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Systematic Surveys of Root-Knot Nematodes from Rice and Soybean Fields of Pakistan

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Abstract: ***Root-knot nematodes (RKN) are economically important pests of a wide range of crops worldwide and several RKN species are known to cause widespread damage to crops. In Pakistan also these nematode play havoc to agricultural crops and cause significant losses amounting billion of rupees annually. To appraise the coalition of root-knot nematodes, the incidence, distribution and percentage occurrence of RKN in rice and soybean plantations were undertaken by commencing detailed and systematic surveys of these crops from different localities of Pakistan. For this purpose a total of 2100 root and soil samples (1500 samples of rice and 600 samples of soybean) were collected from 41 localities across the country (except Balochistan) were undertaken during 2005-2008. The detailed soil analysis showed that two species of root-knot nematodes Meloidogyne incognita and M.javanica (Treub, 1885) Chitwood, 1949 from rice and three RKN species M. incognita, M. javanica and M.hapla Chitwood, 1949 were more frequently recovered from soybean study areas. The overall occurrence of root-knot nematodes was 33.3 % in rice and 67.1% in soybean crops from Pakistan during three years. Locality and host wise overall percentage occurrence of RKN during three years study was 29.3 % %)in Punjab and 36% in Sindh %) from rice plantations; whereas from soybean cultivated areas, the percentage occurrence of root-knot nematodes was 75.4% from Khyber Pakhtunkhwa (KP), 66.6% from Punjab and 59.06% from Sindh.***

Keywords: Root-knot nematode, *Glycine max*, *oriza sativa*, survey and Pakistan

1. Introduction

In Pakistan nematode problem has been recognized as one of the great threats to the agriculture production. Being an agriculture base country, it is extremely important that agriculture sector performs its proper role for substance of national economy. Effective improvement in the agricultural productivity depends on appropriate crop protection from nematodes and other pest diseases.

The root-knot nematodes are the most important factor in reducing agriculture yield of cereals, legumes and vegetables [1]. The root-knot nematodes (Meloidogyne spp) constitute the most widely distributed group among the plant parasitic nematodes. Out of 90 valid species of the world, four Meloidogyne species namely *M.incognita*, *M.javanica*, *M.hapla* and *M.arenaria* are prevalent in Pakistan [2] while *M.graminicola* was reported only from rice from Sheikhupura, Punjab, Pakistan [3]. According to Bridge et al., 2005 following four species of Meloidogyne occur only on upland and hydromorphic rice. While Coyne et al., 1999 and Lopez, 1984 recovered *M.javanica* and *M. arenaria* from rice field, respectively. In Pakistan various researchers reported root-knot nematodes on soybean [4], [5]-[6], [7] and [8].

Rice, an important food and cash crop, is the third largest crop of Pakistan after wheat and cotton. It is planted on an area of over 2.5 million ha (11 % of the total cropped area) and accounts for 17 % of the total cereals produced annually. Soybean (Glycine max) is a globally important oilseed crop (17-24 %) and source of high quality protein (37-42 %) [9] and [10]. In Pakistan, soybean has suffered a setback and has therefore, not been able to attain a respectable position among the oilseed crops. Its cultivation remained limited to a very small acreage and showed a declining trend.in 2005-06 the area of cultivation was 373 thousands/hactares where as in 2009-10 ity declined to 55 thousands/hactares. Its production was 42 tons in 2005-06 and 32 tons in 2009-10 [11]. Biodiversity data of nematode fauna of economically importance crops, including rice, in Pakistan was given by [1]. Infestation of *M.graminicola* and *M.incognita* on rice from Punjab was reported by [3] and [7], respectively. Musarrat et al., (2006) reported *M.incognita* from rice growing area of Sindh and KPK, Pakistan. In Pakistan various researchers reported RKN on soybean [4], [5-6], [7] and [8].

The aim of this study was to determine the coalition of root-knot nematodes, the incidence, distribution and percentage occurrence of RKN in rice and soybean plantations were undertaken by commencing detailed and systematic surveys of these crops from different localities of Pakistan. Detailed analysis revealed that the overall occurrence of root knot nematodes was 67.1 % in soybean as compared to rice 33.3 %.

2. Material and Methods

Survey: Extensive surveys were carried out during the research studies of rice and soybean cultivated areas of Pakistan at different time periods. A total of 277 randomly chosen fields from 41 locations (26 localities of rice fields and 15 localities of soybean fields) were visited and composite soil and root samples were taken from the rhizospheric region of crop plants on each field according to [12].

Screening of soil and root samples for Meloidogyne species: Two different methods were used to determine the

presence of RKN on the field sampled. In the first method both crop and weed roots were examined directly on the field for the presence of root galls. The root-knot infected samples were denoted by a positive (+) sign while those that were not infected were denoted by a negative (-) sign. Direct examination of plant roots for the presence of characteristic root galls is a relatively faster way of detecting root-knot nematodes. The second method involved bioassay of the collected soil samples in the laboratory by rising on susceptible plants i.e., tomato or egg plant. The bioassay method was used to detect low numbers of root- knot nematodes.

Extraction of root-knot nematodes: Infested and uninfected roots of soybean and rice plants were collected and cut into 1-2 cm segments. Short pieces of the roots were placed in a Petri dish containing water and observed under the dissecting microscope. Females were picked by the help of a dropper and were transferred to a glass cavity block for identification. At least 10 perennial patterns of mature females of RKN were prepared for identification [13].

3. Data Analysis

[1] The severity of the infection was determined by estimating the number of galls. The infected samples were denoted by a positive (+) sign while those not infected were denoted by a negative (-) sign.

[2] The percentage incidences, frequency of distribution for each of the tested areas after bioassay and population intensity of RKN in rice and soybean fields of Pakistan were analyzed.

4. Results

Distribution and Percentage Occurrence of Root-Knot Nematodes from Different provinces of Pakistan during 2005-2008

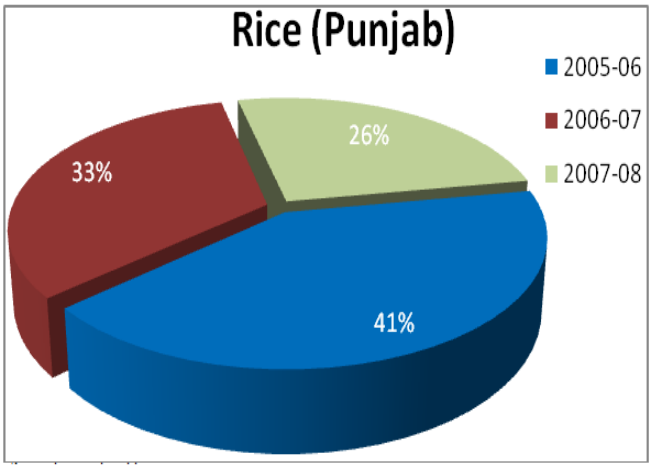
A total of 1500 samples of rice from 26 localities and 600 samples of soybean from 15 localities were collected all over the country (Table 1.1). The detailed analysis about the overall occurrence RKN showed that they were found in 500 samples (33.3 %) from rice and 403 samples (67.1 %) from soybean.

During three year surveys (2005-2008) of rice cultivated zones of Pakistan out of a total 1500 samples, 600 samples were collected from Punjab in which 176 samples were found positive RKN. The percentage occurrence of root-knot nematodes was found to be 29.3 % from Punjab. 900 samples were collected from rice irrigated areas of Sindh. 324 samples showed the presence of root-knot nematode with 36 % of occurrence. Most of the nearby fields were found infected because mostly farmers cultivated same rice cultivar in adjacent fields (Table not included; Fig. 1.1 A-B).

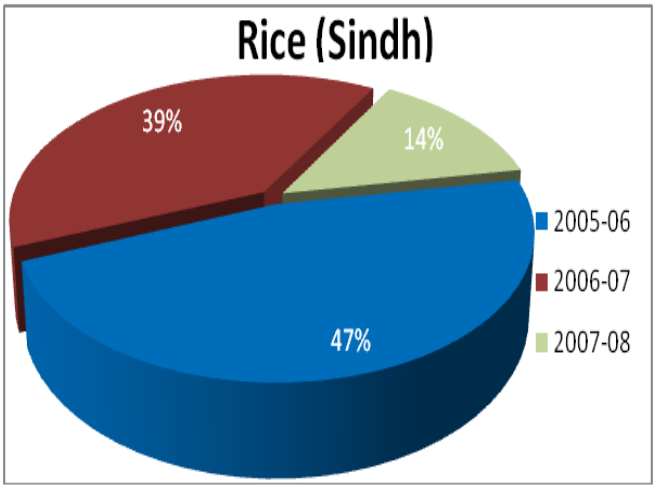
Table 1.1: Surveyed localities of rice and soybean of Pakistan**.**

S.#	*Localities of rice*	*Latitude*	*Longitude*	*S.#*	*Localities of soybean*	*Latitude*	*Longitude*
1	Rahimyar Khan	28°30`N	70°25`E	1	Multan	30°15`N	71°36`E
2	Sialkot	32°30`N	74°31`E	2	Vehari	29°15`N	71°30`E
3	Hafizabad	32°05`N	73°40`E	3	Faisalabad	31°25`N	73°09`E
4	Sialkot	32°15`N	74°52`E	4	Rahimyar Khan	28°30`N	70°25`E
5	Gujranwala	32°10`N	74°12`E	5	Hyderabad	25°23`N	68°24`E
6	Narowal	32°06`N	74°52`E	6	Badin	24°38`N	68°54`E
7	Jhang	31°15`N	74°22`E	7	Sangharh	26°20`N	68°57`E
8	Sheikhupura	30°32`N	71°80`E	8	Thatta	33°35`N	74°14`E
9	Sahiwal	30°45`N	73°80`E	9	Hazara	33°59`N	72°56`E
10	Okara	30°50`N	73°31`E	10	Swat	34°40`N	72°52`E
11	Vehari	29°15`N	71°30`E	11	Dir	35°12`N	71°53`E
12	Kasur	31°07`N	74°27`E	12	Kurram Agency	34°40`N	71°55`E
13	Pakpattan	31°21`N	73°24`E	13	Mansehra	34°25`N	71°50`E
14	Faisalabad	31°25`N	73°09`E	14	Malakand Agency	34°40`N	71°55`E
15	Nawabshah	26°15`N	68°25`E	15	Abbottabad	34°26`N	71°52`E
16	Sukkur	28°55`N	68°55`E				
17	Khairpur	27°06`N	87°44`E				
18	Sangharh	26°20`N	68°57`E				
19	Hyderabad	25°23`N	68°24`E				
20	Badin	24°38`N	68°54`E				
21	Thatta	33°35`N	74°14`E				
22	Dadu	26°06`N	67°45`E				
23	Jaccobabad	28°17`N	68°26`E				
24	Larkana	27°32`N	68°18`E				
25	Nasirabad	27°32`N	69°18`E				
26	Gharo	24°44`N	67°35`E				

Surveys of soybean cultivated areas resulted in the collection of 600 samples. From Khyber Pakhtunkhwa (KPK), where soybean is cultivated on the larger area, 220 samples were collected and 166 samples (75.4 %) were found positive for root-knot nematodes. 165 samples were collected and 110 (66.6 %) samples were positive from Punjab. In Sindh, the percentage occurrence of RKN was 59.06 % in 127 positive samples out of a total of 215 samples which show decline in percentage as compare to both above provinces (Table not included**; Fig.ure 1: 2** A-C).

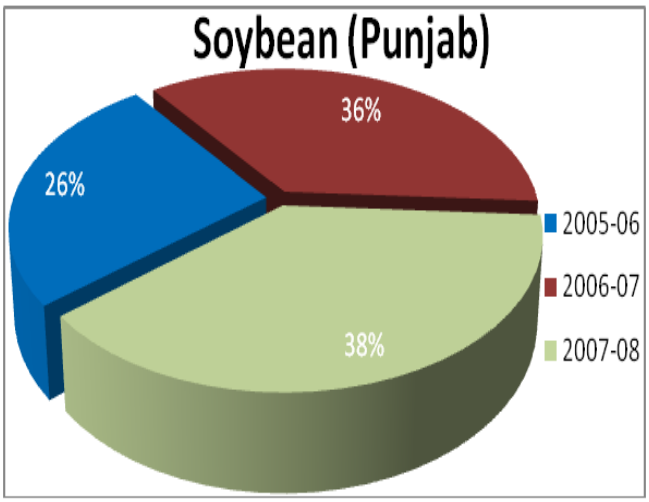


A

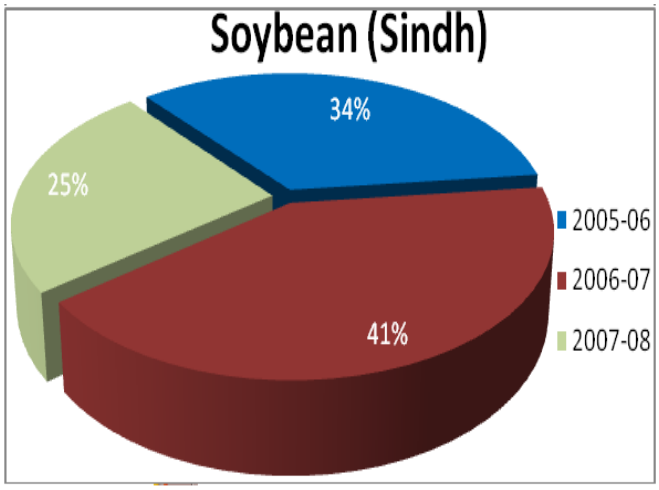


B

Figure 1.1: (A-B). Percentages occurrence of root-knot nematodes from rice cultivated areas of Pakistan; A. Punjab; B. Sindh



A



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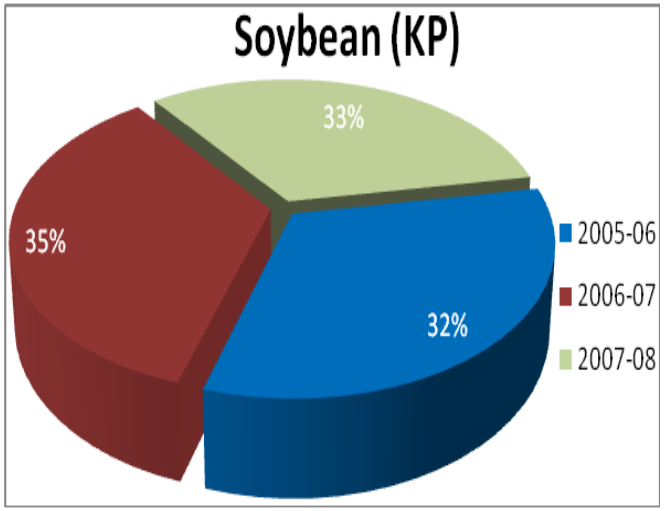


C

Figure 1.2: (A-C). Percentages occurrence of root-knot nematodes from soybean cultivated areas of Pakistan; A. Punjab; B. Sindh; C. Khyber Pakhtunkhwa (KPK)

5. Incidence of Root-Knot Nematodes on Rice and Soybean Localities

Direct examination of roots for root galls / knots indicates presence of root-knot nematodes (RKN) in 68.2 % of the total samples while the bioassay results showed infections from an additional 31.7 % of the samples (Table 1.2). The total percentage incidence of RKN was determined to be 31.7 % taking into account both the incidence recorded via direct examination and bioassay of soil samples from all the 41 different localities of 277 fields of rice and soybean

The sampling and diagnosis method used in the current study includes a more systematic sampling strategy and thorough analysis using direct examination of plant roots and bioassay of soil samples. Direct examination of plant roots for the presence of characteristic root galls is a relatively faster way of detecting RKN and can be concluded by fields.

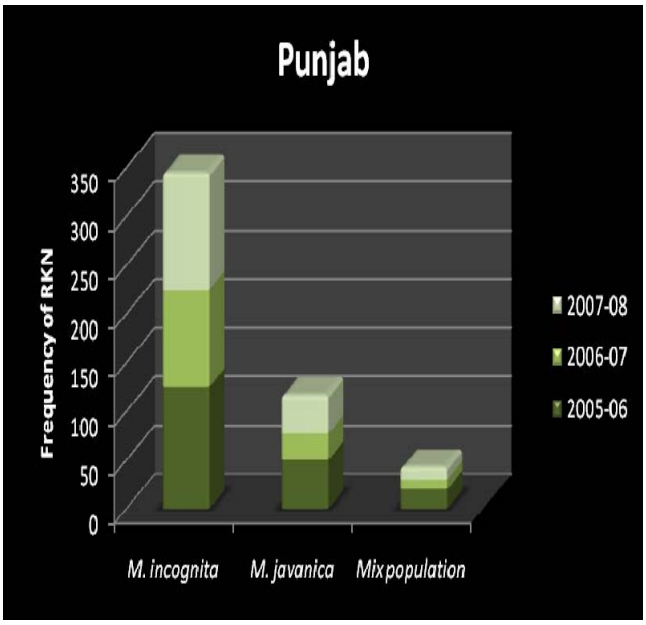
Table 1.2: Incidence of root-knot nematodes from rice and soybean localities

No. of fields sampled (A)	*Direct examination of plant roots (B)*	*Total incidence after bioassay (C)*	*D/F in incidence (C-B)*	*% incidence (C/A) × 100*
48	28	15	13	31.2
39	19	13	06	33.3
32	14	10	04	31.2
20	21	08	13	40.0
18	09	10	01	53.5
20	14	06	08	30.0
36	25	07	18	13.4
14	06	07	01	50.0
25	27	08	19	52.0
25	26	04	22	16.0
277	189	88	105	31.7

6. Percentage Occurrence of Root-Knot Nematode (Meloidogyne) Species in Pakistan

Out of 1500 samples of rice, over all percentage of *M. incognita* was 46.3 % while *M.javanica* was recovered in 9.1 %. Whereas in 39.7% samples mixed population of *M. incognita* and *M. javanica* was encountered. Prevalence of *M. incognita* was 52.5 % out of 600 samples of soybean, *M. javanica* 11.6 % and *M. hapla* was recovered in 20 % whereas *M. graminicola* has not encountered during the present study (Fig. 1.3 - 1.4). Province wise percentage occurrence of *M. incognita* was 31.3% from Punjab and 33.7% from Sindh whereas *M. javanica* was found with 21.3% from Punjab and 25% from Sindh. In case of soybean the highest percentage of occurrence of *M. incognita* was 80% from KPK followed by 72.7% from Sindh and 68% from Punjab. Whereas *M. javanica* was found with 62.5% in Punjab and 44.7% in Sindh, while the least percentage occurrence of *M. hapla* was 20% from KPK only.

A combination of factors is likely to have contributed towards the change in distribution of *Meloidogyne* spp., in Pakistan. Over a period of time, movement of plant materials from root-knot infected fields could have led to spread of *Meloidogyne* spp., to new areas. Natural causes such as flooding and soil erosion also assist in the spread of root-knot nematodes.



A

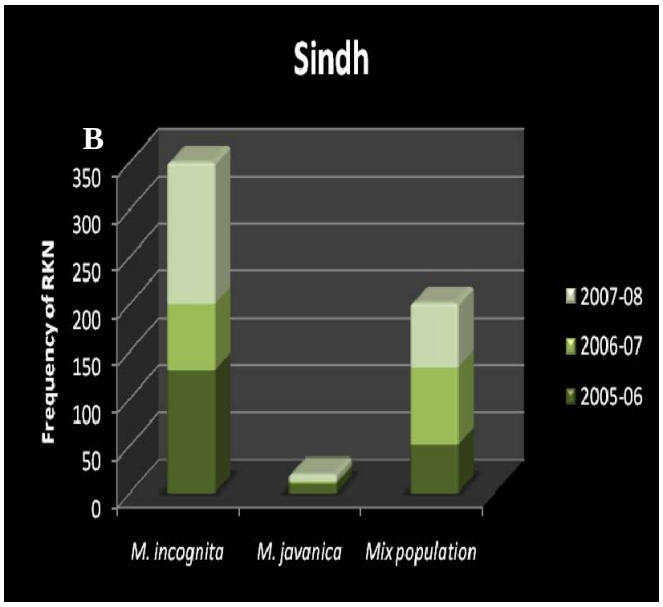


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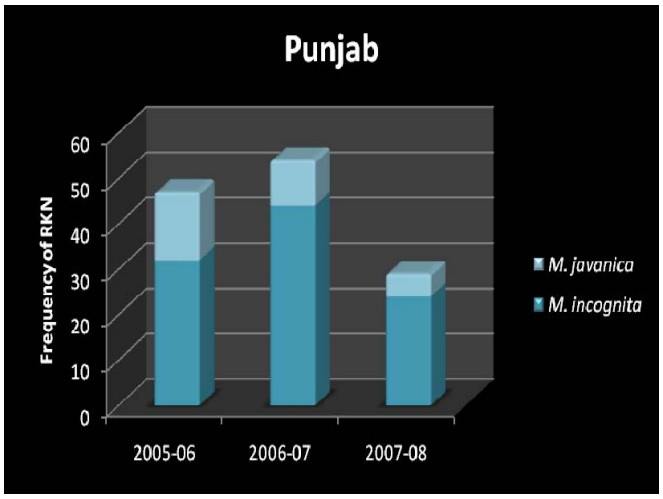
Figure 1.3: (A-B). % occurrence of root-knot nematode species in rice cultivated areas during 2005-2008. A. Punjab; B. Sindh, Pakistan



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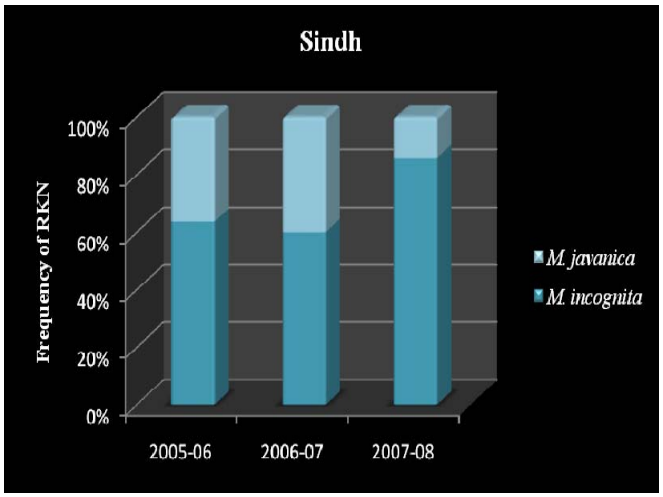

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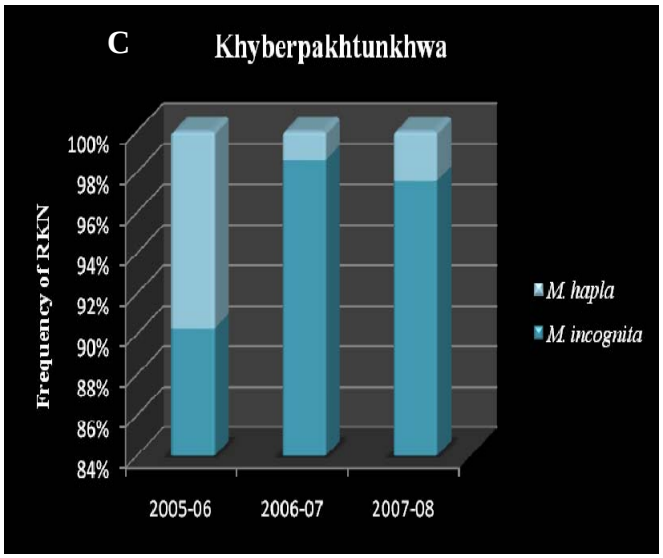

Figure 1.4: (A-C). % occurrence of root-knot nematode species in soybean cultivated areas during 2005-2008. A.Punjab; B. Sindh; C. KP, Pakistan

Table 1.3: Distribution, % occurrence and intensity of root-knot nematode species in rice fields of Pakistan

Locality	*No. of samples collected*	*No. of positive samples*	*Occurrence % of RKN*	*RK I on root system*	*Meloidogyne species and sub species*
Punjab					
Faisalabad	45	10	22.2	< 25	*M. incognita*
Gujranwala	55	28	50.9	26-50	*M. incognita*
Hafizabad	22	3	13.6	1-10	*M. javanica*
Jhang	40	18	45	50-75	*M. i and M. j*
Kasur	65	13	20	< 25	*M. javanica*
Narowal	35	5	14.2	1-25	*M. incognita*
Okara	30	9	30.0	< 25	*M. javanica*
Pakpattan	10	2	20	< 25	*M. incognita*
Rahimyar	33	-	-	-	
Sahiwal	50	12	24	< 25	*M. incognita*
Sheikhupur	150	51	34.0	26-50	*M. incognita*
Sialkot	46	25	54.3	>75	*M. i and M. j*
Vehari	19	-	-	-	-
Sindh					
Badin	50	30	60	50-75	*M. i and M. j*
Dadu	60	30	50	26-50	*M. i and M. j*
Gharo	30	2	6.6	1-10	*M. i and M. j*
Hyderabad	10	3	30.0	<25	*M. javanica*
Jaccobabad	50	21	42.0	26-50	*M. incognita*
Khairpur	10	-	-	-	-
Larkana	300	109	36.33	< 25	*M. i and M. j*
Nasirabad	200	44	22.0	1-10	*M. incognita*
Nawabshah	25	8	32.0	< 25	*M. incognita*
Sanghar	10	-	-	-	-
Shikarpur	70	30	42.8	26-50	*M. i and M. j*
Sukkur	10	2	20	1-10	*M. javanica*
Thatta	75	45	60.0	26-50	*M. incognita*

7. Distribution and Percentage Occurrence of RKN Species in the Surveyed Areas

Detail analysis of the samples, collected from rice and soybean fields, revealed the presence of RKN. These nematodes were identified on the basis of RKN taxonomy (including perineal pattern and morphology of juvenile and male) [14] and [15]. Two species of root-knot nematodes viz., *M. incognita* and *M. javanica* were more frequently recovered from rice and soybean studied areas. From seven localities of rice viz., Sialkot, Jhang (Punjab), Badin, Dadu, Larkana, Shikarpur and Gharo (Sindh) mixed population of two species viz., *M. incognita* and *M. javanica* were encountered. Ten rice localities viz., Gujranwala, Sheikhupura, Narowal, Pakpattan, Sahiwal, Faisalabad (Punjab), Thatta, Jaccobabad, Nawabshah and Nasirabad (Sindh) reproduced only one species *M. incognita*. Whereas from five regions viz., Okara, Kasur, Hafizabad (Punjab), Hyderabad and Sukkur (Sindh) only one species *M. javanica* was isolated. However, from four regions of rice viz., Vehari, Rahimyar Khan (Punjab), Sangharh and Khairpur (Sindh) no RKN species was recovered (Table 1.3).

In the present study root-knot nematode was found infecting both rice and soybean plants, however, the root-knot disease intensity was found low in rice than soybean plantations. Because of stagnant water root-knot nematodes did not produce at high rate of recurrence in rice plantations. In this study out of 26 localities, Sialkot showed the highest disease incidence of root-knot nematodes (>75) on rice root systems followed by two locations viz., Jhung (Punjab) and Badin (Sindh) who shared the same RKI (50-75). However, low index of root-knot was recorded in rest of the localities. During the survey *M. incognita* was found in the highest frequency in rice fields of Badin and Thatta having the same frequency (60 %) in Sindh, while Sialkot (54.3 %) and Gujranwala (50 %) of Punjab showed lower frequency than Sindh (Table 1.3).

Among root-knot nematodes *M. incognita* had been ranked first with respect to geographical distribution and host range. The rate of infection of *M. incognita* has increased as compared to other root-knot species on soybean in Khyber Pakhtunkhwa (KP). However, [6] reported the wide spread occurrence of root-knot species *M. javanica* in a survey of NWFP. So we can say after 16-17 years *M. incognita* has

dominated in KP including soybean crop plants as compared to rice crop according to the scale used by [16].

8. Discussion

During this research many nematodes were encountered from rice and soybean plants, but very little information is available on the importance of these host associated nematodes [6], [1],[17] and [2]. *Meloidogyne* is the most important nematode genus that may cause damage in the production of both crops in the country. As observed the fields of soybean were found damaged by *Meloidogyne* spp., with heavy galling on the roots. While in rice fields less infestation of RKN was found. The reports are similar to those reported earlier for soybean and rice [18] and [19].

In the present study varying degree of frequency of root-knot nematodes in rice and soybean crops was recorded from different localities of Pakistan. In soybean crop variation in incidence may be due to many factors, which contribute to the infection and reproduction of the nematodes in field [20] , [21], as well as climate variation at the localities [22],[23], type of soil [24], [25] and sequence of cropping [26], [27] while in rice field it may be due to the stagnant water in the field.

However, present findings are in confirmation of the results of previous surveys carried out by many researchers that worked on similar objectives viz., [28],[5]-[6], [29]-[33].

During the present study over all occurrence of root-knot nematodes (*M.incognita*) were found 33.3 % in rice and 67.1 % in soybean. These results are also in accordance with the following workers: [34]-[38]. [39] reported occurrence of root-knot (35 %) of the fields and can cause losses in upland rice. [40] reported that the root-knot nematode infect and establish in all the rice ecosystems and cause severe damage to deepwater rice in Asam. [41] observed that transplantation of infected seedlings may results in greater infection and damage to rice plants in the field. Yield losses associated with *M. incognita* on soybean were suppressed up to 90 % in Florida [42]-[44]. [45] and Maung and Jenkins (1959) noted the effect of root-knot nematodes on the yield in soybean. Sinclair and Backman (1989) reported the greatest yield losses of soybean by *M. incognita*. While 10-30 % yield losses was reported by the [46].

9. Future Scope or Recommendations

The present study provides important information to extension specialists, which can be used to create alertness among growers.

i. It should aware the plant scientists to consider nematodes major damaging pests of crops and start searching for resistant cultivars as an option in plant parasitic nematode management.
ii. Awareness campaigns about RKN disease launched on a large scale to disseminate the knowledge among the farmers or growers community.

1) Continue surveys with pathogenic races, e.g. of *M. incognita.*
2) More investigations into pathogenic capabilities and economic threshold infestation levels.
3) Extended nematology consultancies, both international and local level.
4) Emphasis should also be specified to the control of nematodes by biological means and the use of resistant cultivars.

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