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Incidence of Cereal Cyst Nematode Prevailing in Wheat-rice Growing Regions of Punjab Province, Pakistan

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ABSTRACT

Background: Cereal Cyst Nematodes (CCN) are prevailing in all type of agricultural lands and responsible for enormous losses of cereal crops. The understandings on population densities and management of these nematode are required to improve crop health and productivity.

Methods: A study was designed to assess the incidence of cereal cyst nematodes in wheat and rice monoculture cropping regions of eight districts of Punjab province, *viz*., Jhang, Khushab, layyah, Hafizabad, Sheikhupura, Narowal, Gujranwala and Sargodha in Pakistan. Population densities of cereal cyst nematodes were studied by extracting the cysts and second stage juveniles from soil samples and identified the nematode species.

Result: The CCNs were found in 80% of the total 250 samples collected from wheat and rice monoculture fields of at least one crop. In the soil samples collected from wheat fields, an average of 7 to 38 cysts/100g of dry soil with eggs and J2 population of 142-771 were recorded. Whereas from the soil samples of rice fields, 17 to 25 cysts/100g soil were found with 345 to 508 eggs and juveniles. Among the wheat fields, the lowest incidence of 5.5% was recorded in kot–momin and highest incidence of 16.88% was recorded in Silanwali tehsils of Sargodha region. In rice fields, 24.69 to 27.00% incidence of CCNs was noticed in soil samples of various surveyed regions. Three different species of *Heterodera* genus were morphologically identified from the collections. The species includes *Heterodera oryzae*, *Heterodera avenae* and *Heterodera graminophila*. *H. oryzae* was more abundant in rice growing regions while *H. avenae* and *H. graminophila* were present dominantly in wheat growing regions. This study provides an inclusive information regarding cereal cyst nematode densities and species in wheat-rice growing regions of Punjab province of Pakistan.

Key words: Cereal cyst nematode, *Heterodera* spp., Population densities, Soil samples, Wheat-rice monoculturing.

INTRODUCTION

Cereals are the most important food source in the world and 58 per cent of the annual cultivation has been allocated to wheat, corn and rice. By the year 2030, the world population will reach about 8 billion people and grain consumption will be increased significantly (Curtis, 2002). Among cereals, wheat and rice are the main cereals grown in Pakistan. During 2017-18 marketing year, wheat production was estimated as 19.1 million metric tons with an area of cultivation of 6.56 million hectare. The rice is the third largest crop in terms of area sown, after the wheat and cotton crops. Rice production in 2017-18 marketing year was estimated as 3.89 million metric tons with an area of 1.85 million hectare (Anonymous, 2017-18). The production of wheat and rice is expected to increase in coming years.

Cereal cyst nematodes (CCNs) are known as the utmost vital nematode pathogens that reduce the growth of cereal crops. Cereal cyst nematode parasitize the newly developing roots of plants and reduce the crop vigor by producing patches of stunted plants with pale green leaves. Young plants develop less number of tillers and root system turns shallow and have a “bushy-knotted” look (Nicol *et al*. 2010; Smiley and Nicol, 2009). Cereal Cyst nematodes (CCNs) of genus *Heterodera* include approximately 70 species, having a multipart of 12 species known as from the group of *Heterodera avenae* (Rumpenhorst *et al*. 2003). Twelve species from *Heterodera avenae* species complex *viz*., *H. australis, H. filipjevi, H. mani, H. pratensis, H. riparia, H. sturhani and H. ustinov, H. arenaria, H. latipons, H. avenae, H. mothi* and *H. hordecalis* have been characterized by Subbotin *et al*. (2001). Maqbool (1981) noticed dominance of cereal cyst nematodes such as *H. avenae, H. zeae* and *H. mothi* in the soil samples of wheat fields. Maqbool and Kerry (1997) confirmed the presence of cereal cyst nematodes and identified six new cyst nematode species associated with grain crops in Pakistan. During 2003-2004, Shahina and Erum, (2007) conducted a survey in the southern regions of Pakistan and found that 39% of total studied area was infected by cereal cyst nematodes through huge dispersal and host choice of corn cyst nematode (*H. zeae*). The cereal cyst nematodes have shown significant yield losses in specific research trials by as much as 50%

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in Australia, 20% in Pakistan, 90% in Saudi Arabia and 50% in Turkey (Riley *et al*. 2009). More than half of the growing areas of cereals are detected to be infected by CCNs in certain cereal growing areas of Turkey (Abidou *et al*. 2005; Rumpenhorst *et al*. 1996), Europe (Cook, 1974) and USA (Smiley *et al*. 2009). It is also observed that approximately most of the wheat fields are infected by cereal cyst nematodes in China (Peng *et al*. 2010).

The present study was conducted by keeping in view the sparse data on distribution of cereal cyst nematodes, their population densities and most abundant species in specific cereal growing regions. During the surveys, samples were collected from the wheat-rice monoculture fields of Punjab province, Pakistan. The study provided comprehensive information on cereal cyst nematodes population and species.

MATERIALS AND METHODS

Survey and sampling

The survey was conducted in the cereal growing regions of Punjab province for two years 2018 and 2019 for collection of soil samples. Totally eight districts of Punjab province *viz*., Jhang, Khushab, layyah, Hafizabad, Sheikhupura, Narowal, Gujranwala and Sargodha were selected in which wheat-rice cropping pattern is followed with some minor cereal crops (Fig 1). A brief history of all fields such as; cropping pattern, duration of cropping pattern and agronomic practices were collected from the farmers of selected fields. Soil samples were collected at crop harvesting from different aged monoculture 32 wheat and 18 rice fields from different locations (Table 1). The 5-30 cm core of soil was collected in polythene zipper bags and brought to laboratory for nematode extraction. Totally, 250 soil samples were collected from all sites by following the zig-zag sampling method. The research work was carried out at the Laboratory of Plant Pathology, College of Agriculture, University of Sargodha.

Cysts and nematodes extraction

Soil samples collected from the fields were homogenized manually and 100g of soil from each sample was weighted. The samples were processed by using Cobb's shifting and gravity method by 350µm mesh sieve over laid with 20µm mesh sieve for debris collection followed by 75% sucrose floatation (Chen, 2007). The cysts obtained through sieving were observed under stereomicroscope and number of cysts in each sample were measured. The cysts were crushed by using cyst crushing glass device and cyst suspension along with eggs and juveniles were observed under inverted-microscope for counting.

Measuring the population densities of cereal cyst nematodes (CCNs)

The nematode suspension of 1ml from each processed sample were observed and counted in cell culture 12 well plates in three replicates. Number of nematodes were counted and their averages were calculated for 1 ml of suspension. Total number of nematodes were calculated from representative sample by the given formula:

Total no. of nematodes =
No. of nematodes in 1 ml suspension × Total volume of suspension.

Total number of cysts were counted from each sample and their averages were calculated for each locality separately. Total number of cysts were calculated from representative locality by the given formula:

$$\text{Average no. of cysts in one locality} = \frac{\text{Total no. of cysts in one sample}}{\text{Total no. of samples of locality}}$$

Morphological identification of cereal cyst nematodes

The extracted cysts of nematodes from wheat and rice fields were used for egg hatching assay separately. Totally 20 cysts from the samples were placed over microsieves having 300µm pore size and kept the sieves in 0.1% $ZnCl_2$ solution. The hatched juveniles were picked and fixed on glass slide and observed under microscope. The fixed slides were observed under digital camera fitted microscope (Scopus). Physical and anatomical features of juveniles and cysts were observed at different magnifications.

RESULTS AND DISCUSSION

Population densities of CCNs in wheat and rice growing regions

Cereal cyst nematodes (CCNs) were found in 80% of wheat and rice fields surveyed. The results showed that in 160 soil samples collected from wheat fields, 20 samples (12.5%) were noticed to have infection. The number of cysts in wheat fields were varied from 7-38 cysts/100g soil, the eggs and juveniles varied from 142-771 (Table 2). Out of 90 rice fields sampled during the survey, on average 22.5 (25%) samples were found infested with CCNs. Population density of CCNs was varied from 17 to 25 cysts/100g of soil with 345-508 of eggs and juveniles. The results revealed that cereal cyst nematodes are present in all the localities surveyed *viz*., Jhang, Khushab, layyah, Hafizabad, Sheikhupura, Narowal, Gujranwala and Sargodha with abundant population densities.

Prevalence of cereal cyst nematodes across regions

The prevalence of cereal cyst nematodes (CCNs) were assessed from all wheat-rice growing regions surveyed. In the wheat fields, 6.25% and 16.88% are the lowest and highest incidence of CCNs recorded respectively in Sargodha district. In rice fields, 24.69% to 27% incidence was recorded in Gujranwala and Hafizabad respectively. The average numbers of cysts, eggs and juveniles per 100 g of soil in wheat fields was higher than in rice fields (Table 2). The presence of abundant cysts with healthy eggs and juveniles were observed in most of the samples (Fig 2). Moreover, the aged fields were found to be highly infected with CCNs (Fig 3). The results showed that in Punjab province, nematode population density index indicates that

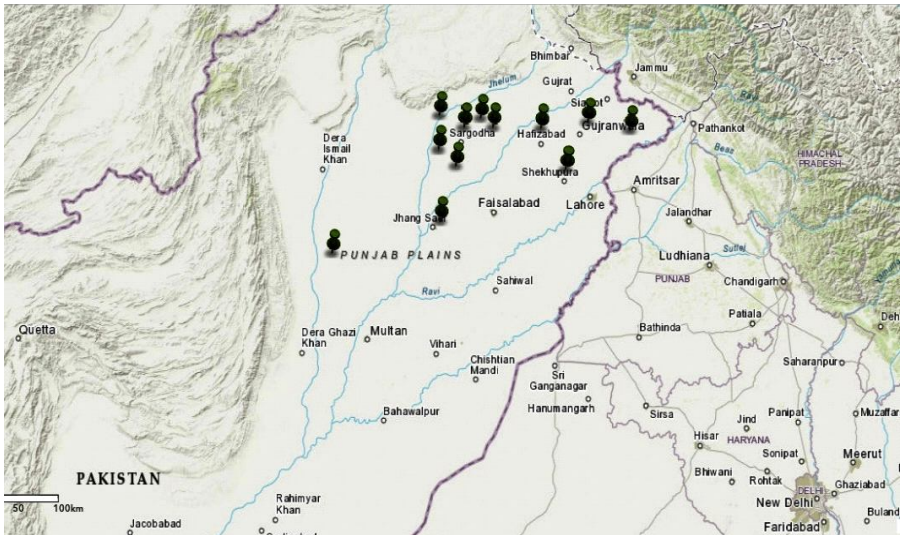

Fig 1: The distribution map of cereal cyst nematodes across rice and wheat growing regions of Punjab, Pakistan.

about half of the wheat and rice fields are moderate to highly infected and proper management practices need to be adopted.

Morphological identification of cereal cyst nematodes

The morphological based identification of CCN species was conducted by picking the second stage juveniles from the hatched cysts. The results showed the presence of three *Heterodera* species in all the samples collected from rice and wheat fields. The *Heterodera oryzae* was more abundant than other species in rice fields and *Heterodera avenae* was found more abundant in wheat fields. *Heterodera graminophila* was present in both rice and wheat fields with other two species. *Heterodera oryzae* is certainly the only species of *Heterodera* to keep cysts with cone vulvae and juvenile in which the hyline portion of the tail is clearly longer than the stylet (Fig 4). While, cysts are lemon shape, dark brown to black, whose dimension was ranged from 0.31-0.81×0.22-0.69mm (average 0.57×0.45mm), with having zig-zag ornamentation without order defined and partially covered by semi-crystalline layer. The length of second stage juvenile (J2) ranges from 0.37 to 0.50mm with an average of 0.44mm long. The second stage juveniles (J2) of *Heterodera avenae* are vermiform and cylindrical with a sharp pointed tail as shown in (Fig 4). Stylet shape is slender and strong with large anteriorly flattened to concave basal knobs. Head section is hemispherical and offset. Middle bulb is round with a large valvuae apparatus inhabiting about half of the corresponding diameter of body (Fig 4). Tail is 3-4 times anal body thickness long. Tail is conoid, gradually narrowing to a finely rounded station. The body of *Heterodera graminophila* was observed as pearly white, usually rounded shaped, with protruding vulva and neck. Thick cuticle with zig-zag pattern, subcuticular punctation and usually a granular surface obscuring details of pattern. *H. graminophila* have strong stylet and curving dorsally, with sharp-developed knobs sloping posteriorly. Moreover, esophageal part appearing about as illustrated, with excretory pore normally

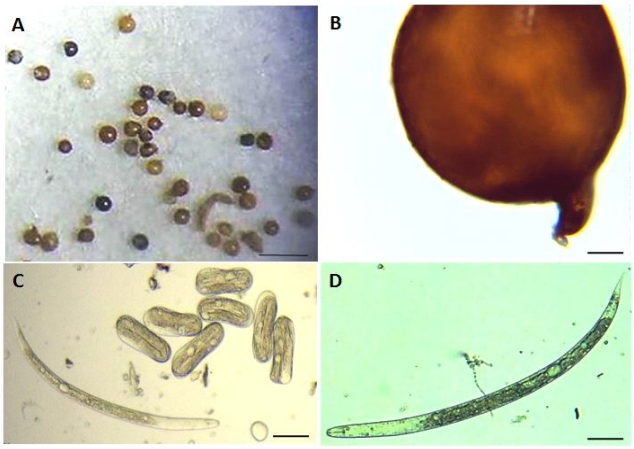

Fig 2: The morphological characters of cereal cyst nematodes in rice and wheat fields. (A) cysts of CCN (B) cyst showing typical appearance (C) healthy eggs of CCN (D) active juvenile of CCN.

Table 1: The selected fields with cropping history and years of monoculturing of wheat/rice for sample collection.

Year range	Cropping pattern	Category (Year)
1	Wheat + Sorghum + Rice	1
3-5	Legume + Rice	3
2-5	Wheat + Legume + Rice	5
1-6	Wheat + Rice	7
8-10	Rice + Sugarcane	9
Above 10	Sorghum + Rice	11

at base of neck. Small anus, inconspicuous and located about 20% of body length from vulva (Fig 4). Cyst surface in *H. graminophila* usually granular, with conspicuous irregularly arranged function. The morphological observations revealed the presence of major *Heterodera* species in all the rice and wheat cropping fields.

The present study of cereal cyst nematode (CCN) revealed the incidence of cereal cyst nematode in different

rice and wheat growing regions of Punjab province Pakistan. Some fields surveyed were found to be highly infected with CCNs while some fields recorded less infection. Number of cyst and nematodes per 100gm of soil were varied in different regions ranging from 17-38 cysts and hundreds of eggs and juveniles. Ahmadil *et al*. (2014) reported varied degree of cereal cyst nematodes across the different regions of Iran. The variation in the infestation of CCNs occurs due to the different factors like application of nematicides, soil solarization, cultural practices or the presence of fungal and bacterial pathogens (Chen, 2007). Cereal cyst nematodes have more devastating effect on rain-fed cereal crops than irrigated crops (Smiley and Nicol, 2009).

According to Rivoal and Cook, (1993), now a days, the use of resistant or tolerant varieties to the nematodes is considered as one of the most efficient management

Table 2: Population densities of cereal cyst nematode (*Heterodera* spp.) from eight districts of Punjab province, Pakistan.

Localities of wheat growing regions	Crop history	Means of cyst/ 100g soil	Means of eggs and juveniles/ 100g soil
Sargodha			
College of agriculture	Rice + wheat	29	589
Chak 71 NB	Citrus + wheat	18	365
Chak 87 NB	Sorghum + wheat	18	365
Chak 93 SB	Legume + wheat	22	447
Jhang			
Village 269	Wheat + cotton	18	365
Chakshorwala	Wheat + sugarcane	15	305
Kot Sai sing	Wheat + cotton	16	325
Chakchistan	Wheat + rice	16	325
Layyah			
Kotla haji shah	Wheat + cotton	29	589
Basti sheikh jalu	Wheat + sugarcane	25	508
Basti Jota	Wheat + gram	26	528
Noor abad	Wheat + cotton	18	365
Khushab			
Badliwala	Wheat + rice	19	386
Hafizadevan	Wheat + sugarcane	20	406
Namywali	Wheat + citrus	20	406
Mohalashershah	Wheat + legume	22	447
Hafizabad			
Thatakhokharan	Wheat + rice	20	406
Dhengranwali	Sorghum + rice	24	487
Tharianwala	Sugarcane + rice	20	406
Chakchatha	rice + legume	25	508
Thata Kalian	Rice + wheat	22	447
Sheikhupura			
Bhurianwala	Rice + wheat	21	426
Dhamoke	Rice + sugarcane	20	406
Jandiala	Wheat + rice	20	406
Herdaiv	legume + rice	18	365
Saikham	rice + wheat	23	467
Narowal			
Dhsadiala	Wheat + rice	23	467
Kotsalakhan	Chickpea + rice	23	467
Bhebalwali	Lentil + wheat + rice	24	487
Siran	Rice + sorghum	17	345
Gujranwala			
Kot Shahan	Legume + rice	20	426
Kotlimughlan	Sugarcane + rice	20	406
Jandiala	Wheat + rice	21	426
Rahwali	Wheat + rice	23	467

strategies for controlling the CCNs and is broadly used in some countries such as Australia, Denmark, Sweden, England and France. However, during the survey, the CCNs were observed in different local varieties of wheat and rice under field conditions, it would be helpful to examine reaction of a wider range of wheat and rice cultivars under controlled conditions. CCNs population was found high in wheat fields than the rice fields. Andersson (1982), reported that spring wheat is a more suitable host for *H. avenae*.

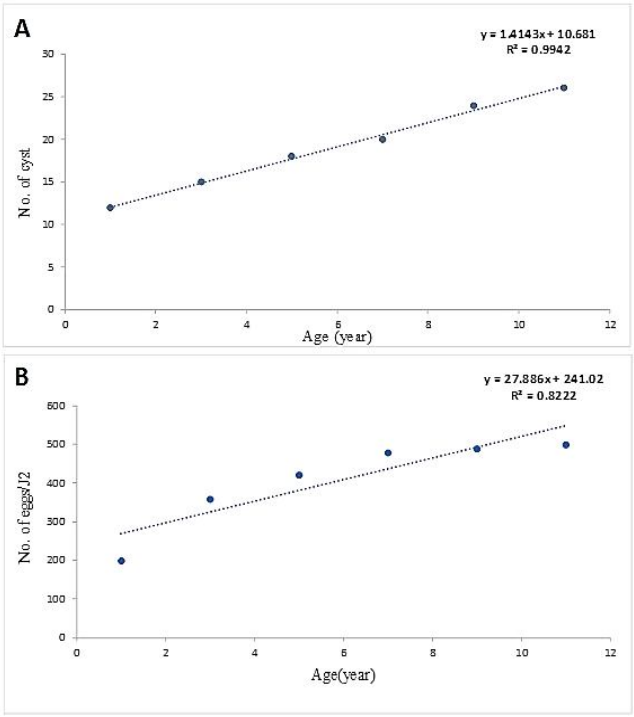

Fig 3: The overall population densities of CCN across rice and wheat growing regions with respect to years of monoculturing. (A) mean number of cysts in fields (B) mean number of eggs in cysts.

The given results showed the trend of increasing population densities of CCNs with respect to years. The level of population was found to be related with the age of fields. Aged fields were found to have more population density of CCNs. The study showed that crop rotation and fallow effects the population of cereal cyst nematode in wheat crop (Taya *et al*. 2000). Three CCNs species *Hetrodera avenae*, *Heterodera oryzae* and *Heterodera graminophila* were found in these cereal growing regions. The microscopic observations of anatomical features of these nematodes described the specialized body parts in all species. The observations of cysts were also made and vulva slit on cyst approximately about 45µm in length (Kumari, 2017). The morphological observations of cereal cyst nematodes were clearly demonstrated by Golden and Bircfield, (1972). The number of J2 and eggs of CCNs in some regions were greater than that measured as damage threshold level for this nematode (Gill and Swarup, 1971; Meagher and Brown, 1974) and is expected that these populations could cause economic yield loss. The most effective control method for CCNs is crop rotation with non-host crops. Some other control methods includes clean fallow and deep ploughing of 2-5 times during May to June in India (Swarup and Sosa-Moss, 1990), also early planting of wheat in order to increase the plant tolerance and vigor against nematode attack and application of nematicides in planting (Brown and Kerry, 1987). This study will provide a comprehensive data on cereal cyst nematode population and distribution across

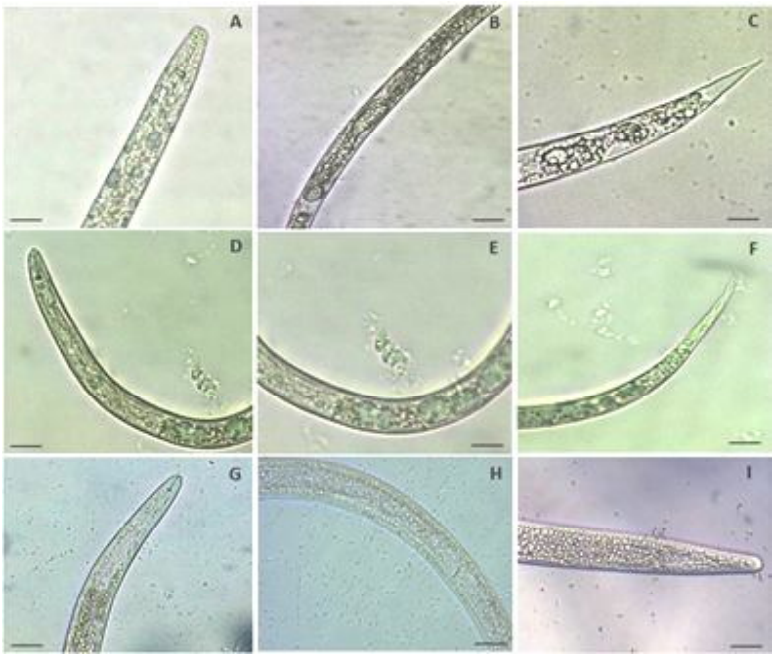

Fig 4: Morphological characterization of *Heterodera* spp. from wheat and rice fields (A, B, C) *Heterodera avenae* (D, E, F) *Heterodera oryzae* (G, H, I) *Heterodera graminophila*.

wheat-rice growing regions of Punjab province of Pakistan and development of resistant cultivars and management strategies can be designed to improve the yield of these both important cereal crops.

CONCLUSION

The soil samples were collected from wheat-rice growing regions of Punjab province of Pakistan to study incidence of cereal cyst nematodes (CCNs). The incidence of 6.25% to 16.88% was found in wheat fields. In rice growing areas, the incidence was 24.69% to 27.00%. Through this study, incidence and prevalence of *Heterodera* spp. were recorded for the first time from wheat- rice growing regions of Punjab province, Pakistan.

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