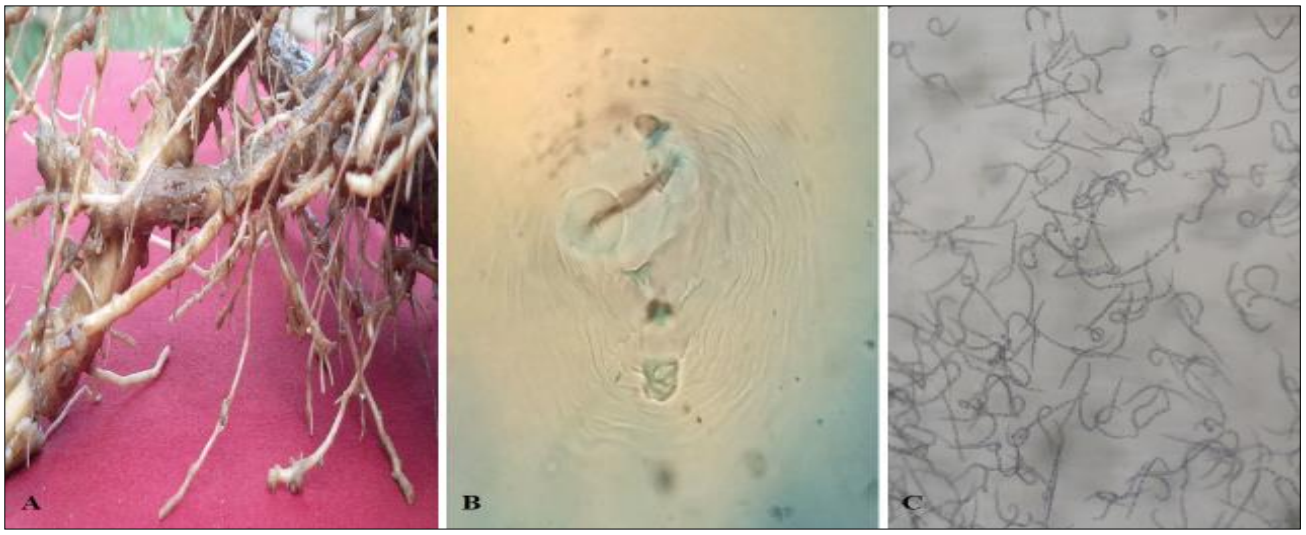

Fig 1 Root-knot nematode infected root of eggplant (A), Perineal pattern of *M. incognita* (B) and freshly hatched second stage juveniles of *M. incognita* (C).

Fig 2 Comparison in root-knot nematode, *M. incognita* infestation on cabbage cultivars root, Mahi cultivar, susceptible with under develop root (A), Parvati super cross cultivar, resistant with healthy root (B).

Cabbage cultivars screening and inoculation technique

Evaluation of cabbage cultivars against *M. incognita* was achieved in earthen pots (25 cm diameter). Before sowing, three seeds of each cultivar were sterilized with 1.0% NaOCl for 15 minutes and washed in DW than shown in earthen pots having 3kg sterilized sandy loam soil and manure (3:1 ratio). Ten to fourteen days after seedling emergence each pot was maintained with one healthy seedling and 3000 freshly hatched J2s of *M. incognita* were inoculated in each pot by making small hole near the root and uninoculated plants consider as control. The experiment was designed on the basis of completely randomized design (CRD) having five replicates for each cultivar. Proper watering and care needed to plants for reducing the error during sampling.

Observations and data collection

After 10-12 week of J2s inoculation, cabbage roots of each cultivar were uprooted and washed in water to remove extra soil with proper care to avoid losses of egg masses during whole process than observation were listed. The estimation of the experimental data was taken on the basis of

various parameters viz. growth (length, fresh and dry weight), yield (no. of leaves, leaf area and fruit circumference), and photosynthetic pigments (chl. a, b and carotenoid). Nematode diseased parameters such as egg masses/root, eggs/egg mass and nematode population /250 g of soil were considered. Percent reduction or increase was calculated of different parameters by following mathematical formula [20-21].

$$\% \text{ Reduction or increase} = \frac{\text{Uninoculated} - \text{Inoculated}}{\text{Uninoculated}} \times 100$$

Nematode population extraction

For knowing the exact population of *M. incognita* in the soil after the completion of the experiment, nematodes extracted with the help of Cobb's sieving and decanting method then Baermann's funnel technique [22-23].

Cabbage cultivars grouping for their resistance

Cabbage cultivars are grouped for their resistance or susceptibility on the basis of degree of *M. incognita* infection, i.e., no. of galls on root which was calculated by using rating scale [24].

Data analysis

Data were evaluated statistically by conducting one-way analysis of variance (ANOVA) with the help of SPSS-17 statistical software (SPSS Inc., Chicago, IL, USA). Duncan's Multiple Range Test (DMRT) uses for comparison of mean values at $P \leq 0.05$. Microsoft Excel 2019 was used to perform the linear regression.

RESULTS AND DISCUSSION

The results of our study indicated that all the cultivars of cabbage against *M. incognita* showed different behavior in respect of various parameters viz., plant growth (shoot and root length, fresh weight and dry weight), yield (no. of leaves, leaf area and fruit circumference), photosynthetic pigments (chl. a, b and carotenoid) and nematode infestation (no. of egg masses/root, no. of eggs/egg mass, population of nematode and no. of galls). However, none of the cabbage cultivars was found immune in all tested cultivars on the basis of rating scale (Table 1). Among tested cultivars, 'Mahi' cultivar was found to be susceptible against *M. incognita* whereas 'Parvati super cross' showed resistant behavior. While other cultivars like E-55, Quick ball and Golden acre shows moderately susceptible behavior against *M. incognita*.

Table 1 Rating scale for the assessment of level of resistant of plant cultivars against root-knot nematode, based on the number of galls (Sasser and Taylor, 1978)

Root index	No. of galls/root system	Resistant rating
0	0	Immune
1	1-2	Highly Resistant
2	3-10	Moderately Resistant
3	11-30	Moderately Susceptible
4	31-100	Susceptible
5	>100	Highly Susceptible

Mahi cultivar showed maximum no. of egg masses (65) per root, no. of eggs (156) per egg mass, nematode population (2500) per 250 gm of soil and maximum no. of galls (41) (Table 4, Fig 2A), such observation on Mahi cultivar indicates that the more no. of J2s enter inside the root and finished their life cycles successfully. Maximum reduction in shoot and root length (47.62 and 40.13%), shoot and root fresh weight (33.49 and 31.90%) and shoot and root dry weight (45.12 and 34.18%) were also observed in Mahi cultivar than followed by Early-55, Quick ball and Golden acre showed 34.51%, 31.96% and 29.87% reduction in shoot length respectively (Table 2). Khan *et al.* [25] were also obtaining similar findings viz., reduction in growth and yield in susceptible carrot cultivars when screened against *M. incognita*.

Table 2 Effect of root-knot nematode, *Meloidogyne incognita* on growth performance of five cabbage cultivars

Cultivars	Treatments	Plant length (cm)				Fresh weight (g)				Dry weight (g)			
		Shoot	% Reduction	Root	% Reduction	Shoot	% Reduction	Root	% Reduction	Shoot	% Reduction	Root	% Reduction
Mahi	Control	10.52^{b}	47.62%	7.50^{c}	40.13%	104.5^{d}	33.49%	12.85^{de}	31.90%	21.85^{e}	45.12%	5.85^{d}	34.18%
	Inoculated	5.51^{d}		4.49^{d}		69.5^{h}		8.75^{f}		11.99^{h}		3.85^{e}	
Early-55	Control	11.01^{b}	34.51%	9.85^{b}	34.51%	109.50^{c}	26.49%	14.95^{d}	20.73%	25.55^{d}	37.96%	7.51^{bc}	26.49%
	Inoculated	7.21^{c}		6.45^{c}		80.49^{g}		11.85^{e}		15.85^{g}		5.52^{d}	
Quick ball	Control	12.2^{b}	31.96%	10.35^{b}	32.27%	111.49^{bc}	24.22%	17.75^{c}	17.18%	28.10^{c}	35.23%	7.85^{bc}	24.84%
	Inoculated	8.3^{c}		7.01^{c}		84.48^{f}		14.70^{d}		18.20^{f}		5.90^{d}	
Golden acre	Control	12.12^{b}	29.87%	10.45^{b}	29.28%	113.95^{b}	21.10%	22.20^{b}	16.66%	30.22^{b}	32.27%	8.95^{ab}	22.90%
	Inoculated	8.50^{c}		7.39^{c}		89.90^{e}		18.5^{c}		20.25^{ef}		6.90^{cd}	
Parvati super cross	Control	16.5^{a}	6.06%	12.89^{a}	16.29%	119.25^{a}	6.54%	27.09^{a}	9.37%	36.7^{a}	16.89%	9.55^{a}	15.18%
	Inoculated	15.5^{a}		10.79^{b}		111.45^{bc}		24.55^{ab}		30.5^{b}		8.10^{abc}	

Each value is the mean of five replicat;es. Means in each column with letters show significant and non-significant differences on the basis of Duncan's Multiple Range Test at $P \leq 0.05$

Parvati super cross cultivar showed less no. of egg masses (10) per root, no. of eggs (15) per egg mass, nematode population (850) per 250 gm of soil and less no. of galls (6) (Table 4, Fig 2B). Minimum reduction in shoot and root length (6.06 and 16.29%), shoot and root fresh weight (6.54 and 9.37%), shoot and root dry weight (16.89 and 15.18%) were also observed in Parvati super cross cultivar (Table 2). Due to resistant ability of Parvati super cross, less no. of J2s penetrated inside the root with less no. of galls as shown in (Table 4). However minimum reduction

in yield viz., no. of leaves (5.74%), leaf area (6.74%), fruit circumference (13.07%) and photosynthetic pigments viz., chl. a (12.85%), chl. b (8.51%) and carotenoid (7.69%) respectively were observed in Parvati super cross, as compare to Mahi cultivar showed maximum reduction in yield viz., no. of leaves (41.21%), leaf area (20.25%), fruit circumference (25.45%) and photosynthetic pigments like chl. a (27.77%), chl. b (26.47%) and carotenoid (25%) respectively (Table 3). According to Vovlas *et al.* [26] the population of J2s reached their maximum and responsible for more reduction in growth and yield in the susceptible host and produces more no. of galls in comparison to resistant host that inhibit the reproduction as well the development of nematodes.

Table 3 Effect of root-knot nematode, *Meloidogyne incognita* on yield and photosynthetic pigments of five cabbage cultivars

Cultivars	Treatments	Yield						Photosynthetic pigments (mg/g) fresh leaves					
		No. of leaves	% Reduction	Leaf area (cm^2)	% Reduction	Fruit circumference (cm)	% Reduction	Chl. a	% Reduction	Chl. b	% Reduction	Carotenoid	% Reduction
Mahi	Control	5.12^{cd}	41.21%	34.12^{d}	20.25%	16.5^{d}	25.45%	0.54^{d}	27.77%	0.34^{cd}	26.47%	0.28^{de}	25%
	Inoculated	3.01^{e}		27.21^{f}		12.3^{f}		0.39^{f}		0.25^{e}		0.21^{f}	
Early-55	Control	5.99^{bc}	31.05%	36.45^{cd}	15.36%	17.75^{d}	21.97%	0.59^{c}	22.03%	0.38^{bc}	21.05%	0.31^{cd}	19.35%
	Inoculated	4.13^{de}		30.85^{e}		13.85^{ef}		0.46^{e}		0.30^{d}		0.25^{e}	
Quick ball	Control	6.90^{ab}	28.40%	41.95^{b}	14.54%	19.85^{c}	20.40%	0.61^{bc}	19.67%	0.39^{bc}	17.94%	0.33^{bc}	15.15%
	Inoculated	4.94^{cd}		35.85^{cd}		15.8^{de}		0.49^{e}		0.32^{d}		0.28^{de}	
Golden acre	Control	7.12^{ab}	26.26%	43.91^{ab}	13.18%	21.85^{b}	18.12%	0.64^{b}	17.18%	0.45^{a}	15.55%	0.36^{ab}	13.88%
	Inoculated	5.25^{cd}		38.12^{c}		17.89^{cd}		0.53^{d}		0.38^{bc}		0.31^{cd}	
Parvati super cross	Control	8.01^{a}	5.74%	45.95^{a}	6.74%	25.85^{a}	13.07%	0.70^{a}	12.85%	0.47^{a}	8.51%	0.39^{a}	7.69%
	Inoculated	7.55^{a}		42.85^{b}		22.47^{b}		0.61^{bc}		0.43^{ab}		0.36^{ab}	

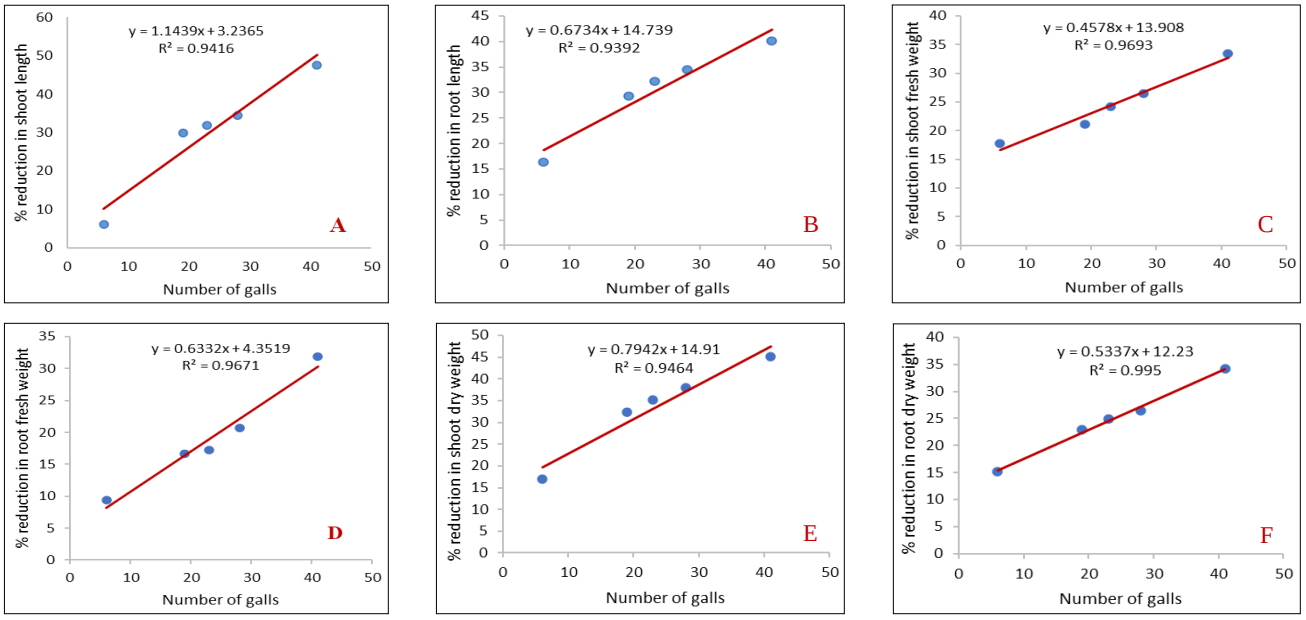

Fig 3 Relationship between number of galls and percent reduction in different growth parameters of cabbage cultivars

Table 4 Effect of root-knot nematode, *Meloidogyne incognita* on different cultivars of cabbage in relation to nematode infestation parameters

Cultivars	Treatments	No. of egg masses/root	No. of eggs/egg mass	Nematode population/250 g soil	No. of galls	Reaction
Mahi	Control	-	-	-	-	Susceptible
	Inoculated	65^{a}	156^{a}	2500^{a}	41^{a}	
Early-55	Control	-	-	-	-	Moderately susceptible
	Inoculated	49^{b}	127^{b}	1850^{b}	28^{b}	
Quick ball	Control	-	-	-	-	Moderately susceptible
	Inoculated	42^{c}	118^{c}	1650^{c}	23^{c}	
Golden acre	Control	-	-	-	-	Moderately susceptible
	Inoculated	35^{d}	109^{d}	1200^{d}	19^{d}	
Parvati super cross	Control	-	-	-	-	Resistant
	Inoculated	10^{e}	15^{e}	850^{e}	6^{e}	

Each value is the mean of five replicates. Means in each column with letters show significant and non-significant differences on the basis of Duncan's Multiple Range Test at $P \leq 0.05$

The result of the experiment revealed that significant differences among all the cultivars of cabbage against *M. incognita*. The cultivars E-55, Quick ball and Golden acre were found to be less or moderately susceptible, undergo less damage compared to susceptible cultivar Mahi. However, Khan *et al*. [27], reported significant reduction in plant growth and yield parameters of Broccoli (Cole crop) at 1000J2s/kg soil of root-knot nematode, *M. javanica*. Other scientists like Pathak *et al*. [28], Khan and Dar [29] also observed the direct relationship between inoculation level of root-knot nematode and the reduction in growth parameters on Cole crops.

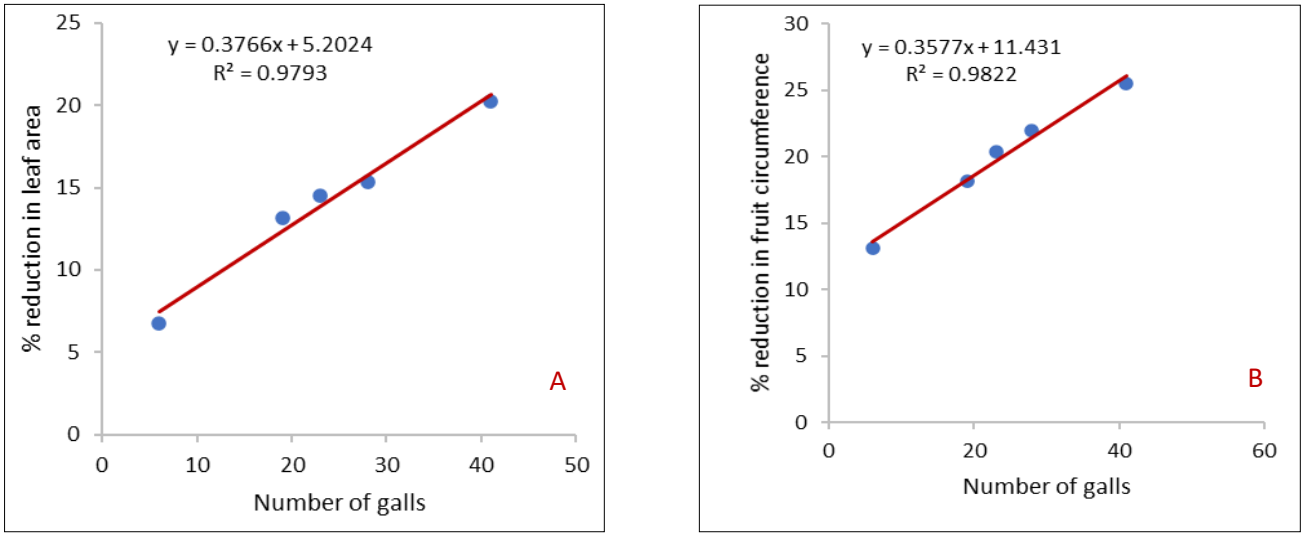

Fig 4 Relationship between number of galls and percent reduction in yield parameters of cabbage cultivars

Regression analysis revealed that a positive relation found between no. of galls and percent reduction in parameters of cabbage cultivars viz., growth and yield i.e., a positive relation occurred between no. of galls and % reduction in shoot length (R^2 = 0.9416), root length (R^2 = 0.9392), shoot fresh weight (R^2 = 0.9693), root fresh weight (R^2 = 0.9671), shoot dry weight (R^2 = 0.9464) and root dry weight (R^2 = 0.995) (Fig 3A-F) and yield parameters, leaf area (R^2 = 0.9793), fruit circumference (R^2 = 0.9822) (Fig 4A-B). All equations of regression are significant.

CONCLUSION

Study of experiment revealed that five of the mostly cultivated cultivars of cabbage in India was found susceptible to moderately resistant against *M. incognita*. Penetration of J2s of *M. incognita* in the roots of susceptible cabbage cultivars causes galling due to which absorption of water and mineral nutrients is affected that result in unhealthy and poor-quality cabbage crop production which ultimately affects its market value and industry that could be a subject of worry in the future with a huge population. In our study the main focus was to find out the level of resistant/susceptibility in all screened cultivars of cabbage against *M. incognita* because the use of resistant cultivar is an important nematode management approach without affecting the environment, soil, human health and enhances the sustainable agriculture, biodiversity and keeps management processes more economical. Thus, the cultivation of resistant cultivars in a nematode-infested field would help to inhibit nematode development and reproduction. Resistant cultivars could also be utilized in a breeding system for making new resistant varieties against PPNs in future cultivation.

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