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Behavior of Plant Parasitic Nematodes Associated With Mulberry Plants with Special Emphasis to the Family Hoplolaimidae from Manipur, India

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Abstract – A study for the population behavior of plant parasitic nematodes associated with mulberry plants was conducted as a part of a thesis submitted to Manipur University in four mulberry farms located at valley districts of Manipur, India. Out of the parasitic nematodes, tylenchid nematodes belonging to the family Hoplolaimidae were found abundantly from the all studied farms. Population changes of the nematodes in relation to soil pH, organic carbon, nitrogen, phosphorous and potassium contents were found. The study showed increased nematode population with increased in soil organic carbon, nitrogen, phosphorous and pH contents and decreased population with increased soil potassium content. Highest nematode population was found at clayey- loam soil type with greater contents of nitrogen, phosphorous, potassium and pH whereas least population was found at sandy loam soil with least nitrogen and phosphorous contents. Among the nematode species of the family Hoplolaimidae, the most abundant *Helicotylenchus graminophilus* increased its population with increase in soil organic carbon, nitrogen, phosphorous and pH contents while the population decreased with increased soil potassium content. The least abundant nematode population of *Rotylenchus minutus* increased with increased of potassium content while its population decreased with increased in soil pH, organic carbon, nitrogen and phosphorous contents.

Keywords – Population behavior, nematodes, Hoplolaimidae, soil parameters, Manipur.

I. INTRODUCTION

Plant parasitic nematodes are thread-like, pseudocoelomate, transparent, triploblastic and bilaterally symmetrical invertebrate animals. They are considered as foe- and friends of farmers as their movements in the soil texture create better aeration of soil minerals. They in other hand are considered as Hidden enemies of farmers in the view that they cause drastic losses in the vegetable and horticultural crops while they are unable to see with naked eyes. Plant parasitic nematodes infected roots show disorganization and collapse of cells of cortical tissues. Sloughing off of epidermal cells was also observed. After penetration the nematode made a pathway through root tissue by destroying cells in its path. The necrotic damage to cells in the area of feeding site also extended to cells some distance away from feeding site. Sometimes the nematodes penetrated deep into cortex and approached the stele. All such infected roots show disorganization of cortical cells and brown lesions. Sometimes galls in rhizospheric roots of plants are produced by root gall nematodes, *Meloidogyne* spp. *H. dihystera* with its head end embedded in cane roots showed secondary infection generally around their feeding sites [1]. Blunt, malformed roots and a reduction in the number of small branch rootlets were found in cane sugars due to feeding of *H. dihystera* [2]. *Rotylenchus* spp. and *Scutellonema* spp. generally feed externally on roots but sometimes some of them embedded anterior end of body in root tissue. Reduction in length, weight of leaves and number of leaf buds were characteristic symptoms of infection of spiral nematodes in mulberry [3]. *Scutellonema* spp. feed on peripheral tissues and cause necrosis on tubers even during storage, thus causing huge qualitative loss as well. Necrosis also leads to secondary attack by fungi and bacteria [4]. The magnitude of crop losses depends upon factors such as susceptibility of crop and environmental condition [5].

As a part of thesis submitted to Manipur University considering the ecological aspects of plant nematodes, the population of nematode, soil pH, organic carbon, phosphorous and potash contents of four mulberry farms in topographically different areas were recorded for their correlations. The four mulberry farms were Regional Tasar Research Station, Chingmeirong, Imphal West district; Sericulture Training Station, Kwata, Bishnupur district; Khangabok Wangbal Government Silk farm, Wangbal, Thoubal District and Mulberry farm, Kalika village, Irilbung, Imphal East district. Imphal West district had a latitude of 24º30´ to 25º0´ North, longitude 93º45´ to 94º15´ East and an altitude of 790.0 (above Mean Sea Level); Imphal East district had a latitude of 24º30´ North and a longitude of 93º45´ East with an altitude of 790.0

(above Mean Sea Level); Bishnupur District had a latitude of 24º15´ North, longitude 93º30´ to 94º0´ East and an altitude of 828.2 (above Mean Sea Level) and Thoubal district had a latitude of 23º 45´ to 24º45´ North, longitude of 93º45´ to 94º15´ East and an altitude of 781.0 (above Mean Sea Level). During the course of study of nematodes associated with mulberry plants, the plant parasitic nematode species belonging to family Hoplolaimidae was most frequently encountered. The encountered nematode species were spiral nematodes-*Helicotylenchus* spp., *Rotylenchus* sp. and *Scutellonema* sp. Study on population behavior of the family in relation to the physico – chemical parameters of soil is also incorporated.

The study is undertaken to find the relationship between nematode population with soil NPK content, organic carbon content and pH respectively so that strategies for adoption of Integrated Management of plant parasitic nematodes infecting mulberry plants could be work out.
The present research paper is organized as – section I contains the introduction of the work carried out, section II contains the related work, section III contains methodology, section IV is the results and discussion of the work and section V contains conclusions of the work.

II. RELATED WORKS

Fluctuations of nematode populations of *Xiphinema americana* in relation to soil temperature and moisture was studied for crop management of strawberry and cherry [6], root - knot nematodes associated with tobacco [7] and stylet bearing nematodes associated with mango [8]. Study on effect of pH on growth and reproduction of free living nematode, *Caenorhabditis* showed that alkalinity of soil enhanced growth while reproduction of nematodes by acidity of soil [9]. Different works were carried out on the relationship between NPK and nematode population of *Rotylenchus reniformis*, *Rotylenchulus reniformis* and *Meloidogyne incognita* associated with different host plants including *Vigna unguiculata* and showed varying correlations [10, 11, 12]. Total *Heterodera cajani* cyst population associated with cowpea was significantly reduced with fertilizer treatments compared to control. Significant reduction in cyst population was recorded with potash treatments alone and/or in various combinations with phosphorous or nitrogen [13]. Root knot nematode associated with cotton showed decreased in galling and reproduction with increasing level of organic carbon [14].

III. METHODOLOGY

For a detailed study, nematode population, soil pH, organic carbon, phosphorous and potassium content of soil were recorded from the four different farms in valley districts of Manipur for a period of two consecutive years 2009 - 2010. At least 20 sub – samples of 500gm of soil were collected from a farm every month. The sub - samples were put in a polythene bag carrying all necessary information including date of collection and name of collector. These sub – samples were mixed thoroughly in a bucket with hands. From the mixed soil, again 500gm was taken and it served as the sample for the particular farm. Soil samples were processed for collection of nematodes through Cobb's sieving and decanting method followed by modified Baermann's funnel technique. Nematodes collected were put in a 100ml conical flask and made a suspension of 100ml by adding distilled water and bowled with a pipette to homogenized the mixture. 10 ml of homogenized suspension was taken in a Syracause counting disc. Three readings were taken and the mean taken up. The same process was applied for all farms under study. Soil pHs were measured by a pH meter. Soil contents of organic carbon, nitrogen, phosphorous and potassium were measured through rapid titration methods [15, 16, 17] respectively. The results of experiments were presented in tabular forms. Plant parasitic nematodes which were economically important and common to all the sites were selected for the study.

The four mulberry farms of the valley Districts of Manipur selected for study were represented as
Site I - Regional Tasar Research Station, Chingmeirong, Imphal West district
Site II - Sericulture Training Station, Kwata, Bishnupur district
Site III - Khangabok Wangbal Government Silk farm, Wangbal, Thoubal District and
Site IV - Mulberry farm, Kalika village, Irilbung, Imphal East district.

IV. RESULTS AND DISCUSSION

Population behavior of Plant Parasitic Nematodes:
Nematode population collected at the four sites are as, site I - 4292 / 500 gm soil, site II - 3573 / 500 gm, site III - 5594 / 500 gm soil and site IV- 2575 / 500 gm of soil. 17 nematode species were common at site I, II and IV while 19 species were present at site III. Among all parasitic nematodes, *Helicotylenchus graminophilus*, *H. cornurus* and *H. psuedorobustus* were most common and abundant at the four sites. *Aglenchus muktii* was present in all the four sites while its highest population was found at site IV. *Aphelenchoides minor* with population somewhat higher than those of *A. muktii* was also found in all the sites, with the highest population at site IV. *Aphelenchus avenae* population was also highest at site IV while lowest was at site IV. *Boleodorus cylindricus* was most abundant at site II and least at site I. In all the four sites studied, *Caloosia luci* was absent except in site III. *Coslenchus tausifi* was most abundant at site IV and least at site III. *Criconemella ferniae* was abundant at site I and least at site III. *Discocriconemella limitanea* was abundant at site I and least at site II. *Ditylenchus microdens* was found only at site III. *Helicotylenchus cornurus* was the second most abundant nematode species whose population was highest at site I and least at Site IV. *Helicotylenchus dihystera* was the 3rd most abundant nematode whose population was highest at site III and least at Site IV. *Helicotylenchus graminophilus* was most abundant of the all the nematode species recorded, with its population highest at site I and

least at site IV. *Helicotylenchus pseudorobustus* was fourth most abundant nematode which population at site I was the highest and least at site IV. Juveniles of *Meloidogyne javanica* were found only at Site II where there was least soil pH and organic Carbon content and lesser Nitrogen, Phosphorous and Potassium contents of soil. *Pratylenchus penetrans* was most abundant at site II and least at site I. *Rotylenchus minutus* was most abundant at site II and least at site I. *Scutellonema aberrans* most abundant at site II and lowest at site I. *Tylenchorhynchus elegans* had highest population at site I while lowest at site II. Only two aphelenchid were encountered like *Aphelenchoides minor* and *Aphelenchus avenae*. *A. minor* was most abundant at site IV with a total population of 244 / 500 gm soil with a soil organic Carbon of 3.31 p.c., Nitrogen 1003.52 p. c., with least Phosphorous and Potassium contents of 12.54 p.c. and 846.72 p.c., soil pH 5.47 with sandy -loam type soil and its population was least at site III with organic Carbon 3.62 p.c., Nitrogen 1254.40 p.c., Phosphorous 15.68 p.c., Potassium 799.68 p.c. and soil pH 5.8 with sandy-loam type soil. *Boleodorus cylindricus* was the least populous nematode species with a population of 10/ 500 gm at site I, 12/500 gm soil at site III and 56 / 500 gm soil at site II. Site II had the highest nematode population with soil pH 5.3, organic Carbon content 3.62 p.c., Nitrogen 1254.40 p.c., Phosphorous 16.68 p.c., Potassium 799.68 p.c. with clay loam type of soil. Site I had the second highest nematode population with soil pH of 6.45, organic Carbon 4.5 p.c., Nitrogen 1568.0 p.c., Phosphorous 21.95, Potassium 470.40 p.c. with sandy-loam type soil. Site IV had least nematode population with soil pH 5.4, organic Carbon 3.31 p.c., Nitrogen 1003.52, Phosphorous 12.54, Potassium 846.72 p.c. with sandy-loam type (Table 1 and 2).

The analysis of correlation co-efficient between the nematodes and the five environmental parameters taken into consideration in the study showed that there was significant positive correlation (significant at 95% level of significance and above) with soil contents of organic Carbon, Nitrogen, Phosphorous and pH. There was significant negative correlation between nematode population and soil potassium content (Table 3).
Most abundant nematode population was found at site II which soil type was clayey-loam with lesser organic Carbon content of 3.6; greater contents of Nitrogen, Phosphorous and Potassium with somewhat higher soil pH value. Least abundant nematode population was found at site IV which soil type was sandy-loam with least Nitrogen and Phosphorous contents. In earlier study nematode population associated with *Prunus salicina* was also abundant in clayey – loam type of soil [18].

Phosphorous content of soil played a decisive role in population dynamics of nematodes. High dosages of Nitrogen were always effective in controlling nematode population if accompanied by Phosphorous [19]. A study in burned and unburned plots of forest that nematode density in unburned plots was not correlated with Carbon content and neither was density and Nitrogen content [20]. Elevated Nitrogen fertilization had significant effects on the abundance and diversity of soil nematodes [21]. The present work also inferred that population densities of root - knot nematodes was lower on Potassium deficient plants than those with adequate Potassium levels.

Table1: Physico-chamical parameters of soil in four mulberry farms

Sites	**Soil type**	**Organic carbon (kg/ha)**	**Nitrogen (kg/ha)**	**Phosphorous (kg/ha)**	**Potassium (kg/ha)**	**pH**
Site I	Sandy-loam	4.55	1568.0	21.95	470.40	6.45
Site II	Sandy-loam	2.79	1160.32	18.81	564.48	5.14
Site III	Clay-loam	3.62	1254.40	15.68	799.68	5.84
Site IV	Sandy-loam	3.31	1003.52	12.54	846.72	5.47
Mean		**3.5675**	**1,246.56**	**17.245**	**670.32**	**5.725**

Table 2: Population of plant parasitic nematodes in the four mulberry farm

Nematode species	**Nematode population per 500 gm of soil Sites**			
	Site I	**Site II**	**Site III**	**Site IV**
Aglenchus muktii	24	58	36	64
Aphelenchoides minor	148	78	64	244
Aphelenchus avenae	66	130	88	144
Basiria aberrans	44	24	48	58
Boleodorus cylindricus	10	56	42	12
Caloosia luci	-	-	820	-
Coslenchus tausifi	80	78	44	84
Criconemella ferniae	120	88	78	68
Discocriconemella limitinae	88	25	30	75
Ditylenchus microdens	-	-	228	-
Helicotylenchus cornurus	820	740	546	312
H. dihystera	790	224	810	128
H. graminophilus	840	720	640	220
H. pseudorobustus	780	328	460	214
Juvenlie Meloidogyne javanica	-	780	-	-

Pratylenchus penetrans	20	90	56	28
Rotylenchus minutus	24	440	380	420
Scutellonema aberrans	340	440	380	420
Tylenchorhynchus elegans	98	54	64	84
Total	**4292**	**3573**	**5594**	**2575**
Total nematode population	**16,034**			

Table 3: Correlation co-efficient and allometric equations of nematode population in relation to soil parameters

Physico-chemical parameter	Correlation co-efficient	Allometric equations
Organic carbon X_1	0.380	$Y= 1680.40 + 652.585 X_1$
NitrogenX_2	0.540	$Y = 416.7 + 2.881 X_2$
PhosphorousX_3	0.319	$Y = 2284.5 + 99.967 X_3$
PotassiumX_4	- 0.084	$Y = 4403.5 - 0.589 X_4$
Soil pH X_5	0.471	$Y = -2092.156 + 1065.617 X_5$

Significant at 0.01 level of significant.

Where X_1 = correlation co-efficient of nematode population with soil organic Carbon

X_2= correlation co-efficient of nematode population with soil Nitrogen

X_3= correlation co-efficient of nematode population with Phosphorous

X_4= correlation co-efficient of nematode population with Potassium and

X_5= correlation co-efficient of nematode population with soil pH

Population behavior of Hoplolaimidae Nematodes:

Nematode species belonging to the species *Helicotylenchus cornurus*, *Helicotylenchus dihystera*, *H. graminophilus*, *H. psuedorobustus*, *Rotylenchus minutus* and *Scutellonema aberrans* were present in all the four sites. These nematodes belong to the family Hoplolaimidae. This group of nematodes seems to adapt all types of soil found in the four studied farms of Mulberry. *H. graminophilus* was the most abundant and *Rotylenchus minutus* had the lowest abundance. The total nematode populations at the sites were presented in table 4. The correlation co - efficient between the nematode species with different physico - chemical parameters of soil are represented in table nos. 5, 6, 7, 8, 9 and 10 respectively.

H. graminophilus was most abundant nematode among all species of the members of family Hoplolaimidae followed by *H. cornurus*. *Rotylenchus minutus* showed lowest nematode population. Correlation coefficient showed that population of *H. cornurus* positively correlated with soil contents of organic Carbon, Nitrogen and Phosphorous. The population of *H. dihystera* also positively correlated with organic Carbon, Nitrogen, Phosphorous and soil pH while negatively correlated with Potassium. The population of *H. graminophilus* increased with increased soil organic Carbon content, Nitrogen, Phosphorous and soil pH and decreased with increased Potassium. *H. psuedorobustus* increased with increased in soil contents of organic Carbon, Nitrogen, Phosphorous and pH and decreased with increased Potassium. The population of *Rotylenchus minutus* and *Scutellonema aberrans* decreased with increased soil contents of organic Carbon, Nitrogen, Phosphorous and pH while increased with decreased Potassium (Table 5, 6, 7, 8, 9, 10).

The population of *H. graminophilous* was deficient at sites where there was high level of potassium but with increase in soil organic carbon content, its population increased while in case of *H. cornurus*, its population was high at potassium abundant soils. The population of *Helicotylenchus dihystera* was deficient in potassium abundant soil, but show high population with high carbon content of soil (Table 4).

Its population was also high in phosphorous abundant soil which was in agreement with earlier work [22]. The population of *H. psuedorobustus* was abundant in Potassium deficient and Carbon abundant soils. In case of *Rotylenchus minutus* and *Scutellonema aberrans*, population was abundant in Potassium abundant soil but deficient organic Carbon, Nitrogen and Phosphorous contents. Their population was also abundant in soil with high pH. Effect of pH on different species of nematodes varied in different level of optimum pH in several species [23]. In the present study there was no great difference in the level of pH.

Table 4: Total nematode population under the family Hoplolaimidae at the four different farms

Nematode species	Site I	Site II	Site III	Site IV	Total
H. cornurus	820	740	546	312	**2,418**
H. dihystera	790	224	810	128	**1,952**
H. graminophilus	840	720	640	220	**2,420**
H. psuedorobustus	780	328	460	214	**1,782**
R. minutus	24	128	95	78	**325**
S. aberrans	340	440	380	420	**1,580**
Total	**3,594**	**2,580**	**2,931**	**1,372**	**10,477**

Table 5: Correlation co-efficient of *H. cornurus* with soil parameters

Physico - chemical parameters	Correlation co - efficient
Organic carbon X_1	0.358
Nitrogen X_2	0.793
Phosphorous X_3	0.979
Potassium X_4	0.257
Soil pH X_5	0.398

Significant at 0.01 level of significant

Where, X_1 = correlation co-efficient of *H. cornurus* with soil organic Carbon

X_2= correlation co-efficient of *H. cornurus* with soil Nitrogen

X_3= correlation co-efficient of *H. cornurus* with Phosphorous

X_4= correlation co-efficient of *H. cornurus* with Potassium

X_5= correlation co-efficient of *H. cornurus* with soil pH

Table 6: Correlation co-efficient of *H. dihystera* with soil parameters

Physico - chemical parameters	Correlation co - efficient
Organic carbon X_1	0.380
Nitrogen X_2	0.8109
Phosphorous X_3	0.493
Potassium X_4	-0.274
Soil pH X_5	0.834

Significant at 0.01 level of significant.

Where, X_1 = correlation co-efficient of *H. dihystera* with soil organic Carbon

X_2= correlation co-efficient of *H. dihystera* with soil Nitrogen

X_3= correlation co-efficient of *H. dihystera* with Phosphorous

X_4= correlation co-efficient of *H. dihystera* with Potassium

X_5= correlation co-efficient of *H. dihystera* with soil pH

Table 7: Correlation co-efficient of *H. graminophilus* with soil parameters

Physico - chemical parameters	Correlation co - efficient
Organic carbon X_1	0.368
Nitrogen X_2	0.828
Phosphorous X_3	0.928
Potassium X_4	-0.834
Soil pH X_5	0.472

Significant at 0.01 level of significant.

Where, X_1 = correlation co-efficient of *H. graminophilus* with soil organic Carbon

X_2= correlation co-efficient of *H. graminophilus* with soil Nitrogen

X_3= correlation co-efficient of *H. graminophilus* with Phosphorous

X_4= correlation co-efficient of *H. gramilophilus* with Potassium

X_5= correlation co-efficient of *H. graminophilus* with soil pH

Table 8: Correlation co-efficient of *H. psuedorobustus* with soil parameters

Physico - chemical parameters	Correlation co-efficient
Organic carbon X_1	0.865
Nitrogen X_2	0.996
Phosphorous X_3	0.823
Potassium X_4	-0.700
Soil pH X_5	0.910

Significant at 0.01 level of significant.

Where, X_1 = correlation co-efficient of *H. psuedorobustus* with soil organic Carbon

X_2= correlation co-efficient of *H. psuedorobustus* with soil Nitrogen

X_3= correlation co-efficient of *H. psuedorobustus* with Phosphorous

X_4= correlation co-efficient of *H. psuedorobustus* with Potassium

X_5= correlation co-efficient of *H. psuedorobustus* with soil pH

Table 9: Correlation co-efficient of *Rotylenchus minutus* with soil parameters

Physico-chemical parameters	Correlation co-efficient
Organic carbon X_1	- 0.947
Nitrogen X_2	-0.694
Phosphorous X_3	-0.380
Potassium X_4	0.325
Soil pH X_5	-0.897

Significant at 0.01 level of significant.
Where, X_1 = correlation co-efficient of *R. minutus* with soil organic Carbon
X_2= correlation co-efficient of *R. minutus* with soil Nitrogen
X_3= correlation co-efficient of *R. minutus* with Phosphorous
X_4= correlation co-efficient of *R. minutus* with Potassium
X_5= correlation co-efficient of *R. minutus* with soil pH

Table 10: Correlation co-efficient of *Scutellonema aberrans* with soil parameters

Physico-chemical parameters	**Correlation co-efficient**
Organic carbon X_1	-0.977
Nitrogen X_2	-0.876
Phosphorous X_3	-0.519
Potassium X_4	0.359
Soil pH X_5	-0.994

Significant at 0.01 level of significant.
Where, X_1 = correlation co-efficient of *S. aberrans* with soil organic arbon
X_2= correlation co-efficient of *S. aberrans* with soil Nitrogen
X_3= correlation co-efficient of *S. aberrans* with Phosphorous
X_4= correlation co-efficient of *S. aberrans* with Potassium
X_5= correlation co-efficient of *S. aberrans* with soil pH

V. CONCLUSIONS

As evident from the study, sandy-loam type of soils retains highest to least organic Carbon, Nitrogen, Phosphorous and Potassium while soil acidity fluctuates in these soil types. In such soil types, total nematode population also fluctuates from moderate to minimum. The farms having clay - loam with moderate sand have moderate contents of soil organic Carbon, Nitrogen, Phosphorous and Potassium while slight acidity of soil prevail. The nematode population remains highest in this type of soil.

There were positive correlations co-efficient between the nematode population with soil organic Nitrogen, pH, organic Carbon and Phosphorous that indicated probability higher population of nematodes with increase in such soil – chemico parameters. Negative correlation between the nematode population and soil Potassium content was found which indicated decrease nematode population with increase soil Potassium content. In case of Hoplolaimidae nematodes, population shows fluctuation from maximum to minimum in sandy- loam type while moderate population is found in clay – loam with moderate sand soil type. Among the plant parasitic nematodes which were frequently encountered around rhizospheric roots of mulberry plants in different soil types, *Helicotylenchus graminophilus* was the most abundant and frequently encountered nematode while *Boleodorus cylindricus* was least abundant though encountered in all the soil types. *Meloidogyne javanica* was found in sandy-loam type of soil. The nematode had potential threat to health and growth of the host plant.

The variation in the nematode population in the four mulberry farms may be due to topographical variations, variations in rainfall, humidity and degree of cultivation accompanied by ploughing frequency and application of fertilizers. An extensive study by incorporating all these parameters could reveal more profound results. In order to adopt Integrated Management System of nematodes in this host plants, plantation of antagonistic plants like *Tagestes* spp., *Eucalyptus* spp. in and around the farming areas could be adopted with application of soil organic amendments like vermicompost, poultry manure, mustard oil cake, neem cake, pongamia cake, castor cake, groundnut cake, saw dust etc. Spraying of botanical extracts of indigenous medicinal plants is also recommended to reduce harmful nematode population and maintain fertility of soil. Laboratory experiments have shown promising results while mass field experiments are needed.

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