

**STUDIES ON VARIABILITY IN THE POPULATIONS  
OF *Heterodera sorghi* (JAIN, SETHI, SWARUP  
AND SRIVASTAVA, 1982) FROM INDIA**

**RAJKUMAR**



**DIVISION OF NEMATODOLOGY  
INDIAN AGRICULTURAL RESEARCH INSTITUTE  
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OF *Heterodera sorghi* (JAIN, SETHI, SWARUP  
AND SRIVASTAVA, 1982) FROM INDIA**

By

**RAJKUMAR**

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**I.A.R.I.**

Approved by :

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Dr. K.K. Kaushal

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**Dr. K.K. KAUSHAL**  
**Principal Scientist**

**Division of Nematology**  
**Indian Agricultural Research Institute**  
**New Delhi-110 012, India**


## **CERTIFICATE**

This is to certify that the thesis entitled "**Studies on variability in the populations of *Heterodera sorghi* (Jain, Sethi, Swarup and Srivastava, 1982) from India**", submitted to the Faculty of the Post Graduate School, Indian Agricultural Research Institute, New Delhi, in partial fulfilment of the requirements for the award of the degree of **Doctor of Philosophy in Nematology** by **Mr. Rajkumar** embodies the results of *bonafide* work carried out by him under my supervision and guidance. No part of the thesis has been submitted by him for any other degree or diploma.

I further certify that any help or information received during the work on this thesis has been duly acknowledged.

Place : New Delhi

Date : 9 March, 2006

  
**(Krishan Kumar Kaushal)**  
Chairman  
Advisory Committee

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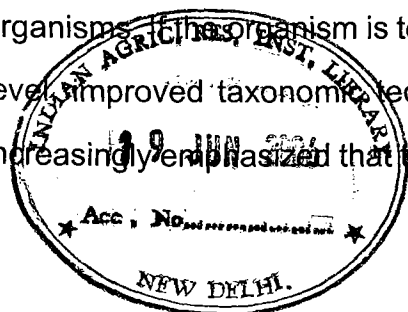
# 1. INTRODUCTION

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The sorghum cyst nematode, *Heterodera sorghi* was identified and described by Jain *et al.*, (1982) from the rhizosphere of sorghum in Ghaziabad district of Uttar Pradesh and subsequent reports from Haryana (Dhawan *et al.*, 1983); Delhi ; Punjab (Sakhuja and Singh, 1985), Himachal Pradesh and Jammu Kashmir (Srivastava and Kaushal, 1986); Andhra Pradesh (Sharma and Sharma, 1998) and Maharashtra (Darekar *et al.*, 1990) have been made. It is known to be associated with sorghum (*Sorghum vulgare*), maize (*Zea mays*), barley (*Hordeum vulgare*), oats (*Avena sativa*), rice (*Oryza sativa*) and wheat (*Triticum aestivum*).

During the last twenty-four years, information has been generated on biology, pathogenecity, host range and ecology (Srivastava, 1985; Srivastava and Sethi, 1987, 1988; Srivastava and Chawla, 1990, 1991; Mittal and Dhawan, 1989, 1991; Chawla and Srivastava, 1995, 2005). But, very little attention has been paid to the biosystematics aspects of this species, which in fact is extremely important for formulating eco-friendly management strategies against this species in sustainable agriculture. Most of the non chemical methods of nematode control (crop rotation, use of resistant varieties, physical and cultural practices, use of bioagents and quarantine regulations) require specific information on host requirement, geographical distribution, temperature tolerance and other biological and ecological features of the target organisms. If the organism is to be accurately identified at species and race level, improved taxonomic techniques are indispensable. It is now being increasingly emphasized that taxonomist must use data form every possible



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source, not only morphological but also ecological, biological, cytological, biochemical and molecular level etc. in order to develop classification and identification schemes of high predictive values (Ferries, 1994; Baldwin and Luc, 1995). But most of these features are often influenced by the environmental factors.

Different environmental factors viz. temperature, moisture and host crops are known to influence the biology and duration of life cycle of this nematode (Srivastava and Chawla, 1991). Morphology, the widely accepted tool in taxonomy may also exhibit variations due to the environmental factors. Such variations have been reported for several plant parasitic nematode species including cyst nematodes such as *Heterodera avenae*.

Intraspecific variations in the naturally occurring field populations are also known for *Ditylenchus myceliophagus* (Fortuner, 1982), *Helicotylenchus pseudorobustus* (Fortuner *et al.*, 1984), *Meloidogyne arenaria* (Cliff and Hirschmann, 1985). Besides morphological features, different geographical populations may exhibit variations in their biological, ecological, physiological or biochemical features etc. which have been reported for several phytonematodes species by various authors (Bird and Wallace, 1966; Das, 1960; Triantaphyllou, 1985; Susser and Carter, 1985; Dropkin, 1988; Hussey, 1990; Mathur *et al.*, 1974; Swarup *et al.*, 1979).

Presently, *Heterodera sorghi*, is known to occur in most of the states of India, except in North Eastern regions and some states in the penninsular India. The widespread distribution in varied agro-climatic conditions showed its highly adaptive capabilities for survival. The morphological variability in the field populations of *H. sorghi* from different geographical regions has not been studied in India. Further, information is also lacking on physiological

specializations and virulence of these populations and also in histopathogenesis induced by *H. sorghi*.

Keeping these lacunas in views, the present investigations were undertaken to :

- (i) study the morphological variations and physiological specializations among different populations of *H. sorghi*
- (ii) study the virulence of these populations on maize
- (iii) study the histopathological changes induced by *H. sorghi* infection in maize roots.

## 2. REVIEW OF LITERATURE

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Literature provides immense wealth of information not only for referral purpose but also for perfection of knowledge and refinement of any work. It also serves as a database for initiating any type of innovative work or for proposing a hypothesis on the basis of historical evidences laid down by previous workers.

For many reasons especially due to the continued addition of large number of taxa and lack of information about intra-specific variability, nematode classification has remained in a fluid state. A better understanding of the extent of intraspecific variability in naturally occurring populations has a great importance in bringing more objectivity in utilization of taxonomic parameters, which are at present not properly defined. Proper identification of nematodes species and populations has importance both in taxonomy and plant protection. Plant parasitic nematodes cause damage to the tune of more than 100 thousand million US \$ annually. Such a huge loss can be minimized, if the causative agent is known much early for advisory works. Nematode management through crop rotation and resistant cultivars is generally specific or race specific. Therefore, it is essential to know the exact identity of the species and host range of the nematode population being tested before development or introduction of resistant cultivars. Similarly crop rotation recommendations can't be made unless the identities of the prominent nematode species or the races are known. If the identity of a population is unknown, the crop selected for use in a rotation scheme may cause dramatic increase in nematode population.

Any taxonomic approach begins with the study of populations as species are composed of widely variable populations. Study of variability gives us an idea of divergence and evolution in populations and the exchange of genetic material between the populations. This also gives us an idea on the reasons for a particular host preference, changes occurring in the phenotype and genotype of a population as a result of geographical isolation.

The review of literature pertaining to the present investigation on cyst nematodes has been documented under following heads :

1. Variation in morphology
2. Variation in physiological specializations
3. Influence of *Heterodera* species on plant growth
4. Histopathological studies.

### **Variation in morphology**

The first cyst nematode was reported on sugar beet and named as *Heterodera schachtii* (Schmidt, 1871). By the turn of the century it became apparent that cyst nematodes caused similar effect on the other crops also with the notation that the sugar beet nematode was involved in every case. Cyst nematodes discovered on potato and wheat were called as the races or variety of *H. schachtii*. This trend was changed with the discovery of *H. goettingiana* as it did not affect sugar beet, a recognized host of *H. schachtii* at that time. The characters such as size, volume, colour of the cyst and presence of sub crystalline layer were used to differentiate it from *H. schachtii*. Following the recognition of host specificity, the potato cyst

nematode, *H. rostochiensis*, the oat cyst nematode, *H. avenae*, the clover cyst nematode, *H. trifolii*, and the hop cyst nematode, *H. humuli*, Filipjev, 1934 were distinguished. And also used characters like, spherical body, long neck, short juvenile stylet and dimensions of the eggs to separate *H. rostochiensis* and long neck, shorter stylet than *H. rostochiensis* to separate *H. avenae* from *H. schachtii*. Thorne (1928) described the grass cyst nematode, *H. punctata* from Canada on the basis of spherical shape of the cyst. Franklin (1951) published a monograph on the cyst-forming nematodes wherein she identified 10 species. Though host range remained the main criterion for the designation of *Heterodera* species for several years few workers also laid stress on morphological differences. Franklin (1951) summarized the diagnostic characters used by earlier workers, including the size and shape of the cyst, the presence or absence of regularly arranged punctations on the cyst wall, sub crystalline layer, egg-sac or yellow cyst phase, the pale colour of *H. humuli*, the anal fenestration of *H. punctata*, some of the dimensions of the juvenile and for *H. goettingiana*, the tridentate distal end of the spicules of the males. Oosterbrink and den Ouden (1954) successfully used the shape of the stylet knobs of the juveniles as diagnostic characteristics. They introduced the characters of the cone-top of lemon shaped cysts for the first time for characterization of the species. They also demonstrated the taxonomic significance of the fenestrae in the cone terminus as on the basis of the length of the fenestrae, they differentiated *H. schachtii*, and *H. trifolii*. Hirschmann (1956) used the distance of the dorsal gland orifice from the base of the stylet to distinguish *H. glycines* from *H. trifolii*. Similarly, Oosterbrink (1960) used this character for differentiating *H. glycines* from *H. galeopsidis*.

Granek (1955) made use of the anus-vulval distance relative to the diameter of the vulva opening to separate *H. tabacum* from *H. rostochiensis*. He later substituted the vulva opening with light thin areas or fenestra in the cyst. Hesling (1973) found it be more accurate and suggested that length rather than diameter of the fenestra should be used as was done by Mulvey (1959). So this ratio can be derived by dividing the distance from the anus to the nearest edge of the fenestra by the length of the fenestra. This ratio is of much value in separating certain species like *G. rostochiensis* (4.5) and *G. achilleae* (1.6).

Cooper (1955) was the first person to introduce a variety of terminology for various structures in the vulval cone of the cyst which were as follows :

**Cone** : The tapered terminal end or the protuberance in the posterior end of the cyst. The species without a cone top are classified as having a terminal area.

**Semifenestra** : The translucent region or the thin-walled area (mostly circular or a half circle) at the posterior - most region of the vulval-cone present on either side of the vulva.

**Underbridge** : Structure composed of muscles bands, which supports the whole vulval cone. It is usually present below the fenestra and runs parallel with the vulval bridge.

**Vulval bridge** : A line of contact between the two semi-fenestrae containing the vulval slit. The width of the vulval bridge differs significantly in the bi-fenestrate cones.

**Bullae** : The knob-like, dark brown structures around vulva and anus inside the vulval cone, situated below the fenestral level or at the level of and below the underbriduge. They vary in size, shape and numbers. Cysts with bullae are termed bullate and without them as abullate. Bullae in *Heterodera zae* are finger shaped and called as cross bullae.

**Basin** : The thick area (in a circular band) surrounding the fenestrae, distinctly present in some of the species.

**Fenestration** : There are three types of fenestration present in the cysts : Circumfenestration, Ambifenestration and Bifenestration.

**Circumfenestration** : It is the type of fenestration where there is only a single fenestra present in the cyst. It is present in round cysts like *Globodera*, *Punctodera* and *Cactodera*.

**Ambifenestration** : It is the type of fenestration where close semifenestrae are separated by a vulval bridge in which is embedded the vulval slit. The vulval bridge may be short and wide (*H. avenae*, the cereal cyst nematode) or it may be long and slender (*Heterodera zae*, the maize cyst nematode and *H. sorghi*, the sorghum cyst nematode). The fenestrae are mostly 'D' shaped in these cyst.

**Bifenestration** : It is the type of fenestration where two well-shaped fenestrae occur side by side. They are not 'D' shaped but circular. Such type of fenestration is present in *H. humuli*, the hop cyst nematode. It is only after 1959 that cyst-forming species with circumfenestration and having round shape were included in the separate genus, *Globodera* (Skarvilovich, 1959) Behrens, 1975; those with additional fenestration around the anus in the genus, *Punctodera* (Mulvey and Stone, 1976); those with slight

protuberance in the genus, *Cactodera* (Krall and Krall, 1978), those with vulva in the contour of the body in *Dolichodera* (Mulvey and Ebsary, 1980) and the rest lemon shaped, ambi-/bi-fenestrated cysts remained in the genus, *Heterodera*.

Various diagnostic characters used in identification of cyst nematodes include the shape of the cyst, which is of taxonomic value in identification of several species. Cyst belonging to the genera *Globodera*, *Cactodera*, *Punctodera* and *Dolichodera* are mostly round in shape and the cysts of genera *Heterodera* and *Afenestrata* are mostly lemon in shape. Size of the cyst is a variable character with populations. *Globodera rostochiensis*, *Globodera pallida*, *Heterodera avenae*, *Heterodera moths* and *Heterodera sorghi* have larger cysts whereas *Heterodera zae*, *Heterodera cajani*, *Heterodera graminis* and *Heterodera skohensis* have smaller sized cysts. Colour of the cyst, presence or absence of sub crystalline layer and patterns on cyst wall are other characters that can be used for identification. All these methods described can only be used at the species or generic level on supplementary characters and is of not much use in population studies as such. The characters that can be used include vulval slit length fenestral shape, presence or absence of bullae and underbridge, stylet length and total length of  $J_2$ , shape and size of stylet knobs, tail length and hyaline portion of the tail (Mulvey, 1972; Mulvey and Golden, 1983).

### **Cone top structure**

Use of cone top structures to differentiate species and genera of cyst nematodes was reported by Mulvey (1972). He studied the posterior ends of the cysts of 39 species of *Heterodera* and arranged them into 5 major groups based on variations in cone top structures and cysts (Mulvey, 1972).

The structure of the vulval cone and associated internal and external characters have been used reliably for the precise identification of cyst nematodes species and in studying intra-specific variations. Vulval cone of cyst nematodes comprises the vulval slit, vulval bridge, underbridge, fenestrae, anus and surrounded by folds of primary and secondary bullae. In case of *Heterodera zae* and *H. cajani*, the bullae are finger shaped. Commonly the species with a strong underbridge have few (*H. sorghi*, *H. mothi*) or no bullae (*H. graminis*) whereas species with prominent and numerous bullae have no bridge (*H. avenae*) or a weak underbridge (*H. filipjevi*). In species like *H. zae* and *H. cajani* which have not many bullae but strong and finger-like bullae are provided with a medium sized underbridge which is equal to the length of vulval bridge. Mulvey (1959) recorded another bridge-like structure crossing the vulval cone in newly formed cysts of *H. trifolii*. This bridge is possibly hardened muscle tissue, run at right angle to the underbridge and was found just below the level of fenestra. Although no two vulval patterns are exactly alike i.e. the cone top structure of individual and populations within a species vary, and this is exploited for population studies. But the basic species character does not change significantly.

**Females** : Diagnostic characters used in case of females of the cysts include shape, size, length of the stylet, position of the excretory pore etc. are taken into consideration while examining them for identification. The width and shape of the stylet knobs are other characters, compared between species.

**Males** : They have been used little in the identification of cyst nematodes. The reason may be the absence of males in some species and also difficulty in obtaining them. Males have rarely been reported in *Heterodera zae*

(Koshiy *et al.*, 1970; Golden and Mulvey, 1983). Similarly, in *H. sorghi* also they have rarely been reported. Nevertheless, the following characters are of some morphological importance : body length and width, head set-off or not, stylet length and shape of the knobs, excretory pore position, spicules length and their tip structure and finally tail shape and size.

**Juveniles** : They are second only to cyst in their importance in identification and systematics. The characters in juveniles that have great systematic values are the stylet length, genital primordium-position, tail length, position of anus and length of hyaline portion of the tail, total body length and width. These are highly variable characters and are thus of high taxonomic value in differentiating species and populations (Mulvey and Golden, 1983).

**Eggs** : The length and width of the egg and L/W ratio are few characters which may vary from species to species and even within a population and thus are of little diagnostic value.

### **Variation in physiological specializations**

Plant being susceptible or resistant to any specific pathogen depends on the source of the seed and the isolates of the organism involved. A particular cereal plant or cultivar may be resistant to one population of *H. sorghi*, but susceptible to and be damaged by another population of *H. sorghi*. Thus, each of these two populations represents a different biological type.

Duggon (1958) conducted population studies of *H. avenae* and suggested the presence of two strains, one specific to cereals and the other to grasses. Andersen (1959, 1961) also found marked difference between Danish field populations in their pathogenicity towards barley and oats

varieties and distinguished two biotypes of the nematode, namely, Race 1 which was non-aggressive and unable to produce cysts on barley varieties , Drost and Alfa, and Race 2 which produced cysts on Drost and Alfa as also on Major and Herta varieties. He claimed that continuous growing of Drost and Alfa varieties in a field of non-aggressive individuals would reduce the populations but some may produce cysts and the multiplication of these would change the population to a more aggressive one. Cotton (1962, 1963) demonstrated differences in pathogenicity among British populations of *H. avenae* and suggested the presence of different biotypes. Gair *et al.* (1962) found barley 191 to be an inefficient host as far as two British populations were concerned. Similarly, Fiddian and Kimber (1964) reported the presence of two biotypes of the cereal cyst nematode in England and Wales similar to those found by Andersen (1965) in Denmark. Subsequently, many workers reported different number of biotypes in various countries (Koer *et al.*, 1964; Lucke, 1969; Andersen and Andersen, 1970; Cook and William, 1972; Rivoal, 1975). Howard and Cotton (1976) considered the complexity of the pathotype situation in Europe to be more due to the non-standardization of testing methods. They observed that the population fall into three categories (i) Pathotype 1 (Netherland A) against which a number of West European cultivars, including Fero, Drost and Alfa are resistant, (ii) Pathotype 2 (Netherland C) consisting of populations which are able to break the resistance of Fero, Drost and Alfa but unable to produce on No. 191, and (iii) Pathotype 3 which can produce on No. 191, CI 8334 and CI 3902.

Cook (1975) opined that Swedish populations resembled British pathotype 3 in cyst morphology but differed from the above population in other respects. The Netherland Race B can also reproduce on No. 191 but

not on the other two cultivars. Hayes and Cotton (1971) reported Harlan 43 as resistant to British pathotype 3. According to Saynor (1975) in Britain, pathotype 2 is more prevalent than pathotype 1 which occurs in mixture with pathotype 2 and pathotype 3 is very rare. In Denmark, pathotype 1 and 2 occur most commonly.

In India, Mathur *et al.* (1974) have reported the presence of five biotypes while Swarup *et al.* (1979) reported only two biotypes in the Indian population of *H. avenae*.

Kaushal and Seshadri (1987) studied morphological variations of five different populations of *Heterodera avenae* (Ludhiana, Narnaul, Jaipur, Udaipur and Ghaziabad) and revealed that in spite of variations obtained in some of the characters among five geographically different populations of cereal cyst nematode, they were found to belong to the species, *H. avenae*.

### **Influence of *Heterodera* species on plant growth**

Hesling (1957) studied the effect of different inoculum levels of *Heterodera avenae* on wheat and reported a marked reduction in plant growth with increased eelworm infestation.

Singh (1967) found a population density of 10,000 larvae of *H. avenae* per 500 cc of soil as marginal damaging level for measurable effects on barley and wheat. The effect of initial levels of inoculum on nematode infestation was found to be density dependent.

Brzeski *et al.* (1970) reported that approximately 10 cysts per g of soil could result in patchiness under English conditions. Yields of cabbage and other brassicas were also reduced when heavily infested by *H. schachtii*.

Miller (1971) studied the pathogenic effect of the horse nettle cyst nematode, *H. virginiae* on Var. 312 dark fired tobacco and found that the average diameter at the base of stalk from non-infested plots was 37 mm and 21 mm from infested plots, dry weight of roots from non-infested plots was 46 g and 20 g from infested plots and dry weight of all above ground parts was 205 g from non-infested plots and 76 g from infested plots.

Meagher and Chamber (1971) while studying the effect of *H. avenae* on wheat reported non-pathogenic effects on height and weight of plants till 60 days after nematode inoculation. However, other plant parameters like number of tillers and foliage fresh weight were significantly reduced.

Gill and Swarup (1971) found 20 larvae/g of soil as the marginal damaging level for measurable effects on wheat and barley, using cysts as the source of inoculum. However, when second stage larvae were used, this effect was even noticeable at the level of 2 larvae/g of soil.

Mai *et al.* (1972) conducted studies on the population levels of *H. schachtii* and damage to red table beet and cabbage. They found that by using high inoculum levels of *H. schachtii* (9772, 4886 and 2443 eggs and larvae per 500 ml soil) top and root weights of red table beet were drastically reduced after six weeks. Weights of cabbage plants grown in the same conditions were reduced by the higher level of inoculum.

Bhatti and Sasser (1973) studied the pathogenicity of *Heterodera lespedezae* on *kobe lespedeza*. They found that younger the seedlings, greater was the adverse effect on plant growth. Two weeks after inoculation the growth of *kobe lespedeza* seedlings was significantly less as compared to the uninoculated plants. There was no appreciable difference between the height of inoculated and uninoculated plants within the same age groups.

In studies with wheat in Australia, Meagher, (1968) found 20 and 40 per cent reduction in yields at *H. avenae* population level of 2 and 16 eggs/g soil respectively.

Sharma and Sethi (1976) found that 10 larvae of *H. cajani* as the initial inoculum per gram of soil was sufficient to cause reduction in the shoot length whereas 100 larvae were required for reducing the root and shoot weights of cowpea variety Pusa Barsati.

Gaur and Singh (1977) studied the pathogenicity of *H. cajani* associated with the moong crop. They found that plant height and fresh weight of shoots was significantly poorer than that of plants grown in nematode free soil.

Kalha and Edward (1979) studied the pathogenicity of some isolates of *H. cajani* on local and T9 urd (*Phaseolus mungo* Roxb.) varieties. They found that there was no significant difference in plant growth as measured by root and shoot length and their fresh and dry weights or pod yields between the infected and non-infested controls.

O'Brien and Fisher (1981) considered the effect of density (0, 150, 300 and 450 juveniles) and time of inoculation (0, 7 or 14 days after sowing) of *Heterodera avenae* on the growth of single plant of a susceptible wheat line and observed that only inoculation at sowing consistently affected growth and there as little difference between densities.

Shiabova (1982) studied the pathogenicity of *H. avenae* on wheat and reported an infection level of 2 larvae per g soil. At 10 and 20 larvae per g of soil, the yield may be reduced by 50 per cent.

Maas and Brinkman (1982) studied the biology and pathogenicity of the yellow beet cyst nematode, *H. trifolii* on sugarbeet. The threshold damage was found to be about 5 eggs/cc of soil for sugarbeet with yield reduced by about 35 per cent at 50 eggs/cc of soil. They also found that strands of sugarbeet and legumes were better on plots with small than with large initial nematode population densities; correspondingly smaller number of nematodes were found in the roots in the former than in the latter plots. At 20°C, rate of development of both nematodes was the same, but the multiplication rate of the yellow beet cyst nematode was much higher than that of white beet cyst nematode. The measured threshold level at which damage occurred was about 5000 eggs/litre of soil for sugarbeet.

Besides, life cycle, pathogenicity of grass cyst nematode, *H. bifenestra* was studied by Mass and Heijbroek (1982). They reported that the yields of perennial rye-grass, meadow grass and westerwolds rye-grass were negatively correlated with concentration of inoculated nematodes, the cumulative yields of grasses were 50, 60 and 70 per cent on uninoculated soil for westerwolds rye-grass, meadow grass and perennial rye-grass respectively.

Meagher (1982) reported that plant height, foliage weight, leaf area and length of primary roots were inversely related to population density of *H. avenae* on cereal crops but even the lowest population density (0.5 larvae/g of soil) caused a significant reduction in the mean length of primary roots. The parameters like foliage weight and leaf area provided a more accurate reflection of growth reduction than plant height, as nematode infestation resulted more in reduction of stem diameter and leaf production. In particular, leaf area of healthy seedlings was about 2.5 times greater than that of most

heavily infested plants. Similarly, Simon and Rovira (1982) also recorded a marked reduction in the plant growth with increased infestation of cereal cyst nematode, *H. avenae*.

Gokte (1982) carried out studies on the relationship between initial inoculum levels and reproduction of *H. avenae* on barley. She found that the number of mature female of *H. avenae* in susceptible cultivars was obviously more at the highest level (10,000 larvae/500 g of soil) than at the lowest level, but the total multiplication factor was more at the lowest level than at the highest level. At the same time, the developmental rate was slower at the two lower inoculum levels (100 and 1000). Although more cysts were formed at highest level, a decreasing trend in egg content was evident from lowest to highest level. Multiplication factors were highest (19) at the lowest level and least (1.9) at the highest one.

Babatola (1983) studied the pathogenicity of *H. sacchari* on rice. He found that rice plant growth and tillering was adversely affected at 18, 36 and 72 cysts/litre of inoculum levels. Infested plants were very chlorotic and had retarded growth. Tiller numbers were reduced and grain yield was significantly lower. Rice roots were very necrotic and blackened. Dry straw weight, dry panicle weight and grain yield were significantly lower in the upland conditions, than in the lowland conditions.

Artokhina (1984) while working on the pathogenicity of *H. medicaginis* on Lucerne reported reduced yields at infestation levels of less than 1000 larvae/100 cm<sup>3</sup> of soil. Thus, at 1500 larvae/100 cm<sup>2</sup>, the green mass yield was 34 per cent less than in uninfected plots in the first years and 6 per cent less in the second and third years. The pathogenicity of *H. medicaginis* was also affected by sowing times and was increased when Lucerne was

sown under spring barley or maize. The pathogenicity threshold for seed production and germination was lower (750-800 larvae/100 cm<sup>3</sup>) than for forage production (2000 larvae/100 cm<sup>3</sup>).

Srivastava and Sethi (1984) conducted pathogenicity tests with *H. zae* on two cultivars of maize namely, composite Shakti and hybrid Histarch. They revealed that an initial inoculum of 100 larvae and above per kg of soil for Pusa (Bihar) population and 1000 larvae for Udaipur population significantly reduced various plant growth characters in comparison to uninoculated check plants. These inoculum levels were considered as the minimum damaging threshold. The host infestation and nematode multiplication were density dependent with maximum cyst production and nematode reproduction occurring at 1000 inoculum level.

Dhawan and Nagesh (1987) observed a significant negative correlation between varying levels of nematode and plant growth. Significant decrease in plant growth was recorded at 60, 90 and 130 days. The threshold level was found to be 5 eggs & juvenile/g soil. A positive correlation was recorded with number of larvae penetrating root, development of larvae inside the root and number of cysts formed. Cyst contents, however, showed a negative correlation.

Srivastava and Chawla (1990) observed significant reduction in the shoot length and fresh and dry shoot and root weights of maize cultivar Deccan-103 at an initial inoculum level of 4 J2 of *H. sorghii*/cm<sup>3</sup> of soil and hence, this inoculum level was considered as the minimum damaging threshold level for this host. The cyst population and nematode multiplication increased significantly with the increasing levels of inoculum. Though, the

maximum cysts and total nematode populations were recorded at the highest level of inoculum i.e. 16 J2/cm<sup>3</sup> soil.

Mittal and Dhawan (1991) studied the effect of varying densities of *H. sorghi* on sorghum and revealed a negative correlation between nematode densities and plant growth characters. Fresh weight of shoot and root decreased significantly at the initial inoculum level of 8 larvae/cm<sup>3</sup> soil. A positive correlation was evidenced between initial nematode densities and number of cysts formed and larvae population in soil. Cyst contents (egg & juveniles/cyst) showed a negative correlation with maximum being at the lowest level of nematode density (1 larva/cm<sup>3</sup> soil) and minimum at the highest level (16 larvae/cm<sup>3</sup> soil). Threshold damaging level was found to be 8 larvae/cm<sup>3</sup> soil.

### **Histopathological studies**

Many investigations have been made on the ultrastructural changes induced in the plant hosts by cyst nematodes. Second-stage juveniles (J2) enters the roots, moving intracellularly through the cortical cells and then usually fix their lip region on one endodermal cell from which they can stimulate a syncytium. The maturation of the nematode is directly related to the development and persistence of syncytium (Acedo *et al.*, 1984).

Endo (1964) while studying the anatomical changes in soybean roots (var. Lee) resulting from infection by *H. glycines* observed that in 2-6 days, syncytia were well established in contact with J2 and J3 especially at or near protoxylem restricting the formation of secondary phloem and xylem. In nine days, syncytia associated with males showed signs of degeneration whereas syncytia associated with female appeared healthy up to an beyond

egg deposition at 21 days. During the period of reduced feeding by mature female, parenchymatous cells grew invasively into regions once occupied by degenerated and shrunked syncytia. These findings were further supported by observations made through light microscopy (Endo, 1965; Endo and Veech, 1970; Veech and Endo, 1970; Acedo *et al.*, 1984), transmission electron microscopy (Gibson *et al.*, 1971; Riggs *et al.*, 1973; Endo, 1978, Kim *et al.*, 1987) and scanning electron microscopy (Jones and Dropkin, 1976).

Endo (1978) observed that feeding plug is formed at sites where stylet of nematode pierce through the cell wall, a plug of material forms around the stylet and extends as a collar into the cell cytoplasm. Many workers (Jones and Nothcote, 1972; Jones and Gunning, 1976) observed the fine structure of syncytia induced by cyst nematodes, which contained an increased number of organelles compared to normal cells from which they are derived. At an advanced stage of infection, walls of the syncytia adjacent to xylem vessels are modified into finger-like in growth that resembles those of transfer cells.

Several authors have given detailed description of the ultrastructural features of the syncytia induced in different susceptible and resistant hosts by *Heterodera* species (Rice *et al.*, 1985; Kim *et al.*, 1986; Bleve-Zacheo *et al.*, 1987), following the progressive changes in the root cells starting from 42 h after nematode inoculation (Riggs *et al.*, 1973). In affected cells, J2 were observed to stimulate syncytia formation about two days after initial penetration and no differences were detected between susceptible and resistant roots during the following two days apart from more extended necrosis around the outer limits of syncytia in resistant hosts. Seven days

after penetration the syncytia components of the susceptible roots had expanded and contained dense and granular cytoplasm in contrast to those in resistant roots where the cytoplasmic content were extremely vacuolated and reduced to a thin layer around the cell walls (Rice *et al.*, 1985; Blevé-Zacheo, 1990; Melillo *et al.*, 1990).

Endo (1991) studied the early response of susceptible and resistant cultivars to infection by *H. glycines* and observed the accumulation of smooth and rough endoplasmic reticulum, interspread among numerous mitochondria, plastids and other organelles, within the thick walled initial syncytial cell (ISC). These ISC and adjacent cells forming the syncytium showed slight enlargement at 18 h after inoculation but had disruptions in their connecting cell walls. Syncytia in susceptible and resistant cv. 18 h and 4 days after inoculation were hypertrophied with hyperplasia, with expanded regions of cell wall dissolutions.

### 3. MATERIALS AND METHODS

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During the course of investigation to assess the variability between populations of *Heterodera sorghi*, five populations were collected from maize fields in different states of Northern India. Following materials and methods were used to achieve the various objectives of the present investigation.

#### 3.1 Collection of *Heterodera sorghi* populations

Sorghum cyst nematode, *Heterodera sorghii* populations were collected from the rhizosphere of maize crop from three different states in North India. The details of the five populations collected are given below :

Locality	State
Chamba	Himachal Pradesh
Kulu	Himachal Pradesh
Tihri	Himachal Pradesh
Ghaziabad	Uttar Pradesh
Kud	Jammu and Kashmir

#### Source of Seed

Seeds of sorghum (*Sorghum bicolor* (L.) Moench) cultivars / genotypes were obtained from the sorghum entomologist, Division of Entomology and seeds of maize, *Zea mays* cultivars / genotypes were procured from Project Director, Directorate of Maize Research, IARI, Pusa Campus, New Delhi.

### **Maintenance of pure culture**

Pure culture of sorghum cyst nematode, *Heterodera sorghi* was raised on maize (cultivar Shaktiman) in the Division of Nematology glass house. The pure culture of different populations was maintained in isolation till sufficient number of cysts was obtained for various trials.

### **Sterilization of sand and soil mixture**

Field soil sieved through 16-mesh sieve, and sand was autoclaved separately at 1.0546 kg pscm for four hours. Subsequently, the soil and sand were exposed to open air for at least 24 hours before using for different trials.

### **Collection of cysts and larvae**

For obtaining cysts in bulk, the soil from pot cultures was processed and sieved by using 30 and 60 mesh sieves. Cysts were subsequently hand-picked under a stereoscopic binocular microscope from the residue collected on 60 mesh sieve. Numbers of larvae, required for different trials, were obtained from healthy cysts placed in 5 cm petri dishes, containing water in the incubator at 28-29°C.

### **Morphological variations of populations**

The morphometric variations of various stages of *Heterodera sorghi* were studied using the compound research Leica DMRB microscope having drawing tube attachment. The Camera Lucida sketches of the selected characters were made.

### **Morphometric variations of eggs**

A single mature cyst was taken and eggs were removed from the cysts. Twenty five eggs from each population were measured for length, width and length width ratio was calculated.

### **Second stage juveniles**

Few mature cysts from each population were placed in fresh water at room temperature and the juveniles (J2's) were allowed to emerge. After about a week the freshly emerged J2's were picked from the suspension and concentrated. The concentrated suspension from each population was killed separately in hot water bath and fixed in 5% formaldehyde solution. Fixed juveniles were processed in glycerin using the method of Seinhorst (1959) and their permanent mounts were prepared in dehydrated glycerin. These permanent mounted specimens were then used for studying the following morphometric characters.

### **Morphometric characters of second stage juveniles (J2)**

Body Length	-	L ( $\mu\text{m}$ )
Maximum body width	-	W ( $\mu\text{m}$ )
Length of stylet	-	S ( $\mu\text{m}$ )
Head width	-	HW ( $\mu\text{m}$ )
Head height	-	Hh ( $\mu\text{m}$ )
Dorsal esophageal gland opening - D.O.G. ( $\mu\text{m}$ )		
Distance from the head to the excretory pore 0 D-H - Ex ( $\mu\text{m}$ )		
Distance from the head to the median bulb valve - D-H - Mb ( $\mu\text{m}$ )		
Distance from head to esophageal gland lobe -D-H - Og ( $\mu\text{m}$ )		
Tail length ( $\mu\text{m}$ )		

Hyaline tail length ( $\mu\text{m}$ )

Anal body width ( $\mu\text{m}$ )

Total body length / Maximum body width = "a" ratio

Total body length / Head to esophageal gland = "b" ratio

Total body length / tail length = "C" ratio

### **Cone top structure**

Cone top structures of mature cysts from each of the five populations were studied in detail. The posterior portion i.e. cone top of 25 cysts of each population were cut with the help of a sharp blade and carefully trimmed so that the cone top structure was at the center of the piece. The tissues adhering to the structure were removed carefully using a pick. The cone top structure was then dehydrated in alcohol and allowed to clear in clove oil for some time and then permanently mounted in canada balsam. The permanent mounts were later subjected to detailed microscopic study. The following morphometric observations were recorded.

Vulval slit length ( $\mu\text{m}$ )

Vulval bridge length ( $\mu\text{m}$ )

Vulval bridge breadth ( $\mu\text{m}$ )

Underbridge length ( $\mu\text{m}$ )

Length of fenestra ( $\mu\text{m}$ )

Breadth of fenestra ( $\mu\text{m}$ )

Distance from the anus to fenestra ( $\mu\text{m}$ )

### **Statistical analysis**

The statistical methods were applied to assess the variability in different morphometric characters and to assess their taxonomic significance.

Morphometric characters of J2, eggs, and cone top observations for each of the population were analyzed statistically to compute their respective range, arithmetic mean, coefficient of variation and standard deviation.

### **Physiological variations of populations**

For physiological specialization studies 10 cultivars / genotypes of sorghum and maize were taken as follows :

#### **Sorghum cultivars**

1. Desi Chari
2. Badi Jowar
3. Safed Jowar
4. Pgn-26
5. Pfgs-97
6. Pfgs-98
7. Kc-1
8. Pfgs-100
9. GJ-39
10. SU-1

#### **Maize cultivars**

1. Karnal (536)
2. Karnal (295)
3. Karnal (323)
4. Karnal (1105)
5. Karnal (1344)
6. Karnal (1352)
7. Karnal (163-1-2)

8. Karnal (1348-6-2)
9. Karnal (CM-141)
10. Karnal (193-1)

Sorghum and maize cultivars / genotypes, (each of 10) were evaluated for physiological specialization in 10 cm earthen pots containing autoclaved sandy loam and sand mixture in the ratio of 3:1. Initially, four seeds of each cultivars were sown in each pot and after germination these were thinned down to one seedling per pot. Ten days old seedlings were inoculated with 4 J2/cc soil. For each cultivar, fifteen inoculated plants were kept. A total of 300 pots were kept for both the crops.

#### **Virulence study**

Pre-soaked and germinated seeds of maize (cv. Prabhat) were planted singly in 15 cm earthen pots filled with a mixture of autoclaved sandy loam soil and sand in the ratio of 3:1. Six days old seedlings were inoculated with three levels of inoculum namely, 2, 4 and 6 J2/cc 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 1 ml each. The juveniles were then placed as per treatment - wise, near the roots of seedlings after carefully removing the top layer of soil which in turn was covered with autoclaved soil. This was followed by light watering of the pots. The trial comprised of four treatments including checks and each treatment was replicated five times for all the populations. The pots were watered regularly till termination of the trial.

## **Observations**

Plant growth characters, namely, fresh root weight, fresh shoot weight, dry root weight, dry shoot weight, shoot height, no. of cysts (at 60 days) and egg & juveniles content / cyst (at the time of harvest) were recorded after nematode inoculation for virulence study. For physiological specializations study, only final cyst number as observed after 60 days of nematode inoculations.

The data obtained were analysed statistically and are presented in the succeeding chapter.

## **Histopathological changes induced by *Heterodera sorghi* infection in maize roots**

To study the histopathological changes, two populations were selected taking one virulent and another less virulent population based upon the virulence study.

## **Raising of plants**

Pre-soaked and germinated seeds of maize (*Zea mays* cv. Prabhat) were planted singly in 10 cm earthen pots filled with a mixture of autoclaved soil and sand in the ratio of 3 :1 respectively. The seedlings were inoculated with 4 J2/g of soil for both the populations.

## **Inoculation**

Both the virulent and less virulent populations of *Heterodera sorghi* comprising of second stage juveniles was determined by counting separately in one ml aliquots five times after thoroughly mixing. Based on the average number of counts the required number of juveniles was poured near the

roots of the plant after removing the surface soil and covering it again with the same soil. The pots were given light irrigation to keep the soil moist. After 6 days of planting the plants were inoculated with 4 J2/g of soil. The roots were collected after 9, 18 and 27 days of nematode inoculation. The root portion showing infection of *H.sorghi* under a stereoscopic binocular microscope, was cut gently with the help of a blade and fixed in FAA (4;1) in 5 ml glass vials. The processing of root material was carried out following the method of Johansen (1940) with slight modifications.

### **Dehydration**

The dehydration of root samples was carried out by running them through ethyl alcohol series consisting of 30, 50, 70 and 90 per cent followed by two changes in absolute alcohol in the end. Subsequent to this, the materials were processed through alcohol : xylene series (3:1, 1:1 and 1:3) followed by two changes in pure xylene. At each grade material was exposed for about 1 hour.

### **Infiltration of wax**

Pure paraffin wax (m.p. 60<sup>0</sup>C) was used for infiltration as well as embedding. Wax infiltration was carried out at 60<sup>0</sup>C in an oven, by transferring the materials from pure xylene to pure wax. The paraffin was replaced after every 4 h till xylene was completely replaced by paraffin (5-6 replacements).

### **Embedding**

Molten paraffin was poured up to a desired height in a paper boat and the lower layer of wax was allowed to cool and solidify. A warm forcep was used to place the root material of less than 1 cm length, horizontally in

the paper boat, followed by carefully pouring some more molten wax over the material which enabled it to get embedded in wax from all sides. The wax was allowed to solidify before it was plunged into cold water.

Subsequently, small pieces containing the material were partitioned for microtomy. Sections were cut after adjusting the microtome at 15 and 20 micron thickness for histopathological studies. Gelatin adhesive was used on glass slides for holding the paraffin ribbons, which were warmed to allow the expansion of sections and then for 24 hours before further processing was done.

### **Staining**

Safranin-fast green method was adopted for staining the sections for histopathological studies. Slides with sections were dipped in xylene for 10 minutes to deparaffinize the sections. These deparaffinized sections were then passed through absolute alcohol followed by a series of 80, 70 and 50% alcohol successively keeping for 2-3 seconds in each grade before finally transferring them in 0.5% aqueous safranin for 1 hour. The extra stain was removed by slight washing with distilled water. Next, sections were destained by dipping them in 70% and 90% alcohol for 2-3 seconds each, before counterstaining with fast green (0.5% fast green in 95% alcohol) for 30 seconds. Sections were then differentiated in 95% alcohol for 2-3 seconds, cleared in xylene (2 changes) and finally mounted in Canada balsam for microscopic observations.

## 4. RESULTS

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Sorghum cyst nematode, *Heterodera sorghi* causes significant damage to sorghum, maize and other cereal crops in India. It holds a potential for increasing its host range which is of major concern. No information is available, on phenotypic and physiological variation existing in field populations in India. When a nematode disperses across boundaries naturally or human aided, they encounter such conditions and we may find same or new hosts on which these populations have to survive. Adaptation to new set of environmental conditions, results in variants from the original population. These variations may be genetic and/or phenotypic and either way exhibit a selective advantage for the organism. So understanding these variations have great implications in understanding the adaptations that permit the successful way of life for different populations under different climatic conditions. This also helps in better understanding the relatedness among populations. Some populations are resistant to a particular control method than other populations. So understanding these variations has implications for its management too. So studies were undertaken to unearth the diversity in sorghum cyst nematode populations. Both morphological and physiological characteristics were studied.

### **Morphological Studies.**

Microscopic observations were recorded from second stage juveniles, cysts, cone top structure of cysts and eggs from five populations of the sorghum cyst nematode, *H.sorghii*. The studies were conducted on single egg mass progeny.

Results from the morphometric observations on five populations [Ghaziabad (Uttar Pradesh), Kulu, Tihri, Chamba (Himachal Pradesh) Kud (Jammu & Kashmir)] are given in Tables 1-4 . The measurements are given in  $\mu\text{m}$  or as stated. The measurements of cone top , second stage juveniles and cysts of *H.sorghi* as per Jain *et al.* (1982) are also given for comparison.

### **Morphological characters of mature cysts**

The five populations of *H.sorghi* were found to have the typical shape for the cysts of the species, with light to dark brown cysts that are lemon shaped with prominent neck and vulval cone. Vulval cone was prominent in this species and was found well developed in all the populations studied . Fenestra on the cone was ambifenestrate, separated by a narrow vulval bridge. Vulval slit was shorter than vulval bridge length. Underbridge strongly developed without fingerlike projections in the middle, length varied with each population. Anus is small, and indistinct and located fairly away from the vulval slit. Bullae were dark brown in colour, very few at the level of underbridge.

### **Morphological characters of second stage juvenile**

Body slightly curved ventrally when heat relaxed ; slightly tapering anteriorly more attenuated at the posterior end. Dorsal oesophageal gland opening situated 4.0-5.0  $\mu\text{m}$  behind the stylet base, median oesophageal bulb ovoid, with well developed valvular apparatus. Tail tapering posteriorly, conical.

### **Morphometric characters of second stage juveniles (J2 )**

Observation on mean values, range, standard deviation and coefficient of variation of each morphometric character are given in Table 1.

**Table 1. Morphometric characters of second stage juveniles of five populations of *Heterodera sorghi***

(Mean  $\pm$  SD; Range; C.V. %; N = 15)

Characters	Ghazia- bad (Type)	Kulu (HP)	Ghaziabad (UP)	Tihri (HP)	Kud (J&K)	Chamba (HP)
Length	461 $\pm$ 6.19 (420-525)	474 $\pm$ 3.90 (430-510) 0.99	462 $\pm$ 6.20 (421-527) 0.93	461 $\pm$ 6.39 (420-528) 1.4	484.33 $\pm$ 4.1 (450-520) 0.89	490 $\pm$ 3.61 (460-560) 0.76
Body width	—	19 $\pm$ 0.29 (18-22) 3.25	17 $\pm$ 0.95 (16-18) 5.61	16.47 $\pm$ 0.74 (15-18) 4.51	19.27 $\pm$ 1.28 (17-21.5) 6.64	20.07 $\pm$ 0.76 (19-21.5) 3.77
Stylet length	21 $\pm$ 0.16 (20-22)	22.5 $\pm$ 0.90 (20-25) 4.47	22 $\pm$ 0.16 (21-23) 4.49	22.02 $\pm$ 0.18 (20-23) 4.32	21.60 $\pm$ 0.76 (20-23) 3.52	21.07 $\pm$ 0.76 (19-24) 4.64
Head width	—	8.65 $\pm$ 0.32 (8-9) 3.64	8.96 $\pm$ 0.14 (8.50-9) 1.61	8.43 $\pm$ 0.42 (8-9) 4.94	9.09 $\pm$ 0.30 (8.50-10) 3.35	8.86 $\pm$ 0.98 (8-9) 4.10
Head height	—	6.92 $\pm$ 0.28 (6-7) 6.13	7.13 $\pm$ 0.31 (6.50-7.5) 4.36	6.97 $\pm$ 0.35 (6.50-7.50) 5.05	7.38 $\pm$ 0.37 (7-8.20) 5.00	6.93 $\pm$ 0.36 (6.50-7.50) 3.86
DGO	—	4.23 $\pm$ 0.26 (4-4.50) 6.13	4.79 $\pm$ 0.26 (4.50-5.00) 5.37	4.73 $\pm$ 0.26 (4.50-5.0) 5.65	4.21 $\pm$ 0.26 (4-5) 6.92	4.82 $\pm$ 0.25 (4.50-5.00) 5.16
Head to excretory pore	—	80 $\pm$ 1.2 (75-85) 1.79	70 $\pm$ 2.96 (65-80) 4.41	77.13 $\pm$ 1.77 (74.5-80) 2.29	70.73 $\pm$ 2.62 (67-75.50) 3.71	75.64 $\pm$ 2.43 (73-80) 3.21
Head to median bulb valve	—	69.85 $\pm$ 1.21 (68-73) 1.74	59.58 $\pm$ 2.50 (55-62) 4.20	70.33 $\pm$ 1.45 (68-73) 2.06	63.93 $\pm$ 1.87 (62-68) 2.92	68.07 $\pm$ 1.82 (65-70) 2.21
Head to oesopha- geal gland lobe	—	152 $\pm$ 1.80 (140-160) 1.87	150.79 $\pm$ 2.24 (146.5-153) 1.49	155.3 $\pm$ 2.40 (150-160) 1.54	157 $\pm$ 1.90 (145-165) 1.95	153 $\pm$ 1.98 (147-160) 2.49
Tail length	52 $\pm$ 0.85 (42-60)	51 $\pm$ 0.6 (42-63) 4.42	52 $\pm$ 0.85 (40-60) 2.18	51.9 $\pm$ 0.70 (42-60) 2.55	55 $\pm$ 0.8 (48-70) 2.33	53 $\pm$ 0.9 (45-60) 2.44
Hyaline tail length	31 $\pm$ 0.39 (28.35)	30.23 $\pm$ 0.99 (29-32) 3.28	29.17 $\pm$ 1.59 (28-32) 5.44	31.73 $\pm$ 0.88 (30-33) 2.78	29.80 $\pm$ 1.03 (28-32) 3.46	29.61 $\pm$ 1.10 (29-33) 3.70

Table 1 contd...

N = 15

Characters	Ghazia- bad (Type)	Kulu (HP)	Ghaziabad (UP)	Tihri (HP)	Kud (J&K)	Chamba (HP)
Anal body width	—	10.50±0.68 (10-12) 6.45	10.67±0.98 (10-13) 9.23	10.90±0.47 (10-11.5) 4.32	10.70±1.07 (8-12) 9.96	10.25±0.78 (9-12) 7.59
a	23±0.33 (20-26)	24.6±0.2 (21-28) 3.15	23.1±0.44 (20-27) 5.28	23±0.33 (20-26) 4.61	26.2±0.3 (22-32) 7.20	26±0.3 (23-30) 4.12
b	9±0.2 (8-12)	4.76±0.1 (6.5-10.5) 2.32	9.2±0.3 (9-13) 2.01	9±0.2 (8-12) 2.05	4.6±0.2 (4-5.6) 2.03	8.5±0.3 (8-10) 2.55
C	—	8.6±0.9 (7.9-9.2) 4.35	8.19±0.22 (7.80-8.44) 2.70	8.91±0.27 (8.49-9.30) 2.97	8.7±0.1 (8-9.5) 2.52	8.6±0.1 (8.2-9.2) 2.60

- a = Total body length/ Maximum body length  
b = Total body length/ Head to oesophageal gland lobe'  
C = Body length / Tail length.

### **Body Length**

The maximum mean value for body length was recorded in Chamba population ( $490 \pm 3.61$ ) followed closely by Kud population ( $484 \pm 4.1$ ). The smallest in terms of body length were individuals from Tihri population ( $461 \pm 6.39$ ). Range for body length of these populations varied from 461-490  $\mu\text{m}$ . The coefficient of variability of these populations was within 1.4%.

### **Maximum body width**

The value of maximum body width ranged from 15-21.5  $\mu\text{m}$  across all the populations. Highest mean value for this character was recorded in Chamba population ( $20.07 \pm 0.76$ ) followed by Kud population ( $19.27 \pm 01.28$ ). The lowest value for body width was observed in Tihri population to ( $16.47 \pm 0.74$ ) The coefficient of variability ranged from 3.25 in Kulu population to 6.64 in kud population. Coefficient of variability in all populations except Ghaziabad and Kud fell below 5%.

### **Stylet length**

Mean values for stylet length is almost same in all the populations with the highest mean value observed in Kulu population ( $22.5 \pm 0.90$ ) followed by Tihri population ( $22.02 \pm 0.18$ ). The lowest value was observed in Chamba population ( $21.07 \pm 0.76$ ). The range for this character varied from 19-25  $\mu\text{m}$ . The coefficient of variability varied from 3.52% in Kud population to 4.64 in chamba population. The coefficient of variability limited within 4.64%.

### **Head Width**

Measurements of head width appeared more or less similar in all the populations studied. Mean value for head width showed only fractional

differences. The range values for this characters was found to lie between (8-10  $\mu\text{m}$ ). Mean head width values in Kud was  $9.09 \pm 0.30$  and in Ghaziabad  $8.96 \pm 0.14$  which were the highest values. In Tihri population this value was lowest ( $8.43 \pm 0.42$ ). Coefficient of variability varied from 1.61-4.94%.

### **Head height**

Head height value ranged between 6-8.2  $\mu\text{m}$ . There were again only fractional differences in mean values with the highest mean value recorded in Kud population ( $7.38 \pm 0.37$ ) and the lowest in Kulu population ( $6.92 \pm 0.28$ ). There was a notable difference in the coefficient of variability ranging from the lowest observation recorded in Chamba population to the highest value in Kulu population (6.13%).

### **Dorsal gland orifice opening (DGO)**

The distance from the base of stylet knobs to the dorsal oesophageal gland opening is an important taxonomic character in many nematodes. There was not much difference in the mean values and range for this character in all the five populations. Highest mean value was recorded in Chamba population ( $4.82 \pm 0.25$ ) and the lowest value was observed in Kud population ( $4.21 \pm 0.26$ ). The coefficient of variation was ranging from 6.13% in Kulu population to 5.16% in Chamba population.

### **Distance from head to excretory pore**

There were significant differences in mean values for distance from head to excretory pore in five populations studied with the highest value recorded in Kulu ( $80 \pm 1.20$ ) and Tihri population ( $77.13 \pm 1.77$ ). The lowest value was observed in Ghaziabad population ( $70 \pm 2.96$ ). The range in this

character also showed significant variation (70-80  $\mu\text{m}$ ). The coefficient of variability was maintained within 5% in all the populations.

#### **Distance from head to median bulb valve**

The range in this character did not vary significantly and was also overlapping (68-73  $\mu\text{m}$ ). Mean value for the distance from head to median bulb valve was highest in Tihri population (70.33 $\pm$ 1.45) and the lowest value was recorded in Ghaziabad population (59.58 $\pm$ 2.50). The coefficient of variability was limited to 4.2%.

#### **Distance from head to oesophageal gland lobe**

The mean highest value was observed in Kud population (157 $\pm$ 1.90) and the lowest in Ghaziabad population (150.79 $\pm$ 2.24). These values ranged in all the populations from 140-165 showing significant variations in this character .

#### **Tail length**

Significant variation was observed in the range for this character in the five populations studied and the value ranged between 40 and 70  $\mu\text{m}$ . Significant differences were also observed in the mean values , which varied from a lowest value in Kulu population (51 $\pm$ 0.6) to the highest value in kud population (55 $\pm$ 0.8). The coefficient of variability varied from 2.18-4.42%.

#### **Hyaline tail length**

The mean value in this character differed marginally and was highest in Tihri population (31.73 $\pm$ 0.88). The range values for hyaline tail length were between 28-33  $\mu\text{m}$ . The lowest coefficient of variability was observed in Tihri

in Tihri population (2.78%) and the highest variability was found in Ghazibad population (5.44%).

### **Anal body width**

The highest mean values for anal body width was observed in Tihri population ( $10.90 \pm 0.47$ ) and the lowest value was observed in Chamba population ( $10.25 \pm 0.78$ ). The coefficient of variability ranged from 4.32% in Tihri population to 9.23 % in Ghaziabad population.

### **'a' value (total body length \ maximum body width**

The highest mean value for the 'a' ratio was observed in Kud population ( $26.2 \pm 0.3$ ) and the lowest in Tihri population ( $23 \pm 0.33$ ). The value of this ratio ranged between 20 and 32 and showed variation even within a population. The coefficient of variability was within 7.20% and it ranged from 3.15-7.2%.

### **'b' value (Total body length \ head to oesophageal gland lobe**

The highest mean 'b' value was observed in Ghaziabad population ( $9.2 \pm 0.3$ ) and the lowest was recorded in Kud population. The coefficient of variability was low in Ghaziabad population 2.01% and highest CV was observed in Chamba population.

### **'C' value (Total body length \ Tail length)**

There was not much difference in 'C' ration calculated for different populations (Table 1). The range of this character was found to lie between 7.80 and 9.5. The coefficient of variability was lowest (2.52%) as observed in Kud population and highest (4.35%) in Kulu population.

### **Morphometric characters of cone top structure**

Data obtained from morphometric measurements of the cone top structure of the five populations of *H.sorghii* are given in Table 2.

#### **Length of vulval slit**

The highest mean value of length of the vulval slit was observed in Tihri population ( $33\pm 1.5$ ) and the lowest in Kud populations and it ranged from 28-40  $\mu\text{m}$  in all the populations. The coefficient of variability was below 9% except in case of Chamba population in which variability percentage within the population was high (13.32%).

#### **Vulval bridge length**

The vulval bridge length exhibited the highest mean value in Chamba population ( $44.45\pm 2.0$ ) followed by Kud population ( $41.9\pm 0.8$ ), The minimum value of  $36.6\pm 2.6$  was observed in Kulu population. The coefficient of variability in the populations was limited below 7%.

#### **Vulval bridge width**

The values for vulval bridge width ranged between 4-12  $\mu\text{m}$  in all the populations under study. Mean value of this width recorded as highest in Kud population ( $19\pm 0.9$ ) and the lowest value was observed in Ghaziabad ( $6.4\pm 0.7$ ). The coefficient of variability was very high in all the populations ranging from 4.11-15.0%.

#### **Under bridge length**

The value for the under bridge length ranged between 70 and 155  $\mu\text{m}$  in all the populations under study. Highest mean value was observed in

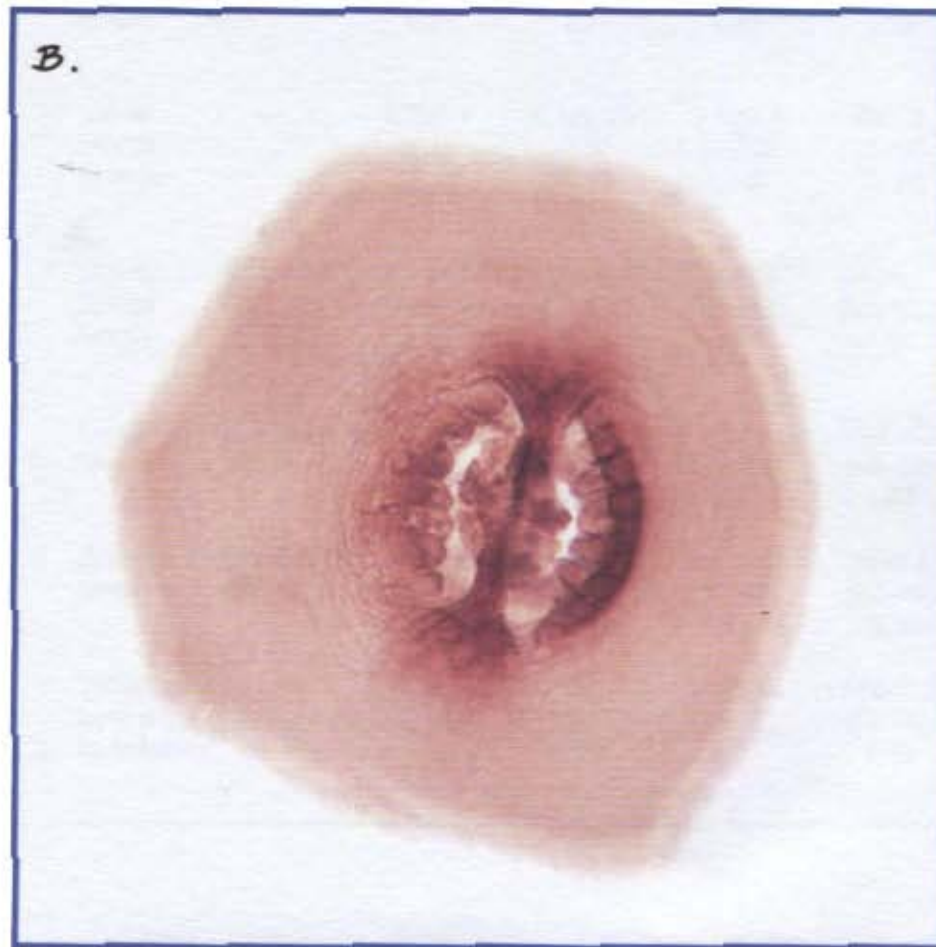


Plate 1A. Cyst of *H. sorghi*

Plate 1B. Cone top of *H. sorghi*

**Table 2. Morphometric characters of conetops of five populations of *Heterodera sorghi***

(Mean  $\pm$  S.D.; Range; C.V. %; N = 15)

Characters	Ghazia- bad (Type)	Kulu (HP)	Ghaziabad (UP)	Tihri (HP)	Kud (J&K)	Chamba (HP)
Vulval slit length	31.8 $\pm$ 1.30 (28-39)	32.04 $\pm$ 2.76 (28-36) 8.63	32 $\pm$ 1.32 (28-40) 8.86	33 $\pm$ 1.5 (30-40) 6.19	30.05 $\pm$ 0.6 (28-35) 7.97	32.10 $\pm$ 0.8 (28-38) 13.32
Vulval Bridge length	—	36.6 $\pm$ 2.1 (36-48) 4.47	40.6 $\pm$ 2.93 (39-49) 6.7	39.87 $\pm$ 2 (36-52) 4.41	41.9 $\pm$ 0.8 (45-58) 5.60	44.2 $\pm$ 2 (49-61) 5.52
Vulval Bridge breadth	—	6.5 $\pm$ 0.7 (5-9) 10.76	6.4 $\pm$ 0.70 (4-9) 15.01	7.5 $\pm$ 0.5 (5-10) 12.86	9 $\pm$ 0.9 (6-12) 12.93	8 $\pm$ 0.5 (4-12) 4.11
Under Bridge length	79.3 $\pm$ 4.39 (70-115)	120 $\pm$ 1.1 (102-138) 6.26	79.6 $\pm$ 4.40 (71-116) 5.59	79 $\pm$ 4.4 (70-115) 4.85	120.5 $\pm$ 1.7 (95-155) 7.65	80.01 $\pm$ 4.1 (80-110) 6.93
Length of fenestra	47.4 $\pm$ 1.99 (40-56)	47.21 $\pm$ 0.9 (40-56) 9.87	47.1 $\pm$ 2 (41-55) 7.38	47 $\pm$ 2 (40-56) 6.22	41.8 $\pm$ 0.9 (30-50) 3.62	54 $\pm$ 0.6 (48-59) 5.24
Breadth of fenestra	38.4 $\pm$ 2.08 (32-56)	40 $\pm$ 0.4 (30-48) 12.70	38.6 $\pm$ 2.15 (33-57) 11.86	38.3 $\pm$ 0.7 (30-45) 13.11	38 $\pm$ 0.7 (27-48) 10.60	39 $\pm$ 2.38 (32-60) 6.89
Distance from anus to fenestra	—	12.71 $\pm$ 1.60 (10-15) 12.59	11.50 $\pm$ 1.12 (10-13) 9.72	13.00 $\pm$ 1.07 (11-15) 8.25	13.05 $\pm$ 0.76 (12-14) 5.79	11.65 $\pm$ 1.27 (10-13.50) 10.87

Kud population ( $120.5 \pm 1.7$ ) and Tihri population had the shortest under bridge ( $79 \pm 4.4$ ). The coefficient of variability ranged from 4.88 and 7.65.

### **Length of fenestra**

The length of fenestra ranged from 30-59  $\mu\text{m}$  in all the five populations of *H.sorghi*. Marginal differences were observed in the mean values of this character except in Chamba ( $54 \pm 0.6$ ) and Kud population ( $41.8 \pm 0.9$ ). The lowest mean value of  $41.8 \pm 0.9$  was observed in Kud population. The value for coefficient of variability was between 3.62 and 9.87%.

### **Width of fenestra**

Highest mean value for the breadth of fenestra was observed in Kulu population ( $40 \pm 0.4$ ) followed by Chamba population ( $39 \pm 2.0$ ) where as the lowest mean value of  $38 \pm 0.7$  was recorded from Kud population. The range in this character was between 27 and 60  $\mu\text{m}$ . Coefficient of variability was consistently high in all populations (6.89-13.11%).

### **Distance from anus to fenestra**

The distance from anus fenestra value ranged from 10-15  $\mu\text{m}$ . Mean value for this character ranged from a high in Tihri population ( $13 \pm 1.07$ ) to low in Ghaziabad population ( $11.50 \pm 1.12$ ).

### **Morphometric characters of eggs**

Data obtained on morphometric characters of eggs in five populations of *Sorghum* cyst nematode *H.sorghi* are presented in Table 3.

**Table 3. Morphometric characters of eggs in five populations of *Heterodera sorghi***

(Mean  $\pm$  S.D.; Range; C.V. %; N = 20)

Characters	Ghazia- bad (Type)	Kulu (HP)	Ghaziabad (UP)	Tihri (HP)	Kud (J&K)	Chamba (HP)
Length	—	109 $\pm$ 0.8 (88-113) 5.08	104.30 $\pm$ 1.12 (98-125) 3.95	109.10 $\pm$ 2.1 (95-120) 6.93	110.90 $\pm$ 7.61 (99-122) 6.86	111.55 $\pm$ 0.9 (102-130) 5.81
Breadth	—	39 $\pm$ 0.40 (30-49) 13.40	38 $\pm$ 0.9 (28-48) 8.32	43 $\pm$ 1.19 (35.55-55.0) 14.70	36 $\pm$ 1.2 (31-47) 13.08	38.5 $\pm$ 0.65 (30-48) 12.62
Length/ Breadth	—	2.42 $\pm$ 0.29 (2.02-3.01) 12.03	1.95 $\pm$ 0.22 (1.78-3.50) 11.51	2.50 $\pm$ 0.35 (1.98-3.09) 14.03	2.44 $\pm$ 0.30 (2.18-3.04) 12.14	2.37 $\pm$ 0.27 (2.05-2.97) 11.57

### Length of egg

The range for length of egg varied from 88-130  $\mu$ m. Mean value for this character varied with the highest value observed in Chamba population (111.55 $\pm$ 0.9) followed by Kud and Tihri population.

The lowest value for mean egg length was observed in Ghaziabad population (104.30 $\pm$ 1.12). Coefficient of variability in all the populations were below 7%.

### Width of egg

Mean value for this character showed a similar trend except in Tihri population (43 $\pm$ 1.19), which peaked out with the highest mean value. The lowest value was observed in Kud population (36 $\pm$ 1.2). Coefficient of variability values ranged from 8.32-14.7%.

### Egg length/ width ratio - L/W

There was only marginal difference in the mean values among all the five populations. The L/W ratio ranged from 1.95 to 2.50. The coefficient of variations was highest in Tihri population (14.3%) and the lowest in Ghaziabad population (11.5%).

### Morphometric characters of cysts

Data obtained on morphometric characters of cysts in five populations of sorghum cyst nematode *H.sorghi* are presented in Table 4.

**Table 4. Morphometric characters of cysts in five populations of *Heterodera sorghi***

(Mean  $\pm$  S.D.; Range; C.V. %; N = 20)

Characters	Ghazia- bad (Type)	Kulu (HP)	Ghaziabad (UP)	Tihri (HP)	Kud (J&K)	Chamba (HP)
Length (exclud- ing neck)	738.6 $\pm$ 25.2 (550-910)	684.2 $\pm$ 1.28 (465-798) 3.89	738.9 $\pm$ 23.8 (551-910) 2.60	741 $\pm$ 11.9 (511-949) 8.94	740 $\pm$ 25.30 (552-909) 4.22	653 $\pm$ 25.8 (523-730) 4.47
Breadh	492 $\pm$ 142 (400-600)	448 $\pm$ 40.2 (375-652) 12.87	493 $\pm$ 66.92 (400-610) 12.75	519.9 $\pm$ 22.7 (400-580) 12.33	498.4 $\pm$ 93.87 (410-605) 18.61	448.1 $\pm$ 62.45 (370-580) 12.59
Length/ Breadth	1.4 $\pm$ 0.01 (1.2-1.8)	1.6 $\pm$ 0.02 (1.1-1.10) 14.93	1.42 $\pm$ 0.05 (1.3-1.8) 15.01	1.4 $\pm$ 0.02 (1.2-1.8) 18.05	1.23 $\pm$ 0.24 (1.3-1.9) 19.59	1.75 $\pm$ 0.16 (1-1.5) 13.15

### Length

The maximum mean value for length of cyst was recorded in Tihri population (741 $\pm$ 11.9) followed by closely by Kud population (740 $\pm$ 25.30). The smallest cyst length was obtained in individuals from Chamba population (653 $\pm$ 25.8).

The range for cyst length in these populations varied from 465-949  $\mu\text{m}$ . The coefficient of variability in length of cysts of these populations was limited within 9%.

### **Width**

The value of Breadth of cyst ranged from 350-655  $\mu\text{m}$  across all populations. Highest mean value for this character was recorded in Tihri population ( $519.9 \pm 22.77$ ) followed by Kud population ( $498 \pm 93.87$ ). The breadth of cyst was observed in Kulu population  $448 \pm 40.2$  the coefficient of variability ranged from 12.33-18.61%.

### **Cyst length/ breadth ratio - L/W**

There was only marginal differences in the mean values of L/W ratio among all the five populations. This ratio ranged from 1.23 to 1.75. Coefficient of variation was highest in Kud population (19.29%) and the lowest in Chamba population (13.15%).

### **Physiological specializations study**

Ten cultivars/lines each of maize and sorghum were tested for physiological variations among five populations of *H. sorghi*. All the tested cultivars/lines gave varying number of cyst production (5 to 200) against different populations studied in maize and sorghum [Table 5 (a) and 6(a)]. The cultivars/lines which produced less than or equal to 10 cysts per pot were considered as resistant and that produced more than 10 cysts/pot were considered as susceptible as given in Table 5(b) and 6(b). Based on Table 5(b) two cultivars \ lines namely, Deshi chari was found resistant against Kulu and Tihri populations and PGN 26 was resistant against Kud and Chamba populations. Hence it clearly showed existence of three groups

**Table 5(a). Reaction of sorghum cultivars to *H. sorghi* populations (average of three replications)**

<b>Sorghum cultivars</b>	<b>Kulu (HP)</b>	<b>Ghaziabad (UP)</b>	<b>Kud (J&amp;K)</b>	<b>Tihri (HP)</b>	<b>Chamba (HP)</b>
Deshi chari	07	63	147	08	123
Badi Jowar	67	29	123	39	46
Safed Jowar	153	90	29	86	137
PGN-26	35	75	06	55	09
PFGS-98	87	18	95	105	65
PFGS100	19	110	139	98	57
KC-1	105	51	63	120	65
GJ-39	110	45	67	78	88
SU-1	90	169	75	163	10
PFGS-97	73	50	85	67	60

**Table 5(b). Reaction of sorghum cultivars to *H. sorghi* population**

<b>Sorghum cultivars</b>	<b>Kulu (HP)</b>	<b>Ghaziabad (UP)</b>	<b>Kud (J&amp;K)</b>	<b>Tihri (HP)</b>	<b>Chamba (HP)</b>
Deshi chari	R	S	S	R	S
Badi Jowar	S	S	S	S	S
Safed Jowar	S	S	S	S	S
PGN-26	S	S	R	S	R
PFGS-98	S	S	S	S	S
PFGS100	S	S	S	S	S
KC-1	S	S	S	S	S
GJ-39	S	S	S	S	S
SU-1	S	S	S	S	S
PFGS-97	S	S	S	S	S

**Table 6(a). Reaction of maize cultivars to *H. sorghi* population  
(Average of three replications)**

<b>Maize cultivars</b>	<b>Kulu (HP)</b>	<b>Ghaziabad (UP)</b>	<b>Kud (HP)</b>	<b>Tihri (J&amp;K)</b>	<b>Chamba (HP)</b>
Karnal-536	126	175	10	58	165
Karnal-295	07	89	65	67	47
Karnal-323	93	120	70	58	18
Karnal-1105	28	79	175	100	88
Karnal-1344	86	180	58	19	173
Karnal-1352	200	10	67	175	75
Karnal-163-1-2	120	17	128	65	118
Kaarnal-193-1	97	98	125	87	155
Karnal-1348-6-2	119	89	105	57	72
Karnal-m-141	55	79	90	05	78

**Table 6(b). Reaction of maize cultivars to *H. sorghi* population**

<b>Maize cultivars</b>	<b>Kulu (HP)</b>	<b>Ghaziabad (UP)</b>	<b>Kud (HP)</b>	<b>Tihri (J&amp;K)</b>	<b>Chamba (HP)</b>
Karnal-536	S	S	S	S	S
Karnal-295	R	S	S	S	S
Karnal-323	S	S	S	S	S
Karnal-1105	S	S	S	S	S
Karnal-1344	S	S	S	S	S
Karnal-1352	S	S	S	S	S
Karnal-163-1-2	S	S	S	S	S
Kaarnal-193-1	S	S	S	S	S
Karnal-1348-6-2	S	S	S	S	S
Karnal-m-141	S	S	S	R	S

among populations studied in sorghum namely, Kulu and Tihri; Ghaziabad; Kud and Chamba populations. But in maize no clear grouping was possible because different cultivars showed resistance against different populations.

### **Effect of varying levels of *H. sorghi* on plant growth characters and nematode multiplication on maize**

A replicated trial in 15 cm earthen pots containing a mixture of sandy loam and sand (3:1) was initiated to study the growth parameters of maize and multiplication of *H. sorghi* at 4 levels of nematode inoculum (0, 2, 4 and 6 J2/cc soil). Of all these five populations (Kulu, Ghaziabad, Kud, Tihri and Chamba), observations on different plant growth parameters and nematode multiplication were recorded after 60 days of nematode inoculation.

#### **Plant growth (Table 7-11)**

The data of all the five populations revealed that all the plant growth characters decreased at different inoculum levels of nematode in comparison to check. Decrease in shoot lengths were found to be non-significant. However, reduction in fresh weights of shoot and root was found to be significant at an initial inoculum level of 4 J2/cc soil and above. While in case of the dry weights of shoot and root, significant reduction occurred at an initial inoculum level of 2 J2/cc soil and above. The maximum per cent reduction of fresh shoot weight of 35.47, 35.27, 22.02, 27.16 and 24.50 in Ghaziabad, Kud, Kulu, Tihri and Chamba respectively. In fresh root weight maximum reduction of 43.94, 39.23, 19.79, 26.56 and 34.85 in Ghaziabad, Kud, Kulu, Tihri and Chamba respectively. While in dry root weight maximum reduction of 49.33, 68.84, 43.06, 63.10 and 47.75 in Ghaziabad, Kud, Kulu, Tihri and Chamba respectively. Similarly in dry root weight maximum

Table 7. Effect of different inoculum levels of *H. sorghi* on shoot height (cm) in maize cv. Prabhat (Average of five replications)

Initial inoculum level (J2/g of soil)	Ghaziabad (UP)		Kud (J&K)		Kulu (HP)		Tehri (HP)		Chamba (HP)	
	Mean	% decrease over check	Mean	% decrease over check	Mean	% decrease over check	Mean	% decrease over check	Mean	% decrease over check
0	119.64	0	122.48	0	124.80	0	120	0	114.96	0
2	118.96	0.56	119.91	9.20	122.44	1.89	117	2.50	114.00	0.83
4	118.02	1.35	119.20	13.95	122.02	2.22	117.50	2.08	112.05	2.53
6	117.40	1.87	117.50	22.02	120.04	3.81	118.0	1.66	111.02	3.42
SEm (±)	1.07		1.20		0.66		0.97		0.89	
CD at (5%)	NS		NS		NS		NS		NS	

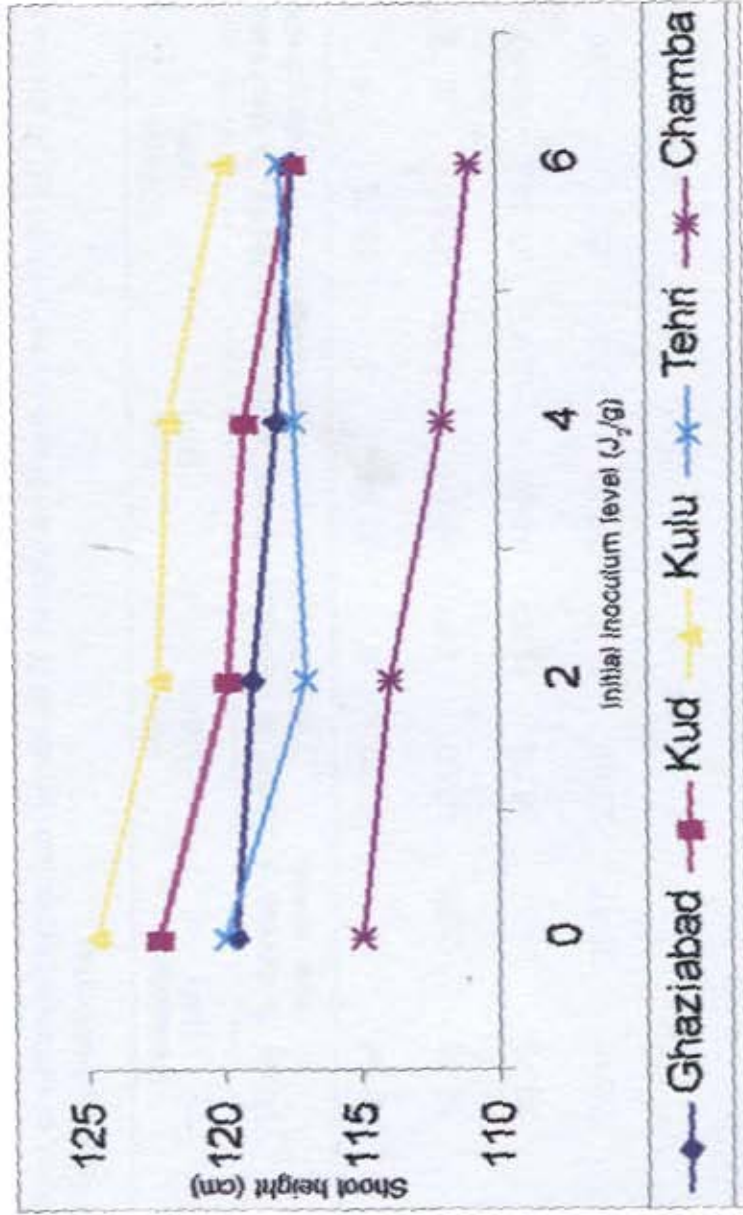


Fig. 1. Effect of different inoculum levels of *H. sorghi* on shoot height in maize cv. Prabhat

**Table 8. Effect of different inoculum levels of *H. sorghi* on fresh shoot weight (g) in maize cv. Prabhat (Average of five replications)**

Initial inoculum level (J2/g of soil)	Ghaziabad (UP)		Kud (J&K)		Kulu (HP)		Tehri (HP)		Chamba (HP)	
	Mean % decrease over check	Mean % decrease over check	Mean % decrease over check	Mean % decrease over check	Mean % decrease over check	Mean % decrease over check	Mean % decrease over check	Mean % decrease over check	Mean % decrease over check	Mean % decrease over check
0	40.54	0	43.20	0	46.58	0	44.72	0	42.20	0
2	39.46	2.66	42.00	2.77	45.01	3.37	43.50	2.72	40.00	5.21
4	30.70	24.97	38.02	11.99	40.08	13.95	37.38	16.41	36.32	13.93
6	26.16	35.47	27.96	35.27	36.32	22.02	32.57	27.16	31.86	24.50
SEm ( $\pm$ )	0.53		0.54		0.45		0.52		0.82	
CD at (5%)	1.55		1.56		1.76		1.50		2.42	

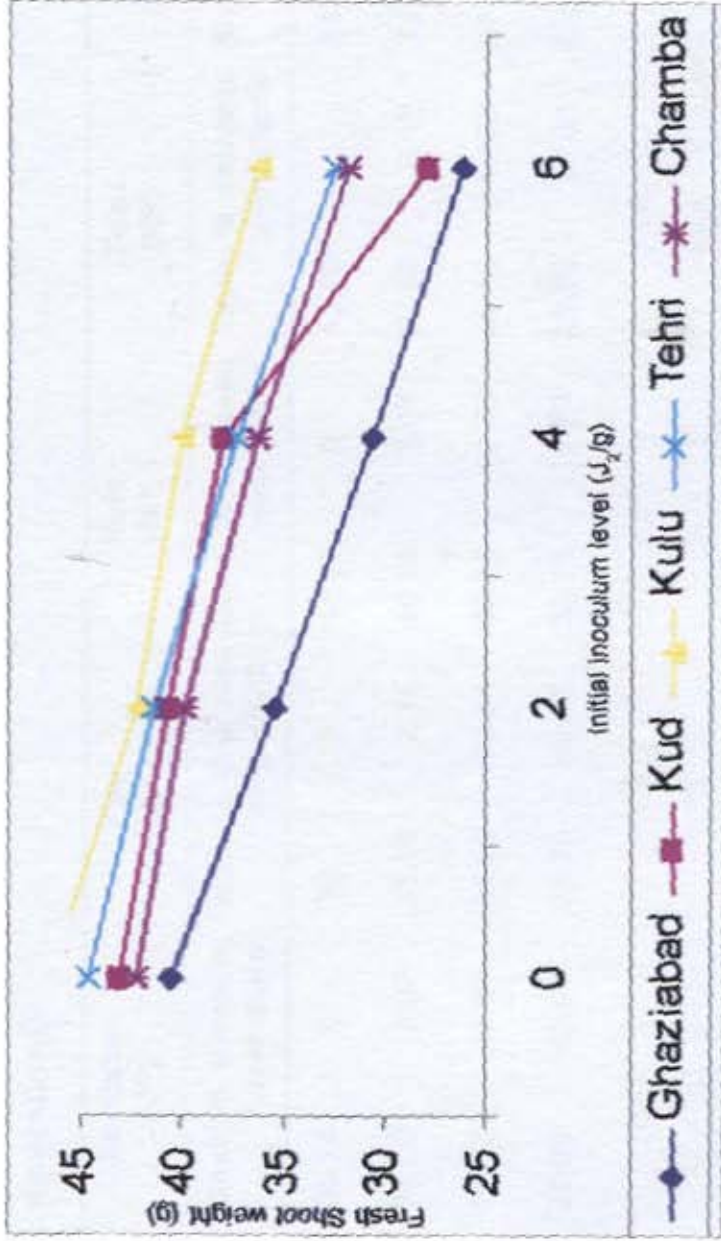


Fig. 2. Effect of different inoculum levels of *H. sorghii* on fresh shoot weight (g) in maize cv. Prabhat

**Table 9. Effect of different inoculum levels of *H. sorghi* on fresh root weight (g) in maize cv/ {Prabhat (Average of five replications)**

Initial inoculum level (J2/g of soil)	Ghaziabad (UP)		Kud (J&K)		Kulu (HP)		Tehri (HP)		Chamba (HP)	
	Mean % decrease over check	Mean % decrease over check	Mean % decrease over check	Mean % decrease over check	Mean % decrease over check	Mean % decrease over check	Mean % decrease over check	Mean % decrease over check	Mean % decrease over check	Mean % decrease over check
0	35.74	0	39	0	41.27	0	40.80	0	34.94	0
2	35.00	2.07	38.16	2.15	40.00	3.07	39.50	3.18	33.80	3.26
4	26.12	39.80	33.60	13.84	36.50	12.76	35.12	13.92	27.50	21.29
6	22.00	43.94	23.70	39.23	33.10	19.79	29.96	26.56	22.76	34.85
SEm ( $\pm$ )	0.47		0.60		0.57		0.50		0.44	
CD at (5%)	1.36		1.76		2.23		1.46		1.29	

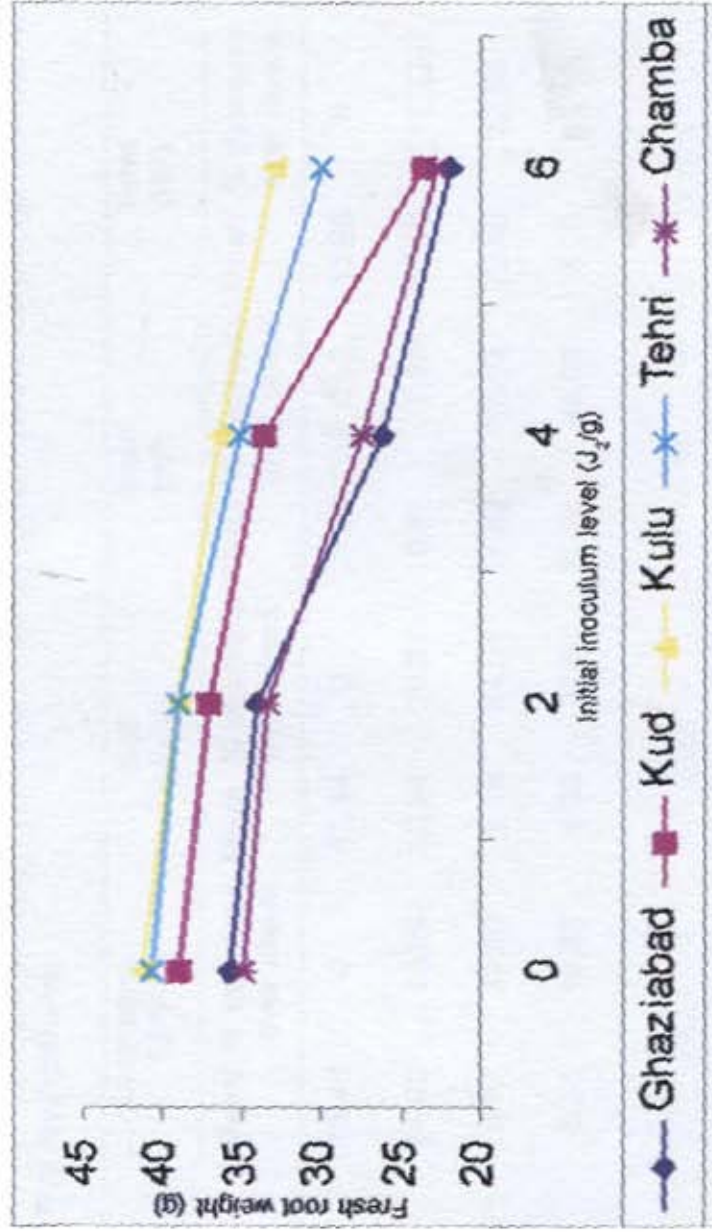
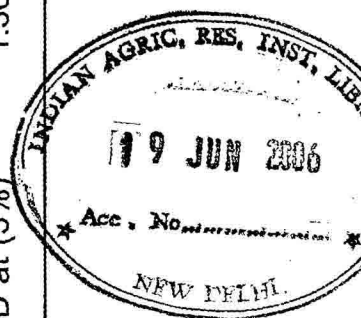


Fig. 3. Effect of different inoculum levels of *H. sorghi* on fresh root weight (g) in maize cv. Prabhat

Table 10. Effect of different inoculum levels of *H. sorghi* on dry shoot weight (g) in maize cv. Prabhat (Average of five replications)

Initial inoculum level (J2/g of soil)	Ghaziabad (UP)		Kud (J&K)		Kulu (HP)		Tehri (HP)		Chamba (HP)	
	Mean % decrease over check	Mean % decrease over check	Mean % decrease over check	Mean % decrease over check	Mean % decrease over check	Mean % decrease over check	Mean % decrease over check	Mean % decrease over check	Mean % decrease over check	Mean % decrease over check
0	16.48	0	12.84	0	21.50	0	13.96	0	16.96	0
2	14.00	15.04	10.24	20.24	19.81	7.86	12.10	13.32	15.02	11.43
4	9.92	39.80	7.18	44.08	17.62	18.04	9.40	32.66	11.96	29.48
6	8.35	49.33	4.00	68.84	12.24	43.06	5.15	63.10	8.86	47.75
SEm ( $\pm$ )	0.47	0.48	0.27	0.19	0.59	0.78	1.56	1.72		
CD at (5%)	1.36	1.89	0.78	1.56	1.72					



T-7607

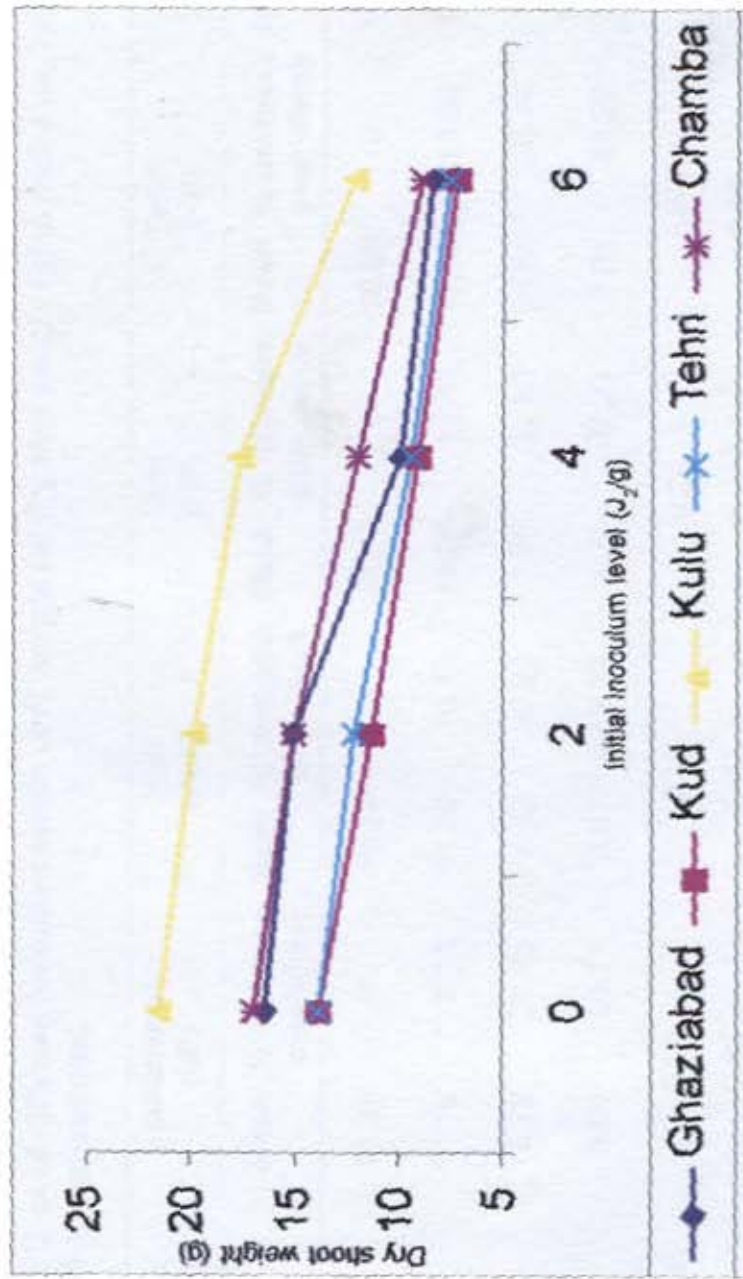


Fig. 4. Effect of different inoculum levels of *H. sorghi* on dry shoot weight (g) in maize cv. Prabhat

Table 11. Effect of different inoculum levels of *H. sorghi* on dry root weight (g) in maize cv. Prabhat (Average of five replications)

Initial inoculum level (J2/g of soil)	Ghaziabad (UP)		Kud (J&K)		Kulu (HP)		Tehri (HP)		Chamba (HP)	
	Mean % decrease over check	Mean % decrease over check	Mean % decrease over check	Mean % decrease over check	Mean % decrease over check	Mean % decrease over check	Mean % decrease over check	Mean % decrease over check	Mean % decrease over check	Mean % decrease over check
0	19.36	0	12.24	0	11.52	0	12.36	0	12.72	0
2	18.16	6.19	11.00	10.13	10.20	11.45	10.00	19.09	12.00	5.66
4	14.28	26.23	8.52	30.39	7.76	32.63	8.56	30.74	9.02	29.08
6	8.96	53.71	6.87	43.87	4.35	62.23	7.01	43.28	7.02	44.81
SEm (±)	0.30		0.22		0.26		0.52		0.17	
CD at (5%)	0.89		0.87		0.75		1.51		0.51	

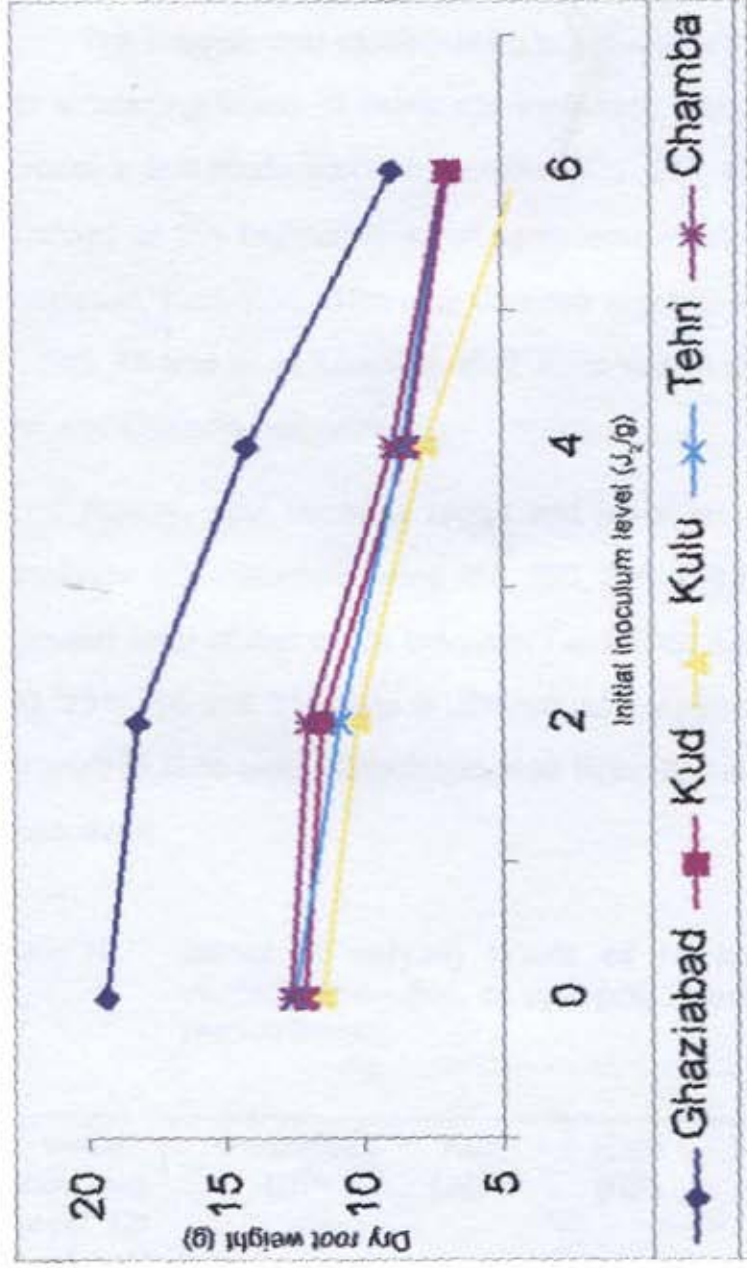


Fig. 5. Effect of different inoculum levels of *H. sorghii* on dry root weight (g) in maize cv. Prabhat

reduction of 53.71, 43.87, 62.23, 43.28 and 44.81 in Ghaziabad, Kud, Kulu, Tihri and Chamba population, respectively.

### **Nematode multiplication (Table 12-13)**

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 J2/cc soil) in Ghaziabad, Kud, Kulu, Tihri and Chamba respectively, and minimum 93, 87, 105, 75 and 97 at lowest level (2 J2/cc soil) in Ghaziabad, Kud, Kulu, Tihri and Chamba respectively.

Finally, cyst contents (eggs and juveniles) showed a negative correlation with maximum being 288, 260, 255, 276 & 291 eggs & J2/cyst) at lowest level of nematode inoculum i.e. 2J2/cc soil and minimum (232, 200, 231, 198 and 210 eggs & J2/cyst), at the highest level of nematode inoculum (6 J2/cc soil) in Ghaziabad, Kud, Kulu, Tihri and Chamba population respectively.

**Table 12. Effect of varying levels of *H. sorghi* on nematode multiplication (No. of cyst/pot) in maize (Average of five replications)**

<b>Initial inoculum level J2/g of soil)</b>	<b>Ghaziabad (UP)</b>	<b>Kud (J&amp;K)</b>	<b>Kulu (HP)</b>	<b>Tihri (HP)</b>	<b>Chamba (HP)</b>
2	93	87	105	75	97
4	168	173	153	230	225
6	307	278	155	260	215

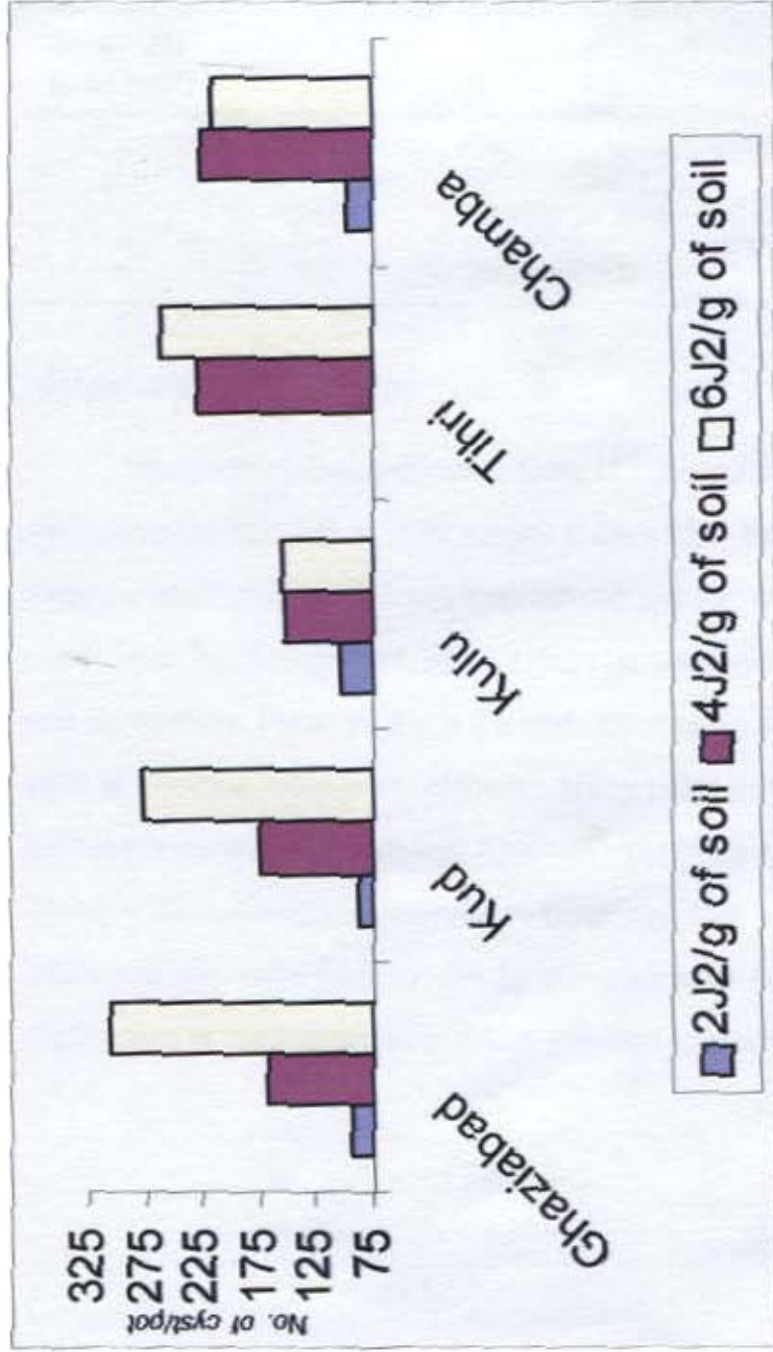


Fig. 6. Effect of varying levels of *H. sorghi* on nematode multiplication in maize cv. Prabhat

**Table 13.** Effect of inoculum levels of *H. sorghi* on egg and larval content per cyst (No. of eggs & J2/cyst) in maize (Average of five replications)

Initial inoculum level J2/g of soil)	Ghaziabad (UP)	Kud (J&K)	Kulu (HP)	Tihri (HP)	Chamba (HP)
2	288	260	255	276	291
4	240	252	238	248	228
6	232	200	231	198	210

### Histopathological studies

Histopathogenesis of maize roots of cultivar Prabhat on virulence and less virulence population of *H. sorghi*, 9 days after inoculation revealed that large number of J2 had penetrated the roots within this period. Most of the penetration by J2 occurred, by and large, in the region of cell differentiation and elongation. Hypertrophy in the cortex and rapid proliferation of pericycle cells in infected roots were evident. Many phloem cells encountered with nematode were hypertrophied. The cell cytoplasm was more granular and dense in the cells close to nematode head than that of the surrounding cells. The syncytia, were formed due to dissolution of adjacent cell walls and coalescing of their cytoplasm which resulted in multinucleate condition.

## 5. DISCUSSION

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Measurements of morphometric characters of conetop structures, second stage juveniles, cyst and egg characters were sufficient in distinguishing between populations of *H. sorghi*. Data on second stage juveniles characters of *H. sorghi* clearly indicated that body width, stylet length, head width, head height, dorsal esophageal gland orifice, distance from the head tip to median bulb valve, tail length, hyaline tail length, anal body width, 'a' ratio and 'c' ratio are conforming characters of original population of Ghaziabad in populations studied. But other characters like head to excretory pore, head to esophageal gland lobe distance and 'b' ratio are showing variations among populations in their respective range.

In case of second stage juvenile characters, Kulu population showed variations from other populations in stylet length, tail length and 'b' ratio, while Chamba populations showed a variations in the six characters out of fifteen characters studied from the second stage juvenile but were almost in the same range of the type measurement of the species as per Jain *et al.* (1982). All the other populations characters studied were in line with the type measurement of *H.sorghi*.

Morphometric measurements of character of conetop structure of five populaton of *H.sorghi* showed higher values in chamba population for vulval bridge length and length of fenestra, whereas Kud population showed higher values for vulval bridge length, under bridge length and distance from anus to fenestra. Vulval slit length and breadth of fenestra were found to be stable characters whereas other characters were found to be least variable.

Observations on eggs of different population revealed that the eggs of Tihri populations were the biggest and Ghaziabad population had one of the smallest eggs in the five populations studied. Breadth of eggs in all populations was found to be stable characters.

Similarly, the cysts of Tihri population had one of the biggest sized cysts in the five populations studied and chamba populations were the smallest size found in the studies.

The morphometric observations showed that Ghaziabad and Tihri populations shared most of the characters with the measurement of the type species, where as Kulu, Kud and Chamba populations exhibited deviations in many characters but they were almost in the same range of original population. Similar trend was also observed by Kaushal and Seshadri (1987), who found similar results in morphological variations of five different populations of the cereal cyst nematode (*H. avenae*). In spite of the variations in some of the characters among five geographically different populations of cereal cyst nematode, they were found to belong to the species, *H. avenae*. All the variations were within the range of reported species. Meagher (1974) has compared the Australian populations with recorded measurements of European and Canadian populations and suggested that variations may be because of environmental factors rather than distinctness as morphologically different species.

### **Physiological specializations studies**

It is an established fact that different varieties/lines of a plant species respond differently to attack by a given nematode species or its populations. A study of the response of 20 cultivars/lines of maize and sorghum against

*H. sorghi* showed that none of the tested cultivars exhibited any resistant reaction to the different populations of *H. sorghi*.

All the tested cultivars/lines gave varying degree of susceptibility of the varieties to nematode infestation in terms of cyst population build up was clearly discernible. The highest cyst multiplication in sorghum was found to be in/on CV SU-1 (169 cysts/plant) in Ghaziabad population and lowest in cv. PGN-26 (6 cyst/plant) of Kud population. While in maize all tested cultivars/lines gave susceptible reactions, highest being found in Karnal-1352 (200 cyst/plant) of Kulu population and lowest cyst/plants found in Karnal-295 (7 cyst/plant) of Kulu population. While testing 24 maize cultivars/lines when evaluated for their response to *H. sorghi*, Chawla and Srivastava (1995) did find moderately resistant reaction's through in the basis of the number of cyst/plant in six cultivars namely, D-851, R-17, W-101, Navin, VL-88 and D-765 supporting as low as 15.3 to 20 cysts/plant against 310.3 on highly susceptible cultivar, Navjot. Earlier, Srivastava and Sethi (1987) also reported differential degree of susceptibility of maize cultivars to *H. sorghi*. Response of sorghum cultivars to *H. sorghi* studied by Srivastava and Sethi (1988) and Mittal (1988) also resulted in similar trend of differential response of varieties to *H. sorghi*.

### **Virulence studies**

The virulence studies of different populations using varying levels (0, 2, 4 and 6 J2/g.of soil) on single cultivar of maize (CV. Prabhat) showed the destructive potential of this parasite in terms of adverse effect on plant growth characters. Except for plant height, all other growth parameters were significantly affected in comparison to check. Somewhat similar results have

been reported by Meagher and Chamber (1971), and Dhawan and Nagesh (1987), who also did not find any pathogenic effect of *Heterodera avenae* on height of wheat plants till 60 days after nematode inoculation. However, other plant growth characters were significantly reduced at 60 days. In the present investigations too, significant reduction in fresh and dry weight of shoots as well as in fresh weight of root, was obtained at 60 days after nematode inoculations. Shoot and root weights were also affected than the plant height. The results also confirmed the findings of Gill and Swarup (1973), Meagher (1982) and Dhawan and Nagesh (1987), who also observed greatest reduction in weights of shoot and root in wheat infested with *H. avenae* and Chawla and Srivastava (1990) reported in maize.

The results further suggested that plant height was not a reliable growth characters for studying the pathogenic effect of *H. sorghi*. Instead, characters like fresh and dry weights of shoot are important and get influenced greatly by an increase in the inoculum levels. Similar inferences in height were drawn by Gill and Swarup (1973), Meagher (1982) and Dhawan and Nagesh (1987), who also arrived at more or less the same conclusions when they reported that characters like fresh weights of shoot and root provided a more accurate reflection of growth reduction than plant height,

Pathogenicity results further suggested that an initial inoculum level of 4 J2/cc soil was considered as the minimum damaging threshold at which significant damage appeared in fresh shoot and root weights. However, maximum percent reduction in fresh shoot weight 35.47, 35.27 22.02, 27.16 and 24.50 occurred at the highest level of inoculum 6 J2/cc soil in Ghaziabad, Kud, Kulu, Tehri and Chamba populations respectively. The

present findings are somewhat 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 which was observed at an initial inoculum level of 4 J2 of *H. sorghi* / cm<sup>3</sup> of soil. However, Mittal and Dhawan (1991) reported significant suppression of fresh weights of shoots and root which occurred at 8 larvae/cm<sup>3</sup> soil and above in sorghum. Shiabova (1982), observed 50 per cent reduction in wheat yields at 10 and 20 larvae of *H. avenae* / g soil. Similarly, Mass and Brinkman (1982) reported 35 per cent reduction in sugarbeet yield at 50 eggs of *H. trifolii* per gram of soil.

In the present investigations, the data on final cyst multiplication in soil showed a positive correlation. Maximum number of cysts in soil were recorded at the highest level of inoculum (6 J2 /cc soil) and minimum at 2 J2 / cc soil. At each level of nematode inoculum used, there was a marked increase in the population, suggesting that cyst multiplication is not density dependent. Similar trend was also observed by Srivastava and Chawla (1990) who reported the cyst population and nematode multiplication increased significantly with the increasing levels of inoculum. Though, the maximum cysts and total nematode populations were recorded at the highest level of inoculum i.e. 16 J2/cm<sup>3</sup> soil in maize. 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. Rao (1984), Gokte and Swarup (1985) and Dhawan and Nagesh (1987) also reported positive correlation between cyst multiplication and levels of initial inoculum 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. zaeae* on maize.

The data on cyst contents (eggs and larvae/cyst), however, showed a negative correlation. With increase in number of cysts there was a corresponding decrease in the cyst contents. This was because of the fact that with the higher production of cysts, greater nutrient stress occurred which resulted decrease in the cyst contents. These results corroborate with the finding of Mittal and Dhawan (1991) which showed that the cyst contents (eggs and larvae/cyst) had a negative correlation with maximum being at the lowest level of nematode density (1 larvae/cm<sup>3</sup> soil) and minimum at the highest level (16 larvae/cm<sup>3</sup> soil). Rao (1984), Gokte and Swarup (1985), Dhawan and Nagesh (1987) and Srivastava and Chawla (1990) also reported negative correlation between initial inoculum level and egg contents of *H. avenae* and *H. zea*e respectively.

Wallace (1963) and Oostenbrink (1966) were of the opinion that the increase in nematode population and subsequent reduction in plant growth characters including crop yield or other manifestations of pathogenic effects are directly influenced by the initial density of the nematode in soil. However, the decrease in cyst production and nematode multiplication was perhaps due to competition for nutrition among the developing nematodes within a given root system. The present investigations indicated that shoot and root weights were better parameters for assessing the pathogenic potential of *H.sorghii* as compared to plant height. Based on above studies Ghaziabad population showed more virulent reactions while Kulu population was found to be less virulent.

### **Histopathological studies**

The histopathological studies of virulence and less virulence populations of *H. sorghi* on maize cultivar (cv. Prabhat) showed the

destructive potential of this parasite in terms of distinct number and size of syncytia formed by both the populations. The syncytia were formed due to dissolution of adjacent cell walls and coalescing of their cytoplasm which resulted in multinucleate condition.

The syncytia were found in contact with adult female nematodes and were occupied with clusters of nuclei, surrounded by dense granular cytoplasm indicating an active condition. Similar results were given by several authors. The detailed features of the syncytia induced in different susceptible and resistant hosts by *Heterodera* spp. (Wyss *et al.*, 1984; Rice *et al.*, 1985).

## 6. SUMMARY

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The present studies on the variability in the populations of *Heterodera sorghi* (Jain *et al.*, 1982) from India was undertaken with the following objectives :

1. To study the morphological and physiological variations among different populations of *H.sorghi*.
2. To study the virulence of these populations on maize
3. To study the histopathological changes induced by *H. sorghi* infection in maize roots.

Morphometric variations studies using five different populations, namely Kulu, Chamba and Tihri from Himachal Pradesh, Kud (Jammu & Kashmir) and Ghaziabad (Uttar Pradesh) undertaken on second stage juveniles, eggs, cyst and cone top structures revealed that Ghaziabad and Tihri populations shared most of the characters with the measurement of the type species, whereas Kulu (H.P.), Chamba (H.P.) and Kud (J&K) populations exhibited deviations in many characters but they were almost in the same range of original population.

The physiological variations studies indicated that sorghum cultivar, Deshi Chari showed resistance against both Kulu (H.P.) and Tihri (H.P.) population while cultivar PGN-26 indicated resistance against Chamba (H.P.) and Kud (J&K) population. Hence there were three groups namely Kulu and Tihri; Ghaziabad; Kud and Chamba populations. But such groups were not found in maize because of different cultivars showing resistant reactions against different populations.

The pathogenicity test conducted on maize (cv.Prabhat), grown in 15 cm earthen pots, revealed that with increasing levels of nematode inoculum, there was a reduction in the plant growth characters. The fresh weights of shoot and root decreased significantly at an initial inoculum level of 4 J2/cc soil. However, dry shoot and root was found to decrease at an initial level of 2 J2/cc soil. All the plant growth characters showed negative correlation with different levels of nematode inoculum.

The data on cyst multiplication in soil, showed a positive correlation. Maximum multiplication of the nematode was recorded at the highest level of nematode inoculum (6 J2/cc of soil) and minimum at the lowest level (2 J2/cc soil). On contrary, the cyst contents (egg & J2/cyst) showed a negative correlation with maximum being at lowest level of nematode inoculum, that is, 2 J2/cc of soil and minimum at the highest level of nematode inoculum (6 J2/cc soil).

The histopathogenesis were characterized by cortical hypertrophied cells, rapid proliferation of pericycle cells, large syncytium consisting of 6-10 enlarged nuclei, enlarged cells and dense granular cytoplasm. However, no major differences were observed with regard to histopathogenesis induced by both the virulence and avirulence populations of *H. sorghi* in maize roots.

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