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Survey to Assess the Absolute Frequency, Relative Frequency, Relative Density, Absolute Density and Prominence Value of different Nematodes Associated with Gerbera and Capsicum in Polyhouse condition

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ABSTRACT: Identification of nematode problem on crops grown under polyhouse is problematic to nematologist as well scientific research community. The present study was carried out with the purpose to assess the occurrence of prevailing plant pathogenic nematode associated with gerbera and capsicum grown in protected cultivation in north Karnataka. Survey on different genera of plant parasitic nematodes associated with gerbera grown in polyhouses of Kelageri, Navaloor and Pachapur revealed that, *Meloidogyne incognita* and *Aphelenchoides* sp. occurred more frequently followed by *Helicotylenchus dihystera*, *Tylenchus*-like PPN and *Rotylenchulus reniformis* with absolute frequencies of 83.3, 66.7, 50, 50, 16.7 respectively. Whereas nematodes associated with capsicum in polyhouses in Dharwad and Chabbi showed maximum diversity in genera of plant pathogenic nematodes such as *Meloidogyne incognita* and *Rotylenchulus reniformis* occurred more frequently followed by *Pratylenchus* sp., *Tylenchus*-like PPN, *Hirschmanniella* sp., *Helicotylenchus dihystera* and *Aphelenchoides* sp. with absolute frequencies of 66.7, 38.9, 16.7, 16.7, 11.1, 5.6 and 5.56 respectively. Based on prominence values, it was evident that in gerbera polyhouses surveyed, *Aphelenchoides* sp., *H. dihystera*, *Tylenchus*-like PPN and *R. reniformis* were prominent after *M. incognita.* Whereas, in the capsicum polyhouses surveyed, *M. incognita* was the most prominent nematode associated with capsicum followed by *R. reniformis, Pratylenchus* sp., *Tylenchus-like* PPN, *Hirschmanniella* sp., *H. dihystera* and *Aphelenchoides* sp.

Keywords: Gerbera, Capsicum, *Meloidogyne,* prominence value.

INTRODUCTION

Protected cultivation is the cultivation of various crops under controlled environmental conditions resulting in higher yields per unit region and as well as increased crop productivity with better quality produce. It serves as an indirect support for the creation of acceptable nursery or planting material and floriculture (Singh, 2005). According to reports, India has over 25,000 hectares of land under protected farming, compared to about 2,000 ha of greenhouse vegetable growing (Hickman, 2011). On an average, in India, a domestic loss of 21,068.73 millions is predicted due to plant parasitic nematodes. In polyhouse vegetable cultivation, loss due to *M. incognita* spreads from 10 to 60 per cent of the region due to mono-cultivation. In major horticultural crops, average annual yield loss due to nematodes goes up to 60 per cent under due to continuous cultivation of the same crop leads to increase the issue of soil-borne organisms (and illnesses) including plant parasite nematodes (Desaeger and Csinos 2006; Engindeniz and Engindeniz 2006; Minuto *et al.,* 2006).

Gerbera (*Gerbera jamesonii* Hook) belongs to Asteraceae family is indigenous to South Africa and Asia. It is the seventh highest produced cut flower among the world's top ten cut flowers. In India gerbera is grown under an area of 1150 ha with a production of 5.02 thousand tonnes of loose flowers and 20.53 lakh numbers of cut flowers. In Karnataka, gerbera is grown under an area of 100 ha with 2.37 lakh number cut flower production (Anon., 2017). In India, yield losses due to *M. incognita* in gerbera is estimated at 30 per cent (Nagesh and Parvatha Reddy 2000) and also involvement between *Phytophthora parasitica* and *M. incognita* is known to cause a disease complex leading

to enormous yield losses. Further, root-knot nematode infection and its interaction with other soil-borne pathogens, exotic varieties of gerbera from Europe suffered 40-60 per cent mortality (Nagesh and Parvatha Reddy 1996).

Capsicum (*Capsicum annuum* L. var. *grossum* Sendt.) is belongs to the family Solanaceae and is also called Bell Peppers. Its species are indigenous to the Americas, where they have been grown for thousands of years. In India capsicum is grown under an area of about 45.85 thousand hectares with production of 327.02 thousand tonnes. In Karnataka, capsicum is grown under an area of 4.13 thousand hectares and with annual production of 81.67 thousand tonnes. (Anon., 2017). In case of capsicum, infection by *M. incognita* makes it highly susceptible to infection by *Fusarium oxsporum* f. sp. *dianthi*. In capsicum, interaction between *Ralstonia solanacearum* and root-knot nematode, *M. incognita* produces wilting symptom. (Rao *et al.,* 2012)

The various nematodes that may evolve in polyhouse-grown ornamental and vegetable plants are Burrowing nematode (*Radopholus similis*), Foliar nematode (*Aphelenchoides fragariae*), Lesion nematode (*Pratylenchus* spp.), Reniform nematode (*Rotylenchulus reniformis* and Stem and bulb nematode (*Ditylenchus dipsaci*). No contribution is made on the community analysis of soil and plant parasitic nematodes associated with gerbera and capsicum. The present study was carried out in view of investigating the type of nematodes which are mostly affecting the polyhouse crop plants (Capsicum and gerbera).

MATERIAL AND METHODS

Collection of nematode samples. A survey on major nematode parasites associated with crops, *viz.,* gerbera and capsicum grown in protected cultivation of northern Karnataka including Dharwad, Belagavi, Bagalkote and Haveri districts was carried out during 2018-19. Samples comprising of soil and roots were collected from rhizospheres of gerbera and capsicum grown in polyhouses. Observations pertaining to the nematode disease symptoms exhibited in these crops were made. Identification of important plant parasitic nematodes in protected cultivation (especially gerbera and capsicum) was done. The information collected during the survey to the different polyhouses of capsicum and gerbera located in northern Karnataka has been presented in the Table 1 and 2 respectively.

Table 1: Plant parasitic nematodes encountered in different locations surveyed and their absolute densities (capsicum).

District	Taluk	Village	*Meloidogyne incognita*	*Helicotylenchus dihystera*	*Rotylenchulus reniformis*	*Aphelenchoides* sp.	*Pratylenchus* sp.	*Tylenchus* like-PPN	*Hirschmaniella* sp.
Dharwad	Dharwad	Dharwad	-	1.65	50.00	-	-	1.65	-
		Kelageri	242.32	-	-	-	156.00	-	-
		COE, Dwd	475.83	-	-	-	-	-	-
	Hubli	Chabbi	-	-	630.52	29.48	44.00	-	-
		Unkal	1024.49	-	282.90	-	-	-	-
	Kundagol	B. Aralikatti	-	-	-	-	64.32	-	127.68
		Hanchinala	-	-	30.15	-	-	165.15	-
		Hireharkuni	255.00	-	561.00	-	-	-	-
Belagavi	Belagavi	Kanabargi	-	-	86.45	-	-	--	43.55
		Sulebhavi	1350.00	-	-	-	-	-	-
	Hukkeri	Pachapur	423.53	-	-	-	-	68.06	-
	Gokak	Arabhavi	459.00	-	-	-	-	-	-
	Raibagh	Mughalkod	322.52	-	-	-	-	-	-
		Kanadal	2542.50	-	-	-	-	-	-
Bagalkote	Badami	Kelawadi	-	-	934.83	-	-	-	-
		Guledgudda	2012.50	-	-	-	-	-	-
Haveri	Haveri	Haveri	1325.00	-	-	-	-	-	-
	Ranebennur	Ranebennur	1533.00	-	-	-	-	-	-
Total			**11965.69**	**1.65**	**2575.85**	**29.48**	**264.32**	**234.86**	**171.23**

Table 2: Plant parasitic nematodes recorded in different locations surveyed and their absolute densities (gerbera).

District	Taluk	Village	*Meloidogyne incognita*	*Helicotylenchus* sp.	*Rotylenchulus reniformis*	*Aphelenchoides* sp.	*Tylenchus* like-PPN
Dharwad	Dharwad	Kelageri	240.00	26.80	-	40.00	-
		Navaloor	201.35	-	-	77.67	15.35
		Hitech Hort. DWD	-	99.00	-	-	33.00
Belagavi	Hukkeri	Paschapur	220.00	132.00	-	44.00	29.04
		Hukkeri	561.00	-	255.00	-	-
Bagalkote	Bagalkote	UHS, Bagalkote	104.00	-	-	80.00	-
		Total	**1326.35**	**257.80**	**255.00**	**241.67**	**77.39**

Sampling of root and soil. Composite soil samples were collected from the depth of 15-20 cm in rhizosphere region of 10-15 capsicum and gerbera plants/spots at random with the help of a scoop, representing one whole polyhouse. Each sample consisted of 50 g of soil and 1 g of root. Later, these samples were pooled to draw a representative sample of 200 grams of soil and five grams of root. All the samples were stored in polythene bags and sealed tightly with a rubber band. A tag containing relevant information *viz.,* date of collection, soil type, cropping history, locality etc., was kept with each bag. The soil and root samples were processed on the same day or kept in the refrigerator at 4°C for a couple of days.

Extraction of nematodes from soil. For extraction of nematodes from the soil, modified Cobb's sieving and Baermann funnel technique (Christie and Perry 1951) was followed by using a series of sieves (of 250, 45, 37 µm pore size). The nematode genera extracted were identified by comparing the characters given by Mai and Lyon (1975). The nematode genera were tentatively identified by comparing the characters given by Mai and Lyon (1975). They were confirmed by making semi-permanent and permanent slides.

Killing and fixing of nematodes. Concentrated nematode suspensions were placed in a glass vial containing minimum quantity of water and the nematodes were killed by adding equal quantity of boiled 40 per cent formalin. After killing and fixing, the nematodes were carefully stored in screw-capped vials as wet collection with proper labels.

Preparation of permanent slides for identification of prevailing nematode. The nematodes extracted from Cobb's sieving and decanting method were taken in a beaker and were used for making the permanent slides for identification of prevailing nematode. The permanent slides were prepared by killing and fixing method given by Seinhorst (1966). The details of nematode species and host were written on slide.

Community analysis. Nematode suspensions obtained upon processing the soil samples from rhizospheres of different polyhouses were used for community analysis. The different plant parasitic nematodes present in the suspension were identified to genus level. Their numbers present in the suspension were determined by taking the average number of nematodes present in five different one milliliter aliquots of nematode suspension. The population densities of different nematode species in the samples were calculated using the formulae (Norton, 1978).

$$\text{Absolute frequency} = \frac{\text{Number of samples containing a species}}{\text{Number of samples collected}} \times 100$$

$$\text{Relative frequency} = \frac{\text{Frequency of a species}}{\text{Total of all species frequency}} \times 100$$

$$\text{Relative density} = \frac{\text{Number of individuals of a species in a sample}}{\text{Total of all individuals in a sample}} \times 100$$

$$\text{Absolute density} = \frac{\text{Number of individuals of a species in a sample}}{\text{Volume or mass or units of the sample}} \times 100$$

$$\text{Prominence value} = \frac{\text{Absolute density} \times \sqrt{\text{Absolute frequency}}}{100}$$

RESULTS AND DISCUSSION

A survey was conducted to find out the prevailing plant pathogenic nematode species associated with gerbera and capsicum grown in protected cultivation in north Karnataka. Twenty four soil/plant samples were collected from Dharwad, Belagavi, Bagalkote, Haveri districts of Karnataka (Fig. 1). Out of twenty four, six samples were from gerbera and eighteen were from capsicum.

Symptomatology of nematode infection in gerbera and capsicum. Survey result indicated that Gerbera infected by nematode showed symptoms of yellowing of leaves, wilting of plant, root rotting and presence of small to medium sized root galls. In case of capsicum almost all stages of capsicum growth were susceptible to nematode infection by showing patchy infestations. The capsicum plants affected with nematodes showed yellow foliage, stunting and appeared unhealthy. Wilting and death occurred in some capsicum plants which were severely infested with nematodes. The plant showed reduced fruit and leaf lamina size with consequent low yield due to the infestation (Plate 1B). Below ground symptoms included the primary and secondary root galling (Plate 1C). Egg masses were visible on the root surface and the female nematode could be seen when the roots were stained and observed under microscope. Plants affected by root knot nematodes were easily infected by soil-borne fungi and bacteria. Galls were seen on the root system affected by the root knot nematode on capsicum and gerbera (Plate 2A, 2B and 2C).

The information on plant pathogenic nematode genera (*Helicotylenchus dihystera, Hirschmanniella* sp., *Rotylenchulus reniformis, Pratylenchus* sp., *Meloidogyne incognita, Tylenchus*-like PPN and other Dorylaimids) associated with gerbera and capsicum were collected during survey depicted in Table 1 and 2. The distribution of nematode diversity in the gerbera and capsicum grown in polyhouses surveyed and their absolute densities in different locations are presented in Table 3 and 4 respectively. Survey on different genera of plant parasitic nematodes associated with gerbera grown in polyhouses of Kelageri, Navaloor and Pachapur revealed that *Meloidogyne incognita* and *Aphelenchoides* sp. occurred more frequently followed by *Helicotylenchus dihystera*, *Tylenchus*-like PPN and *Rotylenchulus reniformis* with absolute frequencies of 83.3, 66.7, 50, 50, 16.7 respectively (Table 3 Fig. 2) . Whereas nematodes associated with capsicum in polyhouses in Dharwad and Chabbi showed maximum diversity in genera of plant pathogenic nematodes such as *Meloidogyne incognita* and *Rotylenchulus reniformis* occurred more frequently followed by *Pratylenchus* sp., *Tylenchus*-like PPN, *Hirschmanniella* sp., *Helicotylenchus dihystera* and *Aphelenchoides* sp. with absolute frequencies of 66.7, 38.9, 16.7, 16.7, 11.1, 5.6 and 5.56 respectively (Table 4, Fig. 3).

Based on prominence values, it was evident that in gerbera polyhouses surveyed, *Aphelenchoides* sp., *H. dihystera*, *Tylenchus*-like PPN and *R. reniformis* were prominent after *M. incognita* (Table 3). Whereas, in the capsicum polyhouses surveyed, *M. incognita* was the most prominent nematode associated with capsicum followed by *R. reniformis, Pratylenchus* sp., *Tylenchus-like* PPN, *Hirschmanniella* sp., *H. dihystera* and *Aphelenchoides* sp. (Table 4).

A random survey was conducted to find out pathogenic nematode species associated with gerbera and capsicum crops and their rhizospheres in protected cultivation located in north Karnataka. Twenty four soil/plant samples were collected from different polyhouses structures located in Dharwad, Hubballi, Belagavi, Kundagol, Bagalkote and Haveri districts. Of these, eighteen were from capsicum and six samples were from gerbera.

On capsicum and gerbera crops, the plant pathogenic nematode genera encountered were *Aphelenchoides* sp., *Helicotylenchus dihystera, Hirschmanniella* sp., *Rotylenchulus reniformis, Pratylenchus* sp., *Meloidogyne incognita, Tylenchus*-like PPN.

Community analysis studies showed that plant pathogenic nematodes such as *Meloidogyne incognita* and *Rotylenchulus reniformis* occurred more frequently than *Pratylenchus* sp., *Tylenchus*-like PPN, *Hirschmanniella* sp., *Helicotylenchus dihystera* and *Aphelenchoides* sp., with absolute frequencies of 66.66, 38.88, 16.66, 16.66, 11.11, 5.55 and 5.55 respectively as far as capsicum crop is considered. In gerbera, *M. incognita* and *Aphelenchoides* sp., occurred more frequently followed by *H. dihystera*, *Tylenchus*-like PPN and *R. reniformis* with absolute frequencies of 83.33, 66.66, 50, 50, 16.66 respectively. Based on prominence values, it was clear that root knot nematode, *M. incognita* was the most prominent nematode associated with capsicum followed by *R. reniformis, Pratylenchus* sp., *Tylenchus*-like PPN, *Hirschmanniella* sp., *H. dihystera* and *Aphelenchoide*s sp. In case of gerbera, *Aphelenchoides* sp., *H. dihystera*, *Tylenchus*-like PPN and *R. reniformis* were prominent after *M. incognita.* Plant parasitic nematodes (*Meloidogyne* spp.) are the most destructive and difficult to control in protected cultivation system (Sharma *et al.,* 2007).

The survey results are in accordance with the findings of Singh and Kumar (2015) who collected 412 root and rhizosphere soil samples from vegetable growing areas of Western Uttar Pradesh. The most frequently occurred pathogenic nematodes was *Meloidogyne incognita* with highest frequency of 82.2. Nagesh and Reddy (2000) reported yield loss of 26 and 30 per cent in carnation and gerbera respectively, other nematodes which may develop in vegetable and ornamental crops grown under polyhouses are Reniform nematode (*Rotylenchulus reniformis)*, Lesion nematode (*Pratylenchus* spp.), foliar nematode (*Aphelenchoides fragariae*), burrowing nematode (*Radopholus similis*) and bulb and stem nematode (*Ditylenchus dipsaci*). Bala and Hosein (1996) also recorded the same nematodes from anthurium and other tropical ornamental crops in Trinidad, West Indies. Singh and Kumar (2015) from Banwari locality of Gurgaon recorded maximum RKN population (129 J_2/200 cc soil) on capsicum, bottlegourd and tomato crops. The frequency of occurrence of *Meloidogyne* and *Xiphinema* spp. was 50.0 in the capsicum. Singh and Khanna (2015) reported that *M. incognita* occurred more frequently with a population range of 37- 1200 per 200 cc of soil in Himachal Pradesh, India. They also reported that the said nematode was more prevalent in capsicum, cucurbits and tomato.

Table 3: Community analysis of plant parasitic nematodes associated with gerbera in polyhouse conditions.

Nematode	Absolute frequency	Relative frequency	Absolute density	Relative density	Prominence value
Meloidogyne incognita	83.33	31.25	265.27	38.31	24.22
Rotylenchulus reniformis	16.66	6.25	255.00	36.83	10.41
Helicotylenchus dihystera	50.00	18.75	85.93	12.41	6.08
Aphelenchoides sp.	66.66	25.00	60.42	8.73	4.93
Tylenchus like-PPN	50.00	18.75	25.80	3.73	1.82

Table 4: Community analysis of plant parasitic nematodes associated with capsicum in polyhouse conditions.

Nematode genera	Absolute frequency	Absolute density	Relative frequency	Relative density	Prominence value
Meloidogyne incognita	66.66	997.14	78.50	60.50	81.41
Rotylenchulus reniformis	38.88	367.98	16.90	22.33	22.94
Pratylenchus sp.	16.66	88.11	1.73	5.35	3.60
Tylenchus like-PPN	16.66	78.29	1.54	4.75	3.20
Hirschmaniella sp.	11.11	85.62	1.12	5.19	2.85
Aphelenchoides sp.	5.55	29.48	0.19	1.79	0.69
Helicotylenchus dihystera	5.55	1.65	0.01	0.10	0.04

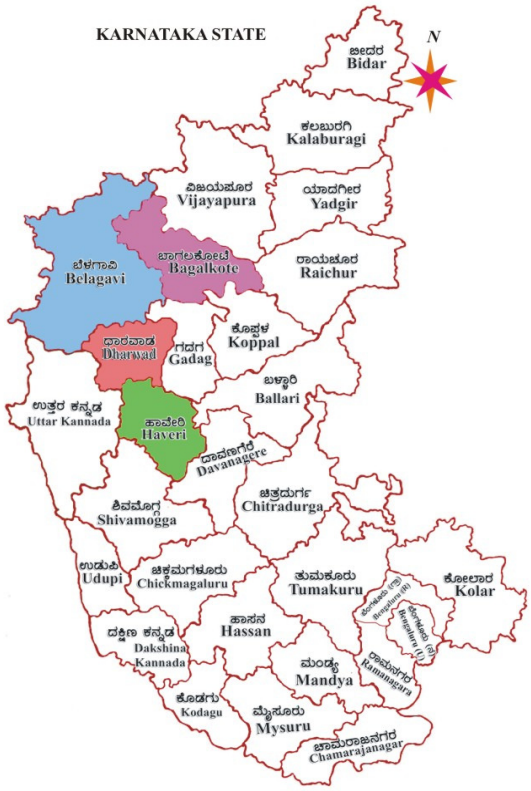

Fig. 1. Survey area in northern Karnataka for the associated plant parasitic nematodes of capsicum and gerbera grown in polyhouse during 2018-19.

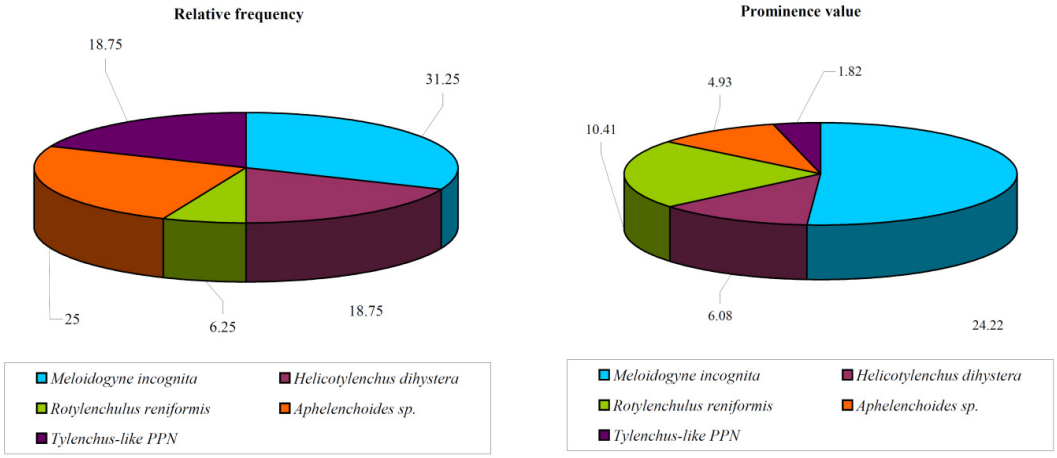

Fig. 2. Relative frequencies and prominence value of plant parasitic nematodes associated with gerbera in polyhouses.

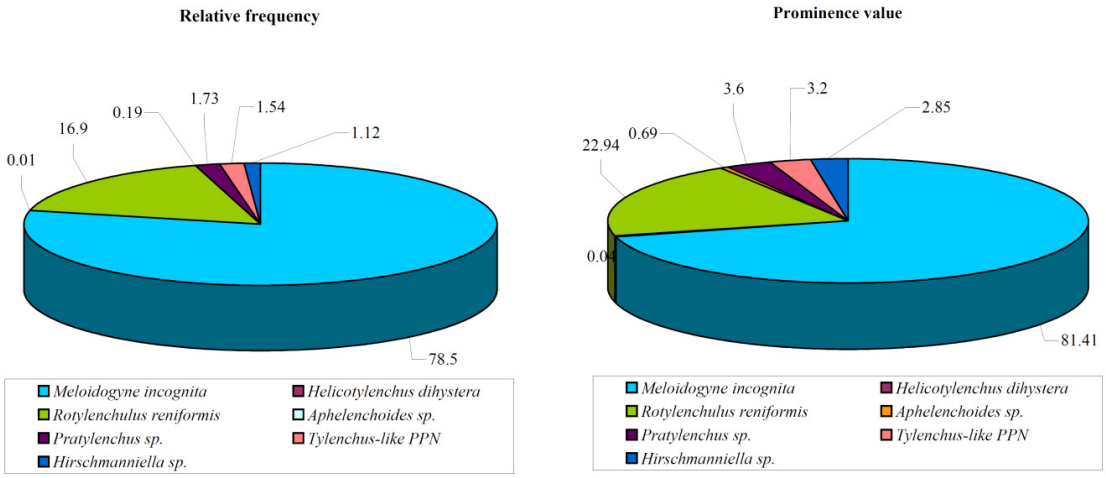

Fig. 3. Relative frequencies and prominence value of plant parasitic nematodes associated with capsicum in polyhouses.

(A) Capsicum polyhouse

(B) Capsicum crop showing yellowing andstunting

(C) Capsicum root showing root galls symptoms at Kanadal, Raibagh (Belagavi dist.)

Plate 1: Polyhouse capsicum crop showing nematode symptoms.

(A) Gerbera crop patches showing nematode symptoms in polyhouse located atKelageri (Dharwad dist.)

(B) Collection of soil and root samples from

(C) Gerbera crop showing yellowing rhizosphere of gerbera affected crop

Plate 2: Polyhouse gerbera crop showing nematode symptoms.

CONCLUSIONS

Efforts were made to know the pathogenic nematode species associated with gerbera and capsicum crops grown in polyhouses located in North Karnataka. Twenty four soil/plant samples were collected from different polyhouses structures located in Dharwad, Belagavi, Bagalkote and Haveri districts. Totally twenty four polyhouses of both gerbera and capsicum were surveyed for the plant pathogenic nematode incidence. The collected rhizosphere samples of gerbera and capsicum were further subjected to soil processing and extraction of plant pathogenic nematodes. Morphological identification of the different plant parasitic nematodes and perineal pattern studies for *Meloidogyne* spp. was done and the prevalent nematodes

were identified to genus level by and large, although species diagnosis was made with respect to the most prevalent/predominant nematode pathogen (and a few others).

The most common plant pathogenic nematodes present were *Helicotylenchus dihystera, Hirschmanniella* sp., *Rotylenchulus reniformis, Pratylenchus* spp., *Meloidogyne incognita, Tylenchus*-like PPN on capsicum and gerbera grown in the polyhouses.

FUTURE SCOPE

Knowing status of different nematodes associated with gerbera and capsicum creates variety of ways to assess the loss caused by these associated nematodes this leads to aid in the disease knowing of temporal and spatial distribution of nematodes in vegetable crops grown in polyhouse condition.

Author contributions. The following are the author contributions: Sunil Awati and S Linagaraju designed the research. Sunil Awati carried out the research activities, analyzed the data, writing-original draft preparation, Software handling. Virupaksha Prabhu H and S. M. Mantur provided technical direction during the experiment Sunil Awati and Manthesha H D prepared the final draft of the document and was Title Page Click here to access/download;Title Page;Front Page.docx modified and submitted. Manthesha H D-Supervised the submission. For publication, each author has read and given their approval.

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Conflict of Interests. None.

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