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First report and morphological characterization of *Meloidogyne javanica* from Una district of Himachal Pradesh

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Abstract

The present study was conducted during the period of 2015-16 (June-September) to characterize morphological profiles of *Meloidogyne javanica* from Papaya and Pumpkin in Himachal Pradesh, India. Affected plants displayed poor growth and significant galling on roots. Serious infections resulted in formation of large numbers of galls, wilted leaves, root rot, and then plant death. Morphological as well as morphological studies were carried out for confirmation of referred species. The present specimens have greater body length (550-789 vs. 541-804 μm), body width (320-550 μm vs. 311-581 μm) and longer spear length (16-22 vs. 14-20 μm) as compared to the earlier described populations by several authors. Morphometrics and female perineal pattern of the isolates fit within the ranges for *M. javanica*. Perineal patterns were oval to squarish in shape, usually with coarse, broken striae and with conspicuous lateral lines. Females had robust stylet with a dorsally curved cone and large transversely ovoid knobs that are offset from the shaft. This species of root-knot nematode was reported for first time from the state.

Keywords: *Meloidogyne javanica*, papaya, pumpkin and morphometrics

1. Introduction

Among the top five plant pathogens affecting world's food production, root-knot nematodes are one of the most devastating pathogens of crops and the most important species, sometimes referred to as the four major species^[8] are the tropical *M. arenaria*, *M. incognita* and *M. javanica*, and the temperate *M. hapla*. In India, the first report of the root-knot nematode (tea eelworm) on tea was initially identified as *Heterodera radicola* from Kerala^[1]. As of now, 14 species of root-knot nematode have been documented from different states of India and out of which four species viz. *M. incognita*, *M. javanica*, *M. graminicola* and *M. arenaria* are most common and damaging species^[8]. Overall, the most common species together parasite more than 5500 plant species, including annual and perennial crops^[12]. Several weed species (226 species belonging to 43 families) are known to act as hosts of root knot nematodes^[5]. Their worldwide damage in terms of reduced yield and quality is estimated to be > \$US 100 billion/year^[2]. The damage symptoms caused by nematode infection may be apparent on parts of the plant both above and below the ground. Above-ground symptoms include varying degrees of stunting, yellowing of foliage, wilting, symptoms of nutrient deficiency and distortion of plant parts. Below-ground symptoms include galls, lesions, stunting, malformation, bifurking and excessive formation of side roots (root beard). The main objective of the study is to determine the prevalence and incidence of Javanese root knot nematode (*M. javanica*) in Himachal Pradesh.

2. Materials and Methods

Survey and Sampling

A systematic survey was conducted in Una district of Himachal Pradesh. About thirty root and soil samples were collected randomly from different cultivated crops viz., Capsicum, Pumpkin, Papaya, Okra, Castor, Tomato and Cucumber. Each sample (250 cc) was put in polythene bag and tied with rubber band to check the evaporation. Supporting data regarding name of the host, locality, date of collection, etc. were tagged to the bag. The samples were then brought to the laboratory for further processing.

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Morphological identification of root-knot nematodes

Fresh gall roots with mature females were selected to prepare perineal pattern. About twenty females from each location were removed by teasing the galls with fine needle. The posterior end was cut under a stereoscopic microscope with the help of a sharp blade. Cut portion was trimmed with a nylon bristle to remove the inner tissue. The perineal pattern was aligned in such a way that it was in a straight line with anus oriented down. The perineal pattern was gently pressed against the glass with hair brush. A cover slip was placed on it gently. Excess glycerine was removed with the help of blotting paper, sealed with nail polish and labeled. *Meloidogyne* spp. females were dissected from the galls using a stereomicroscope (Leica, S8APO) at 40 × magnifications. Females were placed in a drop of 45% lactic acid and the perineal regions were cut and cleaned. Perineal patterns were

prepared following the procedures suggested by [6]. Morphological observations and photographs were completed within 24 hours following slide preparation with the help of a light microscope equipped with a digital camera (Leica, DM2500).

Measurements and identification keys of root-knot nematodes

Twenty specimens of adult females, ten adult female perineal patterns, ten specimens of second stage juveniles and five specimens of males from each population under reference were observed under compound research microscope for their morphological characters. Morphometric measurements of various body parts and their ratios of the individual populations were taken using ocular micrometer.

Morphometric characters used for identification of species of <i>Meloidogyne</i>			
Perineal pattern	Adult female	Juvenile	Adult male
LVS (length of vulval slit)	L (Body length)	L (Body length)	L (Body length)
AVS (anus to vulval slit)	W (Body width)	Stylet length	W (Body width)
ATT (anus to tail terminus)	Stylet length	'c' ratio	LMB (length of median bulb)
IPD (Interphasmidial distance)	Neck length	Tail	WMB (width of median bulb)
Dorsal arch	LMB (length of median bulb)	-	Stylet length
Lateral field	WMB (width of median bulb)	-	Spicule
Striae	-	-	Gubernaculum

3. Results and Discussion

Morphological characters observed in two populations of *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949

In all, twenty mature females were extracted separately from each population of root-knot nematodes recovered from two locations viz., Rampur and Santoshgarh in district Una and observed for their morphological and morphometric characters. Perineal patterns of these females were cut and studied under microscope for confirming the identification at species level.

Description

Rampur population

Females: Pear shaped, body filled with eggs, no distinct posterior protuberance, L = 550-789 µm, Stylet 16-20 µm long, stylet shaft cylindrical, stylet knobs small, rounded not deeply indented, anteriorly protruding neck, 179-240 µm in length. Oesophagus with a large muscular median bulb, 12-15 µm in diameter and 15-25 µm in length.

Perineal Patterns: Typical of *M. javanica* with rounded low dorsal arch, smooth striae and distinct parallel lateral lines delineating the dorsal and ventral regions of the pattern. Distance between anus to vulval slit (AVS) 18-26 µm, distance from anus to tail terminus (ATT) 12-15 µm. Two distinct phasmids having inter-phasmidial distance (IPD) of 19-24 µm.

Second stage Juveniles: L= 335-445 µm, lip region not offset, labial disc elevated. Lateral lips absent. Stylet 10-18 µm long with clear basal knobs, tail = 25-60 µm, distinct hyaline tail terminus, long narrow tapering tail with roundish terminus.

Males: L = 890-1300 µm, Lip region not offset, labial disc elevated, lateral lips absent, stylet 20-24 µm long, rounded to transversely elongate, bluntly rounded tail with slightly curved spicules measuring 23-37 µm.

Santoshgarh population

Females: Pear shaped body, L = 578-750 µm, Stylet 18-22

µm long, basal knobs small, rounded and clear. Neck extends anteriorly 180-252 µm in size. Median bulb 16-30 µm in length and 12-17 µm in width.

Perineal patterns: Oval with low dorsal arch, striae smooth, lateral field distinct and very clearly demarcated by more or less parallel lines. Distance between anus to vulval slit (AVS) 15-20 µm and distance between anus to tail terminus (ATT) 12-16 µm. Two distinct phasmids having inter-phasmidial distance (IPD) of 20-25µm.

Second stage Juveniles: L= 342-450 µm, labial region not offset, Lateral lips absent, Stylet 12-15 µm long, tail = 30-47 µm, distinct shorter hyaline tail terminus, long narrow tapering tail with roundish terminus.

Males: Labial region not offset, lateral lips absent, stylet 18-26 µm long, basal knobs offset and rounded, bluntly rounded tail with slightly curved 23-36 µm long spicule.

Morphological characteristics of observed populations were consistent with those described by [7] for identification of *M. javanica* in which females had rounded perineal patterns with low, trapezoid shape dorsal arch, striae smooth interrupted by a pair of incisures on both sides, corresponding to lateral fields, clearly demarcated from striae by more or less parallel lines, with often distinct tail whorl. The morphological observations on *M. javanica* populations recorded during the present studies are in close proximity with the revelations of [10] who described this species for the first time from Brazil. Also, the second stage juveniles of two populations of *M. javanica* showed morphological similarities with respect to the species specific taxonomic characters in head and tail regions among themselves as well as with the original description given by Treub in 1885.

Morphometric variations in females of two populations of *Meloidogyne javanica*

Morphometric variations as recorded in adult females, their perineal patterns, second stage juveniles and males of root-

knot nematode populations (hence identified as *M. javanica*) encountered in Una district of the state were recorded to ascertain the validity of different characters within the species. Twenty females of each population were visualised for studying their morphometric characters.

Description: based on females, juveniles and males of each population

Measurements

Female: Body length = 550-789 μm ; body width = 320-550 μm ; Stylet length = 16-22 μm ; neck length = 179-252; LMB = 15-30 μm ; WMB = 12-17 μm

Perineal pattern: LVS = 17-23 μm ; AVS = 15-26 μm ; ATT = 12-16 μm ; IPD = 19-25 μm

Second stage juvenile (J2): L = 335-450 μm ; Stylet length = 12-15 μm ; Tail = 25-60 μm ; c = 6.5-15 μm

Male: L = 965.9-1051.4 μm ; W = 17-23.5 μm ; Stylet length = 21-25 μm ; LMB = 17.2-25 μm ; WMB = 9-13 μm , Spicule length = 21-32.5 μm ; Gubernaculum = 9-12 μm

Host plants: Litchi (Rampur population) and papaya (Santoshgarh population)

Studies on morphometric variations in adult females, perineal patterns, second stage juveniles and adult males of two *M. javanica* populations encountered during the present investigations were undertaken so as to ascertain the validity of taxonomic significance of different characters within and

between these populations. The accumulated measured values have been given in Tables 1-5.

Numerical character variations in two populations of adult females of *M. javanica* presented in Table 1 reveal interesting differences between the two populations of the same species. The range of female body length in Rampur population recovered from litchi roots was bigger (550-789 μm) as compared to Santoshgarh population recovered from papaya in which female body size varied between 578-750 μm . However, mean body length in females from litchi (Rampur) remained smaller at 624.5 μm as compared to 653.4 μm attained in females infecting papaya in Santoshgarh. Interestingly, whereas, body length seemed to be a variable character within Rampur population (CV = 13.4 per cent), it remained comparatively stable (CV = 8.8 per cent) in Santoshgarh population. Corresponding to body length, mean female body width was also lesser (447.1 μm) in litchi population of *M. javanica* as compared to 463.3 μm in papaya population. This character was assessed to be 'Variable' as coefficient of variance in both the populations exceeded 12 per cent.

The population of *M. javanica* females harbouring papaya (Santoshgarh population) had a longer stylet in the range of 18-22 μm as compared to that harbouring litchi (Range of stylet length = 16-20 μm). Accordingly, the mean stylet length in two respective populations was 20.2 μm and 17.8 μm . Despite, difference in stylet length in two populations, this taxonomic character was found to be 'Stable' within the population with coefficient of variance much below 12 per cent (7.8 per cent in litchi and 6.4 per cent in papaya population) in both the populations.

Table 1 Morphometric variations in two populations of mature females of *M. javanica* (dimensions of characters in μm)

Character	Rampur (Una) n=20	Santoshgarh (Una) n=20	CR
L	*624.5 \pm 83.7 ** (550-789) 13.4	653.4 \pm 57.7 (578-750) 8.8	Moderately Variable
W	447.1 \pm 83.2 (320-540) 18.6	463.3 \pm 75.3 (325-550) 16.2	Moderately Variable
Spear	17.8 \pm 1.4 (16-20) 7.8	20.2 \pm 1.3 (18-22) 6.4	Stable
Neck length	215.1 \pm 30.3 (179-240) 14.0	221.3 \pm 28.6 (180-252) 12.9	Moderately Variable
LMB	20.2 \pm 1.3 (15-25) 6.4	21.1 \pm 2.3 (16-30) 10.9	Stable
WMB	13.6 \pm 1.1 (12-15) 8.0	14.8 \pm 1.4 (12-17) 9.4	Stable

L = body length, W- body width, LMB = Length of median bulb, WMB = Width of median bulb

*Mean \pm SD, ** Range, CV per cent,

CR- character ranking: stable (<12 per cent); variable (12-20 per cent); highly variable (>20 per cent)

Neck length in individuals of both populations differed significantly in the range of 179-252 μm . This character was found to be 'Variable' within the populations with CV exceeding 12 per cent in both the populations under consideration.

Length of median bulb (LMB) was appreciably close to similar in both the populations under consideration. The mean LMB in individuals harbouring litchi was 20.2 μm \pm 1.3 as compared to 21.1 μm \pm 2.3 in nematodes infecting papaya. Coefficient of variance remained far below 12 per cent in both the populations under study. Hence, LMB has been ranked as a 'Stable' character within the populations of *M. javanica* and thus has significance as a taxonomic character. On the lines of LMB, width of median bulb (WMB) is also a stable character. The mean WMB of 13.6 μm in litchi populations and 14.8 μm

in papaya population not only were close to each other but also their per cent variance factor at 8.0 and 9.4 in corresponding populations were very close.

Table 2, relates to the measurements of various characters of perineal patterns thought to be taxonomically significant in characterization of root-knot nematode. Characters like LVS, AVS, ATT and IPD in perineal patterns of ten females each from both the populations were measured at and evaluated for their taxonomic significance. The mean lengths of vulval slit (LVS) were recorded at 19.3 and 20.8 μm in respective populations infecting litchi (Rampur) and papaya (Santoshgarh). Variations were non-significant within the population in both the locations. Hence, LVS is a stable character that can be used in characterization of *M. javanica* populations.

Table 2: Morphometric variations in two populations of perineal pattern of mature females of *M. javanica* (dimensions of characters in μm)

Character	Rampur (Una) n = 10	Santoshgarh (Una) n = 10	CR
LVS	*19.3 \pm 1.4 ** (17-21) 7.2	20.8 \pm 1.4 (18-23) 6.7	Stable
AVS	21.3 \pm 2.7 (18-26) 13.0	22.6 \pm 1.9 (15-20) 8.4	Variable
ATT	13.6 \pm 1.2 (12-15) 8.9	14.4 \pm 1.5 (12-16) 10.4	Stable
IPD	22.0 \pm 2.9 (19-24) 13.1	23.7 \pm 2.8 (20-25) 11.8	Variable

LVS = length of vulval slit, AVS = anus to vulval slit, ATT = anus to tail terminus, IPD = inter-phasmidial distance

*Mean \pm SD, ** Range, CV per cent

CR- character ranking: stable (<12 per cent); variable (12-20 per cent); highly variable (>20 per cent)

Average distance between anus to vulval slit (AVS) showed slight difference between the two populations under investigations. Mean AVS value of 21.3 μm \pm 2.7 in females infecting litchi was close to 22.6 μm \pm 1.9 in those infecting papaya roots. However, while, the character showed variability in litchi population (CV = 13.0 per cent), it was found to be 'Stable' in females harbouring the papaya roots (CV = 8.4 per cent). Distance between anus to tail terminus (ATT) as calculated in the perineal patterns of the two populations revealed slight variance at 13.6 μm \pm 1.2 and 14.4 μm \pm 1.5 in respective populations harbouring litchi and papaya roots. Both the populations showed stability of this

character within the population with respective CV's of 8.9 and 10.4 per cent, thus ranking it as a 'Stable' taxonomic character. Inter-phasmidial distance in two populations ranged between 19-24 μm (litchi population) and 20-25 μm (papaya population). Calculated coefficient of variance at 13.1 per cent in litchi population indicated moderate variance in this character but in papaya population CV remained below 12 per cent (11.8 per cent) to consider it as a 'Stable' character within the population.

Morphometric characters viz., body length, stylet length, tail length and 'c' value of second stage juveniles of two populations of *M. javanica* have been demonstrated in Table 3.

Table 3: Morphometric variations in two populations of juvenile (second stage) of *M. javanica*. (Dimensions of characters in μm)

Character	Rampur (Una) n = 10	Santoshgarh (Una) n = 10	CR
L	*385.6 \pm 42.7 ** (335-445) 11.0	400 \pm 48.4 (342-450) 12.0	Slightly variable
Spear length	13.4 \pm 0.9 (12-15) 6.7	13.9 \pm 1.1 (12-15) 7.9	Stable
Tail	36.5 \pm 4.5 (25-60) 12.3	37.4 \pm 6.5 (30-47) 17.3	Moderately Variable
C	9.2 \pm 1.7 (6.5-12) 18.4	12.6 \pm 2.5 (7.4-15) 19.8	Moderately Variable

L = body length, 'c' = body length/tail length

*Mean \pm SD, ** Range, CV per cent

CR- character ranking: stable (<12 per cent); moderately variable (12-20 per cent); highly variable (>20 per cent)

The range of body length of J2s in two populations (335-445 and 342-450 μm) corresponded to each other. Body length didn't vary significantly within the population with CV's of 11.0 and 12.0 per cent in litchi and papaya populations respectively. Stylet length of J2's ranged between 12-15 μm in both the populations. The mean stylet lengths of 13.4 μm \pm 0.9 and 13.9 μm \pm 1.1 measured in individuals infecting litchi and papaya respectively also corresponded to each other. With the coefficient of variance of 6.7 per cent in individuals infecting litchi and 7.9 per cent in those infecting papaya, it is assessed to be a 'Stable' character of significance in characterization of *M. javanica* populations.

Appreciable variations were recorded in tail length of juveniles which ranged between 25-60 and 30-47 μm in

respective populations infecting litchi and papaya roots. However, the two populations were very close to each other as far as values of mean tail lengths are concerned (36.5 and 37.4 μm in litchi and papaya population, respectively). This character has been evaluated as 'Variable' within the population owing to its CV exceeding 12 per cent in individual populations. Accordingly, 'c' value showing CV exceeding 12 per cent (CV = 18.4 and 19.8 per cent in respective populations from litchi and papaya) has also been assessed as a 'Variable' character within the population.

Data recorded in Table 4, signifies the taxonomic value of various morphometric characters observed in adult males of two populations of *M. javanica* collected from litchi and papaya in Rampur and Santoshgarh regions of district Una.

Table 4: Morphometric variations in two populations of adult males of *M. javanica*. (Dimensions of characters in μm)

Character	Rampur (Una) n = 5	Santoshgarh (Una) n = 5	CR
L	*1051.4 \pm 153.9 ** (890-1300) 14.6	965.9 \pm 128.6 (800-1150) 13.3	Moderately Variable
W	22.3 \pm 2.8 (20-23.5) 12.5	19.7 \pm 2.7 (17-22.5) 13.7	Moderately Variable
Stylet	23.1 \pm 1.2 (20.0-24) 5.1	23.2 \pm 1.4 (21-25) 6.0	Stable
LMB	19.9 \pm 1.8 (17.2-25) 9.0	21.3 \pm 2.5 (18-24.5) 11.7	Stable
WMB	11 \pm 0.7 (10-12) 6.3	11.1 \pm 1.2 (9-13) 10.8	Stable
Spicule	25.4 \pm 1.5 (23-27) 5.9	24.3 \pm 1.2 (23-26) 4.9	Stable
Gubernaculum	10 \pm 0.7 (9-11) 7.0	11.4 \pm 0.9 (10-12.5) 7.8	Stable

L = body length, W = body width, LMB = length of median bulb, WMB = width of median bulb

*Mean \pm SD, ** Range, CV per cent

CR- character ranking: stable (<12 per cent); moderately variable (12-20 per cent); highly variable (>20 per cent)

Observations on body length of adult males of *M. javanica* recorded from two locations indicate a wide range of variation in size within the population. Body length of individuals recorded in the rhizosphere of litchi roots (Rampur population) varied between 890-1300 μm and those observed

in the rhizosphere of papaya roots ranged between 800-1150 μm . Body length of males is a variable character within the individual population (CV = 14.6 per cent in litchi and 13.3 per cent in papaya population). Similarly, a big variation was observed in body width which varied from 17-23.5 μm in

two populations. Due to high percent coefficient of variance of 12.5 per cent and 13.7 per cent quantified in the referred populations. Body width has also been found to be a 'Variable' character. Stylet length was measured to be in the range of 20-24 μm in Rampur population collected from rhizosphere of litchi plants and 21-25 μm in Santoshgarh population collected from papaya rhizosphere. Despite, variations in range the mean stylet size in both the populations were very close to the tune of 23.1 and 23.2 μm , respectively. Stylet lengths were recorded to be nearly similar within and between the populations under reference with the low coefficient of variance of 5.1 and 6.0 per cent, thus, making it a 'Stable' taxonomic character in the populations of *M. javanica*. Length and width of median bulbs in the adult males of referred populations of *M. javanica* fall in the range of stable characters within the populations of *M. javanica* due to CV values below 12 per cent in both the populations. It is despite the fact that range of length of median bulbs (17.2-25.0 μm in Rampur population and 18-24.5 μm in Una population) in two populations was quite big. Observations inferred similar results with the width of median bulb.

The average lengths of spicules measured in two populations were 25.4 and 24.3 μm in Rampur and Santoshgarh populations respectively. The range of spicule length between 23-27 μm was more or less overlapping in both populations. Low per cent coefficients of variance to the tune of 5.9 and 4.9 per cent were recorded within the populations under observation indicating towards the stability of this character in *M. javanica* populations. Interestingly, the population from litchi having larger mean spicule length had a smaller

gubernaculum in the range of 9-11 μm and the population having smaller mean spicule length bore a larger gubernaculum of 10-12.5 μm range. The mean gubernaculum lengths in litchi and papaya populations were 10.0 and 11.0 μm , respectively. The low coefficient of variance to the tune of 7.0 and 7.8 per cent in respective populations were supportive of the stability of this character in the observed populations of root-knot nematode.

On the basis of aforesaid observations, stylet length, LMB, WMB, spicule and gubernaculum size have been found to be 'Stable' characters in the adult male populations of *M. javanica* and can thus be used in morphometric characterization of this nematode.

Comparative description of morphometric values of *M. javanica* populations calculated during present investigations with that of the original work has been summarised in Table 5.

Data placed in referred table reveals similarities in the measurements of various characters of females recorded during the studies with the observations recorded in original description of *M. javanica*. The present observations on *Meloidogyne javanica* ^[11] Chitwood, 1949 are in conformity with the original description by Orton Williams, (1972) except some variations which may be due to ecological differences. The present specimens have greater body length (550-789 vs. 541-804 μm), body width (320-550 μm vs. 311-581 μm) and longer spear length (16-22 vs. 14-20 μm) as compared to the earlier described population. However, the morphometric measurements of species were in accordance with the results given by recent authors ^[3, 10].

Table 5: Comparative description of *M. javanica* (Treub, 1885) Chitwood, 1949 with the original species

Sr. No.	Characters	After Orton Williams, 1972	Author's observations
1	Female Length (μm)	541-804	550-789
2	W (μm)	311-581	320-550
3	Spear length (μm)	14-20	16-22
4	Neck length (μm)	-	179-252
5	LMB (μm)	14-24	15-30
6	WMB (μm)	10-15	12-17
Perineal pattern			
8	LVS (μm)	-	17-23
9	AVS (μm)	-	15-26
10	ATT (μm)	-	12-16
11	IPD (μm)	-	19-25
Second stage juveniles (J2)			
12	Body length (μm)	387-459	335-445
13	Spear length (μm)	9.4-11.4	12-15
14	Tail (μm)	26-42	25-60

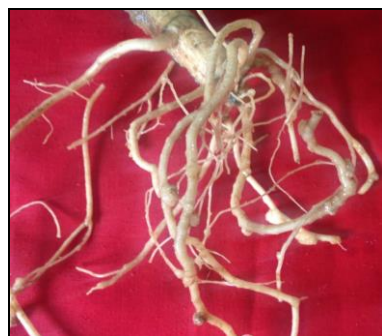


Fig 1: *M. javanica* galling on Papaya roots



Fig 2: *M. javanica* galling on Pumpkin roots

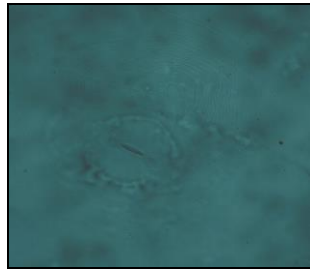


Fig 3: Perineal pattern



Fig 4: Adult female of *M. javanica*

4. Conclusion

The preceding manuscript dealt with the research investigations conducted to ascertain the status of *Meloidogyne javanica* in the state of Himachal Pradesh. From the results it was concluded that two populations recorded from Rampur and Santoshgarh in district Una were identified to be *M. javanica* on the basis of morphological and morphometric characters as well as on the basis of perineal patterns of females which were rounded with low dorsal arch with distinct lateral fields. This species of root-knot nematode was recorded for first time from the state.

5. Future Prospects

Lack of accurate and current data on various *Meloidogyne* spp. present in different parts of the country and the ubiquitous nature of pathogen poses a greater risk to the future of food production in country. To adequately address emerging and other *Meloidogyne* spp., accurate identification of *Meloidogyne* spp. is an essential and crucial step in deciding control measures which would be most suitable for crop management in different crops.

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