Virulence studies of indian populations of sorghum cyst nematode, *Heterodera sorghi* in Maize

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Abstract

Five sorghum cyst nematode, Heterodera sorghi *populations, that cause nematode disease in maize, were collected from Kulu, Chamba (Himachal Pradesh), Tihri (Uttarakhand), Ghaziabad (Uttar Pradesh) and Kud (Jammu and Kashmir) to determine the degree/pattern of virulence in a collection of five populations against maize. Maize plants were inoculated with juveniles and assessed for the nematode multiplication and reduction in growth parameters. The results showed that decrease in shoot lengths was found to be non significant. However, significant reduction in fresh shoot and root weight was observed in maize due to all five populations at the initial inoculation level of 4 and 6 second stage juveniles (J_2)/g soil compared to uninoculated control and which were significantly different from each other. Significant reduction in dry shoot and root weight of maize was recorded at the initial inoculum level of 2, 4 and 6 J_2/g soil in all five populations except Ghaziabad population, where significant reduction of dry root weight was observed at initial inoculation level of 4 and 6 J_2/g soil compared to uninoculated control. Maximum multiplication of the nematode was recorded at the highest level of nematode inoculum (6 J_2/g of soil) and minimum at the lowest level (2 J_2/g of soil). The Ghaziabad population was found to be more virulent among the five populations on maize and may be helpful in developing region oriented management strategy.*

Keywords: Maize, sorghum cyst nematode, *Heterodera sorghi,* virulence

Introduction

Cyst-forming nematodes (*Heterodera* spp.) are highly specialized and economically important soil-borne parasites attacking numerous agricultural crops (Hamzeh *et al*., 2016). Yield losses caused by cyst nematodes in cereals could be up to 90% in severely infested fields in many countries of world (Rivoal and Cook, 1993; Riley *et al*., 2009). Crop losses due to cyst nematodes have been documented by Bridge *et al.* (2005), *Heterodera elachista* decreases yield by 7-19% and even higher yield losses have been attributed to *H. oryzicola* in India. The sorghum cyst nematode, *Heterodera sorghi*, one of the important cyst forming nematodes, was first recorded and described from India on the roots of sorghum (*Sorghum vulgare*) from Raispur in Ghaziabad and Naini in Allahabad districts of Uttar Pradesh (Jain *et al.,* 1982), subsequently from Gurgaon district of Haryana by Dhawan *et al.* (1983). Besides sorghum, other crops like maize (*Zea mays*), barley (*Hordeum vulgare*), oats (*Avena sativa*), rice (*Oryza sativa*) and wheat (*Triticum aestivum*) are good hosts for nematode. Maize has been found to be highly preferred host relative to other recorded hosts for *H. sorghi*, supporting high number of nematode population (Srivastava and Sethi, 1987; Srivastava and Gautam Chawla, 1991). Srivastava and Kaushal (1986) reported its presence in the Una district of Himachal Pradesh; and also reported from parts of Delhi and Jammu and Kashmir, Ludhiana district of Punjab (Sakhuja and Inderjit Singh, 1985), Andhra Pradesh (Sharma and Sharma 1988) and Maharashtra (Darekar *et al.,* 1990) states of India. It is found to be widely distributed now in the maize growing states of the country causing significant damage to the maize crop (Srivastava and Sethi, 1984). Annual yield loss caused by plant parasitic nematodes in India ranged from 10 to 80 per cent in cereals and other crops (Bhatti, 1992) and in world causing annual monitory loss of more than $ 215.77 billion to many lifesaving crops (Abd-Elgawad, 2014); however**,** damage caused by them has not received adequate attention.

Maize is one of the top ranking cereals in the world agricultural economy and ranks third in importance among India's cereals, after rice and wheat, for human food. Its cultivation has become common practice throughout the year in most of its states for various purposes that include grain, feed, fodder and industrial products. In the country, more than three-fourths of the area to maize production is contributed by eight states, *viz.,* Andhra Pradesh, Bihar, Karnataka, Madhya Pradesh, Maharashtra, Rajasthan, Uttar Pradesh, Tamil Nadu and North Eastern plains (Singh *et al.*, 1997; Reddy *et al*., 1999). Plant parasitic nematodes *viz.,* cyst nematodes (*Heterodera* sp.), lesion nematodes (*Pratylenchus* sp.), root-knot nematodes (*Meloidogyne* sp.), stunt nematode (*Tylenchorhynchus* sp.) and spiral nematode (*Helicotylenchu*s sp.) have been found to be associated with maize (Norton, 1984) and are responsible for causing 10.2% loss in maize (Sasser and Freckman, 1987). Among them, cyst nematodes (*Heterodera* sp.) is key nematode pest in India and world. An attempt was, therefore, made to determine the degree/pattern of virulence of five populations of *H. sorghi* collected from different maize growing locations in India on maize crop under pot conditions. The virulence studies can provide development and reproduction potential of a pathogen that are fundamental factors for efficient management (Patil *et al.,* 2015) and the economic impact of the nematodes on crops (Mustafa *et al*., 2014)) and therefore, it provides essential data for nematode management. Such information is very important for the management of *H. sorghi* on maize in India.

Materials and methods

The stock culture of *H. sorghi* was maintained on maize. Five different populations of *H. sorghi* were collected from four different maize growing states, Kulu, Chamba (Himachal Pradesh), Tihri (Uttarakhand), Ghaziabad (Uttar Pradesh) and Kud (Jammu and Kashmir) of India. The experiment was conducted in 15-cm-diameter earthen pots containing 1000 g steam-sterilized loamy soil and sand mixture in the ratio of 3:2. The seeds of maize cultivar (Prabhat) were first surface sterilized in 0.1% mercuric chloride for 5 minutes, rinsed in distilled water 3-4 times and then sown. Six days old seedlings were inoculated with freshly hatched second stage juveniles (J_2) of *H. sorghi* at an initial inoculum level of 2, 4 and 6 J_2/g soil. Besides, uninoculated seedlings without nematode served as check. Prior to nematode inoculations, average larval number of the known suspension was ascertained by counting five times aliquot of 1ml each. The juveniles were then placed as per treatment-wise, near the roots of seedlings after carefully removing the top layer of soil. The pots were arranged according to a randomized block design in a glass-house and there were five replicates per population. Plants were regularly watered throughout the experiment to record observation on plant growth characters. Sixty days after nematode inoculation, the soil from each pot was thoroughly mixed and a 250 g sub-sample was processed with Cobb's sieving and decanting technique (Cobb, 1918) using 20 and 60 mesh sieves and nematode multiplication was recorded. The nematode cysts were observed under a stereoscopic microscope and counted. The method described by (Mittal and Dhawan, 1989; 1991) was adopted to study the effect on plant attributes namely, plant height, fresh root weight and fresh shoot weight. The dry root weight and dry shoot weight were recorded by air drying at room temperature (28 °C). Only final cyst number (at 60 days) from (root + soil) was recorded in relation to the initial inoculums levels. Egg and juvenile's content/cyst (at the time of harvest) were recorded after inoculation.

Data analysis

The data obtained were subjected to analysis of variance (ANOVA) and means were compared detect the significant difference among the treatments.

Results and discussion

Plant growth characters

Results revealed that all the five populations (Kulu, Ghaziabad, Kud, Tihri and Chamba) significantly reduced the plant growth character except plant height (Table 1).

Table 1. Effect of different inoculum levels *of H. sorghi* on shoot height (cm) in maize cv. Prabhat

Initial inoculums level (J_2/g of soil)	Ghaziabad (UP)		Kud (J & K)		Kullu (HP)		Tehri (Uttarakhand)		Chamba (HP)	
	Mean	Decrease over check (%)	Mean	Decrease over check (%)	Mean	Decrease over check (%)	Mean	Decrease over check (%)	Mean	Decrease over check (%)
0	119.6	0	122.5	0	124.8	0	120.0	0	115.0	0
2	119.0	0.6	119.9	9.2	122.4	1.9	117.0	2.5	114.0	0.8
4	118.0	1.4	119.2	13.1	122.0	2.2	117.5	2.1	112.1	2.5
6	117.4	1.9	117.5	22.0	120.0	3.8	118	1.7	111.0	3.4
SEm (±)	1.07		1.20		0.66		0.97		0.89	
CD (P=0.05)	NS		NS		NS		NS		NS	

Present findings are in confirmation with Dhawan and Nagesh (1987) who found negative correlation between cyst nematode densities and plant growth characters. Significant reduction in fresh shoot and root weight was observed in maize due to all five population at the initial inoculation level of 4 and 6 J_2/g soil compared to uninoculated control which were significantly different from each other (Table 2 and 3). The increase in initial population density resulted in decrease in fresh shoot and root weight in maize. At highest inoculam level (6 J_2/g soil), maximum reduction (35.5%) in fresh shoot weight was recorded in Ghaziabad population followed by Kud (30.5%), Tehri (27.2%), Chamba (24.5%) and Kulu (22.0%) population (Table 2).

Table 2. Effect of different inoculum levels *of H. sorghi* on fresh shoot weight (g) in maize cv. Prabhat

Initial inoculums level (J_2/g of soil)	Ghaziabad (UP)		Kud (J & K)		Kulu (HP)		Tehri (Uttarakhand)		Chamba (HP)	
	Mean	Decrease over check (%)	Mean	Decrease over check (%)	Mean	Decrease over check (%)	Mean	Decrease over check (%)	Mean	Decrease over check (%)
0	40.5	0	43.2	0	46.6	0	44.7	0	42.2	0
2	39.5	2.7	42.0	2.8	45.0	3.4	43.5	2.7	40.0	5.2
4	30.7	25.0	38.0	12.0	40.1	14.0	37.4	16.4	36.3	13.9
6	26.2	35.5	28.0	30.5	36.3	22.0	32.6	27.2	31.9	24.5
SEm (±)	0.53		0.54		0.45		0.52		0.82	
CD (P=0.0)	1.55		1.56		1.76		1.50		2.42	

Similarly, maximum reduction (43.9%) in fresh root weight was recorded in Ghaziabad population (43.9%) followed by Kud (39.2%), Chamba (34.9%), Tehri (26.6%), and Kulu (19.8%) population (Table 3).

Table 3. Effect of different inoculum levels *of H. sorghi* on fresh root weight (g) in maize cv. Prabhat

Initial inoculums level (J_2/g of soil)	Ghaziabad (UP)		Kud (J & K)		Kullu (HP)		Tehri (Uttarakhand)		Chamba (HP)	
	Mean	Decrease over check (%)	Mean	Decrease over check (%)	Mean	Decrease over check (%)	Mean	Decrease over check (%)	Mean	Decrease over check (%)
0	35.7	0	39.0	0	41.3	0	40.8	0	34.9	0
2	35.0	2.0	38.2	2.2	40.0	3.07	39.5	3.2	33.8	3.3
4	26.1	39.8	33.6	13.8	36.5	12.8	35.1	13.9	27.5	21.3
6	22.0	43.9	23.7	39.2	33.1	19.8	30.0	26.6	22.8	34.9
SEm (±)	0.4		0.60		0.57		0.50		0.44	
CD (P=0.05)	1.36		1.76		2.23		1.46		1.29	

Table 4. Effect of different inoculum levels *of H. sorghi* on dry shoot weight (g) in maize cv. Prabhat

Initial inoculums level (J_2/g of soil)	Ghaziabad (UP)		Kud (J & K)		Kullu (HP)		Tehri (Uttarakhand)		Chamba (HP)	
	Mean	Decrease over check (%)	Mean	Decrease over check (%)	Mean	Decrease over check (%)	Mean	Decrease over check (%)	Mean	Decrease over check (%)
0	16.5	0	12.8	0	21.5	0	14.0	0	17.0	0
2	14.0	15.0	10.2	20.2	19.8	7.9	12.1	13.3	15.0	11.4
4	9.9	39.8	7.2	34.1	17.6	18.0	9.4	32.7	12.0	29.5
6	8.4	49.3	4.0	38.8	12.2	43.1	5.15	43.1	8.9	47.8
SEm (±)	0.47		0.48		0.27		0.19		0.59	
CD (P=0.05)	1.36		1.89		0.78		1.56		1.72	

Significant reduction in dry shoot and root weight of maize was recorded at the initial inoculum level of 2, 4 and 6 J_2/g soil in all five population compared to uninoculated control (Table 4 and 5). Increase in initial inoculum level resulted in significant decrease in dry shoot and dry weight of maize. At highest inoculum level (6 J_2/g soil), maximum reduction (49.3%) in dry shoot weight was recorded in Ghaziabad population (49.3%) followed by Chamba (47.8%), Tehri (43.1%) and Kulu (43.1%) population (Table 4).

Whereas maximum reduction (53.7%) in dry root weight was recorded in Ghaziabad population followed by Chamba (44.8%), Kud (43.9%), and Tehri (43.3%) population (Table 5).

Srivastava and Chawla (1990) also reported reduction in shoot weight and root weight in Maize due to *H. sorghi*. Similar findings on *Heterodera* sp. were recorded by Anamika (2015) and Mukesh *et al.* (2015). Adel Al- Abed *et al.,* 2013 confirmed reductions in root and shoot weights and growth of maize was directly correlated with increase in initial nematode population density. The negative correlation could be due to the competition for feeding sites and the greater damage to infected roots with increasing initial population.

Virulence results further suggested that an initial inoculums level of 4 J_2/g soil was considered as the minimum damaging threshold at which significant damage occur in reduction of plant growth characteristics. The present

Table 5. Effect of different inoculum levels *of H. sorghi* on dry root weight (g) in maize cv. Prabhat

Initial inoculums level (J_2/g of soil)	Ghaziabad (UP)		Kud (J & K)		Kullu (HP)		Tehri (Uttarakhand)		Chamba (HP)	
	Mean	Decrease over check (%)	Mean	Decrease over check (%)	Mean	Decrease over check (%)	Mean	Decrease over check (%)	Mean	Decrease over check (%)
0	19.4	0	12.2	0	11.5	0	12.4	0	12.7	0
2	18.2	6.2	11.0	10.1	10.2	11.5	10.0	20.0	12.0	5.7
4	14.3	36.2	8.5	30.4	7.8	32.6	8.6	30.7	9.0	30.0
6	9.0	53.7	6.9	43.9	4.4	42.2	7.0	43.3	7.0	44.8
SEm (±)	0.30		0.22		0.26		0.52		0.17	
CD (P=0.05)	0.89		0.87		0.75		1.51		0.51	

findings are similar to Srivastava and Chawla (1990) who reported significant reduction in the shoot length and fresh shoot and root weights of maize cultivar Deccan -103 at an initial inoculums level of 4 J_2 of *H. sorghi*/cm^3 of soil.

Cyst multiplication

The data on cyst multiplication in soil showed a positive correlation with increasing levels of nematode inoculum, there was an appreciable increase in cyst production with maximum 307, 278, 155, 260 and 215 being recorded at the highest level of nematode inoculum (6 J_2/g soil) in Ghaziabad, Kud, Kulu, Tihri and Chamba population, respectively (Fig. 1).

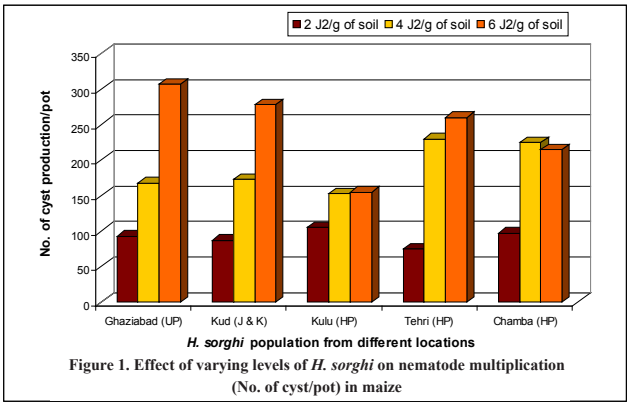

Figure 1. Effect of varying levels of *H. sorghi* on nematode multiplication (No. of cyst/pot) in maize

At each level of nematode inocula used, there was a marked increase in the population, suggesting that cyst multiplication is not density dependent. These findings are similar with the results of Srivastava and Chawla (1990) who reported the cyst population and nematode multiplication increased significantly with the increasing levels of inoculum. Mittal and Dhawan (1991) reported that a positive correlation was evidenced between initial nematode densities of *H. sorghi* and number of cysts formed and larval population in the soil. Dhawan and Nagesh (1987) also reported positive correlation between cyst multiplication and levels of initial inoculums of *H. avenae* on wheat and recorded highest cyst production at the highest inoculum level. Similar trend was also recorded by Srivastava and Sethi (1984) for *H. zeae* on maize.

Cyst content

Cyst contents (eggs and juveniles) showed a negative correlation with increasing levels of nematode inoculum; maximum being 288, 260, 255, 276 and 291 eggs and J_2/cyst at 2 J_2/g soil and minimum (232, 200, 231, 198 and 210 eggs and J_2/cyst), at 6 J_2/g soil in Ghaziabad, Kud, Kulu, Tihri and Chamba populations, respectively (Fig. 2).

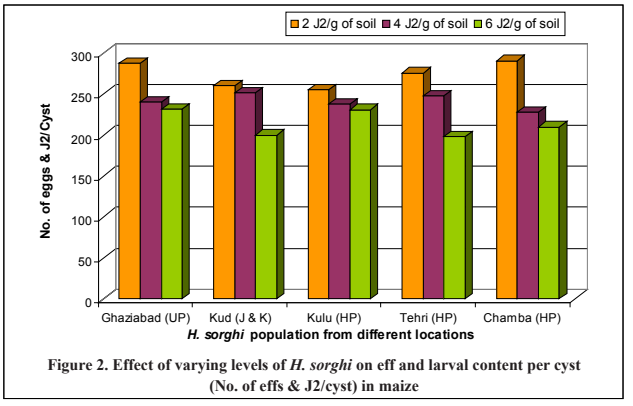

Figure 2. Effect of varying levels of *H. sorghi* on eff and larval content per cyst (No. of effs & J2/cyst) in maize

This was because of the fact that with the higher production of cysts, greater nutrient stress occurred which resulted decrease in the cyst contents. Findings are in conformity with results of Mittal and Dhawan (1991) who showed that the cyst contents (egg and larvae/cyst) had a negative correlation with maximum being at the lowest level of nematode density (1 larvae/cm^3 soil).

Based on above studies. Gaziabad population was found more virulent among the five populations on maize and may be helpful in developing region oriented management strategy.

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