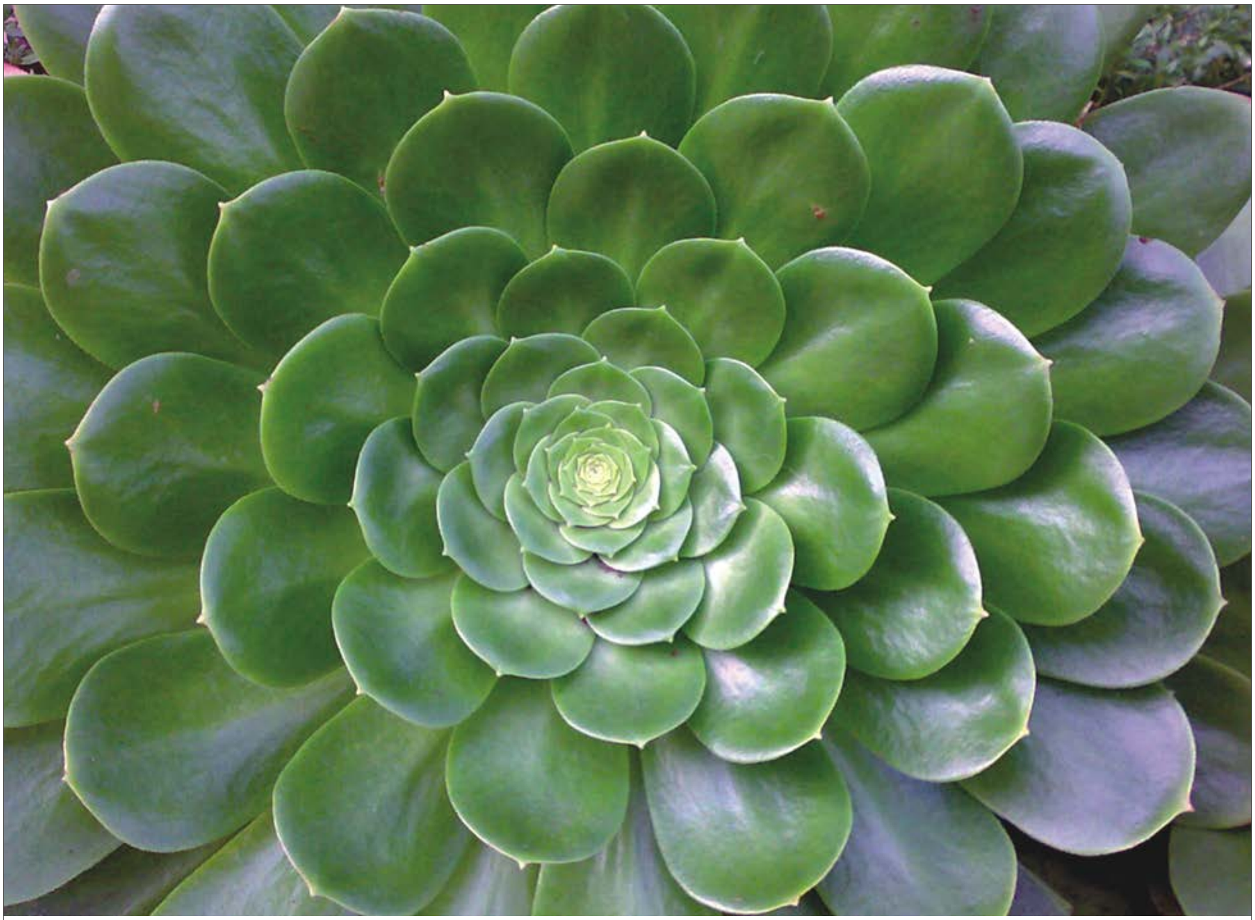

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Effect of Various Physico-Chemical Factors on the Incidence of Root Knot Nematode *Meloidogyne* spp. Infesting Tomato in District Aligarh (Uttar Pradesh) India

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ABSTRACT
Root-knot nematodes, (*Meloidogyne* spp.) are main pathogens of tomato in India. A survey was undertaken to find out the effect of physico-chemical properties of soil on the incidence and infestation of two root-knot nematode species namely *Meloidogyne javanica* and *Meloidogyne incognita* on tomato growing fields in Aligarh. Maximum disease frequency (87.5%) was found in Shah Jamal and Mahraval and minimum (25%) was found in Chherat Sudhal. Population density of *Meloidogyne* spp. was found to be the highest (750 J2 100 cm^{-3}) in Mahraval while as the lowest (170 J2 100 cm^{-3} in Chherat Sudhal. The highest nematode population observed in Mahraval having greater nitrogen, phosphorous and potassium with somewhat higher moisture content in the sandy loamy type of soil. Least nematode population was observed in Chherat Sudhal with least nitrogen, phosphorous and moisture content. Analysis of correlation coefficient showed that population of *Meloidogyne* spp. had significantly positive correlation with sand percentage, soil moisture, organic carbon, nitrogen, phosphorous and potassium while negative correlation with the silt, clay, soil temperature and soil pH at ($p \leq 0.01$). A total of 59 (61.45%) samples out of 96 were found infested with root knot nematode *Meloidogyne* spp. showing 83 and 75% distribution of *M. javanica* and *M. incognita*.

Key words: Correlation coefficient, disease frequency, *Meloidogyne* spp. survey

INTRODUCTION
Tomato (*Solanum lycopersicum* L.) is one of the most important and widely grown vegetable crop in the world, ranking second after potato, with an annual production of nearly 100 million t tomatoes in 3.7 million ha worldwide. India is the second largest producer of tomato after china covering the area of 865 ha with the production of 16,826 metric t tomatoes which is 11.5% of the total tomatoes production (IHB., 2011). The species is thought to be originated from Andes region of South America. Tomatoes eaten all over the world are important source of vitamins, minerals, dietary fibers and carbohydrates. Beside they contain carotene, lycopene natural antioxidant found to prevent prostate cancer (whfoods.org) and also protect from harmful UV rays. Seeds are medicinally important that promotes gastric secretion, acts as blood purifier which helps in maintaining healthy condition of intestine. The yield of tomato per hectare in India is very low in comparison to other developing countries due to various factors. Numerous obstacles of low yield are diseases caused by bacterial, fungal, viral and nematode pathogens. Among these

Root knot nematode *Meloidogyne incognita* and *Meloidogyne javanica* are widespread and recognized as a damaging pathogen of tomato that causes yield losses ranging from 35-39.7% (Jonathan *et al*., 2001). Moreover, these parasites also interact with other disease causing organisms to produce disease complexes and break down resistance against other pathogens and reduce plant tolerance to environmental stress (Begum *et al*., 2012). It is estimated that plant parasitic nematodes *Meloidogyne* spp. cause annual economic loss of US$157 globally (Abad *et al*., 2008). Among these, the root-knot nematodes, *Meloidogyne* spp. are major pests affecting quality and quantity of the crop. In a survey the root-knot nematodes have been found the most predominant group targeted by 48% of global nematicides used (Haydock *et al*., 2006). Root-knot nematode shows characteristic below ground symptoms in the root system, characterized by the formation of typical galls, these galls leads to extensive swelling which cause distortion and give unhealthy appearance to the root that limit the fruit production. They altered tissue at feeding site also disrupt the vascular tissue hampering the upward transport of water and dissolved nutrients by the xylem and translocation of photosynthesis to the other region of the plant by phloem (Anwar, 1995; Hajra *et al*., 2009). Nutrient and water uptake are substantially reduced due to damaged root system resulting in weak and low yielding of plants (Abad *et al*., 2003). Hence, present studies were conducted with the objective to determine and document the disease incidence, occurrence and intensity of root knot nematodes in tomato cultivations in the vegetable growing areas of Aligarh district.

MATERIALS AND METHODS

A systematic survey was carried out to find out reliable estimate of tomato field infested with root knot nematode in excessive vegetable growing area of Mahraval, Shah Jamal, Talaspur Khurd, Idgah Madrak, Gabhana, Sasni, Akrabad, Panethi, Harduaganj, Jawan and Chherat Sudhal of Aligarh district Uttar Pradesh.

Field sampling: During surveys root, soil and plant samples were carefully collected from 12 different localities having extensive tomato cultivation from randomly selected fields to access the incidence, intensity and distribution pattern of root-knot nematode. Root and soil samples were kept in polythene bags and date of collection and locality were labeled. A total of 96 samples were collected and brought to the department of Botany Aligarh Muslim University Aligarh for critical examination.

Root sampling: Infected roots were washed with tap water. It was dried and weighed and then immersed in an aqueous solution of phloxin B (0.15 g L^{-1}) for 15 min. The roots were again washed with tap water to stain egg masses. Root system was visually rated for galling and egg masses indices on a 0-5 scale where, 0 = no galls/egg masses, 1 = 1-2 galls/egg masses, 2 = 3-10 galls/egg masses, 3 = 11-30 galls/egg masses, 4 = 31-100 galls/egg masses and 5 = >100 galls/egg masses per root system.

Composite soil sample 100 cm^3 was thoroughly mixed in 2 L water and stirred the soil suspensions that allow the heavy soil particles to settle down for about 5 sec. Nematode isolation was done by employing sieving-cum-modified Baermann funnel techniques. The nematodes were counted under the stereo-binocular microscope. The frequency of occurrence (percentage) of individual tomato fields in all localities was calculated by the following equation:

$$\text{Frequency of occurence} = \frac{\text{No. of samples with RKN infection}}{\text{Total No. of samples}}$$

The incidence (percentage) of root knot nematode of individual tomato field was determined by following equation:

$$\text{Incidence (\%)} = \frac{\text{Total No. of infected plants}}{\text{Total No. of observed plants}} \times 100$$

Soil analysis: During survey soil samples collected from different sites marked and packed in polythene bags brought to the laboratory for the estimation of physico-chemical properties of the soil to find out the effect of these soil parameters on root-knot nematode population. Total organic carbon was estimated by the method. The texture of soil as well as particle size were determined by hydrometer method, total nitrogen by Micro-Kjeldahl method, total phosphorus by molybdenum blue method and the pH of the soil was estimated with pH meter. The soil characteristics are depicted in tables.

Moisture content: Moisture content in the soil was usually expressed in terms of percentage on dry weight basis. A known amount of soil sample was taken from the different locality and it was dried in oven at 80°C for 48 h and weighed again. The percentage of moisture content was calculated by using the following equation:

$$\text{Moisture content (\%)} = \frac{\text{Loss of weight on drying}}{\text{Dry weight of soil}} \times 100$$

Soil temperature: Soil temperature reading was taken up by the thermometer during the survey while collecting the soil samples at a depth of 5-20 cm below the ground.

Maintenance of inoculum: The inoculum of some selected fields from each locality was maintained on tomato cv. 'K-21' in a greenhouse by inoculating three weak seedlings in pots containing autoclaved soils with chopped infected root collected from the field.

Identification of species: Identification of *Meloidogyne* spp. collected from different localities was done by applying perineal pattern method. Mature females were dissected out from galls present on the roots of tomato plants. Perineal patterns slides (5-10) from each sample or locality were prepared and examined under microscope to study their characteristics.

RESULTS AND DISCUSSION

Population density of *Meloidogyne* spp. differs as per the locality. Maximum population density of *Meloidogyne* J2 750 per 100 cm^3 and 130 g^{-1} of the soil and root in Mahraval and minimum population density 170 J2 per 100 cm^{-3} and 39 per g of the soil and root, respectively in Chherat Sudhal. Population of nematode depends upon the season. The increase in nematode populations with season would be probably due to moisture (Jordaan *et al*., 1989) and ease of movement of the nematodes through the large soil pore diameter and soil particle size (Van Gundy, 1985). The soil temperature was 20-25°C in Mahraval, Shah Jamal, Talaspur khurd, Idgah, Madrak, Gabhana, Sasni, Akrabad, Panethi, Harduaganj, Jawan, Chherat Sudhal, respectively. Higher soil temperature cause desiccation and dryness of soil because they necessary thin film of water around

Table 1: Status of root-knot disease incidence, frequency and intensity on tomato in Aligarh district Uttar Pradesh

				Meloidogyne spp. density	
Localities	Soil temperature (°C)	Soil moisture (%)	pH	100 cm^3 of the soil	Per g of root
Mahraval	20	18.45	6.00	750±38	130±28
Shah Jamal	20	14.70	5.90	670±43	128±32
Talaspur Khurd	21	11.00	6.10	595±36	125±13
Idgah	21	10.50	6.30	425±30	122±22
Madrak	21	10.80	6.40	628±24	118±15
Gabhana	22	8.20	6.20	400±21	110±27
Sasni	22	6.25	6.60	620±29	103±18
Akrabad	23	5.38	7.00	645±41	90±23
Panethi	23	5.45	7.30	422±18	79±16
Harduaganj	24	4.55	7.60	460±13	62±18
Jawan	24	4.65	8.00	235±16	54±24
Chherat Sudhal	25	4.40	7.80	170±10	39±13

them for the movement and in low soil moisture content the connection of soil in soil solution increased and nematode are subjected to increased stress and during this nematodes consume a considerable amount of energy stored and reduce their population density (Gaur, 1994). The corresponding figure for soil moisture percentage ranged from18.45% in Mahraval to 4.40% Chherat Sudhal and the Hydrogen ion potential (pH) ranged from 5.90 Shah Jamal to 8.00 Jawan. The results presented in Table 1 clearly indicate that multiplication of root knot nematode was found to be highest in Mahraval where soil moisture were also more (18.45%) and multiplication of root knot-nematode decrease as the moisture content decrease. It represent that there is correlation between nematode multiplication and moisture content. These results are in confirmation with Siddiqui (2007) who observed that higher soil moisture is favorable for nematode multiplication. Root-knot nematode population increased when the pH was in the range of 5-7.0 and as the pH goes more than 7 nematode population decrease but the pH do not show direct influence on nematode population. Out of the 96 samples analyzed, 59 (61.45%) samples were found infested with root knot nematode *Meloidogyne* spp. which reveals that tomato is the good host for root knot nematodes. Disease frequency varied locality wise. Maximum frequency (87.5%) was observed in Mahraval and Shah Jamal, closely followed by Talaspur Khurd, Idgah and Madrak localities. In all the three localities the frequency was 75%. It was followed by 62.5, 62.5, 50, 50, 50 and 37.5% in Gabhana, Sasni, Akrabad, Panethi, Harduaganj and Jawan, respectively. Minimum diseased frequency (25%) was found in Chherat Sudhal. Disease incidence varied in entire districts and ranged from 9-59%. The maximum disease incidence (59%) of root-knot nematodes was found in Mahraval followed by Idgah (53%) and Madrak (42%). The lowest incidence was recorded from Chherat Sudhal (9%) followed by Jawan (12%) and Panethi (13%). The intensity of the disease in tomato in different localities in Aligarh based upon average gall and egg mass index was generally high (Table 2). Likewise the variation in disease frequency disease intensity also showed variation as per the area wise. Both gall and egg mass index varied in between 2-5. Highest disease intensity, average gall and egg mass index was found in Mahraval and Shah Jamal where disease frequency was also high. The gall and eggmass index were 4 in Talaspur khurd, Idgah, Madrak and Gabhana localities. It was found 3 in Sasni, Akrabad, Panethi and Jawan. Minimum average gall and egg mass index 2 was found in Harduaganj and Chherat Sudhal where disease frequency was found minimum. Therefore, the intensity of the disease was highest in Mahraval and Shah Jamal and minimum disease intensity was observed in Chherat Sudhal (Table 2). The highest nematode population was found in Mahraval with the organic carbon (0.36%) nitrogen 165, phosphorous 34.3 and potassium 267.4 kg ha^{-1}. The second highest nematode population in sandy loamy type of soil was found in Shah Jamal with the organic carbon (0.38%) nitrogen 189.9 kg ha^{-1} phosphorous

Table 2: Data of soil temperature, moisture and pH and *Meloidogyne* spp. density in soil and around the roots of tomato in different localities of Aligarh district Uttar Pradesh

			Disease incidence (%)			
Localities	Sample analyzed	Infected sample	Range	Average	Disease frequency (%)	GI/EMI
Mahraval	8	7	11-82	59	87.5	5/5
Shah Jamal	8	7	16-42	26	87.5	5/5
Talaspur Khurd	8	6	5-33.3	23	75.0	4/4
Idgah	8	6	0-86	53	75.0	4/4
Madrak	8	6	0-76	42	75.0	4/4
Gabhana	8	5	0-46	24	62.5	4/4
Sasni	8	5	0-53	27	62.5	3/3
Akrabad	8	4	0-33	19	50.0	3/3
Panethi	8	4	0-36	13	50.0	3/3
Harduaganj	8	4	0-26	14	50.0	2/2
Jawan	8	3	1-23	12	37.5	3/3
Chherat Sudhal	8	2	0-22	9	25.0	2/2

pH: Hydrogen ion potential, ±Represent to the standard error of nematodes per 100 cc soil and per g root, GI: Gall index, EMI: Egg mass index on Taylor and Sasser's scale

Table 3: Soil texture and physico-chemical properties around the rhizosphere of tomato plants in different localities of Aligarh district Uttar Pradesh

		Particle size (%)			Available nutrients (kg ha^{-1})			
Localities	Soil texture	Organic carbon (%)	Sand	Silt	Clay	N	P	K
Mahraval	Sandy loam	0.36	78.20	15.0	6.8	165.0	34.3	267.4
Shah Jamal	Sandy loam	0.38	77.30	15.5	7.2	189.9	46.0	236.2
Talaspur Khurd	Sandy loam	0.28	74.60	17.5	7.9	142.0	25.0	212.0
Idgah	Sandy loam	0.26	72.40	18.4	9.1	132.0	19.3	193.3
Madrak	Sandy loam	0.29	76.00	16.7	7.3	138.3	31.0	216.0
Gabhana	Sandy loam	0.30	71.00	19.0	10.0	124.0	14.4	177.0
Sasni	Sandy loam	0.27	75.30	17.0	7.7	141.3	24.5	210.0
Akrabad	Sandy loam	0.34	76.50	16.5	7.0	151.3	26.6	223.3
Panethi	Sandy loam	0.26	71.80	18.8	9.4	120.0	18.4	148.3
Harduaganj	Sandy loam	0.28	73.30	18.6	8.1	128.6	17.4	204.6
Jawan	Sandy loam	0.25	69.40	19.4	11.2	118.6	13.3	161.5
Chherat Sudhal	Sandy loam	0.24	68.00	20.0	12.1	116.0	11.2	196.2

46 and potassium 236.2 kg ha^{-1}. Lowest nematode population was found in Chherat Sudhal in sandy loamy type of soil with the organic carbon content (0.24%) nitrogen 116 kg ha^{-1}, phosphorous 11.2 and potassium 148.3 kg ha^{-1}. The highest nematode population was observed in Mahraval having greater nitrogen content, phosphorous and potassium with somewhat higher moisture content in the sandy loamy type of soil. Least nematode population was observed in Chherat Sudhal with least nitrogen, phosphorous and moisture content (Table 3). However, even in the same ecosystem confliction was observed. Nitrogen fertilization significantly increased the relative abundance of herbivores and decreased that of omnivores in a subtropical farm in Florida, US (Wang *et al*., 2006) but had no significant impact on soil nematode communities in a temperate farm in northeast China (Liang *et al*., 2009). Higher level of nitrogen present in the soil commonly reduces soil pH and causes ammonium and aluminum toxicity or introduces sufficient salt to harm soil biota (Wei *et al*., 2012). Similar to the nitrogen there is the contradiction in the observation of phosphorous (Zhao *et al*., 2014). Phosphorus addition did not significantly affect *Meloidogyne incognita*. Li *et al*. (2007) reported that elevated nitrogen fertilization had significant effects on the abundance and diversity of soil nematodes.

Physico-chemical properties of soil during the experiment in the experimental fields comprised a very high proportion of sand (above 75%), with relatively low clay and slit making it characteristic sandy-loamy type of soil. Nematode population is positively correlated with the sand percentage

Table 4: Occurrence and identity of *Meloidogyne* spp. infecting tomato in different localities of Aligarh district Uttar Pradesh

Localities	*M. incognita*	*M. javanica*
Mahraval	*M. incognita*	-
Shah Jamal	*M. incognita*	*M. javanica*
Talaspur Khurd	*M. incognita*	*M. javanica*
Idgah	*M. incognita*	*M. javanica*
Madrak	-	*M. javanica*
Gabhana	*M. incognita*	-
Sasni	*M. incognita*	*M. javanica*
Akrabad	-	*M. javanica*
Panethi	*M. incognita*	*M. javanica*
Harduaganj	*M. incognita*	*M. javanica*
Jawan	*M. incognita*	*M. javanica*
Chherat Sudhal	-	*M. javanica*
Meloidogyne spp. frequency (%)	75	83.33

-: Represent absence of *Meloidogyne* spp.

where nematode population increase with increase in the sand percentage. Maximum nematode population with the highest sand percentage (78.2%) was found in Mahraval and minimum population with the lowest sand percentage (68%) was found in Chherat Sudhal. Nematode population is negatively correlated with the silt plus clay percentage where nematode population decrease with increase in the silt and clay percentage. Maximum nematode population with the lowest silt plus clay percentage (15.00+6.8 = 21.8%) was found in Mahraval and minimum population with the highest silt and clay percentage (32.10%) was found in Chherat Sudhal. A significant difference was observed in nematode population in the soil which contained silt plus clay 21.8% and in the soil which contained 30.6 and 32.2% silt plus clay (Table 3). These results are in conformity with the Prot and van Gundy (1981) who stated that penetrate tomato roots decreased as the percentage of clay and silt increased and higher percentage of silt plus clay inhibit the nematode migration and penetration. Another factors influence the damaging potential may be climatic factor, edaphic factor and growing of nematode susceptible varieties of tomato. Among them soil type is a primary edaphic factor that may influence the damage potential of *Meloidogyne* spp. (Desaeger and Rao, 2000). Soil texture clearly had an impact because it influenced reproduction, build up of population densities in fields, relationship between pre-plant population densities and crop productivity (Koenning and Barker, 1995). Crop production areas with soils containing higher percentages of sand had higher population of *M. incognita* compared to those fields with low sand contents (Ogbuji, 1981). There are reports which confirmed that distribution, frequency and severity of root-knot nematodes are affected by varying agro-climatic conditions of the areas, soil type, moisture, soil pH and particular cropping sequence (David, 1985).

During survey two species of root knot nematode *M. incognita* and *M. javanica* were identified on the basis of perineal pattern. These species of root knot nematode were found either singly or in combination. Out of these two *M. javanica* was found most frequently. *Meloidogyne javanica* was observed in 10 (83.3%) locations and *M. incognita* was found at 9 (75%) location (Table 4). The study of analysis of correlation coefficient shows that population of *Meloidogyne* spp. has significantly positive correlation with soil moisture, organic carbon, nitrogen, phosphorous and potassium while negative correlation with the soil temperature and soil pH at 0.01 level of significance. Scattered points present in the graphs represent weather the two variables are having a relation or not. Maximum scattered points with Minimum correlation was found in between *Meloidogyne* spp. density and soil moisture (R^2 = 0.452, R = 0.672) with positive correlation. Maximum correlation with highest condensed points was found between *Meloidogyne* spp. density and phosphorous (R^2 = 0.718, R = 0.8473) which is depicted in Table 5 and Fig. 1. The distribution and occurrence

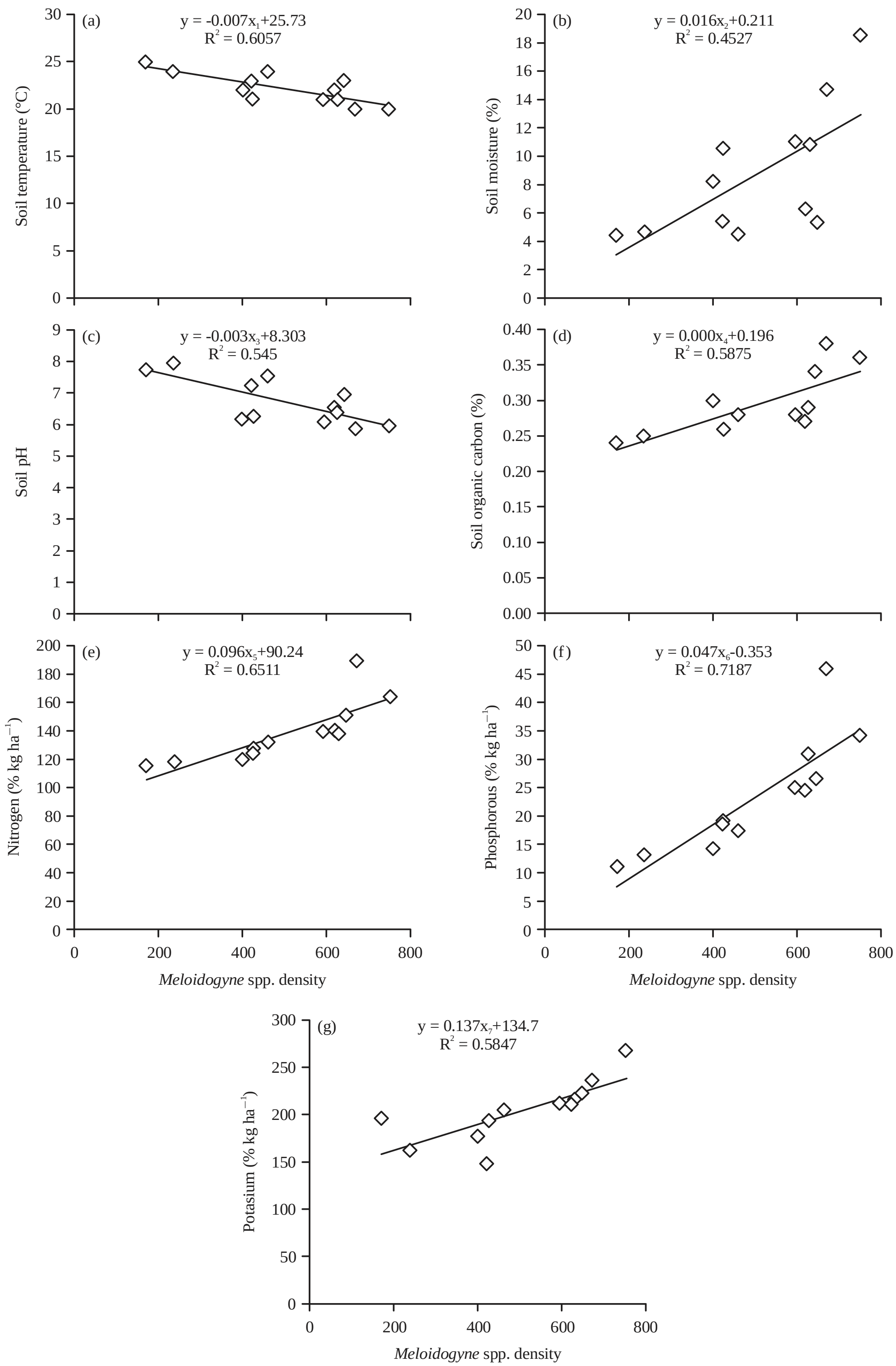

Fig. 1(a-g): Relationship between different physico-chemical parameters and population density of *Meloidogyne* spp. (a) Soil temperature, (b) Mosture, (c) pH, (d) Organic carbon, (e) Nitrogen, (f) Phosphorous and (g) Potassium

Table 5: Correlation co-efficient of root-knot nematode population in relation to physico-chemical properties of soil

Physico- chemical parameters	Correlation co-efficient	Allometric equation
TemperatureX_1	0.605	$Y = 0.007X_1+25.73$
Soil moistureX_2	0.452	$Y = 0 .016X_2+0.211$
Soil pHX_3	0.545	$Y = 0.003X_3+8.303$
Organic carbonX_4	0.587	$Y = 0.00X_4+0.196$
NitrogenX_5	0.651	$Y = 0.096X_5+90.24$
PhosphorousX_6	0.718	$Y = 0.047_6-353$
PotassiumX_7	0.584	$Y = 0.137X_7-134.7$

*Values are significant at 0.01 significant levels. X_1: Relation of *Meloidogyne* spp. density with soil temperature, X_2: Relation of population of *Meloidogyne* spp. density with soil moisture, X_3: Relation of population of *Meloidogyne* spp. density with soil pH, X_4: Relation of population of *Meloidogyne* spp. density with organic soil carbon, X_5: Relation of population of *Meloidogyne* spp. density with nitrogen, X_6: Relation of population of *Meloidogyne* spp. density with phosphorous, X_7: Relation of population of *Meloidogyne* spp. density with potassium

of *M. incognita* and *M. javanica* on singly or concomitantly sample collected from 12 different sites apparently manifest the prevalent distribution of these species. The high disease frequency and intensity of nematode showed the importance and reflect the view of crop under threat, causes yield losses ranging from 20-33% (Sasser, 1989). The frequency distribution of *M. javanica* is excessively large in number in tomato infected area as compared to *M. incognita*. This represent that the species is momentously important and to be conceived a serious threat to the host. The frequency of distribution of *Meloidogyne* spp. is very high due to growing of same crop for several years in the same field that lead to nutrient depletion, decrease soil fertility and increase harmful agriculture pest that reduce the productivity. Present findings of the survey are in conformity with the observation of Esfahani (2009) who observed the common occurrence of these species with dominance of *M. javanica* and their mixed population in the area. Disease frequency was found to be 87.5% in Mahraval and Shah Jamal location either due to availability of suitable host, growing of susceptible varieties and high temperature which provide favorable condition for the nematode multiplication and infection of root knot nematode in the crop (Cuc and Prot, 1992). In the location of Chherat Sudhal and Jawan the frequency of root knot nematode is very low due to high organic matter and fallow of the field for the last few years that lead to decrease the nematode populations and other possibilities might be due to persistence of dry conditions and climatic factors which may temper the population increase of the two species (Taylor *et al*., 1982). Rotation to non-host crops can substantially reduce the nematode population (Viaene and Abawi, 1998). Reliable crop loses estimate are important for establishing research, extension and budget priorities (Dunn, 1984). Specific estimate of vegetable crop loss due to *M. incog*nita and *M. javanica* ranged to 17-20% eggplant, 18-33% melon and 24-38% in tomato (Kathy, 2000).

CONCLUSION

During the survey it was analyzed that root-knot nematodes (*Meloidogyne* spp.) found in all fields but the locality viz., Mahraval and Shah Jamal were found heavily infested. It furnishes the baseline distribution of root-knot in correlation with tomato. In the recent finding *M. incognita* and *M. javanica a*re found in different proportion in all the localities in which *M. javanica* was found prevalent. Physico-chemical parameters, soil temperature, soil pH, silt and clay have significant impact on nematode population and shows negative linear correlation, while organic soil carbon, Nitrogen, phosphorous and potassium have positive correlation with reference to root knot-knot nematode density. This study gives an idea regarding the relationship between the physico-chemical properties and nematode species by altering the ratio of chemical elements present in the soil by the addition of chemicals fertilizer that effect the nematode population by bring into existence of unfavourable soil flora and fauna without disturbing the ecosystem. Survey not only provides information about the association of root knot nematode with the tomato but also furnishes report about the incidence, frequency of occurrence, density, geographical distribution and potential damage and influence on crop and also help in making government policy for the nematode management and increase in crop yield. Which revels estimate yield loss can be enhanced by better information of nematode distribution by creating awareness among farmers via print, electronic media through private and public organizations. Therefore, it is necessary that the researchers should pay direct attention towards widespread distribution of root knot nematodes and interaction with other soil microorganism.

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