Vegetable Science (2026) 53(1): 131-141
doi: 10.61180/vegsci.2026.v53.i1.17
ISSN- 0970-6585 (Print), ISSN- 2455-7552 (Online)
www.isvsvegsci.in

RESEARCH ARTICLE

Prevalence and molecular characterization of root-knot nematodes (*Meloidogyne* spp.) and pepper mottle virus (PepMoV) infecting chilli in Punjab

Amandeep Kaur[1], Sukhjeet Kaur[2*], Abhishek Sharma[2], Anupam Sekhon[1] and Harwinder Singh Buttar[2]

Abstract

Chilli (*Capsicum annuum* L.), an economically important crop of the Solanaceae family, is highly susceptible to various biotic stresses, particularly root-knot nematodes (RKN) and viral diseases, leading to significant yield losses. A comprehensive survey was conducted across major chilli-growing districts of Punjab to assess the prevalence of RKN and pepper mottle virus (PepMoV). The highest incidence of RKN (70%) was recorded in Malerkotla, whereas the lowest incidence (19.20%) was observed in Ferozepur. Similarly, PepMoV incidence was highest in Malerkotla (74%) and lowest in Bathinda (10.67%). Morphological characterization of RKN populations was performed through perineal pattern analysis, and species identification was further confirmed using sequence characterized amplified region (SCAR) species-specific primers targeting *Meloidogyne incognita* and *M. javanica*. Detection of PepMoV was initially carried out using enzyme-linked immunosorbent assay (ELISA) and subsequently validated through immunocapture reverse transcription polymerase chain reaction (IC-RT-PCR) using potyvirus-specific primers. The findings reveal the widespread occurrence and high incidence of RKN and PepMoV in Punjab, emphasizing the urgent need for developing and implementing integrated disease management strategies to minimize yield losses and ensure sustainable chilli production.

Keywords: Chilli, ELISA, IC-RT-PCR, pepper mottle virus, root-knot nematodes, SCAR markers

[1]Department of Plant Pathology, Punjab Agricultural University, Ludhiana – 141004, Punjab, India

[2]Department of Vegetable Science, Punjab Agricultural University, Ludhiana – 141004, Punjab, India

[*]**Corresponding author;** Email: sk-randhawa@pau.edu

Citation: Kaur, A., Kaur, S., Sharma, A., Sekhon, A., & Buttar, H.S. (2026). Prevalence and molecular characterization of root-knot nematodes (*Meloidogyne* spp.) and pepper mottle virus (PepMoV) infecting chilli in Punjab. Vegetable Science, 53(1), 131-141.

Source of support: Nil

Conflict of interest: None.

Received: 02/05/2026; **Revised:** 31/07/2026; **Accepted:** 31/07/2026

Introduction

Chilli (*Capsicum annuum* L.) is one of the most important members of the Solanaceae family, the genus *Capsicum* comprises five domesticated species, namely *C. annuum*, *C. frutescens*, *C. chinense*, *C. baccatum* and *C. pubescens*, among these *C. annuum* is the most widely cultivated across the world (Barboza et al., 2022). This species traces its origin to South America (Tripodi et al., 2021) and was brought to India by Portuguese traders in the late 15th century, where it subsequently evolved as a secondary centre of diversity (Dhaliwal et al., 2014). Chilli is cultivated for diverse purposes such as fresh consumption, spice production, oleoresin extraction, industrial processing, and medicinal uses (Reddyet al., 2024; Saleh et al.,2018). Green fruits are also valued as a rich source of natural antioxidants, particularly vitamins A, C and E (Bal et al., 2022). With substantial export potential, *C. annuum* provides an important income-generating crop option, especially for small and marginal farmers. In India, chilli occupies about 423.72 thousand hectares with an annual production of 4.59 million tonnes (Anonymous, 2023-24). In the state of Punjab, chilli is grown over an area of 10.61 thousand hectares, with an average yield of 20.18 quintals per hectare,

resulting in a total production of 21.42 thousand tonnes per annuum (Anonymous, 2024).

However, chilli production is constrained by several biotic stresses, including fungal, bacterial, viral, phytoplasmal, and nematode pathogens which cause huge yield losses (Muthukumar & Bhaskaran, 2007). Plant-parasitic nematodes of the genus *Meloidogyne*, commonly referred to as root-knot nematodes (RKNs), are among the most destructive nematode pests, responsible for yield reductions of up to 15% in chilli, translating into annual economic losses of nearly ₹744.90 million in India (Kumar et al., 2020). These nematodes are particularly prevalent in regions with warm climates or mild winters (Kaur et al., 2025). Management of root-knot nematodes is particularly challenging due to their rapid multiplication, short generation time, and ability to parasitize a wide range of hosts (Azlayet al., 2023).

In addition to root-knot nematodes, several begomoviruses and potyviruses, including pepper veinal mottle virus (PVMV), pepper vein-banding virus, pepper mottle virus (PepMoV), and pepper mild mottle virus (PMMoV), have been reported to infect *Capsicum* species worldwide, leading to considerable economic losses (Kim et al., 2009, Cheng et al., 2011). PepMoV was first described as a distinct potyvirus strain in Arizona, USA, in 1969 (Nelson & Wheeler, 1972). In India, Sandhu and Chohan (1979) recorded its occurrence in chilli, referring to it as the Indian PepMoV, and subsequent studies in Punjab confirmed its association with chilli crops (Kaur et al., 2014). The virus, transmitted by aphids in a non-persistent manner, has been detected in major chilli-growing regions of Punjab with an incidence of 15 to 21% (Sharma, 2018). Under field conditions, PepMoV infection leads to pronounced symptoms on leaves and fruits, hampers plant growth, and can cause severe yield reductions (Kaur et al., 2014; Sharma, 2018). Considering the economic importance of chilli and the production challenges posed by RKN and PepMoV, the present study was undertaken to see the prevalence and severity of nematodes and viruses in the major chilli-growing districts of Punjab.

Materials and Methods

Surveys were carried out during 2024-25 across various chilli growing districts of Punjab, including Ludhiana, Sangrur, Jalandhar, Tarn Taran, Malerkotla, Bathinda, Patiala and Ferozepur, to evaluate the incidence and prevalence of RKN and PepMoVin chilli crop. In case of nematode, yellowing of plants, patchy appearance of dwarf and malnourished plants in the field, plants showing wilting type external symptoms were taken into consideration along with along with root gall severity and soil nematode population. The percentage of root knot nematode disease incidence (%) was determined by randomly uprooting 8 to 10 symptomatic plants from four corners and one central patch within each field. The number of plants exhibiting typical root knots was then counted, and the incidence was calculated using the previously mentioned formula. The root gall index for individual plants was assessed using a 0 to 10 scale as described by Bridge and Page in 1980, where 0 = no galls; 1 = few small galls difficult to detect; 2 = small but clearly visible galls, main roots clean; 3 = some larger galls, main roots clean; 4 = larger galls predominate, main roots clean; 5 = about 50% of roots galled with some main root involvement; 6 = galling on main roots; 7 = majority of main roots galled; 8 = all main roots galled with few clean roots; 9 = all roots severely galled and plants usually dying; and 10 = all roots severely galled with no functional root system. Soil samples collected during surveys were analyzed to determine RKN populations. A working sample of 250 cc soil was taken from the thoroughly mixed composite sample and processed using Cobb's sieving and decanting technique (Goodey, 1957). The sample was prepared in a 250 ml beaker to estimate the nematode population and the number of root-knot nematodes was counted using a counting dish under a stereo zoom binocular microscope.

For virus incidence, crop samples exhibiting characteristic viral symptoms, *viz*., vein banding, leaf puckering, misshapen leaves, leaf yellowing, mottling of yellow and green colour on leaves, reduction in size of leaves etc. were taken into consideration while collecting the samples. The viral disease incidence was calculated by taking 10 to 12 samples from four corners and one central patch of each field using the formula given below:

$$\text{Per cent disease incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants observed}} \times 100$$

Identification and characterization of root-knot nematodes infecting chilli

Identification and characterization of root-knot nematode spp. infecting chilli crop under Punjab conditions was done by both morphological and molecular studies. Single egg mass progenies from different populations collected during the survey were maintained on susceptible chilli genotype 'Punjab Surkh' for morphological and molecular studies. Juveniles and females required for morphological analysis were obtained from the single egg mass progenies. These samples were carefully processed for detailed examination. The perineal pattern of mature females served as the primary criterion for initial identification in morphological detection. For perineal pattern studies, the galled portions of the roots were stained with acid fuchsin, following the method described by Eisenback et al., (1981). The perineal patterns of five females were analyzed for each population.

Morphometric measurements of juveniles and females were also recorded. Observations were conducted using a Kyowa microscope at 10 x and 40 x magnifications. Images were captured with the built-in Kyowa digital camera,

and morphometric measurements were performed on the images using the Kyowa image analysis application system. Nematode species were identified by comparing morphometric measurements with the help of a key given for identification of *Meloidogyne* species by Jepson (1987).

Further, confirmation of morphologically identified root-knot nematode species was carried out through PCR using sequence characterized amplified regions (SCAR) primers specific to major *Meloidogyne* species: *M. incognita* and *M. javanica*. Total DNA was extracted from morphologically identified *M. incognita* and *M. javanica* progenies using the Proteinase K method (Williamson et al., 1997) followed by PCR amplification using the specific primers (Zijlstra et al., 2000). Juveniles and egg masses were transferred to 1.5 mL microcentrifuge tubes containing 50 µL lysis buffer (1X PCR buffer with 100 µg mL^{-1} Proteinase K), crushed using a sterile micropestle, and frozen at –20°C for 1 hour. Samples were incubated at 60°C for 1 hour, followed by enzyme inactivation at 94°C for 10 minutes. After centrifugation at 13,000 rpm for 2 minutes, the supernatant was collected, and DNA was precipitated by adding two volumes of chilled absolute ethanol and incubating at –20°C for 1 hour. The DNA was pelleted (14,000 rpm, 3 minutes), washed with 70% ethanol, air-dried, and re-suspended in 50 µL 1× TE buffer. The extracted DNA was stored at –20°C and used as a template for PCR.

The isolated DNA was subjected to PCR amplification with SCAR markers specific to major *Meloidogyne* species. PCR was initially performed in a 10 µL reaction volume and subsequently scaled up to 30 µL for gel purification. Each reaction consisted of 2X GoTaq Green Master Mix (Promega), species-specific primers, nuclease-free water, and template DNA (1.5 µL). Amplification was carried out in a ProFlex thermal cycler (Applied Biosystems). Species-specific SCAR markers were used for identification of root-knot nematodes. *Meloidogyne incognita* was amplified using primers Finc (5′-CTCTGCCCAATGAGCTGTCC-3′) and Rinc (5′-CTCTGCCTCACATTAAG-3′), producing an amplicon of ~1200 bp at an annealing temperature of 54°C (2 minutes). *Meloidogyne javanica* was identified using primers Fjav (5′-GGTGCGCGATTGAACTGAGC-3′) and Rjav (5′-CAGGCCCTTCAGTGGAACTATAC-3′), yielding an amplicon of ~670 bp at 64°C (1-minute) (Zijlstra et al., 2000). PCR products were resolved on 1% agarose gel prepared in 1X TAE buffer using agarose low EEO (SRL). Electrophoresis was performed under standard conditions, and bands were visualized under a UV transilluminator (Syngene) and documented using an Alpha Imager HP gel documentation system (USA).

Identification and confirmation of pepper mottle virus infecting chilli

Chilli (*Capsicum annuum* L.) leaf samples exhibiting virus-like symptoms were collected during field surveys and immediately transported to the laboratory under chilled conditions. Initial detection of pepper mottle virus (PepMoV) was performed using double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). The assay was conducted using PepMoV-specific polyclonal antisera (Agdia Inc., USA) according to the manufacturer's instructions. Absorbance values were measured at 405 nm using a Tecan microplate reader, and samples were considered positive when the optical density (OD) exceeded at least twice that of the healthy control. For molecular confirmation, representative samples (5 to 7 per district) showing high ELISA absorbance values were selected for immunocapture reverse transcription polymerase chain reaction (IC-RT-PCR). Immunocapture was performed using virus-specific antibodies to selectively bind virions, followed by reverse transcription. First-strand cDNA synthesis was carried out using oligo(dT) primers to target the polyadenylated viral RNA genome (Kaur et al., 2018). Subsequent PCR amplification was performed using potyvirus-specific primers Nib2F and Nib3R, targeting a conserved region of the nuclear inclusion body (NIb) gene. The amplification generated a product of approximately 350 bp. PCR reactions were carried out under optimized cycling conditions, and amplified products were resolved by electrophoresis on 1% agarose gel prepared in 1X TAE buffer. The gels were stained, visualized under UV illumination, and documented using a gel documentation system.

Results and Discussion

Prevalence and incidence of root-knot nematodes

A survey of chilli crops across various districts of Punjab revealed notable differences in the incidence and severity of root knot nematodes (RKNs) influenced by the chilli varieties cultivated and the crop rotation being practiced by farmers in different regions (Table 1). The root knot nematode infested plants showed characteristic galls/knots on the root system (Figure 1a), the highest incidence of RKN was recorded in regions where chilli was cultivated in rotation with crops such as potato, capsicum, brinjal, cucurbits, and cauliflower. In contrast, the lowest incidence was observed in areas where chilli followed rice and wheat crop rotation. The reduced RKN incidence following rice cultivation may be attributed to the practice of puddling, commonly employed in rice fields. Similar findings were reported by Katti and Ch (2022), who observed significantly lower RKN populations in puddled soils compared to non-puddled ones. Additionally, fields planted with the chilli hybrid CH-27 also exhibited lower RKN incidence, likely due to the hybrid's reported resistance to RKN (Anonymous, 2024). Among the surveyed districts, Malerkotla reported the highest mean RKN incidence of 70%, followed by Jalandhar (55.29%) and Ludhiana (47%) (Figure 2a). Whereas, the lowest incidence was observed in Ferozepur district (19.20%). The highest RKN

Table 1: Survey of chilli growing areas for incidence of root-knot nematode and pepper mottle virus

District	*Name of Village*	*Area*	*Latitude Longitude*	*Name of the variety*	*Crop rotation*	*RKN* incidence (%)*	*Soil population/ 250 cc soil*	*RGI** (0-10) Scale*	*PepMoV*** incidence (%)*	*ELISA Reaction*
Sangrur	Chhahar	1.50 acre	N-30°2′29.9″ E-75°54′41″	CH-10	Rice-Chilli and Capsicum	60%	1250	7.80	30%	+
	Chhahar	1.00 acre	N-30°2′12.1″ E-75°54′21.2″	Local	Rice- Chilli	Nil	Nil	Nil	20%	+
	Benra	1.00 acre	N-30°19′41.4″ E-75°51′52″	Local	Chilli-Potato-Chilli	40%	850	5.60	60%	++
	Ubhia	1.25 acre	N-29°59′42.1″ E-75°57′59.5″	CH-27	Basmati rice - chilli	10%	200	1.33	45%	+
	Ubhia	0.50 acre	N-29°59′43.7″ E-75°56′41.9″	CH-27	Rice – chilli	08%	150	1.15	30%	+
	Ladda	1.00 acre	N-30°18′54.1″ E-75°49′53.5″	CH-1	Cabbage-Chilli-Cucurbits	65%	986	6.33	45%	+
Mean						30.50	572.67	3.70	38.33	
Range						0–65%	0–1250	0–7.80	20–60%	
Malerkotla	Bhaini	1.50 acre	N-30°32′50.3″ E-75°56′0.5″	H1 pepsi	Cauliflower/ Brinjal -Chilli	80%	1050	7.33	60%	++
	Sandhaur	0.50 acre	N-30°34′18″ E-75°44′41.9″	MS-12	Chilli-Sweet pepper	65%	950	6.86	83%	++
	Dhuri	2.00 acre	N-30°22′29.9″ E-75°50′4.8″	CH-10	Chilli- Potato-Chilli	65%	2000	8.50	80%	++
Mean						70.00	1333.33	7.56	74.00	
Range						65–80%	950–2000	6.86–8.50	60–83%	
Patiala	Birarhwal	1.00 acre	N-30°25′52.6″ E-76°10′47.5″	CH-3	Chilli- Potato	35%	650	6.33	40%	+
	Birarhwal	4.00 acre	N-30°25′52.6″ E-76°10′47.5″	CH -10	Chilli- Potato-Bottleguard	40%	450	5.35	35%	+
	Khokh	1.25 acre	N-30°26′33″ E-76°10′23.6″	CH-12	Chilli- Potato – Muskmelon	25%	650	5.86	30%	+
	Asarpur	1.00 acre	N-30°19′29.7″ E-76°29′8.8″	CH-12	Chilli-Potato-Wheat	12%	350	2.65	25%	+

Mean						28.00	525.00	5.05	32.50	
Range						12–40%	350–650	2.65–33	25–40%	
Jalandhar	Adampur	2.00 acre	N-31°29′51.7″ E-75°37′15.7″	Sahiba	Potato- Chilli and Guava	90%	1500	8.50	15%	+
	Chachowal	3.00 acre	N-31°13′29.5″ E-75°39′10.1″	Sahiba	Potato-Chilli	50%	950	7.33	10%	+
	Garibaksha	3.00 acre	N-31°29′59.77″ E-75°37′38.86″	HPH 5531	Potato-Cauliflower-Chilli	75%	1100	7.50	10%	+
	Garibaksha	3.00 acre	N-31°29′59.77″ E-75°37′38.86″	Sahiba	Pea-Chilli	80%	1350	8.00	20%	+
	Awan Khalsa	0.50 acre	N-31°00′6.83″ E-75°32′44.11″	CH-27	Rice- Chilli	5%	120	1.00	15%	+
	Adraman	2.00 acre	N-31°01′28.77″ E-75°15′36.81″	HPH 5531	Potato-Cauliflower-Chilli	80%	1360	8.10	10%	+
	Adraman	1.00 acre	N-31°01′28.77″ E-75°15′36.81″	CH-27	Potato - Chilli	7%	135	1.10	15%	+
Mean						55.29	930.71	5.93	13.57	
Range						5–90%	120–1500	1.00–8.50	10–20%	
Ferozepur	MallanWala	2.00 acre	N-31°2′46.3″ E-74°49′33.7″	United Hybrid 8307	Rice- Chilli	20%	300	2.86	60%	++
	Mallan Wala	1.50 acre	N-31°2′46.6″ E-74°49′33.8″	United Hybrid8307	Rice- Chilli	10%	250	2.15	55%	++
	Sumna Village	1.00 acre	N-31°2′46.5″ E-74°49′34″	United Hybrid8307	Rice- Chilli	11%	300	2.66	45%	+
	Challan wala	3.00 acre	N-31°2′2.02″ E-74°50′24.3″	Local	Pea-Chilli	20%	450	3.45	25%	+
	Badhani	3.50 acre	N-31°2′44.8″ E-74°49′32.9″	UnitedHybrid8307/ Sahiba	Rice-Chilli and Capsicum	35%	300	2.66	30%	+
Mean						19.20	320.00	2.76	43.00	
Range						10–35%	250–450	2.15–3.45	25–60%	

Ludhiana	PAU	2.00 acre	N-30°53′57″ E-75°47′37.9″	Germplasm lines	Chilli-Cucurbits	58%	550	4.33	65%	++
	Boparai Kalan	2.00 acre	N-30°48′14.6″ E-75°38′30.9″	Local	Bittergourd -Chilli	50%	450	4.15	65%	++
	Dhatt	1.50 acre	N-30°49′4.6″ E-75°37′10.6″	Local	Bittergourd -Chilli-Onion	40%	250	2.45	30%	+
	Jodhan	1.00 acre	N-30°47′55″ E-75°45′37.5″	Local	Chilli-tomato	40%	600	5.5	45%	+
Mean						47.00	462.50	4.11	51.25	
Range						40–58%	250–600	2.45–5.5	30–65%	
Tarn Taran	Nabipur	2.00 acre	N-31°12′48.2″ E-74°53′16.8″	Local	Rice-Chilli	60%	350	3.66	40%	+
	Nabipur	1.00 acre	N- 31°12′48.15″ E-74°53′16.7″	Local	Rice-Chilli	40%	250	2.45	40%	+
	Tharu	1.50 acre	N-31°28′48.1″ E-74°52′19.4″	Soldier	Rice - Chilli	50%	300	3.11	15%	+
	Bhoru	1.00 acre	N-31°33′8.7″ E-74°49′24.4″	Veer ji	Rice - Chilli-Berseem	7%	150	1.33	25%	+
	Gohlwar	1.00 acre	N-31°31′0.6″ E-74°53′51.7″	Local	Rice - Chilli	10%	200	1.66	35%	+
	Chabal Kalan	2.00 acre	N-31°28′40.41″ E-74°47′59.83″	CH-10	Cauliflower – Chilli	25%	300	2.11	40%	+
Mean						32.00	258.33	2.39	32.50	
Range						7–60%	150–350	1.33–3.66	15–40%	
Bathinda	Mehma sarja	0.50 acre	N-31°18′34.9″ E-74°50′36.25″	Local	Rice-Chilli	10%	200	1.86	2%	-
	Dhelwan	0.25 acre	N-31°18′39.9″ E-74°2′29″	Local	Chilli-Cauliflower	25%	250	2.00	10%	+
	Dhelwan	0.50 acre	N-31°18′40.5″ E-75°2′8.5″	Local	Chilli-Cauliflower	40%	350	2.65	20%	+
Mean						25.00	266.67	2.17	10.67	
Range						10–40%	200–350	1.86–2.65	2–20%	

*RKN- root-knot nematode; **RGI- Root galling index; ***PepMoV- Pepper mottle virus

Figure 1 (a) Root knots/galls due to root knot nematode infection in chilli

Figure 1 (b) Symptom variability on chilli leaves due to pepper mottle virus

Figure 1: Root-knot nematode infected chilli plants showing characteristic galls on roots (a); Pepper mottle virus infected chilli plants showing different types of symptoms (b)

incidence (90%) was recorded in the chilli variety 'Sahiba' under the potato–chilli and guava crop rotation system in Jalandhar, followed by an 80% incidence in the same variety under the "Pea–Chilli" rotation and in the hybrid 'HPH 5531' under the potato–cauliflower–chilli rotation in Jalandhar. Similarly, in Malerkotla district, an 80% incidence was also observed in the variety 'H1 Pepsi' grown under the cauliflower/brinjal–chilli rotation system (Table 1).

Laboratory analysis of soil samples collected during the survey revealed significant variation in soil nematode populations across districts. The highest overall mean soil nematode population of RKN was recorded in Malerkotla district (1333.33 J_2/250 cc of soil). In contrast, the lowest nematode population was observed in Tarn Taran (258.33 J_2/250 cc soil). Soil nematode populations varied considerably across locations (Table 1; Figure 2b). The maximum population density (2000 J_2/250 cc of soil) was recorded in Malerkotla under the "Chilli–Potato–Chilli" rotation for the variety 'CH-10'. The observations on the root galling index (RGI), which reflects the severity of nematode damage, also indicated significant variation across districts. The highest mean RGI was recorded in Malerkotla (7.56), followed by Jalandhar (5.93). Whereas, lowest RGI values were observed in Bathinda (2.17) and Tarn Taran (2.39). The root gall index (RGI) ranged from zero in certain fields of Sangrur where the 'Local' variety was cultivated, to a maximum of 8.5 in severely infested fields of Malerkotla & Jalandhar (Table 1).

Kaur, (2015) conducted a survey in five major chilli-growing districts of Punjab and reported the highest incidence of RKN in Sangrur district (80%), while the lowest was recorded in Ferozepur (40%). The corresponding soil nematode populations ranged from 615 J_2/250 g of soil in Sangrur to 104 J_2/250 g in Ferozepur. Similarly, Chaudhary et al., (2011) carried out the survey of 13 localities across nine different crops in the Hamelmalo subzone of Eritrea, found the highest RKN density in chilli (73.5%) and the lowest in *Eruca sativa* (12.24%). In another study, Zarina et al., (2015) investigated 15 chilli-growing regions in Sindh, identifying 52 nematode species belonging to 33 genera. Among these, *Meloidogyne javanica* was the most dominant species found infecting chilli plants, particularly in NNRC (Karachi) and Sukkur regions. Gautam et al., (2014) also reported a 67% incidence of root-knot nematode in chilli crops from the central plains of Chhattisgarh. More recently, Siddiq et al., (2023) surveyed the Musakhail district in Balochistan province for nematodes incidence in fruits and vegetable crops. During the study they identified five genera (*Heterodera*, *Meloidogyne*, *Aphelenchus*, *Helicotylenchus* and *Ditylenchus*) of phytonematodes associated with the crops and *Meloidogyne* genus was most prevalent in all the studied sites.

Prevalence and incidence of pepper mottle virus

During the survey for PepMoV incidence, a range of characteristic symptoms were observed. These included mottling on the upper leaves, mosaic initiation, vein banding, leaf narrowing, puckering and deformation of both the leaf lamina and fruits (Figure 1b). A noticeable reduction in fruit number was also recorded. Malformed fruits displaying dark and light green streaks were observed to be a common occurrence. Leaf samples exhibiting typical symptoms of PepMoV were collected from various districts during the survey and transported to the laboratory in an ice box for further analysis. The incidence of PepMoV varied considerably across districts. The highest average incidence was recorded in Malerkotla (74%), followed by Ludhiana (51.25%), Ferozepur (43%), Sangrur (38.33%), Patiala (32.5%), Tarn Taran (32.5%), Jalandhar (13.57%), and the lowest in Bathinda (10.67%). Within individual districts, the incidence ranged from a minimum of 2% in Bathinda for the 'Local' variety to a maximum of 83% in Malerkotla for the 'MS-12' variety (Table 1; Figure 2c).

The widest range was observed in Sangrur, where incidence levels varied between 20 and 60%. Similarly, Ferozepur and Ludhiana showed broad incidence

Table 2: Morphometric observation of *M. incognita* females and juveniles

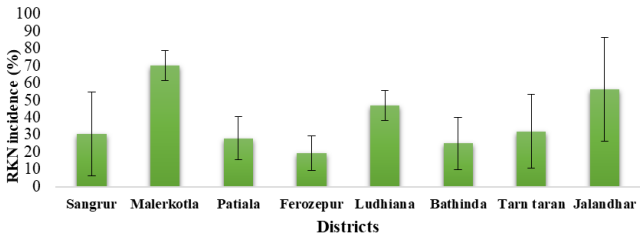
Characters (µm)	*Malerkotla*	*Ludhiana*	*Jalandhar*	*Ferozepur*	*Tarn Taran*	*Sangrur*	*Gross range of population*
Females							
Length	653.16 ± 52.68 (591.40- 714.90)	613.50 ± 58.42 (547.30- 691.10)	527.94 ± 41.32 (487.90- 584.90)	702.60 ± 39.09 (659.20- 751.10)	565.40 ± 45.21 (530.00- 686.00)	657.99 ± 41.41 (520.00- 670.00)	487.90–751.10
Width	478.18 ± 39.86 (409.60- 509.60)	447.22 ± 5.51 (441.20- 455.20)	391.58 ± 54.30 (299.70- 434.90)	562.52 ± 59.96 (502.30- 639.40)	349.10 ± 33.30 (296.00- 421.00)	384.37 ± 37.75 (333.00-429.00)	296.00–639.40
Stylet length	13.42 ± 1.23 (12.00 - 15.20)	13.54 ± 0.61 (12.80 - 14.20)	16.80 ± 1.08 (15.10 - 17.70)	15.36 ± 0.50 (14.80 - 16.10)	14.60 ± 1.15 (13.20 - 15.62)	15.88 ± 1.11 (14.75-17.86)	12.00–17.86
Juveniles							
Length	467.24 ± 32.33 (419.30- 509.70)	377.30 ± 15.59 (361.70- 395.80)	391.80 ± 11.88 (376.60- 405.30)	410.10 ± 26.27 (380.20 - 449.20)	377.37 ± 46.21 (342.00- 415.00)	400.20 ± 28.33 (383.00-432.00)	342.00–509.70
Width	19.24 ± 3.46 (14.80 - 22.90)	17.14 ± 3.55 (14.00 - 21.00)	12.40 ± 0.54 (11.70 - 13.00)	12.88 ± 1.25 (11.70 - 14.30)	12.9 ± 1.33 (12.00 - 13.85)	11.86 ± 1.33 (10.66-13.00)	10.66–22.90
Stylet length	12.36 ± 0.90 (11.00 - 13.20)	12.64 ± 1.21 (11.30 - 14.10)	10.18 ± 0.66 (9.50 - 11.00)	11.44 ± 0.88 (10.30 - 12.50)	11.20 ± 0.46 (10.55 - 12.15)	10.33 ± 0.66 (9.25-12.60)	9.25–14.10
Hyaline tail length	11.28 ± 0.62 (10.50- 12.10)	9.90 ± 1.15 (8.70 - 11.40)	9.28 ± 0.46 (8.80 - 10.00)	9.06 ± 1.16 (7.90 - 10.40)	9.18 ± 1.12 (9.34-11.28)	10.66 ± 1.54 (9.66-11.76)	7.90–12.10
Tail length	56.10 ± 2.21 (53.70 - 58.50)	53.80 ± 3.91 (48.30 - 57.90)	49.30 ± 1.56 (47.00 - 51.20)	53.12 ± 1.99 (50.20 - 55.10)	38.55 ± 2.99 (36.50-46.35)	48.35 ± 3.46 (44.5-56.33)	36.50–58.50

ranges of 25–60% and 30–65%, respectively. In contrast, narrower ranges were recorded in Jalandhar (10–20%) and Patiala (25–40%), indicating relatively more uniform levels of infection within those districts. A wide range of symptom variability associated with PepMoV infection was documented on chilli crop in different studies. These included leaf mottling, mosaic patterns, vein banding, puckering, narrowing or distortion of the leaf lamina, and a reduced number of flowers and fruits (Sharma, 2018). Under Punjab conditions, Kaur et al., (2014) observed PepMoV incidence on chilli crop presenting varied symptoms such as fruit malformation, leaf puckering, blistering, mottling, and vein greening. Additionally, Sharma (2018) noted the presence of PepMoV across multiple districts in Punjab, with the highest incidence reported in Patiala, followed by Sangrur.

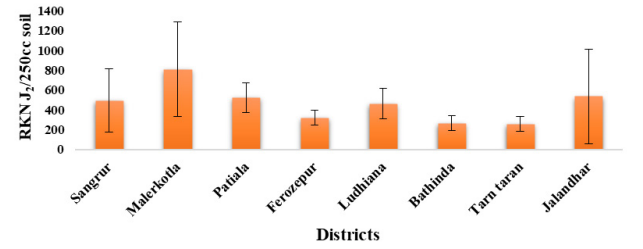
Detection and characterization of root-knot nematodes infecting chilli

Root-knot nematodes are major pests that severely affect chilli crops worldwide. Several species, including *Meloidogyne incognita*, *M. javanica*, *M. arenaria*, and *M. hapla*, have been reported to infect chilli on a global scale (Kaur et al., 2025). The perineal pattern observations of the mature females from the samples collected during the current study revealed the association of two *Meloidogyne* spp., *i.e., M. incognita* and *M. javanica* with the chilli crop under Punjab conditions. The *M. incognita* populations recorded high dorsal arch with no visible lateral lines exhibiting the characteristic features for *M. incognita* sp. while the perineal patterns from *M. javanica* populations showed high dorsal arch but with visible lateral lines depicting the characteristic feature of *M. javanica* sp. (Eisenback, 1985).

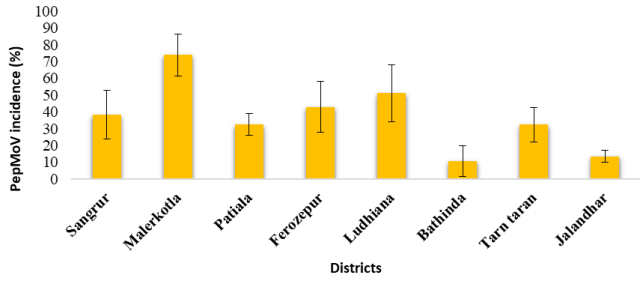
The morphometric observations of the mature females of *M. incognita* exhibited body lengths of 487.90 to 751.10 µm and widths of 296.00–639.40 µm, with stylet lengths of 12.00 to 17.86 µm. Second-stage juveniles (J2) showed body lengths of 342.00 to 509.70 µm and stylet lengths of 9.25 to 14.10 µm. Maximum values were generally recorded in Malerkotla and Ferozepur populations (Table 2). In *M. javanica* populations, female body length ranged from 512.00 to 675.00 µm and width from 315.00 to 456.00 µm, with stylet lengths of 12.66 to 16.67 µm. J2 body length ranged from 365.00 to 476.00 µm and stylet length from 10.20 to 12.16 µm, with higher values observed in Sangrur populations (Table 3). Overall, morphometric observations were consistent with earlier reports (Kaur et al., 2016; Kaur and Attri, 2013), though slightly larger body dimensions were recorded, suggesting possible regional variation. Out of the two root-knot nematode (RKN) species found associated with chilli crop under Punjab conditions. *M. incognita* was predominant and was found prevalent across all surveyed districts (Ludhiana, Jalandhar, Patiala, Sangrur, Tarn Taran, Bathinda, Malerkotla, and Ferozepur), whereas



(a) Root-knot nematode incidence in different chilli growing districts of Punjab



(b) Root-knot nematode soil population in different chilli growing districts of Punjab



(c) Pepper mottle virus incidence in different chilli growing districts of Punjab

Figure 2: Root-knot nematode incidence (%) in different chilli growing districts of Punjab (a) ; District wise mean root-knot nematode soil population in chilli crop (b); Pepper mottle virus incidence in different chilli growing districts of Punjab (c).

M. javanica was confined to Ludhiana and Sangrur district. Morphological and morphometeric analyses revealed variability among the populations collected from different areas.

Morphologically identified populations were confirmed through PCR using species-specific SCAR primers for *M. incognita* and *M. javanica* species. DNA extracted from single egg mass progenies when amplified using Finc/Rinc and Fjav/Rjav primers, all *M. incognita* populations produced the an expected ~1200 bp amplicon confirming the *M. incognita* sp., while *M. javanica* populations yielded ~670 bp fragments confirming the presence of *M. javanica* sp. (Figure 3a &b). PCR products were sequenced and analyzed using BLASTn, showing ~96 to 97% similarity with reference sequences. The sequences were submitted to GenBank with accession numbers PQ858425 and PQ858426.

Detection and confirmation of pepper mottle virus (PepMoV)

The ELISA results confirmed the prevalence of PepMoV on chilli in Punjab, with varying degrees of infection recorded across all surveyed districts (Table 1). To ensure more reliable detection, representative ELISA-positive samples were analyzed using Immuno Capture RT-PCR (IC RT-PCR). In all five to seven high virus titres samples from each district were amplified using IC RT-PCR with potyvirus specific primers. In IC RT-PCR, cDNA synthesised from the RNA isolated from the ELISA positive samples, using oligo(dT) primers when amplified with the potyvirus-specific Nib2F/3R primer set they yielded the expected ~350 bp amplicon on a 1% agarose gel (Figure 3c). The resulting PCR products when Sanger sequenced, and sequence analysis using BLASTn revealed 98% identity with the GenBank accession KJ744259 (*Pepper mottle virus* isolate Ferozepur: India, polyprotein gene, partial cds). In India, Sandhu & Chohan (1979) characterized chilli mottle disease using the micro-precipitin test. Later, Ravi et al., (1997) reported the occurrence of

Table 3: Morphometric observation of *M. javanica* females and juveniles

Characters (µm)	*Ludhiana*	*Sangrur*	*Gross range of population under study*
Females			
Length	567.2 ± 22.19 (512.00–666.00)	618.15 ± 45.17 (596.00–675.00)	512.00–675.00
Width	365.6 ± 34.45 (315.00–448.00)	430.12 ± 46.90 (376.00–456.00)	315.00–456.00
Stylet length	12.80 ± 0.90 (12.66–13.90)	15.36 ± 0.96 (14.80–16.67)	12.66–16.67
Juveniles			
Length	390.16 ± 20.11 (365.00–436.00)	416.30 ± 40.16 (390.00–476.00)	365.00–476.00
Width	11.86 ± 1.13 (11.26–12.86)	11.66 ± 0.90 (10.46–12.86)	10.46–12.86
Stylet length	11.54 ± 1.15 (11.34–12.16)	10.76 ± 0.86 (10.20–11.18)	10.20–12.16
Hyaline tail length	10.86 ± 1.40 (9.86–11.66)	11.31 ± 1.13 (9.76–12.66)	9.76–12.66
Tail length	58.86 ± 33 (48.60–60.00)	54.16 ± 4.12 (48.3–58.90)	48.30–58.90

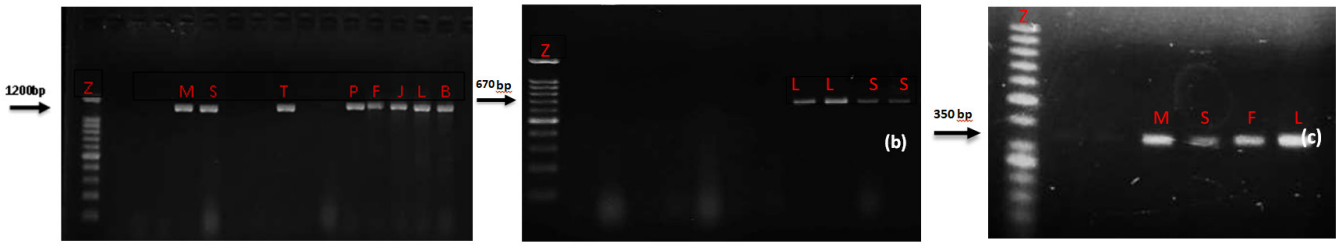


Figure 3: Confirmation of prevalent root knot nematode species and pepper mottle virus using molecular markers**.** (a) Agarose gel (1%) showing amplicon of ~1200 bp with *M. incognita* primer pair; (b) Amplicon of ~670 bp &with *M. javanica* primer pair (M- Malerkotla, S- Sangrur, T- Tarn Taran, P- Patiala, F- Ferozepur, J- Jalandhar, L- Ludhiana, B- Bathinda, Z- 100 bp ladder); (c) Agarose gel (1%) showing amplicon of ~350 bp with Nib primer pair (M- Malerkotla, S- Sangrur, F- Ferozepur, L- Ludhiana, Z- 100 bp ladder.

pepper vein banding virus, pepper veinal mottle virus, and potato virus Y in peppers from Karnataka. Rodriguez-Alvarado et al., (2002) conducted a two-year survey in the primary chilli-growing regions of southern New Mexico to identify viruses associated with severe pepper diseases. The study also examined weeds commonly occurring in and around pepper fields, analyzing a total of 685 pepper samples and 336 weed samples for viral infections. Using indirect enzyme-linked immunosorbent assay (ELISA), the researchers detected PepMoV in plants showing characteristic symptoms, including mosaic patterns, leaf distortion, and fruit malformation. More recently, Kaur et al., (2014) provided the first report from Punjab, India, confirming the presence of PepMoV in peppers through two sensitive assays, ELISA and RT-PCR-based partial sequence characterization.

Conclusion

This study demonstrates the widespread occurrence of root-knot nematodes and Pepper mottle virus (PepMoV) in chilli-growing regions of Punjab, with *M. incognita* identified as the predominant species and *M. javanica* present in specific locations. Morphological and morphometric observations, supported by molecular confirmation using species-specific SCAR markers, ensured accurate identification of nematode populations associated with the chilli crop in Punjab. The variation in nematode incidence across districts suggests the influence of agronomic practices, with reduced infestation observed under rice-based cropping systems and in resistant chilli hybrids. PepMoV was also widely prevalent and associated with characteristic symptoms, and its detection was reliably confirmed through ELISA and RT-PCR assays. The concurrent presence of these pathogens underscores their potential to cause significant yield losses, emphasizing the need for systematic screening of the available germplasm pool for the identification of resistant source(s) and designing appropriate integrated disease management strategies for sustainable chilli production.

Author's Contribution

Conceptualization and designing of the research work (SK, Ab.S); Execution of field/lab experiments and data collection (AK, SK, AS, HSB); Analysis of data and interpretation (SK, Ab.S, AK, AS, HSB); Preparation of manuscript (AK, SK).

Declaration

The authors declare that they have no competing interests**.**

Acknowledgments

The authors are thankful to the Indian Council of Agricultural Research and Punjab Agricultural University, Ludhiana for supporting this work.

References

Anonymous. (2023-24). Available online: http://agriwelfare.gov.in/en/StatHortEst

Anonymous. (2024). Package of Practices for Cultivation of Vegetables. PAU, Ludhiana.

Azlay, L., El Boukhari, M. E. M., Mayad, E. H., & Barakate, M. (2023). Biological management of root-knot nematodes (*Meloidogyne* spp.): a review. Organic Agriculture, 13, 99-117.

Bal, S., Sharangi, A. B., Upadhyay, T. K., Khan, F., Pandey, P., Siddiqui, S., Saeed, M., Lee, H. J., & Yadav, D. K. (2022). Biomedical and Antioxidant Potentialities in Chilli: Perspectives and Way Forward. Molecules, 27, 63-80.

Barboza, G. E., García, C. C., de Bem, Bianchetti. L., Romero, M. V., & Scaldaferro, M. (2022). Monograph of wild and cultivated chili peppers (*Capsicum* L., Solanaceae). PhytoKeys. 200, 1.

Bridge, J., & Page, S. L. J. (1980). Estimation of root-knot nematode infestation levels on roots using a rating chart. Tropical Pest Management, 26, 296-298.

Chaudhary, K. K., Brhane, D., Okube, H., Zaid, T., & Dagnew, E. (2011). Distribution, frequency of occurrence and population density of root knot nematode in Hamelmalo–Eritrea. African Journal of Microbiology Research. 5, 5656-5661.

Cheng, Y. H., Deng, T. C., Chen, C. C., & Liao, J. (2011). First Report of *Pepper mottle virus* in bell pepper in Taiwan. Plant Disease, 95, 617.

Dhaliwal, M. S., Yadav, A., & Jindal, S. K. (2014). Molecular characterization and diversity analysis in chilli pepper using simple sequence repeats (SSR) markers. African Journal of Biotechnology, 13, 3137-3143.

Eisenback, J. B., Hirschmann, H., Sasser, J. N., & Trintaphyllou, A. C. (1981). A guide to the four most common species of root-knot nematode *(Meloidogyne* species*),* with a pictorial key. Department of Plant Pathology, North Carolina State University, USA.

Eisenback, J. D. (1985). Detailed morphology and anatomy of 2nd stage juveniles, males and females of the genus *Meloidogyne* (root-knot nematodes), Pp 47-77. In: Sasser, J. N. and Carter, C. C. (eds.) An Advanced Treatise on *Meloidogyne*, Biology and Control. Raleigh, NC, USA.

Gautam, S. K., Sahu, G., Verma, B. K., & Poddar, A. N. (2014). Status of root-knot nematode (*Meloidogyne* species) disease in vegetable crops of some districts of central plain region of Chhattisgarh State, India. African Journal of Microbiology Research, 8, 1663-1671.

Goodey, J. B. (1957). Laboratory methods for work with plant and soil nematodes. Tech Bulletin, Ministry of Agriculture, London, UK.

Jepson, S. B. (1987). Identification of Root-knot Nematodes *(Meloidogyne* spp.). CAB International, Wallingford, UK.

Katti, G., & Ch, P. (2022). Impact of rice cultivation methods on insect pest incidence and their management. Journal of Rice Research, 15, 84-89.

Kaur, H. &Attri, R. (2013). Morphological and morphometrical characterization of *Meloidogyne incognita* from different host plants in four districts of Punjab, India. Journal of Nematology, 45, 122-127.

Kaur, M., Kumar, S., Yadav, S., Arora, I., Yadav, B., & Kaushik, P. (2025) Screening of chili genotypes against root-knot nematode (*Meloidogyne incognita*) under screen-house conditions. Vegetable Science, 52 92-99.

Kaur, S. (2015). Characterization of Root-knot nematode and viruses infecting pepper (*Capsicum annuum* L.) in Punjab and Identification of Resistant Sources. Ph.D. Dissertation. Punjab Agricultural University, Ludhiana.

Kaur, S., Kang, S. S., Dhillon, N. K., & Sharma, A. (2016). Detection and characterization of *Meloidogyne* species associated with pepper in Indian Punjab. Nematropica, 46, 209-220.

Kaur, S., Kang S S & Sharma A (2018) Molecular characterization and cross infectivity of poty and begomo viruses associated with hot pepper (*Capsicum annuum* L.) in Punjab (India). Research Journal of Biotechnology, 13(10), 14-22.

Kaur, S., Kang, S. S., Sharma, A., & Sharma, S. (2014). First report of *Pepper mottle virus* infecting chilli pepper in India. New Disease Reports, 30, 14.

Kim, Y. J., Jonson, M. G., Choi, H. S., Ko, S. J., & Kim, K. H. (2009). Molecular characterization of Korean Pepper mottle virus isolates and its relationship to symptom variations. Virus Research, 144, 83-88.

Kumar, V., Khan, M. R., & Walia, R. K. (2020). Crop loss estimations due to plant-parasitic nematodes in major crops in India. National Academy Science Letters, 43(5), 409-412.

Muthukumar, A., & Bhaskaran, R. (2007). Tactics to manage disease problems in pepper. Spice India, 20, 20-25.

Nelson, M. R. & Wheeler, R. E. (1972). A new virus disease of pepper in Arizona. Plant Disease Reporter, 56, 731.

Ravi, K., Joseph, J., Nagaraju, N., Krishnaprasad, S., Reddy, H., & Savithri, H. (1997). Characterization of a Pepper vein-banding virus from chilli pepper in India. Plant Disease, 81, 673-677.

Reddy, D. N., Mylabathula, M. M., & Al-Rajab, A. J. (2024). Industrial Demand and Applications of Capsaicin, pp 249-291. In: Swamy, M. K., & Kumar, A. (eds.) Capsaicinoids. Springer, Singapore.

Rodriguez-Alvarado, G., Fernandez-Pavia, S., Creamer, R., & Liddell, C., (2002). Pepper mottle virus causing disease in chile peppers in southern New Mexico. Plant Disease, 86(6), 603-605.

Saleh, B. K., Omer, A., & Teweldemedhin, B. (2018). Medicinal uses and health benefits of chili pepper (*Capsicum* spp.): a review. MOJ Food Processing & Technology, 6(4), 325-328.

Sandhu, K. S., & Chohan, J. S. (1979). Studies on the characterization of the mottle disease of chilli (*Capsicum annuum*) in the Punjab. Indian Journal of Mycology and Plant Pathology. 9: 177-182.

Sharma, S. (2018). Molecular detection, characterization and management of pepper mottle virus. Ph.D. dissertation. Punjab Agricultural University, Ludhiana, India.

Siddiq, M., Zareen, B., Akhtar, B., & Ismial, T. (2023). The study of parasitic nematodes of vegetables and fruit crops in district Musakhail, Balochistan, Pakistan. Pakistan's Multidisciplinary Journal for Arts & Science, 4: 108-127.

Tripodi, P., Rabanus-Wallace, M. T., Barchi, L., Kale, S., Esposito, S., Acquadro, A. & Stein, N. (2021). Global range expansion history of pepper (*Capsicum spp*.) revealed by over 10,000 genebank accessions. The Proceedings of the National Academy of Sciences, 118, e2104315118.

Williamson, V. M., Caswell-Chen, E. P., Westerdahi, B. B., Wu, F. F., & Caryl, G. (1997) A PCR assay to identify and distinguish single juveniles of *Meloidogyne hapla* and *M. chitwoodi*. Journal of Plant Nematology, 29, 9-15.

Zarina, B., Akhtar, S., & Khan, D. (2015). The nematodes of chilli plants of Sindh: Abundance, diversity and the assemblage. FUUAST Journal of Biology, 5, 71-92.

Zijlstra, C., Donkers-Venne, D., & Fargette, M., (2000). Identification of *Meloidogyne incognita, M. javanica* and *M. arenaria* using sequence characterized amplified region (SCAR) based PCR assay. Nematology, 2, 847-853.

सारांश

मिर्च (*कैप्सिकम एनम एल.*), जो सोलेनेसी परिवार की एक आर्थिक रूप से महत्वपूर्ण फसल है, विभिन्न जैविक तनावों, विशेष रूप से रूट-नॉट नेमाटोड (RKN) और वायरल रोगों के प्रति अत्यधिक संवेदनशील होती है, जिसके कारण उपज में भारी नुकसान होता है। पंजाब के प्रमुख मिर्च उत्पादक जिलों में RKN और पेपर मोटल वायरस (PepMoV) की व्यापकता का आकलन करने के लिए एक व्यापक सर्वेक्षण किया गया। RKN रोग की सर्वाधिक घटना (70%) मालेरकोटला में दर्ज की गई, जबकि सबसे कम घटना (19.20%) फिरोजपुर में देखी गई। इसी तरह, PepMoV रोग की घटना मालेरकोटला में सबसे अधिक (74%) और बठिंडा में सबसे कम (10.67%) देखी गई। RKN आबादी का रूपात्मक लक्षण वर्णन पेरिनियल पैटर्न विश्लेषण के माध्यम से किया गया, और प्रजातियों की पहचान की पुष्टि आगे 'सीक्वेंस कैरेक्टराइज्ड एम्प्लीफाइड रीजन' (SCAR) प्रजाति-विशिष्ट प्राइमरों का उपयोग करके की गई, जो मेलोइडोगाइन इनकोग्निटा और एम. जावानिका को लक्षित करते हैं। PepMoV का पता लगाने का कार्य शुरू में 'एंजाइम-लिंक्ड इम्यूनोसॉर्बेंट एसे' (ELISA) का उपयोग करके किया गया था, और बाद में 'इम्यूनोकैप्चर रिवर्स ट्रांसक्रिप्शन पॉलीमरेज चेन रिएक्शन' (IC-RT-PCR) के माध्यम से इसकी पुष्टि की गई, जिसमें पोटिव वायरस-विशिष्ट प्राइमरों का उपयोग किया गया था। ये निष्कर्ष पंजाब में RKN और PepMoV की व्यापक उपस्थिति और उच्च घटना को उजागर करते हैं, जो उपज के नुकसान को कम करने और मिर्च के सतत उत्पादन को सुनिश्चित करने के लिए एकीकृत रोग प्रबंधन रणनीतियों को विकसित करने और लागू करने की तत्काल आवश्यकता पर जोर देते हैं।