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Incidence and New Record of *Aphelenchoides perietinus* (Bastian, 1865) Steiner, 1932 in Rice Fields of Punjab, Pakistan

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ABSTRACT

Surveys were conducted to document infestation of rice by plant parasitic nematodes especially emphasized on *Aphelenchoides* nematodes during 2016-2018 at harvest stage. Results indicate that incidence of *Aphelenchoides perietinus* in rice fields of Punjab was high in both years. Among the district surveyed, Lahore, Sheikhupura and Narowal were severely infested. The samples from these three districts showed 88.8%, 75% and 53.5% respectively. Moreover, resistance also evaluated by the symptoms of cultivated rice varieties. Among the tested cultivars on the bases of symptoms and incidence of *A.perietinus,* Lateefy, Dilrosh-92 and DR-8 showed high resistance. Moderate resistance was identified with Sugdasi Bengalo, IR-6 and Kanwal-95. While Shua -92, Sharshar, Sada hayat, Kangni-27, and Jajai-27 were susceptible to *A.perietinus* nematodes. Susceptible plant expresses varies symptoms such as shortening of plant, reduction of Tiller and grains per plant and low yield (100 seed weight). In few infested fields symptomless but infested plants were also found. In the present survey it is noted that *A.perietinus* was first time recorded from rice fields and first time evaluate resistance source in rice varieties against this nematodes. This survey also confirmed that this nematode is one of the most important and damaging pest which occurred in those fields where fungi infestation was measureable. Further the area infested with this nematode species are potential source for estimation of interaction between fungi and *A.perietinus* nematodes.

KEY WORDS: Survey, resistance, *Aphelenchoides* spp, rice varieties and infestation.

INTRODUCTION

Cultivated rice, *Oryza sativa* L., represents the world's most important staple food crop, feeding more than half of the human population (Lundo *et al*., 2006). Rice is an important food and cash crop, is the third largest crop of Pakistan after wheat and cotton. Pakistan is famous for growing and exporting long grain aromatic Basmati rice. The country ranked 14th in terms of rice production and 6th in rice export in the world.

Rice growing areas have different environments due to this in different countries and regions incidence of nematode disease was different because rice crops attacked by different nematode species. Generally, in irrigated rice, *Hirschmanniella* and *Aphelenchoides* species are frequently found. Nematodes not also causing direct crop loss, nematodes also cause indirect economic losses resulting from export/trade restrictions imposed due to the presence of quarantine nematode pests. Agreement of World Trade Organization revised their regulations and included several pests in the regulatory lists. Varaprasad *et al.*, 2006 reported that amongst main nematode diseases of rice, Ufra and white-tip causing nematodes find a place in regulatory pest lists of numerous countries.

Fungal feeding nematodes definitely affect the balance of endophytic fungi in host plant. Thus, in case of nematode disease incidence cannot depend on plant genotypes but also depend on as much or more on composition of endophyte species which production is enhance by *Aphelenchoides* nematodes (Raghavendra and Newcombe, 2013).

Throughout the world, *Aphelenchoides spp* is widely distributed and now occurs in mainly of the rice growing areas (Ou, 1985). However, nematode found in maximum density even a weekly parasitic species like *Aphelenchoides parietinus* might kill epidermal cells to cause a young luscious root a yellowish to brownish colour. It is external discoloration of the tissues.

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In Pakistan, very rare work have done on nematode disease of rice. Anwar and Khan (1973) first time reported *A. bessyi* nematodes from rice fields of Punjab, Pakistan. Khan *et al*., 1990 studied on seed born fungi, bacteria and nematodes of rice in the Punjab and found 13 seeds out of 170 were infected with white tip disease. Musarrat *et al*., 2015 conducted an extensive survey of rice growing areas of Pakistan except Balochistan and reported different plant parasitic nematodes from rice fields including *Aphelenchoides peritineous* and *A. bessyi*.

The main intentions of this study were construct data on population dynamics and assessing the prevalence and distribution of *A. peritinus* in rice growing localities of Punjab province. Different plant parasitic taxa were occurred but *A.paritinus* found in high density. In many fields pure population of this nematode was found. The species, *Aphelenchoides paritinus* was reported in Pakistan for the first time. While it was identified for the first time in association with rice throughout the world. Earlier, *A.paritinus* had been reported from different regions of world from rhizosphere of different host, the incidence and distribution of it in all rice cultivated areas is unknown. Therefore a survey of *Aphelenchoides paritinus* in Pakistan was undertaken and estimated its symptoms under field conditions, the extent of its distribution in some selected rice areas, the levels of infestation and its effects on yield components were investigated. The main aim of this research was to evaluate resistant rice cultivars and measure infestation of this nematode first time. The data of resistance variety can be useful to find source of resistance.

MATERIAL AND METHODS

Survey: Extensive surveys were taken throughout the consecutively two growing seasons of rice at harvest level. These rice growing areas of Punjab province of Pakistan can be divided in different zones such as Barani area, Mixed zone and Rice -Wheat zone. The main purpose of the sampling was to observe the incidence and prevalence of *A.parietinus* plant root feeder parasitic nematodes.
Survey area 25 x 25 m was marked off by plastic ropes and collected soil and root samples. For this purpose plant was uprooted and plant shoot was cut off. Roots with rhizosphere soil were placed in plastic bags and used for the extraction of root feeder nematodes. Estimated 390 randomly selected fields from 10 locations were surveyed and soil samples were taken from each field of rice.

Screening of soil and root samples for *Aphelenchoides* spp

The root samples were washed in soil suspension having bucket and thoroughly mixed. Extraction of plant parasitic nematodes from soil and rhizosphere of rice was made by Cobb's sieving and Baermann funnel techniques (Baermann, 1971 and Cobb, 1918, respectively).
The nematodes which had moved through the sieve into the water were collected after 24 hrs, and pour off excess amount of water finally 50 mL suspension was obtained, after that the suspensions were observed under a stereoscopic microscope ,juvenile and adult *Aphelenchoides parietinus* were counted. To estimate the susceptibility and resistance of the varieties, the prominence value (PV = nematode population density x √ (frequency of occurrence/10) of *A. parietinus* was measured (De Waele *et al*., 1998).

Quantitative and Qualitative analysis

Nematodes were counted in an open counting chamber by the pouring of 5 ml extracted nematode suspension and repeat three times. For qualitative analysis, concentrated water transferred into glass cavity block and nematodes were killed by pouring hot water. For qualitative analysis heat killed nematode suspension was placed by a dropper on a glass slides and cover with cover slip and sealed with transparent nail polish. Nematode populations were counted on this temporary slide under the compound microscope and identify the juvenile and adult nematodes of *Aphelenchoides* genus.

Identification of *Aphelenchoides* spp

Suspension of killed nematodes immediately fixed and preserved in a TAF solution for 24 hrs. After 24 hrs they were washed with distilled water 3 times. Excess amount of supernatant water were poured off and further processing into glycerine by a slow dehydration method for which specimens were place in a cavity block containing 2 ml of 1.25% glycerine solution and kept in an incubator at 40-55 C for 5-6 days. Processed nematodes were picked with hair needle and placed in a small drop of glycerine on the slide. Paraffin wax was placed as three small lumps around the drop then placed cover slip on the wax lumps. Slide was gently heated on hot plate to melt the wax and filled the space between the slide and cover slip.

Measurement and line drawing of nematodes:
Measurement and line drawing of the nematodes were done by the method given by de Man (1880) ad Hooper (1986) respectively.

Statistical analysis:
Community analysis of phytoparasitic nematodes in rice fields of Pakistan was done by the using of Norton techniques (1978).

RESULTS AND DISCUSSION

Incidence of *Aphelenchoides parietinus*
The total incidence percentage of *A.parietinus* was determined from all soil samples of rice from major rice growing province (Punjab) of Pakistan. Present analysis showed that rice nematode *Aphelenchoides parietinus* was more prevalent (85.61%) in soil (Table.1).

Table 1. Incidence of *Aphelenchoides parietinus* nematodes from rice localities.

Localities	No of fields samples (A)	Extraction of *A.perietinus* by roots (B)	Total incidence After bioassay (C)	D/F in incidence (C-B)	% incidence (C/A) x 100
Sialkot	25	04	26	22	16.0
Lahore	36	32	34	2	88.8
Gujranwala	20	08	21	13	40.0
Sheikhupura	32	24	29	05	75.0
Okara	48	15	28	13	31.2
Jhang	39	13	19	06	33.3
Narowal	18	10	09	01	53.5
Kasur	20	06	14	08	30.0
Sahiwal	105	35	73	38	33.3
Faisalabad	47	15	23	08	31.91
	390	**123 (31.53%)**	**252 (64.61 %)**	**33.58%**	

The fields with high incidence of *Aphelenchoides parietinus* were easily detected through direct examination of root symptoms. In the present study different root symptoms were detected such as surface necrosis of roots as minor symptoms. While in major symptoms yellowish to brownish color of young succulent root. It might be due to the high density of *A.parietinus*. The present investigations indicated that two localities such as Lahore and Sheikhupura were highly infested out of ten surveyed localities of Punjab.

The plants on fields with moderate level of infestation did not always show clear above ground symptoms but soil had few numbers of studied nematodes. Localities i.e Okara, Gujranwala, Jhang, Sahiwal and Faisalabad out of total localities were moderately infested with *A. parietinus.* Whereas in Sialkot fields with low level of infestation i.e 16% often had plant which did not showed any above and underground symptoms and roots are healthy not had *Aphelenchoides* nematodes after direct examination under binocular microscope, but nematodes also found in rhizosphere.

Table 2. Community analysis, Population and nematodes density/250 cm^3 soil after Robbins et al (1989).

S.NO	Localities	Nematode population	Absolute frequency	Relative frequency	Mean density	Relative density	Prominence value
1	**Sialkot**	160	4.7	3.62	0.83	18.4	39.9
2	**Lahore**	1600	65.1	15.18	6.15	50.5	407.5
2	**Gujranwala**	396	54.6	12.7	1.61	30.5	219.5
3	**Sheikhupura**	600	72.5	16.91	2.30	39.8	350.5
4	**Okara**	120	11.3	2.63	1.46	21.4	71.9
5	**Jhang**	80	14.6	3.40	0.30	14.6	55.8
6	**Narowal**	620	74.5	57.4	3.22	40.8	352.2
7	**Kasur**	500	50.5	30.9	2.60	21.3	150.6
8	**Sahiwal**	421	54.6	12.70	1.61	30.5	225.4
9	**Faisalabad**	340	50.9	11.8	1.30	20.2	144.1

Based on the prominence value, frequency of occurrence and abundance, Lahore, Sheikhupura and Narowal was the most infested region. In these regions Rice- Wheat is most common cropping sequence and farmer use the same rice varieties. These both factors might have contributed to the high prominence value of *A. parietinus*. During the survey it was found that most common growing varieties of rice are same in surveyed localities of Punjab. While other varieties grown locally different from region to region based on local market demand and preference.

According to community analysis Population density could be categorized as high in the localities of Lahore, Sheikhupura and Narowal (Table.3).In case of frequency among the all rice fields, the most infested samples encountered from Narowal followed by Sheikhupura and Lahore. Locality Lahore had the highest absolute density of *A.peritinus*, while the least density was observed in Jhang .The prominence value (PV) of *Aphelenchoides* genus varied among all infested zones (Table.2). Lahore (PV= 407.5) was the most infected locality followed by Narowal (352.2) and Sheikhupura (350.5). Whereas the least PV was recorded from Faisalabad i.e 39.9 as compared to other localities of Punjab. It should be noticed that *A.parietinus* was reported for the first time from rice fields in Pakistan.

Table 3. Rating of resistance and quantitative analysis of *Aphelenchoides parietinus* occur in soil around the *Oryza Sativa* L. plant in fields of Punjab.

Cultivars	Reaction	Damage Index	Nematodes/250 g soil		Symptoms in Fields
			Mean	localities	
Jajai-77	S	5.0	1433	2,4,7	Stunted growth, low yields, hollow tillers
Khushboo-95	MS	4.5	1089	3,5,6,9,10	Not accountable symptoms
Lateefy	H.R	0.8	22	1,8,9,10	Healthy
Sonahri-Sugdasi	R	1.5	83	3,5,9	Healthy
Sugdasi bengalo	MR	2.6	255	3,5,6,9,10	Healthy
Sugdasi sadagulab	R	1.8	87	1,8	Healthy
Super basmati	R	2.0	99	1	Healthy
Dilrosh 97	MS	4.0	600	9,10	Not accountable symptoms
Dilrosh-92	H.R	1.0	20	1,8,10	Healthy
DR-8	R	1.1	63	1,8,10	Healthy
DR-83	R	1.7	88	1,8,10	Healthy
IR-6	MR	3.0	499	3,5,6,9	Healthy
Kangni-27	S	5.0	1600	2,4,7	Stunted growth, low yields, hollow tillers
Kanwal-95	MR	2.8	368	3,5,6	Healthy
Sada hayat	S	3.6	544	2,4,7	Not accountable symptoms
Sarshar	S	4.0	600	2,4,7	Stunted growth, low yields, hollow tillers
Sugdasi ratria	R	2.0	99	1,10	Healthy
Shua 92	H.S	5.0	1488	2,4,7	Reduced plant growth without matured tillers and grains
Shadab	R	1.4	74	1,8,9,10	Healthy

Localities: 1. Sialkot, 2. Lahore, 3. Gujranwala, 4. Sheikhupura, 5. Okara, 6. Jhang, 7. Narowal, 8. Kasur, 9. Sahiwal, 10. Faisalabad

Ranking of Resistance: In the present study the most common cultivasr Jajai- 77, Kangni-27, Shua-92 and Shadab were frequently grown in the highly infested localities. While moderate susceptible varieties were Khushboo, Dilrosh-97. Other remaining varieties were ranked as moderately resistant and resistant. The level of resistance varies from region to region. According to damage high damage index was recorded in Jajai-77, Kangni-27 and Shua -92 varieties (Table-3). While low damage index was recorded in Lateefy which ranked as highly resistant variety. With respect to nematode population, maximum population was found from Lahore, Sheikhupura, and Narowal localities where commonly grown varieties were Shua -92, Kangni-27 and Jajai-77. Whereas lowest and ignorable population of *A.parietinus* was recorded from Sialkot, Kasur, Sahiwal and Faisalabad localities.

Plant growth and yield assessment: Data has been analyzed by the factorial test (F-test) at $p < 0.05$ level and presented in table.4. According to plant length and tillers per plant non significant results were observed in all resistant varieties. In case of susceptible genotypes, significant results were obtained in terms of plant length and tillers/plant, the variation in number of nematodes was significant. Jajai-77, Kangni-27 and Shua-92 exhibited more

numbers of nematodes in soil as compared to other susceptible rice genotypes (Table.4). In case of yield assessment significant results were obtained in the weight of 100 seed in all susceptible varieties as compared to control. While maximum 100 seed weight were recorded in resistant varieties viz., Lateefy and Dilrosh-92. Significant results were also observed in moderate resistant varieties. Significantly maximum yield was recorded in all highly resistant varieties followed by resistance varieties. While minimum yield was recovered in five susceptible varieties followed by Shua-92 > Jajai-77 > Sadahayat> Sarshar >Kangani-27.

Table 4. Calculated F values and mean comparison of plant growth and yield parameters of rice by applying ANOVA for single factor.

Cultivars	Plant length	Tillers/plant	Yield assessment	
			100 Seed wt	Total yield
Jajai-77	7.3* b	7.9*b	8.7*a	12.4*a
Khushboo-95	9.8* ab	9.8*b	6.6*b	8.7*b
Lateefy	3.2cd	3.3d	0.2g	2.1d
Sonahri-Sugdasi	3.3cd	1.5e	3.1cd	1.9de
Sugdasi bengalo	4.1c	2.1e	1.5ef	2.1d
Sugdasi sadagulab	3.8c	2.9d	3.0cd	2.5d
Super basmati	4.2c	3.9c	2.6e	3.0c
Dilrosh 97	8.7*ab	9.8*b	4.8c	2.0de
Dilrosh-92	2.6d	3.4c	4.0c	3.3c
DR-8	3.5cd	2.5d	1.0ef	1.3e
DR-83	4.1c	3.6c	1.0ef	2.3d
IR-6	3.8c	4.1c	1.0ef	3.2c
Kangni-27	14.3*a	20.6*a	6.8*b	10.9*a
Kanwal-95	4.0c	2.8d	1.6ef	2.3d
Sada hayat	8.8*ab	6.5*b	10.9*a	14.3*a
Sarshar	8.6*ab	12.3*a	9.4*a	13.8*a
Sugdasi ratria	3.2cd	4.1c	1.0ef	2.2d
Shua 92	20.3*a	6.8*b	2.2e	4.4*c
Shadab	3.3cd	2.1e	1.0ef	3.4c

Morphological identification:
Aphelenchoididae family is identified by its large metacorpus and pharyngeal glands not usually enclosed in a bulb (overlapping) just like other phytoparasitic nematodes. The main morphological characteristic shared by the *Aphelenchoididae* family members is short stylet while other plant parasitic nematodes having large stylet with well developed knobes.

Description: Female body slightly ventrally accurate when relaxed. Cuticle finely annulated. Head slightly rounded. Lateral field marked by four incisures. Stylet 8-8.8 µm long, with small basal swellings, conus a little shorter than shaft. Oesophagous 176-205 µm long. Metacarpus 7.2-11.2 µm wide and 9.6-13.6 µm long. Excretory pore 50-52 µm from anterior end. Nerve ring immediately behind metacarpus. Hemizonid not seen. Ovary single, outstretched, oocytes in a single row except at the tip, vulva transverse. Spermatheca elongate oval filled with rounded sperms, vagina directed anterial. Occupying about 1/3 of vulva body width. Intestine straight, rectum 14.4-22.4 µm long. Post vulval sac elongate, 32-44 µm long, usually containing sperms, tail conoid, ventrally curves, 22.4-26.4 um long with terminal mucro (Table.5).

Table.5. Measurements (µm) of *Aphelenchoides parietinus* (Bastian, 1865) Steiner, 1932.

Morphological characters	Range (Mean ± Sd)	
	Female N= 20	Male= 20
L	410.4-506.4 (457.0±58.9)	376.8-464 (418.3±49.5)
A	24.4-32.8 (27.7±2.4)	21.5-34 (25.6±1.4)
B	6.0-8.8 (7.4±0.86)	6.6-8.2 (6.9±0.50)
C	13-20 (17.3±1.4)	14.7-20.6 (15.8±1.44)
c'	2.1-3.3 (2.7±0.34)	2.1-3 (2.4±0.25)
V	55.9-71.4 (67.5±5.6)	-
Stylet	5.6-8.8 (7.5±0.98)	8.0
Spicules	-	11.2-16 (14.3±1.36)
Tail	22-28.8 (26.0±2.5)	21.6-28.8 (26.1±2.44)
Rectum	13-22.4 (19.3±2.7)	-
Post vulval sac	32-45 (40.5±10.3)	-

Male body curved ventrally more so in the posterior region. Cuticle finely striated. Head offset from the body. Stylet with prominent basal thickening. Nerve ring and excretory pore as in female. Testis single, outstretched reaching interiorly, upto oesophageal gland. Spicules are typically of Aphelenchoides, gubernaculums absent. Tail 24.4-28.8 µm, shape of tail similar to female tail. Three pairs of caudal papillae are present, the first pair near cloacal opening, the second one mid way along the tail and the third at the base of mucro.

Remarks: Specimens were collected from rhizosphere of rice (*Oriza sativa* L.) from Punjab, Pakistan. The population of *A.parietinus* resembles to general morphological and morphometrical characters corresponding to those of the type population (Bastian, 1865) Steiner, 1932. The present population have slight differentiation in some measurements i.e., the length of tail smaller than tail of *A. parietinus* (22.4-28.8 µm vs 32.9 µm), c ratio is slightly greater than *A. parietinus* (15-17 vs 13-19). Tail with mucro and ventrally curves as compare to *A.parietinus* that slightly terminally curved (Fig.1). Description and illustrations were given for the first time from Pakistan.

DISCUSSION

Aphelenchoides are large nematodes genus and economically important pests of several crops especially in rice crop. In previous studies mostly researchers reported White tip disease of rice which causing agent is *Aphelenchoides besseyi* and next spp is *Hirschmanniella graminis* that frequently prevalent in rice fields. While other Aphelenchoides spp are not frequently found in rice fields but when present in large numbers even a weekly parasitic species like *Aphelenchoides parietinus* may kill enough epidermal cells to give a young succulent root a yellowish to brownish color. It is superficial discoloration of the tissues. In the present study high incidence and abundance of *Aphelenchoides perietinus* was observed in Punjab province of Pakistan. This study exhibited that prevalence of this weak nematode was due to same cropping system and grown same varieties in these localities due to the local market demand.

In surveyed areas many researchers focused on reproduction of crop which determining resistance against phytoparasitic nematodes but nematode pathogenicity is not the major criteria. In this case symptom development should be evaluated. Plant length, tillers per plant and yield of 19 rice cultivars indicated that specific cultivars grown in upper Punjab of Pakistan are susceptible to *Aphelenchoides perietinus.* The results of present survey indicate and emphasize the need for breeding local cultivars. In many European countries *A.besseyi* has been controlled principally through the use of resistant cultivars (2, 13).

The present surveys were carried out at harvesting of rice and, therefore, the early symptoms were not encountered. Khan (2010) reported that development of white tip disease and deformation of rice grain is dependent on population density of nematodes in rice plant and other many environmental factors. In the present investigation, population densities were varied among the rice sampling sites. The *A.perietinus* nematode in rice is widely distributed might be due to infested seedlings and soil through water between locations and regions within the same province. Jamali *et al* (2006) conducted survey from rice fields of Iran and he observed that incidence and severity of white tip disease varies from year to year and from variety to variety he concluded that these variations were found due to environmental factor, cultural practices and local rice cultivars. The present study further confirmed incidence and widespread of *A.perietinus* among all surveyed rice fields under Punjab conditions.

Conclusion: The results of the present survey investigations indicate that weak nematodes such as A.perietinus present in excess then cause a disease. During the survey highly infested localities were point out to farmers and also confirm the resistant genotypes of rice that grower should be cultivated in infested fields. This nematode first time reported from rice fields of Punjab and first time gives detailed of infestation, symptoms and infested districts.

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Compliance with ethical standards
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