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Investigation on the impact of varying degrees of *Heterodera avenae* inoculation on the physiological characteristics of wheat

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Abstract
The present study aimed to examine how different levels of *H. avenae* inoculation affect the physiology of wheat. The experiments were carried out in the screen house at the Department of Nematology, CCSHAU, Hisar. We investigated the impact of varying inoculum levels, specifically 5, 10, and 15 eggs and juveniles per gram of soil of *H. avenae*, on the physiological characteristics of wheat by making observations 30 days after sowing. As the inoculum level of *H. avenae* increased, there was a significant reduction in several key parameters, including total chlorophyll, carotenoid content, chlorophyll fluorescence, photosynthetic rate, transpiration rate, and stomatal conductance, at each inoculum level. The most substantial decreases in these parameters were observed at the highest inoculum level, which included reductions of 39.71%, 30.55%, 7.90%, 39.75%, 51.58%, and 64.86%, respectively. Furthermore, at the highest inoculum level, we observed the highest nematode population density and the lowest wheat biomass.

Keywords: Rootknot nematode, *Heterodera avenae*, wheat, chlorophyll, physiology, photosynthetic rate, transpiration rate, stomatal conductance

1. Introduction
Wheat, scientifically known as *Triticum aestivum*, is a significant cereal grain that was initially found in the Levant region. However, it is currently being grown and produced all over the world. Wheat, belonging to the poaceae family and having a chromosome number of 42, is a crop that undergoes self-pollination. The plant can thrive at elevations ranging from below sea level to 5000 m and in regions with rainfall levels ranging from 300-1130 mm. Wheat has been a fundamental component of the global food supply since the inception of human civilisation. It supplies 20% of the everyday protein and caloric intake for a population of 4.5 billion individuals. The composition of this substance consists of around 8-15% protein, 2-2.5% fiber, 1-1.5% fat, 1.5-2% minerals, and 62-71% carbohydrate. The quantity of wheat per person per day has risen from approximately 79 g/capita/day to over 185 g/capita/day, even though the population has doubled since 1961 (Bhardwaj *et al*., 2010) [3].
In the context of India, wheat holds significant agricultural importance, following rice, and has played a pivotal role in the successful implementation of the Green Revolution. India is the world's second-largest wheat producer, trailing only China. Wheat cultivation spans across latitudes from 10°N to 37°N within India. In 2022-23, the total wheat production in India reached 110.55 million tonnes (Anonymous, 2023) [1]. Projections suggest that India's wheat production in 2023-24 is expected to reach approximately 112.7 million tonnes.
Wheat farming encounters numerous challenges, with the primary concern being reduced yields caused by insect and pest infestations. Globally, it has been calculated that such infestations lead to a substantial yield reduction of approximately 28.2% in wheat crops. Research by Dhaliwal *et al.* in 2010 [4] conducted in India revealed that insect pests were responsible for crop losses of 25% in rice and maize, and 5% in wheat. Wheat, as a crop, is susceptible to a diverse range of pests, predominantly including bacteria, fungi, viruses, and plant-parasitic nematodes, all of which can have detrimental effects on both the quality and quantity of the harvest.
In the realm of wheat crop infestations, a number of plant parasitic nematodes are known to be problematic. However, two specific nematodes, namely *Heterodera avenae*, responsible for the 'Molya disease' (as noted by Vasudeva in 1958), and *Anguina tritici*, commonly referred to as the seed gall or ear cockle nematode, stand out as major culprits. Their presence results in

an annual economic loss of approximately Rs. 97.28 million in India (as reported by Jain *et al.* in 2007) [13]. Nematodes belonging to the Heteroderidae family are predominantly responsible for causing damage to various crops. This family is characterized by sedentary endoparasites and encompasses genera such as *Heterodera*, *Globodera* (known as cyst nematodes), and *Meloidogyne* (commonly known as root-knot nematodes). Among these nematodes, it is infestations of *Heterodera* and *Globodera* that lead to significant agricultural losses.

In the presence of a disease, plants experience a loss of vitality primarily due to disruptions in their physiological functions. Attributes such as growth, yield, and development are outcomes of essential physiological processes like photosynthesis, respiration, and transpiration that occur within plants. Nonetheless, there are gaps in our understanding of how nematodes affect plant physiological processes, including photosynthesis, nutrient uptake, respiration, and their role in utilizing energy sources for increased yields. This study aims to explore the connection between cereal cyst nematode parasitism and its impact on the physiological processes of wheat plants.

2. Materials and Methods

2.1 Experimental Site

The studies pertaining to this research were conducted in the screenhouse located in the Department of Nematology, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana (Latitude 29.144425"N, Longitude 75.704296"E).

2.2 Nematode inoculum

The cysts of *Heterodera avenae* Woll. were acquired from soil samples collected from wheat fields in the village of Dharnia, Fatehabad, Haryana, where the fields were naturally infested. The soil was meticulously blended, and many soil samples measuring 200 cc each were extracted from it. The aforementioned samples were subjected to Cobb's method of sieving and decanting. The samples underwent a process of sieving using a 20-mesh sieve. The cysts were acquired by backwashing the detritus on 60 mesh sieves. The cysts were examined under the microscope to ascertain the level of inoculum present within the contaminated soil. The mean cyst population was determined to be 19 cysts per 100 grams of contaminated soil.

2.3 Cyst content estimation

To determine the average number of eggs and juveniles in the cyst, a total of 10 cysts were randomly selected and crushed in a counting dish filled with water. Subsequently, the suspension was transferred into a graduated cylinder and supplemented with water until the total volume reached 25 ml. Using a pipette, one ml aliquots were extracted from the mixture after thorough shaking, in order to count the quantity of eggs and juveniles.

2.4 Preparation of inoculum

There were total four treatments: control, 5 eggs & J2/g soil, 10 eggs & J2/g soil, and 15 eggs & J2/g soil. In order to achieve these amounts of inoculum, the contaminated soil was combined with autoclaved soil (15 lbs/20 min) in a specific ratio. Sterilized autoclaved soil was treated as control.

2.5 Raising and maintenance of wheat plants

The seeds of the susceptible wheat cultivar WH 1105 used were obtained from the Wheat and Barley section of the Department of Genetics and Plant Breeding at Chaudhary Charan Singh Haryana Agricultural University in Hisar, Haryana. To expedite germination, the seeds were soaked in water overnight. Seed sowing was conducted on November 7th. In each earthen pot with a 15 cm diameter and a 1 kg capacity, three pre-germinated seeds were planted. These pots were filled with a mixture of sterilized soil and infested soil. Prior to filling the pots, the recommended quantities of nitrogen (N), phosphorus (P), and potassium (K) in a 150:60:60 kg/ha ratio were incorporated into the soil. The full doses of phosphorus and potassium were mixed in at the time of sowing, while the nitrogen was added in two installments: half at sowing and the remaining half 21 days after sowing. Following the emergence of seedlings, only one plant was retained per pot. Adequate watering was provided to ensure the well-being of the plants.

2.6 Observations

In the experiment, two separate sets were maintained for the independent measurement of physiological parameters and nematode-related parameters. The plant's physiological characteristics included observations of chlorophyll a and b, the ratio between chlorophyll a and b, total chlorophyll content, 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 recorded 30 days after sowing (DAS), while measurements for biomass and the final nematode population were taken at the time of crop maturity.

Chlorophyll Measurement

To estimate chlorophyll levels, both chlorophyll a and chlorophyll b (Chl a and Chl b), along with total chlorophyll, were quantified using the method outlined by Hiscox and Israelstam (1979) [11]. One hundred milligrams of leaf tissue were collected from each plant and washed under running tap water. This leaf tissue was then submerged in 10 milliliters of dimethyl sulfoxide (DMSO) in a test tube and left in the dark for 24 hours. The following day, the test tubes were placed in a hot water bath at 65 °C for 30 minutes. The optical densities of these chlorophyll extracts in dimethyl sulfoxide were measured at wavelengths of 645 and 663 nanometers using a spectrophotometer (MT-129).

The following formulas, as per Arnon (1949) [2], were used to determine the different components, providing chlorophyll measurements in milligrams per gram of fresh leaf weight:

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 = volume of the extract (ml)
W = fresh weight of the sample (g)
A645 = optical density at 645 nm
A663 = optical density at 663 nm

Carotenoid Measurement
The carotenoid content was quantified using the method outlined by Hiscox and Israelstam in 1979 [11]. A hundred milligrams of leaf tissue were collected from each plant and rinsed with running tap water. Subsequently, this leaf tissue was submerged in 10 milliliters of dimethyl sulfoxide (DMSO) within a test tube and left in the dark for 24 hours. The following day, the test tubes were placed in a hot water bath at 65 °C for 30 minutes. The optical densities of the carotenoid extracts in dimethyl sulfoxide were measured at 480 nanometers using a spectrophotometer. To determine the carotenoid content per gram of leaf fresh weight, the following formulas, as described by Arnon in 1949 [2], were employed:

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

Where
V represents the volume of the extract in milliliters
W stands for the fresh weight of the sample in grams
A480 signifies the optical density at 480 nanometers
Gaseous exchange parameters and leaf temperature

The photosynthetic rate, transpiration rate, stomatal conductance, and leaf temperature of a fully grown leaf were assessed using an infrared gas analyzer, specifically the IRGA ADC BioScientific LCi-SD System. The fully expanded leaf was carefully positioned within the gas analyzer chamber to maximize exposure to photosynthetically active radiation (PAR). These parameters were determined by monitoring the fluctuations in CO_2 concentration over a specified time interval. These measurements were conducted during bright and sunny hours.

Chlorophyll fluorescence
The Fv/Fm ratio, which represents the ratio of variable chlorophyll fluorescence to maximal chlorophyll fluorescence, was determined using the Opti-Sciences OS-30P chlorophyll fluorometer under bright sunlight conditions. To prepare the fully expanded leaves for measurement, they were initially acclimated to darkness for 20 minutes by securing them with clips. Subsequently, the dark-adapted samples were subjected to continuous illumination for 1 second, provided by a set of three light-emitting diodes within the sensor. Data collection occurred between 10:30 A.M. and 12:00 Noon.

Biomass
Biomass measurements were taken once the plants had reached their physiological maturity. At this stage, the plants were harvested by cutting the above-ground portions, and their individual weights were determined using a weighing balance, specifically the SF-400 C model.

Final nematode population
Soil samples were collected from each of the inoculated pots in the second set. These samples underwent processing using Cobb's sieving and decanting method. The soil was sifted through 20 and 60 mesh sieves, and the cysts were recovered by rinsing the debris from the 60-mesh sieve into a graduated cylinder. Subsequently, the contents were examined under a microscope to quantify the cyst population. To estimate the average number of eggs and juvenile nematodes within the cyst, ten cysts were randomly selected and crushed in a counting dish filled with water. The resulting suspension was transferred to a graduated cylinder, and water was added to reach a total volume of 25 ml. Using a pipette, one-milliliter aliquots were drawn from this suspension, after thorough agitation, to count the number of eggs and juveniles. The final nematode population was calculated as the product of the cyst population and the average cyst content. The reproduction factor was determined by dividing the final nematode population by the initial nematode population.

3. Results
3.1 Physiological parameters
As shown in the Table 1, When the inoculum level increased from 5 eggs and J2/g soil to 15 eggs and J2/g soil, a noteworthy decrease in chlorophyll a content was observed at each level of inoculation. The most substantial reduction (40.67%) in plants that were inoculated, as opposed to those that were not, was seen when the inoculum level was 15 eggs and J2/g soil, followed by a 27.96% reduction at the 10 eggs and J2/g soil. The smallest reduction occurred at 5 eggs and J2/g soil, amounting to 13.55%. Chlorophyll b content decreased as the inoculum levels increased. The chlorophyll b content in plants inoculated with 5 eggs and J2/g of soil was statistically comparable to that of uninoculated plants but significantly higher than in plants inoculated with 10 or 15 eggs and J2/g of soil. The most substantial reduction (33.33%) in chlorophyll b content in inoculated plants, in comparison to uninoculated plants, was noted at the 15 eggs and J2/g of soil, followed by a 20.83% reduction at the 10 eggs and J2/g of soil. As the inoculum level rose from 5 eggs and J2/g soil to 15 eggs and J2/g soil, there was a notable decrease in the total chlorophyll content at each inoculum level. The most significant reduction (39.71%) in total chlorophyll content in inoculated plants compared to uninoculated plants was seen at the 15 eggs and J2/g soil, followed by a 26.95% reduction at the 10 eggs and J2/g soil, and a 12.05% reduction at the 5 eggs and J2/g soil. As the inoculum level increased, no significant variations were observed in the ratio of chlorophyll a to b at any given inoculum level. As the inoculum level increased from 5 eggs and J2/g soil to 15 eggs and J2/g soil, there was a substantial decrease in carotenoid content at each inoculum level. The most significant reduction (30.55%) in carotenoid content in inoculated plants compared to uninoculated plants was noted at the 15 eggs and J2/g soil, followed by a 22.22% reduction at the 10 eggs and J2/g soil, and a 13.88% reduction at the 5 eggs and J2/g soil. The trend in chlorophyll fluorescence exhibited a decline as the inoculum level increased, going from 5 eggs and J2/g soil to 15 eggs and J2/g soil. The most substantial reduction (7.90%) in chlorophyll fluorescence in inoculated plants compared to uninoculated plants was noted at the 15 eggs and J2/g soil level, followed by a 3.35% reduction at the 10 eggs and J2/g soil level, and a 1.74% reduction at the 5 eggs and J2/g soil level.

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	

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	

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)	2.50
10	158 (12.62)	222 (14.92)	35154 (187.41)	3.51
15	206 (14.40)	208 (14.44)	42898 (207.09)	4.28
C.D at 5%	0.53	0.44	7.25	0.26

3.2 Gaseous exchange parameters

The photosynthetic rate in plants inoculated with 5 eggs and J2/g of soil was not significantly different from that of uninoculated plants. However, a significant decrease in the photosynthetic rate was observed in plants inoculated with 10 and 15 eggs and J2/g of soil compared to uninoculated plants. The most substantial reduction (39.75%) in the photosynthetic rate in inoculated plants, when compared to uninoculated plants, was seen at the 15 eggs and J2/g soil, followed by a 28.13% reduction at the 10 eggs and J2/g soil. As the inoculum level increased from 5 eggs and J2/g soil to 15 eggs and J2/g soil, there was a notable decrease in the transpiration rate at each inoculum level. The most significant reduction (51.58%) in transpiration rate in inoculated plants compared to uninoculated plants was noted at the 15 eggs and J2/g soil, followed by a 48.41% reduction at the 10 eggs and J2/g soil, and a 38.88% reduction at the 5 eggs and J2/g soil. As the inoculum level increased from 5 eggs and J2/g soil to 15 eggs and J2/g soil, there was a significant decrease in stomatal conductance compared to uninoculated plants at each inoculum level. Stomatal conductance in plants inoculated with 10 eggs and J2/g soil and 15 eggs and J2/g soil was not statistically different from the conductance observed in plants with 5 eggs and J2/g soil inoculum level. The most substantial reduction (64.86%) in stomatal conductance in inoculated plants, compared to uninoculated plants, was observed at the 15 eggs and J2/g soil, followed by a 57.14% reduction at the 10 eggs and J2/g soil, and a 42.85% reduction at the 5 eggs and J2/g soil. The leaf temperature measured in plants inoculated with 5 eggs and J2/g of soil was not statistically different from the leaf temperature of uninoculated plants. However, a significant rise in leaf temperature was noted in plants inoculated with 10 eggs and J2/g of soil and 15 eggs and J2/g of soil. The most substantial increase (1.84 ºC) in leaf temperature in inoculated plants, compared to uninoculated plants, was observed at the 15 eggs and J2/g soil, followed by a 0.86 ºC increase at the 10 eggs and J2/g soil.

3.3 Nematode parameters

As the inoculum level rose, there was a proportional rise in cyst population, cyst content, final nematode population, and reproduction factor. The highest nematode population was achieved at the highest inoculum level of 15 eggs and J2/g soil, while the lowest nematode population was observed at the lowest inoculum level of 5 eggs and J2/g soil.

3.4 Biomass

A noticeable decrease in biomass occurred as the inoculum level increased from 5 eggs and J2/g soil to 15 eggs and J2/g soil. The most substantial reduction (54.90%) in plant biomass, compared to uninoculated plants, was observed at the 15 eggs and J2/g soil, followed by a 36.60% reduction at the 10 eggs and J2/g soil, with the lowest reduction occurring at the 5 eggs and J2/g soil (14.50%).

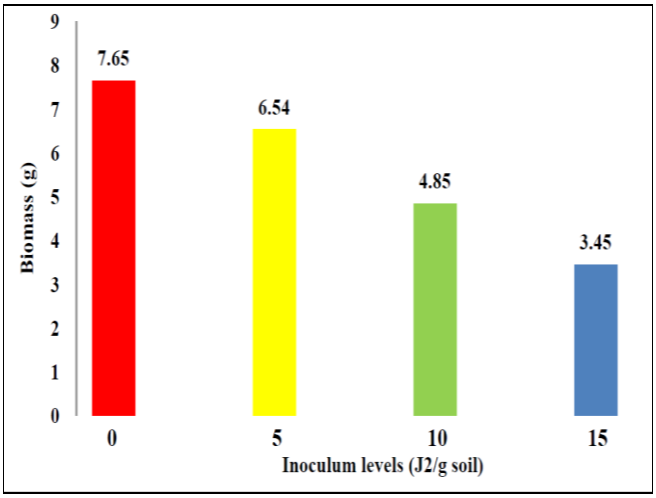

Fig 1: Effect of varying degrees of *H. avenae* inoculation on biomass in wheat

4. Discussion

In our current research, we observed that as the nematode inoculum levels increased, physiological processes and plant growth were negatively impacted. These findings align with prior studies conducted by Hesling (1957) [10] and Gill & Swarup (1973) [6]. Specifically, the 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/g soil. Similar results were documented in the leaf pigments of *Mentha arvensis* by Thakur (2014) [16] and *Ocimum kilimandscharicum* by Haseeb *et al*. (1998) [8] when infected with *Meloidogyne incognita*. The core component of chlorophyll involves a magnesium ion connected to the nitrogen in the 5-ring structure (pheoporphyrins) through methine bridges, accompanied by a lengthy phytol chain (İnanç, 2011) [12]. Additionally, the increasing inoculum level of *H. avenae* led to reduced nutrient uptake in wheat, particularly in nitrogen and magnesium (Nagesh & Dhawan, 1988) [14], resulting in diminished chlorophyll content.

The leaf's capacity for photosynthesis, CO_2 absorption, and levels of photosynthetic pigments all declined as the initial nematode population increased. Consequently, the photosynthetic rate and stomatal conductance decreased as the inoculum level rose. Haseeb & Shukla (1995) [6] obtained similar findings in their study on the photosynthetic rate of *Mentha citrata* infected with *Pratylenchus thornei*. Haseeb *et al*. (1990) [8] also noted a comparable impact on the photosynthetic rate of *Hyoscyamus niger*, and Thakur (2014) [16] 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 (1999) [15] reported a rise in leaf temperature in papaya as the inoculum level of *Meloidogyne incognita* increased.

A higher inoculum level results in increased nematode feeding and greater nematode reproduction, ultimately slowing down physiological growth and adversely impacting the plant's biomass and yield. This outcome aligns with findings from Dhawan & Nagesh (1987) [5], who observed similar effects in wheat when exposed to varying population densities of *Heterodera avenae*, as well as Nagesh & Dhawan (1988) [14], who reported comparable results regarding the final nematode population and wheat growth.

5. Conclusion

Cereal cyst nematode was found to be a be causing significant reductions in the leaf pigments concentration and gaseous exchange parameters. Higher the population of *Heterodera avenae* present in the soil, more will be the reduction in the plant growth.

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