

A SURVEY OF THE NEMATODE PROBLEM OF TOBACCO IN PAKISTAN: (A) NURSERIES

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Seventy nine nurseries of tobacco (*Nicotiana tabacum*, *N. rustica*) were surveyed for the presence of nematodes. Plant parasitic forms recovered were *Helicotylenchus indicus*, *Aphelenchus avenae*, *Paratylenchus* spp., *Meloidogyne* spp. *Helicotylenchus canadensis*, *Hoplolaimus californicus*, *H. indicus*, *Hopolaimus galeatus*, *Helicotylenchus mircolobus*, *Tylenchorhynchus acutus*, *Helicotylenchus pseudorbustus*, *Tylenchorhynchus nudus*, *Malenchus exiguus*, *T. acutoides*, *T. canalis*, *Pratylenchus pratensis*, *P. penetrans*, *Aphelenchoides sacchari*, *Pratylenchus coffeae*, *Scutellonema bradys* and *Rotylenchulus reniformis*. This order also shows the intensity of the occurrence of nematodes. With regards to the frequency of occurrence, *Aphelenchus avenae* was the most frequently encountered species followed by *Helicotylenchus indicus*. The least frequent nematode species was *Rotylenchulus reniformis*. Stunting, unthrifty growth and yellowing of seedlings were the above ground disease symptoms. *Helicotylenchus indicus* and *Pratylenchus* spp. are considered to be important pest of tobacco nurseries in the study area. Occurrence of root-knot nematodes only in low number has been believed to be due to the extreme cold conditions of the months during which nursery seedlings are raised. Free-living nematodes found during the survey have also been listed.

INTRODUCTION

Tobacco (*Nicotiana tabacum*; *N. rustica*) constitutes one of the most important commercial crops of Pakistan. Although it is cultivated in all provinces of Pakistan, over 90% of the total 49869 hectares of cultivation is done in two provinces, namely, the North West Frontier Province (NWFP) and the Punjab. In the NWFP, it is cultivated in the districts/agencies of Peshawar, Mardan, Kohat, Kurram, Khyber, Mohmand, Orakzai, Abbotabad, Mansehra, Kohistan, Malakand, Swat, Dir, Chitral, Bajour, Bannu, D.I. Khan, and NW/SW agency. In the province of Punjab, the principal areas of cultivation are in the districts of Attock, Rawalpindi, Jhelum, Gujrat, Sargodha, Faisalabad, Jhang, Mianwali, Sialkot, Gujranwala, Sheikhpura, Lahore, Kasur, Sahiwal, Vehari, Multan, Muzaffargarh, D.G. Khan, Bahawalpur, Bahawalnager, and Rahim Yar Khan. Tobacco is a good source of earning for small to big farmers and a lucrative export item for the Government and traders.

In Pakistan, tobacco seedlings are raised in November-December and transplantation is done in the months of February and March. While the pest problem is taken care of in the standing crops, hardly any measure is taken to protect the nursery stock in the beds. Since nematodes are disseminated through planting stock and a small number of them may cause significant injury to a seedling, protec-

tion of plants right from the beginning, i.e. the nursery stage, is important. During this period, which constitutes the *Critical Growth Period* of the plant, the protection provided results in the healthy establishment of plants and vigorous yield at a later stage. While there are some reports of nematodes associated with standing crops, no record exists of nematodes found from tobacco nurseries. Hence a survey of tobacco nurseries was undertaken with a view to study the fauna and the transfer of nematodes to the fields.

MATERIALS AND METHODS

Soil samples together with intact seedlings were collected by making cores in the nursery beds. On arrival in the laboratory, they were transferred to plastic pots. Nematodes were isolated by improved Baermann's funnel method. Nematodes isolated from the funnel after 48 hours were relaxed by gentle heat and fixed in 4% formalin. Slides were made from freshly relaxed specimens. After having been studied, the nematodes were preserved and stored in vials. Roots were chopped finely, passed through a coarse sieve and placed over tissue papers for overnight. Since the larvae of some species of *Meloidogyne* may not extract rapidly, bioassays were performed by transferring the samples to the plastic pots for 8 weeks. After this the

plants were checked for the presence of galls on the roots. Seventy-nine nurseries in the following areas were surveyed: (1) Mangha, (2) Wardega, (3) Ameen Khan Pul, (4) Amanat Kot in or near Charsaddah, (5) Village Mahboob Khan in Takht Bai (6) Adeena, (7) Kalu Khan, (8) Fazalabad, (9) Maneri, (10) Shah Mansoor, (11) Amber, (12) Sheikh killi, (13) Foujoon, (14) Lahor, (15) Yar Hussain, (16) Enayatabad, (17) Banda Piran, (18) Buffa Doraha around Mansehra, (19) Inayat killi in Bajour area, (20) PTB Station, Khan Garhi, (21) PTB Station, Mansehra in NWFP and (22) Depalpur, (23) 53 Tubewell area round Okara, (24) Gujrat area, (25) Mandi Bahauddin area, (26) PTB Station, Okara in the Punjab.

RESULTS

An overview of nematode occurrence. The highest total number of nematode species was found in Gujrat area where 11 species of parasitic and seven of non-parasitic nematodes were recovered. This was followed by Mansehra where six parasitic and seven non-parasitic species were found and Yar Hussain which yielded four parasitic and seven non-parasitic species (Table 1). The minimum of two species was recovered in Maneri, Shah Mansoor, 53 Tubewell area, and Amber,

Intensity of occurrence of plant parasitic nematodes. *Helicotylenchus indicus* was found in the highest number, as 97 nematodes per 100 ml. soil were recovered. It was followed by *Aphelenchus avenae* (12-93/100 ml soil), and *Paratylenchus* spp. (14-73); *Pratylenchus* spp. were found in low numbers. The population range of various nematodes is given in Table 2.

Frequency of occurrence of parasitic nematodes *Aphelenchus avenae* was the most frequently encountered nematode species which was found in 51.8% of samples analysed. This was followed by *Helicotylenchus indicus* (40.5%) and *Tylenchorhynchus acutus* (29.1%). The least frequent species was *Rotylenchulus reniformis* which was found in only six out of 79 samples. The frequency of occurrence of other nematodes is given in Table 1.

Aboveground visible symptoms of injury. Stunting, unthrifty growth and yellowing of the seedlings were the aboveground disease symptoms. Sometimes seedlings in the whole bed were found affected while in some cases the effect was noticeable only in patches. In most of the cases, the aboveground disease symptoms could not be correlated with the presence of nematodes. It was only in some areas such as Charsaddah (Fig.1), Bajour (Fig.2), PTB Station of Mansehra (Fig.3), and PTB Station of

Table 1. Species of parasitic nematodes recovered from tobacco nurseries in the N.W.F.P. and Punjab

Nematode	Number of positive samples	Frequency of occurrence (%)	Occurrence* sites
<i>Aphelenchus avenae</i>	41	51.8	3, 4, 5, 8, 13, 14, 24, 26
<i>Helicotylenchus indicus</i>	32	40.5	1, 2, 19, 20, 21, 23
<i>Tylenchorhynchus acutus</i>	23	29.1	14, 24, 25
<i>Helicotylenchus microlobus</i>	20	25.3	26
<i>H. pseudorobustus</i>	20	25.3	1, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 17, 18, 19, 24
<i>Tylenchorhynchus acutooides</i>	19	24.0	17, 21
<i>Basirrolaimus indicus</i> [1]	18	22.7	1, 17, 18, 19, 20, 21, 22, 23, 24
<i>H. galeatus</i>	17	21.5	1, 2, 3, 4, 5, 14, 16
<i>H. californicus</i>	15	18.9	15
<i>Paratylenchus</i> spp.	15	18.9	1, 3, 4, 5, 6, 7, 8
<i>Pratylenchus coffeae</i>	15	18.9	24
<i>P. penetrans</i>	15	18.9	1, 3, 13, 15, 24
<i>Malenchus exiguus</i>	15	18.9	13
<i>Helicotylenchus canadensis</i>	14	17.7	3, 14, 24
<i>Tylenchorhynchus T. nudus</i> ^{canalis}	14	17.7	24
<i>T. nudus</i> ^{canalis}	13	16.4	12, 15
<i>Aphelenchoides sacchari</i>	11	13.9	18
<i>Pratylenchus pratensis</i>	8	10.1	4, 15, 16, 19
<i>Scutellonema bradys</i>	7	8.8	21, 24
<i>Meloidogyne</i> spp.	7	8.8	13, 14, 24
<i>Rotylenchus reniformis</i>	6	7.5	24

*Listed in Materials and Methods

Gujrat (Fig.4) that well-defined disease symptoms were observed.

Underground symptoms of disease. Except Foujoon and Lahore in the NWFP and Okara in Punjab where galls

Table 2. Intensity of the occurrence of parasitic nematodes in the soil

Nematode	Population range (nematodes/100 ml soil)
1. <i>Helicotylenchus indicus</i>	6 – 97
2. <i>Aphelenchus avenae</i>	12 – 95
3. <i>Paratylenchus</i> spp.	14 – 73
4. <i>Meloidogyne</i> spp.	5 – 51
5. <i>Helicotylenchus canadensis</i>	5 – 43
6. <i>Hoplolaimus californicus</i>	4 – 43
7. <i>Hoplolaimus indicus</i>	4 – 26
8. <i>H. galeatus</i>	4 – 25
9. <i>Basirolaimus indicus</i> [1]	3 – 39
10. <i>Tylenchorhynchus acutus</i>	3 – 31
11. <i>Helicotylenchus pseudorobustus</i>	5 – 28
12. <i>Tylenchorhynchus nudus</i>	5 – 26
13. <i>Malenchus exiguus</i>	14 – 23
14. <i>T. acutoides</i>	4 – 20
15. <i>T. canalis</i>	5 – 20
16. <i>Pratylenchus pratensis</i>	6 – 14
17. <i>P. penetrans</i>	6 – 11
18. <i>Aphelenchoides sacchari</i>	4 – 9
19. <i>Pratylenchus coffeae</i>	3 – 9
20. <i>Scutellonema bradys</i>	3 – 7
21. <i>Rotylenchulus reniformis</i>	3 – 4

Table 3. Species of free-dwelling nematodes recovered from survey sites

Nematode species	Occurrence sites
<i>Discolaimus major</i>	2, 15, 21, 22, 23, 24, 25, 26
<i>Chiloplacus</i> spp.	14, 21, 24
<i>Eudorylaimus subjunctus</i>	2, 15, 19, 20, 21, 22, 24, 25
<i>E. varians</i>	15, 24, 26
<i>E. major</i>	26
<i>Rhabditis longicaudatus</i>	15, 21, 24
<i>R. terricola</i>	2, 3, 13, 15, 16, 17, 18, 21, 24
<i>Mononchus papillatus</i>	15, 21
<i>Mylonchulus rosensis</i>	21
<i>Aporcelaimellus</i> spp.	13, 15
<i>A. obscurus</i>	3, 14, 25

produced by root-knot nematodes, and Charsaddah (NWFP) where lesions produced by *Pratylenchus* spp. were found, there were no symptoms of disease on roots in the samples collected.

Free-living nematodes. *Rhabditis* (*R. longicaudatus* and *R. terricola*) was the most frequently encountered free-dwelling nematode genus as it was recovered from 16 out of



Fig. 1. A nursery bed in Charsaddah area showing unthrifty growth of seedlings.



Fig. 2. A nursery bed in Bajor from where lesion and spiral nematode were recovered.

79 samples. It was followed by *Eudorylaimus* (*E. subjunctus*, *E. varians*) which was found in 10% samples. The least common free-living nematode was *Mylonchulus rozensis* which was found only in a solitary sample. (Table 3)

DISCUSSION

Results indicate that a fairly large number of nematode species are associated with tobacco in the area under study.



Fig. 3. A nursery bed in PTB Research Sub-Station, Mansehra from where *Hoplolaimus indicus*, *Scutellonema bradeys* and *Aphelenchoides sacchari*, were recovered.



Fig. 4. PTB sub station, Gujrat which yielded *Tylenchorhynchus canalis*, *Hoplolaimus indicus* and *Helicotylenchus pseudorobustus*.

Both quantitatively and qualitatively large numbers of nematodes were found in Gujrat (Punjab), Mansehra and Yar Hussain in the district Swabi of NWFP.

Occurrence of *Helicotylenchus indicus* in higher numbers is quite understandable as these nematodes are capable of surviving in varied climates and habitats. Though *Pratylenchus* spp. were found in low numbers, in view of their established virulence, they may be considered as serious pests of tobacco in this area. Furthermore, these nematodes are also endoparasitic in nature which enables them to spread to different areas even if marginal precautions of checking dissemination such as simple washing of the roots are taken.

Root-knot nematodes which are among the most serious pests of tobacco were encountered only in few cases (8.8%). This is probably because of the time of raising nursery beds. In this area, nurseries are raised in November to February, the months that are characterised by extreme cold conditions which are not suitable for development of these nematodes, especially the tropical species - *M. incognita* and *M. javanica*. However, the root-knot nematode (*Meloidogyne javanica*) were recovered frequently from samples which were transferred to plastic pots and allowed to grow for 8 weeks. This explains why root-knot nematodes are frequently found in standing crop stage and not in the nursery beds.

No work has previously been done on nematode infestation of tobacco nurseries in Pakistan. Some reports which are available deal only with nematodes of standing crops. Brown [2] was the first worker to have reported nematodes *Heterodera tabacum* and *Pratylenchus* spp. from Peshawar. In our survey, we failed to find any species of *Heterodera* but *Pratylenchus* spp. were found. This may be attributed to the age of the plants (seedlings in our case, grown-up plants in other case), and the time of sampling (February in our case, June in Brown's case). Mohibullah *et al* [3] have mentioned root-knot nematodes affecting tobacco cultivation in the NWFP. However, they have neither given any information about the nematode species nor about the age of plants. Maqbool [4] has reported 15 species of plant parasitic nematodes associated with tobacco in NWFP and Punjab. He also reported *Globodera tabacum* (syn. *Heterodera tabacum*). Again this is obviously due to the age of the plant and the time of sampling. Maqbool failed to recover *Scutellonema*, *Rotylenchus*, *Aphelenchoides* and *Paratylenchus* spp. However, except for the last named, other nematodes are only of academic interest as far as tobacco is concerned. In view of the recovery of several important parasitic nematodes from nursery beds, it may be concluded that both preventive

and curative measures should be taken to check the loss caused to the seedlings. This would help in protecting the plants during their *critical growth period* which would help in their healthy establishment and resultant vigour. It would also check dissemination of nematodes from one place to another. A more detailed account will be presented on this aspect in the second part of this paper which will deal with the nematodes of standing crops.

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FIG. 1. *Caenorhabditis*



FIG. 1. *Caenorhabditis*

Plants which occur in this area, was collected at Manora, Sandhor and Gulsh in January

The internal structure shows a central cell 16-18 µm in dia surrounded by a ring of 8-7 large angular cells. 22-38 µm in dia. (Fig. 2)

Plant which occurs in this area, was collected at Manora, Sandhor and Gulsh in January

INTRODUCTION

Karachi has a very humid marine climate. The marine plants have attracted the attention of phylogenetic and biologists because of their economic value as compared to land plants. seaweeds are composed of a better variety of carbohydrates, protein, fat, vitamins and minerals as investigated by Flax [1], Dean et al [2], Post and Turner [3], Lee [4], Whitlock and Rupp [5], Loring et al [6], Chapman [7], Jansen [8] and Farook et al [10].

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DESCRIPTION OF SPECIES

Caenorhabditis [11, 12]. Plant species commonly identified into prostrate branched horizontal and erect caespitose or with terminal horizontal and erect caespitose, branched, upto 1.2 mm broad, with much branched thallose. Axillillators erect and flat, lanceolate, up to 12 cm long and upto 5 mm broad, terminal laterally compressed, opposite, sickle-shaped, apex mucronate, contracted at the base.