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Impact of Different Inoculum Levels of Cereal Cyst Nematode (*Heterodera avenae*) on the Physiological Traits of Wheat (*Triticum aestivum*)

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Authors' contributions

This work was carried out in collaboration between both authors. Author RSK conceived the idea, designed the experiments and wrote the paper. Author ASS performed the experiments, recorded the observations and analyzed the data. Both authors read and approved the final manuscript.

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ABSTRACT

This study aimed to examine the effects of various inoculum levels of *H. avenae* on wheat physiology. The experiments were conducted in the screenhouse at the Department of Nematology, CCSHAU, Hisar, with inoculum levels set at 5, 10, and 15 eggs and juveniles per gram of soil. Observations on physiological parameters were made 30 days post-sowing. It was found that increasing inoculum levels led to significant decreases in total chlorophyll, carotenoid content, chlorophyll fluorescence, photosynthetic rate, transpiration rate, and stomatal conductance. The

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highest inoculum level showed the most pronounced reductions, with respective decreases of 39.71%, 30.55%, 7.90%, 39.75%, 51.58%, and 64.86%. Highest nematode inoculum level also resulted in the highest reduction in gaseous exchange parameters, biomass and leaf pigments concentrations and maximum increment in nematode population density.

Keywords: Rootknot nematode; Heterodera avenae; wheat; chlorophyll; physiology; photosynthetic rate; transpiration rate; stomatal conductance.

1. INTRODUCTION

Wheat, scientifically termed *Triticum aestivum*, is a crucial cereal grain originating from the Levant region and is now cultivated globally. This self-pollinating crop belongs to the family “Poaceae” and has a chromosome number of 42. It thrives at altitudes ranging from below sea level to 5000 meters and in areas with annual rainfall between 300 and 1130 mm. Wheat has been a staple in the human diet since the beginning of civilization, providing 20% of the daily protein requirement and caloric intake for approximately 4.5 billion people. Its nutritional composition includes 8-15% protein, 2-2.5% fiber, 1-1.5% fat, 1.5-2% minerals, and 62-71% carbohydrates. The per capita daily consumption of wheat has increased from around 79 g/day to over 185 g/day, despite the global population doubling since 1961 [1].

In India, wheat holds significant agricultural importance, second only to rice, and has been instrumental in the Green Revolution. India is the second-largest wheat producer globally, after China, with cultivation occurring between latitudes 10°N and 37°N. In the 2022-23 period, India produced 110.55 million tonnes of wheat [2] with projections for 2023-24 estimating an increase to approximately 112.7 million tonnes.

With the increase in Wheat production year after year, there has been a substantial increase in crop losses as well. Wheat farming faces severe yield reductions caused by insect and pest infestations, which globally account for a substantial 28.2% decrease in yields. Research by Dhaliwal *et al.* [3] in India found that insect pests caused losses of 25% in rice and maize and 5% in wheat. Wheat is vulnerable to a wide range of pests, including bacteria, fungi, viruses, and plant-parasitic nematodes, all of which can adversely affect crop quality and quantity [4].

Among the plant-parasitic nematodes affecting wheat, *Heterodera avenae* (causing 'Molya disease,' as identified by Vasudeva in [5] and *Anguina tritici* (the seed gall or ear cockle nematode) are particularly notable. These nematodes result in an annual economic loss of approximately Rs. 97.28 million in India [6]. The Heteroderidae family, including sedentary endoparasites like *Heterodera*, *Globodera* (cyst nematodes), and *Meloidogyne* (root-knot nematodes), is primarily responsible for crop damage, with *Heterodera* and *Globodera* causing significant agricultural losses. Several reports suggests that crop losses ranging from 15-96 % have been observed in wheat because of *Heterodera avenae* [7].

Heterodera avenae, causing Molya disease in wheat, parasitizes the below ground part of the plant particularly root system. Nematode feeding on roots along with the movement inside the root system leads to disruption of root structure and the potential of water uptake in plants, which further leads to scarcity of water inside the plant system and closure of stomatal aperture [8]. However, the impact of nematodes on plant physiological processes, such as photosynthesis, nutrient uptake, and respiration, remains underexplored. Also, the relationship between the differential population levels of *H. avenae* and the physiological processes of wheat is not yet unfolded. Uncovering the damaging potential of the increasing population levels of *H. avenae* in wheat will further reveal its importance to the scientists as well as farmers for its management in the field. So, this study aims to investigate the relationship between cereal cyst nematode population levels and its effects on the physiological changes of wheat plants.

2. MATERIALS AND METHODS

2.1 Experimental Site

The research was conducted in the screenhouse of the Department of Nematology at Chaudhary Charan Singh Haryana Agricultural University (CCSHAU) in Hisar, Haryana, located at Latitude 29.144425"N and Longitude 75.704296"E.

2.2 Nematode Inoculum

Cysts of *Heterodera avenae* Woll. were obtained from soil samples collected from naturally infested wheat fields in Dharnia village, Fatehabad, Haryana. The soil was thoroughly mixed, and several 200 cc samples were extracted. These samples were processed using Cobb's decanting and sieving method, involving a 20-mesh sieve and backwashing debris on 60-mesh sieve. The cysts were microscopically examined to determine inoculum levels, with an average population of 19 cysts per 100 grams of contaminated soil.

2.3 Cyst Content Estimation

To estimate the number of eggs and juveniles per cyst, 10 cysts were randomly selected and crushed in a counting dish with water [9]. The suspension was transferred to a graduated cylinder, diluted to 25 ml with water, and aliquots of 1 ml were taken for counting eggs and juveniles after thorough mixing.

2.4 Preparation of Inoculum

Sandy loam soil was utilised for the experiment. There were four treatments *viz.* control, 5 eggs & juveniles/g soil, 10 eggs & juveniles/g soil, and 15 eggs & juveniles/g soil. Nematode pre-infested was collected from a farmer's field and population level was estimated. The required inoculum levels were achieved by mixing *H. avenae* pre-infested soil (known population level) with autoclaved soil (15 lbs/20min) in specific ratios for attaining the desired population level. Sterilized autoclaved soil served as the control.

2.5 Raising and Maintenance of Wheat Plants

Seeds of the susceptible wheat cultivar WH 1105 were procured from the Wheat and Barley section of the Department of Genetics and Plant Breeding at CCSHAU. To promote germination, seeds were soaked overnight and sown on November 7th, 2016. Three pre-germinated seeds were planted in each 15 cm diameter, 1 kg capacity earthen pot filled with a mixture of sterilized and infested soil (source of nematode inoculum). Prior to filling the pots, nitrogen (N), phosphorus (P), and potassium (K) were added in recommended doses. Phosphorus and potassium were fully incorporated at sowing, while nitrogen was added in two halves: one at sowing and the other 21 days later. After seedling emergence, one plant was retained per pot and adequately watered.

2.6 Observations

The experiment had two sets: one for physiological parameters and one for nematode-related parameters. Physiological characteristics included chlorophyll a and b, their ratio, total chlorophyll, carotenoid levels, chlorophyll fluorescence, photosynthetic rate, transpiration rate, stomatal conductance, leaf temperature, and plant biomass. The final nematode population was also recorded. Physiological parameters were observed 30 days after sowing (DAS), while biomass and nematode population were measured at crop maturity.

Chlorophyll measurement: Chlorophyll a and b, along with total chlorophyll, were measured using Hiscox and Israelstam's [10] method. Leaf tissue samples (100 mg) were washed, immersed in 10 ml dimethyl sulfoxide (DMSO), and kept in the dark for 24 hours. Samples were then heated in a water bath at 65°C for 30 minutes, and optical densities at 645 and 663 nm were recorded using a spectrophotometer (MT-129). Chlorophyll content was calculated using Arnon's [11] formulas:

- Chlorophyll a (mg/g fresh weight) = [(12.7 × A663) - (2.69 × A645)] * (V/1000 × W)
- Chlorophyll b (mg/g fresh weight) = [(22.9 × A645) - (4.68 × A663)] * (V/1000 × W)
- Total Chlorophyll = (20.08 × A645 + 8.02 × A663) * (V/1000 × W)
- Ratio of Chl a and Chl b = Weight of Chl a (mg) ÷ Weight of Chl b (mg)

Where V is the extract volume (ml) and W is the sample fresh weight (g).

Carotenoid measurement: Carotenoid content was also determined using Hiscox and Israelstam's [10] method. Leaf tissue (100 mg) was washed, immersed in 10 ml DMSO, and processed similarly to chlorophyll samples. Optical density at 480 nm was measured. Carotenoid content was calculated using:

- Carotenoids (mg/g fresh weight) = (1000 × A480 - 1.90ChlA - 63.14ChlB/214) * (V/1000 × W)

Where V is the extract volume (ml) and W is the sample fresh weight (g).

Gaseous exchange parameters and leaf temperature: Photosynthetic rate, transpiration rate, stomatal conductance, and leaf temperature were measured using an infrared gas analyzer (IRGA ADC BioScientific LCi-SD System). Fully expanded leaves were placed in the gas analyzer chamber to maximize exposure to photosynthetically active radiation (PAR). Measurements were taken during bright, sunny hours by monitoring CO_2 concentration changes.

Chlorophyll fluorescence: The Fv/Fm ratio (variable to maximal chlorophyll fluorescence) was measured with an Opti-Sciences OS-30P chlorophyll fluorometer under bright sunlight. Leaves were acclimated to darkness for 20 minutes with clips before continuous illumination for 1 second from LEDs in the sensor. Data were collected between 10:30 AM and 12:00 Noon.

Biomass: At physiological maturity, plants were harvested, and above-ground portions were weighed using an SF-400 C balance.

Final nematode population: Soil samples from inoculated pots were processed using Cobb's decanting and sieving method. Cysts were recovered, and their population was counted under a microscope. To estimate average eggs and juveniles per cyst, 10 cysts were crushed, and the suspension was diluted to 25 ml. One-milliliter aliquots were taken for counting. The final nematode population was calculated as the product of cyst population and average cyst content, with the reproduction factor determined by dividing the final population by the initial population.

3. RESULTS

3.1 Physiological Parameters

Table 1 illustrates that increasing the inoculum level from 5 to 15 eggs and J2/g soil resulted in a significant decrease in chlorophyll a content at each inoculation level. The most substantial reduction of 40.67% was observed at the 15 eggs and J2/g soil level, followed by a 27.96% reduction at 10 eggs and J2/g soil, and the smallest reduction of 13.55% at 5 eggs and J2/g soil. Similarly, chlorophyll b content decreased with higher inoculum levels. Plants inoculated with 5 eggs and J2/g soil had chlorophyll b content statistically comparable to uninoculated plants but significantly higher than those inoculated with 10 or 15 eggs and J2/g soil. The greatest reduction in chlorophyll b content, 33.33%, was noted at the 15 eggs and J2/g soil level, followed by a 20.83% reduction at 10 eggs and J2/g soil.

Total chlorophyll content also decreased notably with higher inoculum levels. The most significant reduction of 39.71% was observed at 15 eggs and J2/g soil, followed by a 26.95% reduction at 10 eggs and J2/g soil, and a 12.05% reduction at 5 eggs and J2/g soil. No significant variations were observed in the ratio of chlorophyll a to b across different inoculum levels.

Carotenoid content also showed a marked decrease with increasing inoculum levels. The most significant reduction, 30.55%, was at 15 eggs and J2/g soil, followed by a 22.22% reduction at 10 eggs and J2/g soil, and a 13.88% reduction at 5 eggs and J2/g soil. Chlorophyll fluorescence exhibited a declining trend with higher inoculum levels. The most substantial reduction in chlorophyll fluorescence, 7.90%, was at the 15 eggs and J2/g soil level, followed by a 3.35% reduction at 10 eggs and J2/g soil, and a 1.74% reduction at 5 eggs and J2/g soil.

3.2 Gaseous Exchange Parameters

As shown in Table 1, the photosynthetic rate in plants inoculated with 5 eggs and J2/g soil did not differ significantly from that of uninoculated plants. However, significant reductions in the photosynthetic rate were observed in plants inoculated with 10 and 15 eggs and J2/g soil. The largest reduction (39.75%) was observed at 15 eggs and J2/g soil, followed by a 28.13% reduction at 10 eggs and J2/g soil. As the inoculum level increased from 5 to 15 eggs and J2/g soil, the transpiration rate decreased significantly at each level. The greatest reduction (51.58%) occurred at 15 eggs and J2/g soil, followed by a 48.41% reduction at 10 eggs and J2/g soil, and a 38.88% reduction at 5 eggs and J2/g soil. Stomatal conductance also decreased significantly with increasing inoculum levels. Although the conductance in plants inoculated with 10 and 15 eggs and J2/g soil did not differ statistically from those inoculated with 5 eggs and J2/g soil, the most substantial reduction (64.86%) was at 15 eggs and J2/g soil, followed by a 57.14% reduction at 10 eggs and J2/g soil, and a 42.85% reduction at 5 eggs and J2/g soil. Leaf temperature in plants inoculated with 5 eggs and J2/g soil did not differ significantly from uninoculated plants, but a significant increase was noted in plants inoculated with 10 and 15 eggs and J2/g soil. The highest increase (1.84ºC) was at 15 eggs and J2/g soil, followed by a 0.86ºC increase at 10 eggs and J2/g soil.

Table 1. Effect of varying degrees of *H. avenae* inoculation on leaf photosynthetic pigments and chlorophyll fluorescence in wheat

Inoculum level (J2/g soil)	Chl a (mg/g f.w.)	% Reduction	Chl b (mg/g f.w.)	% Reduction	Total Chl (mg/g f.w.)	% Reduction	Ratio of Chl a and Chl b	Carotenoid (mg/g f.w.)	% Reduction	Chlorophyll Fluoroscence	% Reduction
0	1.18	--	0.24	--	1.41	--	4.95	0.36	--	0.746	--
5	1.02	13.55	0.23	4.16	1.24	12.05	4.50	0.31	13.88	0.733	1.74
10	0.85	27.96	0.19	20.83	1.03	26.95	4.61	0.28	22.22	0.721	3.35
15	0.70	40.67	0.16	33.33	0.85	39.71	4.60	0.25	30.55	0.687	7.90
C.D at 5%	0.07		0.03		0.08		N.S.	0.02		0.009	

**Mean of 3 replicates*

Table 2. Effect of varying degrees of *H. avenae* inoculation on gaseous exchange parameters and leaf temperature in wheat

Inoculum level (J2/g soil)	Photosynthetic Rate (µmol m^{-2} s^{-1})	% Reduction	Transpiration Rate (mol m^{-2} s^{-1})	% Reduction	Stomatal conductance (mol m^{-2} s^{-1})	% Reduction	Leaf temperature (ºC)	% Increase
0	3.27	--	1.26	--	0.07	--	22.42	--
5	3.06	6.42	0.77	38.88	0.04	42.85	22.46	0.18
10	2.35	28.13	0.65	48.41	0.03	57.14	23.28	3.84
15	1.97	39.75	0.61	51.58	0.03	64.86	24.26	8.21
C.D at 5%	0.88		0.26		0.02		0.25	

**Mean of 3 replicates*

Table 3. Effect of varying degrees of *H. avenae* inoculation on its multiplication in wheat

Inoculum level (J2/g soil)	No. of Cyst (per pot)	Cyst content (per cyst)	Final nematode population (per pot)	Reproduction factor
5	104 (10.25)	241 (15.54)	25071 (158.27)	5.01
10	158 (12.62)	222 (14.92)	35154 (187.41)	3.52
15	206 (14.40)	208 (14.44)	42898 (207.09)	2.86
C.D at 5%	0.53	0.44	7.25	0.26

**Mean of 5 replicates (Final nematode population counts taken at 140 DAS), ** (Figures in the parentheses are square root transformed values)*

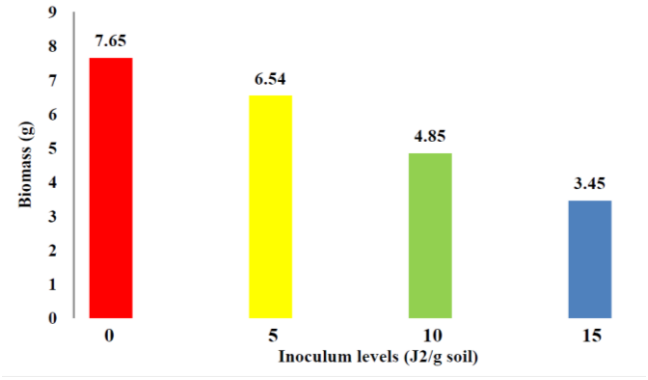

Fig. 1. Effect of varying degrees of *H. avenae* inoculation on biomass in wheat

3.3 Nematode Parameters

As the inoculum level increased, there was a corresponding increase in cyst population, cyst content, final nematode population, while reproduction factor decreased. The highest nematode population was recorded at the highest inoculum level of 15 eggs and J2/g soil, while the lowest population was observed at the 5 eggs and J2/g soil level.

3.4 Biomass

A significant decrease in plant biomass was observed as the inoculum level increased from 5 to 15 eggs and J2/g soil. The largest reduction (54.90%) in biomass occurred at 15 eggs and J2/g soil, followed by a 36.60% reduction at 10 eggs and J2/g soil, and the smallest reduction (14.50%) at 5 eggs and J2/g soil.

4. DISCUSSION

In our current research, we observed that increasing nematode inoculum levels negatively impacted physiological processes and plant growth, consistent with the findings of Hesling [12] and Gill & Swarup [13]. Specifically, levels of chlorophyll a, chlorophyll b, and carotenoids decreased with each subsequent increase in nematode inoculum, with the most significant reductions (40.67%, 33.33%, and 30.55%, respectively) occurring at 15 eggs and juveniles per gram of soil. Similar outcomes were documented in the leaf pigments of Mentha arvensis by Thakur [14], in wheat infected by *Heterodera avenae* by Sharma *et al*. [15], in bread wheat infected by *Heterodera filipjevi* by Ahmadi *et al*. [16] and *Ocimum kilimandscharicum* by Haseeb *et al.* [17] when infected with *Meloidogyne incognita*. The core component of chlorophyll includes a magnesium ion connected to nitrogen in the 5-ring structure (pheoporphyrins) through methine bridges, accompanied by a lengthy phytol chain [18]. Furthermore, the increasing inoculum level of *H. avenae* led to reduced nutrient uptake in wheat, particularly of nitrogen and magnesium [19,20], resulting in diminished chlorophyll content. Similarly, Ghasemzadeh *et al*. [21] reported decreased chlorophyll fluorescence in tomato infected with *M. incognita*.

As the initial nematode population increased, the leaf's capacity for photosynthesis, CO_2 absorption, and levels of photosynthetic pigments all declined. Consequently, the photosynthetic rate and stomatal conductance decreased with higher inoculum levels. Haseeb & Shukla [22] obtained similar results in their study on the photosynthetic rate of *Mentha citrata* infected with *Pratylenchus thornei*. Haseeb *et al.* [23] also noted a comparable impact on the photosynthetic rate of *Hyoscyamus niger,* and Thakur [14] observed a reduction in stomatal conductance in *Mentha arvensis* due to *Meloidogyne incognita*. It is evident that as nematode levels increase and invade the roots, they induce greater water stress in plants, resulting in elevated leaf temperatures at each inoculum level. Ramkrishanan and Rajendran [24] reported a rise in leaf temperature in papaya with increasing inoculum levels of *Meloidogyne incognita*.

A higher inoculum level results in increased nematode feeding and reproduction, ultimately slowing physiological growth and adversely impacting plant biomass and yield. This outcome aligns with findings from Dhawan & Nagesh (1987) and Sharma *et al*., 2020, who observed similar effects in wheat exposed to varying population densities of *Heterodera avenae*, as well as Nagesh & Dhawan (1988), who reported

comparable results regarding the final nematode population and wheat growth.

5. CONCLUSION

Cereal cyst nematode (*Heterodera avenae*) proved to be a deterrent factor in the physiology of wheat. Various physiological processes like photosynthesis, transpiration, stomatal conductance and other factors like leaf pigment concentrations (chlorophyll a, chlorophyll b, carotenoid *etc.*) which are an important factor governing the growth and development of a plant, were severely impacted by the *H. avenae* infestation. Also, with the increase in the population level of the cereal cyst nematode, a substantial decrease in all these physiological parameters were observed. With maximum reduction being obtained in the highest population level of the nematode. So, this proves the worthiness of this nematode to be taken as a serious pest in the wheat cultivation and to devise eco-friendly methods to overcome this serious pest problem in the near future.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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