CHAPTER II
REVIEW OF LITERATURE

This chapter deals with review of molecular characterization of root-knot nematodes, *Meloidogyne* species; study on the morphological and morphometric variations among the populations of root-knot nematode, and effect of temperature on the biology of root-knot nematode. The relevant literatures have been categorized according to the studies conducted, under different sub-headings.

2.1 Molecular characterization of root-knot nematode, *Meloidogyne* species

Needham (1743) made the beginning of the history of Phytonematology by reporting the wheat eelworm. In 1855, Berkeley reported root-knot nematode for the first time from cucumber plant grown in a glass house in England.

Root-knot nematode was earlier known by different names given by different scientist around the world. Berkeley (1855) while reporting named this nematode as *vibrios*. In 1872, Greef called this nematode as *Anguillula radicicola* while Cornu (1879) named it as *Anguillula marioni*. Muller (1884) placed this nematode under the genus *Heterodera* and accordingly named it as *H. radicicola*. Goodey (1932) named this nematode as *Heterodera marioni*. But, it was Goeldi in 1887, which place this nematode under the genus *Meloidogyne*. Kofoid and White, (1919) named this nematode as *Oxyuris incognita*.

Goeldi (1887) gave the type species of *Meloidogyne* as *M. exigua* under the sub-family Meloidogyninae. In 1949, Chitwood considered all the root-knot nematode under the genus *Meloidogyne* supporting Goeldi, 1887 and also classified the species as *M. exigua*, *M. javanica*, *M. incognita* and *M. arenaria* and *M. hapla* and *M. incognita acrita*.

Till date more than 97 species of *Meloidogyne* had been reported from all over the world (Hunt and Handoo, 2009). The most widely occurred and distributed species in this genus are *M. incognita*, *M. javanica*, *M. arenaria*, *M. chitwoodi*, *M. fallax* and *M. hapla* which accounts for more than 95 per cent of occurrences. *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla* are of major damaging species,
being responsible for at least 90 per cent of damages caused by the nematodes (Castagnone Sereno, 2002). The impact of these species was enhanced by their wide host ranges and the most common species among them are estimated to be able to infect more than 5500 plant species (Trudgill and Blok, 2001).

In India, root-knot nematode was first reported by Barber (1901) on tea from Kerela. Till date, 14 species namely *M. incognita*, *M. javanica*, *M. arenaria*, *M. hapla*, *M. africana*, *M. brevicauda*, *M. thamesi*, *M. exigua*, *M. gramminicola*, *M. indica*, *M. gramminis*, *M. lucknowica*, *M. triticoryzae* and *M. piperi* had been recorded from India (Khan et al., 2014). Out of 14 species recorded from different states of India, only four species *viz.*, *M. indica*, *M. lucknowica*, *M. triticoryzae* and *M. piperi* were described first from India. In India, *M. incognita* and *M. javanica* were most widely distributed, attacking a wide range of crops. *M. arenaria* were mostly a problem on groundnut but found to parasitized vegetable crops also. *M. gramminicola* and *M. triticoryzae* were major problems of rice under aerobic conditions as well as in wheat crop while, *M. hapla* were confined to temperate hilly areas.

The first report of a DNA technique used for taxonomic purposes in Nematology was in 1985 when Curran and his co-workers, analyzed fragments of genomic DNA with restricted enzymes to differentiate *M. arenaria* from *M. javanica* and other non-parasitic nematodes. Curran et al. (1986) used the restriction patterns of repetitive DNA to distinguish physiological races within the four major species of *Meloidogyne*.

Powers et al. (1986), and Power and Sandall (1988) proposed a phylogeny of the *Meloidogyne* genus based on the restriction patterns of mitochondria DNA (mtDNA). The approaches used were time consuming therefore, were not practiced for rapid identification.

The polymerase chain reaction (PCR) was a new development that enables the exponential amplification of DNA starting from a single molecule. Using this technique, Harris et al. (1990) amplified a 1.8kb fragment of mtDNA from the DNA contained in a single egg or juvenile of the root-knot nematode. The fragments contained restriction fragment length polymorphism (RFLPs) for the enzyme *Hinf I* that allowed the separation of *M. incognita*, *M. hapla*, *M. arenaria* and *M. javanica* group. Thus, he showed that PCR allowed identification of *Meloidogyne* species during any
life stages. One drawback of this approach was the need to obtain information on nematode DNA sequences for the design of the two primers used in the reaction.

Development in PCR technology had made it possible to use a single random primer of about ten nucleotide long by lowering the annealing temperature during the amplification cycle, primer anneals at random in the genome, allowing the synthesis of highly polymorphic amplification products (Caetano et al., 1991; Wilsh and McClelland, 1990 and Williams et al., 1990).

Williams et al. (1990) reported the use of Random Amplified Polymorphic DNA (RAPD) and extended DNA analysis to characterize a wide range of organisms. The power of RAPD-PCR was in its ability to detect DNA sequence variation without the need of prior DNA sequence information. RAPD-PCR had been used for interspecific and especially intra-specific discrimination among Globodera and Meloidogyne species.

Powers and Harris (1993) demonstrated the use of PCR for identification and characterization between Meloidogyne species based on amplification of mitochondrial DNA. The PCR fragment band size differences between species became apparent when PCR products were separated on agarose gels. The development of the PCR made it possible to find out the discriminate among the species even only minute amounts of material were available, such as, for example, single juvenile of Meloidogyne species.

Cenis (1993) used RAPD-PCR technique with twenty-two primers to evaluate eighteen populations of root-knot nematode collected from different countries of the world and identified the four major species of Meloidogyne. It was observed that out of twenty-two primers, OPA-01 produced amplified DNA bands whose size allowed the separation of the four species, while the other eleven primers could separate one, two, or three species.

Swain et al. (1999) first categorized the M. incognita population collected from different parts of India (Delhi, Bhubaneswar, Jodhpur-1, Coimbatore-1, Sirsa, Coimbatore-2, Jodhpur-2 and Shimla) into four races based on the International host differentiation test and then, they isolated the total genomic DNA of four races of M. incognita from juveniles. Using 34 sets of RAPD primers, PCR analyses were performed on the total genomic DNA to access the extent of genetic variation and established genetic relationships among the races. Based on the RAPD profiling, the
four races of *M. incognita* had been distinguished. Cluster analysis, using UPGMA, demonstrated that race-1 was considerably different from other three races while, race-2 was closer to race-3 than race-4.

Randig *et al.* (2001) performed RAPD-DNA fingerprinting on single female of root-knot nematode, *Meloidogyne* spp., using a new procedure of DNA isolation. One fourth of the total DNA isolated from a single female was proved to be sufficient as a template in a PCR. The electrophoretic patterns of amplified fragments were reproducible between replicates from single females of the same progeny, and identical to those obtained with the genomic DNA purified from a large number of nematodes. Moreover, comparative analysis over the three successive generations showed stability of the amplification patterns, demonstrating the utility of the procedure for the epidemiological and ecological studies on root-knot nematodes.

Dong *et al.* (2001) used single egg mass to identify species- specific sequence tag site of twenty-six different isolates of root-knot nematode. The twenty-two isolates included seven isolates of *M. arenaria*, three of *M. hapla*, eleven of *M. incognita* and five of *M. javanica*. RAPD-PCR was tested to detect species- specific DNA fragments. Nematode isolates of the same species could be grouped unambiguously by most of the polymorphic RAPD patterns where variation among isolates of each species was also observed, especially within *M. arenaria* and *M. hapla*. Potential species- specific DNA fragments from RAPD-PCR were cloned and sequenced, and the species-specific PCR primers pairs for *M. arenaria*, *M. hapla*, *M. incognita* and *M. javanica* were developed.

Umarao *et al.* (2003) characterized the four Indian races of *Meloidogyne incognita* by characterizing them using internal transcriber spacer (ITS) 1 and 2 of rDNA. rDNA amplified from the genomic DNA of four races were cloned and sequenced. Amplification of rDNA from the four races resulted in about 800 base pair DNA fragment (ITS 1 and ITS 2, the 5.8 S rRNA gene and a small portion of the 3´ end of the 18S gene and 5´ end of the 28 S gene). Alignment of ITS sequences of the four races indicated that there was variation in the sequence among the four races resulting in different restriction sites that could be used for differentiating the races. Phylogenetic analysis of the rDNA sequenced data indicated that the race 1 and 4 were the most closely related forming one cluster to which race 2 was related, while race 3 formed entirely a separate cluster.
Abrantes et al. (2004) observed that direct examination of DNA was potentially a more powerful method to characterize inter- and intra-specific variability among nematodes. Development of techniques such as PCR, RFLP, RAPD and AFLP had increased the accuracy and speed of nematode characterization as well as identification.

Qiu et al. (2006) developed a simple PCR assay protocol for detection of root-knot nematode (RKN) species like, *Meloidogyne arenaria*, *M. incognita* and *M. javanica* extracted from soil. Nematodes extracted from the soil were digested with proteinase K, and PCR assay was carried out with the primers specific to root-knot nematode and with universal primers spanning the ITS of rRNA genes. Presences of root-knot nematode J2 could be detected among the large numbers of other plant-parasitic nematodes by following the protocol.

Adam et al. (2007) presented a molecular protocol for distinguishing seven most economically important *Meloidogyne* spp. viz., *M. incognita*, *M. javanica*, *M. arenaria*, *M. mayaguensis*, *M. hapla*, *M. chitwoodi* and *M. fallax*. DNA was extracted from individual J2 of *Meloidogyne* spp. and was amplified by PCR resulting in amplification of rDNA, specific SCAR and RAPD fragments.

Devran and Sogut (2009) collected 95 population of root-knot nematode from green house crops of West Mediterranean region of Turkey. They used species-specific primer for identification and differentiation of *Meloidogyne* population. It was found that SEC-1 F/ SEC-1R and INCK1 4F-INCK1 4R primers were specific and effective tool for identifying *Meloidogyne incognita*, Fjav/Rjav and DJF/DJR primers for *M. javanica* and Far/Rar for *M. arenaria*.

Karajeh et al. (2010) reported that using SCAR and RAPD, DNA were extracted from different developmental stages of Jordanian population of root-knot nematodes of viz., *M. javanica*, *M. incognita* (race1 and 2) and *M. arenaria* (race 2). Using RAPD-PCR primer PA-01, clear DNA bands were produced that clearly distinguished one species from another and allowed the identification of three *Meloidogyne* species.

Naz et al. (2012) studied the prevalence, incidence and molecular identification of root-knot nematodes infecting tomato in Pakistan. DNA amplification of rDNA (D2A-D3B) using SCAR produced characteristic products of 420 bp for *M. arenaria* (Far/Rar), 1200 bp for *M. incognita* (Finc/Rinc), and 670 bp for *M. javanica*
(Fjav/Rjav), while DNA amplification of mtDNA with C2F3/1108 primer yielded 1700 bp product for all the three species of root-knot nematodes. The sequencing of 28S rDNA product generated with the D2A-D3B primers does not differentiate among the three *Meloidogyne* species.

### 2.2 Morphology and morphometric variations of root-knot nematodes

In nematology, identification had been primarily based on light microscope observations (morphology) and measurements of morphological characters (morphometrics) of juveniles, males and females (Coomans *et al.*, 1978). Identification and description of *Meloidogyne* species had been based on morphology of perineal pattern supplemented by other morphological characteristics of females, juveniles and males.

Morphological resemblance (homologies) also formed the underlying basis of evolutionary systematic (Coomans, 2000). The light microscope was usually used for observing the morphology and the morphometrics of males, females and juveniles characters. But a light microscope had its limitations and the, more or less recent, use of the scanning electron microscope had considerably improved the accuracy of observation and description of, especially small, morphological characters. During the past two decades, taxonomists had increasingly used molecular diagnostic tools to confirm the validity of existing species and to assist in the identification and description of new species (De Waele and Elsen, 2007).

Chitwood (1949) provided detailed morphological observations of *Meloidogyne*. He gave greater emphasis on perineal patterns morphology, stylet morphology and distance from the stylet knobs to the dorsal esophageal gland opening (DOG) to described *M. hapla* and *M. incognita acrita*, and redescribed *M. arenaria*, *M. exigua*, *M. incognita* and *M. javanica*.

Davide (1980) reported that in *M. arenaria*, the neck length and width, stylet knob width, length of dorsal esophageal gland opening were highly variable with high coefficient of variation values. His study was conducted on the population of *M. arenaria* collected from fig tree.

Pant *et al.* (1985) reported that *M. incognita* exhibit morphometric and allometric variation in relation to host crop. Different host influenced the female dimensions to a varied degree. Large and robust females were reported on highly susceptible plants like *Trigonella foenum graecum, Pisum sativa* and *Coriandrum*
sativa, while smaller on tolerant plants like Lycopersicon esculentum cv. pelicum, L. pimpinellifolium.

Shiela et al. (1985) reported that M. incognita exhibit clear morphological and morphometric variation with soil pH. Body length and width, neck length, median bulb length and width, dorsal and ventral arches, number of lines on left and right side of the perineal pattern were significantly higher at soil pH 7 than those obtained from plants grown at either acidic or alkaline pH levels. Neck width, stylet length, vulval anal distance, B/A ratio and DOGO remained unaffected by pH. At both acidic and alkaline pH levels, smaller females were observed as against bigger ones at neutral pH.

Subramanian and Sivakumar (1988) observed that Pratylenchus delattrei showed morphometric and allometric variations in relation to different host crops (crossandra, maize and tomato). Stylet length, V per cent and ‘m’ value showed least variation indicating reliable taxonomic characters while, total body length, a, b, b´, c, c´, tail length, number of tail annules, excretory pore position and length of the post-uterine branch showed greater variability. c´ and length of esophagus were found to be most variable amongst all the characters studied.

Singh et al. (1995) studied on influence of hosts (different varieties of tomato) on morphometric and allometric variations of M. incognita female. Smaller females were produced on Lycopersicum esculentum cv. NMR-1, cv. Coldset and L. lycopersicoides which were moderately resistant to nematode. Moderate sized females of root-knot nematode were recorded on L. esculentum host crop (cv. AC-142, AC-238, S-12, Punjab Chhuhara, Punjab Kesri), while highly susceptible host crop (cv. Sioux., Co-1 and Co-2) produced large sized females on L. esculentum.

Das and Bajaj (2005) studied intraspecific variations of Ditylenchus myceliophagus in relation to fungal host (Alternaria alternate, Agaricus bisporus, Fusarium solani, Mucor spp., Pleuotus sajor-caju, Rhizoctonia solani, R. bataticola, Sclerotium sclerotiorum and Trichoderma harzianum), temperature (13, 20, 25 and 30±1°C) and age of host culture (30, 40 and 55 days). They reported that occurrence of males, shape of the stylet knobs, number of lateral lines, junction between corpus and median bulb, arrangement of oocytes in ovary, crustaformia, limit of anterior genital tract, shape of spicules, and covering of bursa were not affected by temperature, type of
host and age of host culture. However, marked variations occurred in the basal esophageal bulb and tail shape.

There are number of reasons stated for using perineal pattern as one of the important characters in species identification of *Meloidogyne*. Perineal pattern is stable and does not change significantly over an extended period of culturing (Eisenback, 1985; Hirschman, 1985). With the invention of scanning electron microscope and improved light microscopy techniques, more accurate and detailed observation on morphological features is considered supplementary to the traditional line drawings. Line drawing, however, is still useful in representing internal features of the nematode which cannot be focused and captured in a single image view.

2.3 Effect of temperature on the biology of root-knot nematode

2.3.1 Effect of temperature on embryogenesis

Nematode embryogenesis is unique in the sense that the cells destined to form a particular organs are laid down in the early cleavages. This type of development is called determinate cleavage. The foundation of nematode embryology was laid down by Boveri (1892) who published embryology of *Parascaris equorum*. His work directly initiated the detailed study of the nematode embryology by many workers. In 1927, Pai worked out the embryogenesis of *Turbatrix aceti* in a very comprehensive manner.

Modern nematologists do not accept the classical model of *Parascaris equorum* because it was a spirurid, but rely upon information obtained from *Caenorhabditis elegans*. Sulston *et al.* (1983) first determined the complete embryonic lineage of *C. elegans*. In *C. elegans*, the first cleavage of the zygote produces two unequal cells, a larger anterior ectodermal founder cell (AB) and smaller posterior cell (P1). AB gives rise to 389 surviving cells, comprising 254 neuronal, 72 hypodermal, 23 muscle and 40 other structural cells. Second cleavage that occurs within 10 minutes of the first cleavage, results in two daughter cells, EMS and P2. EMS further divides into two founder cells, MS and E. MS produces 80 survivors comprising of 48 muscles, 13 neuronal, 9 glands, 2 somatic gonad cells and 8 other cells while, E produces 20 mesenteron cells. P2 divides to produce somatic founder cell (C) and germinal cell (P3). C produces 47 survivors of whom 32 are muscle, 13 hypodermal, and 2 neuronal cells. P3 divides to founder cell D and P4. D produces 20 muscle cells and P4 produces two germinal cells Z2 and Z3. At eclosion stage, the juvenile consists of a total 558 cells.
During this process several cells die, which are eliminated either by suicide or by murder through phagocytosis.

Hechler (1963) reported that eggs of *Seinura tenuicaudata* undergo first cleavage in one and a half to two hours of deposition and total time of development from egg laying to hatching was ranged from 35-45 hours at 28°C. She also noticed that emergence of juvenile always occur at the end of the egg, never at side.

Flegg (1968) studied the embryology of *X. diversicaudatum, X. viuttenezi, Xiphenema sp., Longidorus profundorum* and *L. macrosoma*. He reported that first cleavage of eggs of both the genera occur 24- 48 hours after laying eggs. Hatching occurs between 19-24 and 25-30 days from egg laying in *Xiphenema* and *Longidorus* species, respectively.

Bird (1972) stated that optimum temperature for embryogenesis of *Meloidogyne javanica* lies between 25-30°C. He observed that embryogenesis was slightly more rapid at 30°C (9-10 days), but more eggs complete development at 25°C (11-13 days). At 25, 27.5 and 30°C, embryogenesis was about twice as rapid as at 20°C (23-25 days) and about 4 times as rapid as at 15°C (46-48 days).

The embryology of *Trichodorus similis* was studied by Wyss (1973) with the aid of time lapse photography. At 25°C, the first cleavage began 12-15 hours after deposition of eggs and 2-cell stage lasts for 7 hours. He further reported that with the increase in juvenile activity, the egg membrane eventually splited. The nematode did not use the stylet to split the eggshell. He failed to observe the moult within the egg.

Inserra *et al.* (1983) observed that at 20°C, duration of embryonic development of *M. chitwoodi* and *M. hapla* was about 20 days while at 10°C; it takes 82-84 days for *M. chitwoodi* and 95-97 days for *M. hapla*. Cumulative egg hatch was not greater in root leachates than in distilled water, but temperature did significantly affect egg hatch. Less than 1 per cent of eggs of both nematode species hatched at 4°C while at 15°C, per cent cumulative hatch of both nematode species was significantly lower than that at 20°C and 25°C. The cumulative egg hatched of two species did not differ at 25°C, but was higher at 25°C than at 20°C. At 7°C, the emergence of *M. chitwoodi* juveniles was about seven times greater than that of *M. hapla* in distilled water.
Waele and Wilken (1989) reported that hatching of eggs of *D. destructor* took 8, 4, 3, 2 and 1 days at 16°, 22°, 25°, 28° and 34°C, respectively which narrated that fluctuation of temperature greatly influenced its embryogenesis.

Vovlas and Larizza (1994) reported that *X. index* completed its embryonic development in 10-12 days at 24±2°C.

Charchar and Danto (2001) studied the embryogenic development of *M. chitwoodi* races 1 and 2 and stated that *M. chitwoodi* required 55, 24 and 13 days at 12, 18 and 24°C, respectively, *M. hapla*, took 60, 26, 15 and 10 days at 12, 18, 24 and 30°C, respectively. Eggs of *M. chitwoodi* failed to complete embryonic development at 6 and 30°C, rather eggs were found dead after 13 days at these temperatures. Maximum eggs of *M. chitwoodi* race 1 and 2 hatched at 12-24°C and reduced its hatching per cent at 30 °C while eggs of *M. hapla* increased hatching percentage at 30°C. Optimum temperature for hatching appeared to be 18-24°C for all three populations.

Das and Bajaj (2011) reported that *Ditylenchus myceliophagus* completes its embryonic development in 75 hours and 279 hours at 25°C and 13°C, respectively under controlled condition. Further, a considerable variation in timing of cleavage was recorded at two temperatures, while eggs of *D. myceliophagus* failed to develop at 5° and 35°C.

Morris *et al.* (2011) studied the effect of temperature on hatching of *M. minor* from golf greens in North-West Europe. They recorded high per cent of hatching (46-88%) when eggs were incubated at 20°C, as compared to 15°C (43%). At 20-25°C, hatching of *M. minor* ranged from 63-76 per cent, while the highest hatching were observed at 23°C with an average duration of 7 days compared to 17 days at 15°C.

Khan *et al.* (2014) reported that the optimum temperature for hatching of *M. chitwoodi* and *M. fallax* was 20°C. However, temperatures above 20°C and less than 25°C were more favorable for hatching of *M. chitwoodi* than *M. fallax*, which in contrast hatched in greater numbers below 20°C but not lower than 15°C.

### 2.3.2 Effect of temperature on penetration and multiplication

Plant parasitic nematodes had evolved diverse parasitic strategies and feeding relationships with their host plant to obtain nutrients for their survival. Depending upon genera, they feed from the cytoplasm of unmodified living plant cells or had evolved to modify root cells into elaborate discrete feeding cells. Plant–parasitic
nematodes used a hollow protusible stylet to penetrate the wall of a plant cell, inject gland secretions into the cell, and withdraw nutrients from the cytoplasm. Feeding behavior of all plant parasitic nematodes was similar in nature. It had been characterized by injection of dorsal esophageal gland secretion into the host cell through the stylet prior to the nutrient uptake. Behavior of nematodes that leads to feeding consists of five different phases viz., exploration, penetration, esophageal gland secretion, ingestion and stylet withdrawal and moves away from the feeding site (Doncaster, 1971; Wyss, 1981).

But studies using monoclonal antibodies specific for sub-ventral gland secretions demonstrated that antigens in the nematode stylet secretions originated in the sub-ventral gland cells (Davis et al., 1994; Goverse et al., 1994 and Smant et al., 1997). In addition to that, recent discovery of β-1, 4- endoglucanase (cellulase) were found to be synthesized in the sub-ventral gland cells of cyst nematodes and secreted through the nematode’s stylet in planta unequivocally established a role for sub-ventral gland secretions in plant parasitism (Smant et al., 1998 and Wang et al., 1999). The predicted morphological resistance to anterior flow of sub-ventral gland secretions in the esophageal lumen during maximal pumping of the metacarpus must be minimal during secretion phase of a feeding cycle. Furthermore, production of stylet secretions in vitro by pre-parasitic juveniles and adult females involved very little movement of the metacorporeal pump chamber, also when the tri-radiate pump chamber is closed; there was a slight gap between the sclerotized walls of the pump chamber that might permit anterior flow of the sub-ventral gland cell secretions during phase of the feeding cycle.

Feeding behavior of root-knot nematodes is different from that of ectoparasites. Females feed for a longer period of time on the specific feeding cells known as the giant cells. The nematode undergoes morphological changes from vermiform second stage juvenile to saccate or globular body shape. Feeding by root-knot nematodes take place in repeated cycle and are interrupted only during moulting (Wyss and Zunke, 1986; Sijimons et al., 1994; Hussey and Grundler, 1998). Early report of feeding behavior of Heterodera marioni was made by Linford (1937, 1942). In vitro observations on feeding behavior of H. marioni (Hussey and Grundler, 1998) revealed that by local exploration H. marioni selects a suitable host cell. Once the specific cell was selected, stylet was inserted into the cell and remains protruded for several hours. Following that, nematode withdraws the stylet and again re-inserts it and starts secreting esophageal gland secretion through stylet, which modifies cell phenotype. This phase is followed by nutrient uptake. One feeding cycle is completed
with stylet retraction. This type of feeding process lasts throughout the parasitic phase of the nematode. Wyss et al. (1992) described the feeding behavior of root-knot nematodes into four cyclic phases viz., local exploration, root penetration, migration at permanent feeding site and feeding from multinucleate giant cell. Formation of feeding tubes by sedentary endoparasitic nematodes are unique features (Rumpenhorst, 1984) and been observed in *Rotylenchulus reniformis* (Razak and Evans, 1976; Rebios, 1980), *Heterodera* spp. (Endo, 1991; Wyss et al., 1984) and *Meloidogyne* spp. (Hussey and Mims, 1991; Wyss et al., 1992).

Wong and Mai (1973) reported that temperatures at 21.1 – 26.7°C were more favorable for movement and invasion of *M. hapla* than temperatures at 15.5 and 32.2°C.

Prot and Van Gundy (1981) reported that photoperiod influenced migration of *M. incognita* juveniles towards tomato roots. 33 per cent juveniles migrated vertically up to 20 cm in 7 days inside the roots when exposed to 12 hrs in dark and then exposed to light for 12 hrs. Only 7 per cent juveniles migrated inside the root when kept in light at a constant for 24 hrs.

On tomato cultivars (Rutgers and Sanmarzano), Maqbool and Ghazala (1985) observed that juvenile penetration, development and sex expression towards femaleness were increased with increased susceptibility. They also stated that temperature 25±3°C was more favorable for root-knot nematode infection and root-knot development than higher temperatures (above 25±3°C).

Mishra et al. (1985) observed that juveniles of *Heterodera zeae* penetrated roots within 3 hrs of inoculation, Nematode penetrated just behind the root tip where tissue differentiation had not taken place. After 6 hrs, the juveniles were completely within the root and remained in the cortical region up to 24 to 72 hrs. Juvenile reached the central portion of the roots within 5 days.

Haque and Padmavathy (1985) studied the penetration of roots by *Rotylenchulus reniformis* on tomato varieties. They recorded that *R. reniformis* penetrated both Pusa Ruby and Patriot tomato varieties within 48 to 72 hrs. However, penetration time was found to be significantly different. Highest number of juveniles in roots was invariably observed after 96 hrs.

Pline et al. (1988) reported that eggs of *M. incognita* were acclimated at 23°C. Newly hatched juvenile migrated towards higher temperatures when placed in
shallow thermal gradient averaging 23°C and threshold gradient for that response was found to be below 0.01 C/cm, with a best estimate at 4 X 10^{-4} C/cm.

In 1994, Umesh and Ferris carried out an investigation on influence of temperature on *M. chitwoodi*. They found that maximum number of *M. chitwoodi* penetrated barley crop at 20°C while, minimum at 15°C.

Singh and Goswami (1999) studied the penetration and development of *M. incognita* alone and in presence of wilt fungus on cowpea cultivars *viz.*, Pusa Komal (susceptible to both nematode and wilt fungus) and C-152 (resistant to nematode and susceptible to wilt fungus). It was found that maximum penetration of *M. incognita* in both the cultivars occurred up to 7 days after inoculation.

Khan *et al*. (2014) reported that the optimum temperature for migration of *M. chitwoodi* into host tissue was 20°C and for *M. fallax*, it was 25°C. They further stated that invasion of roots by both the nematode was higher on potato than maize at 15, 20 and 25°C. The degree-days (DD$_5$, base temperature 5°C) required for completing their life cycle were 555-740 DD$_5$ on potato and 705-740 DD$_5$ on maize stating that temperature played a more important role in post-penetration development than the host plant.