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Lochan Sharma
Ph. D. Scholar, Department of Nematology, CCSHAU, Hisar, Haryana, India

RS Kanwar
Principal Scientist, Department of Nematology, CCSHAU, Hisar, Haryana, India

Gurpreet Singh
Assistant Professor, PDM, University, Bahadurgarh, Jhajjar, Haryana, India

Renu Munjal
Principal Scientist, Department of Botany and Plant Physiology, CCSHAU, Hisar, India

Corresponding Author:
Lochan Sharma
Ph. D. Scholar, Department of Nematology, CCSHAU, Hisar, Haryana, India

Effect of *Heterodera avenae* and *Urocystis agropyri* on chlorophyll content of wheat at different sowing times

Lochan Sharma, RS Kanwar, Gurpreet Singh and Renu Munjal

Abstract
A pot experiment was conducted to find out the effect of *Heterodera avenae* and *Urocystis agropyri* on chlorophyll content of wheat at different sowing times in rabi season 2018-19 under screen house conditions. SPAD (Soil Plant Analysis Development) chlorophyll content was measured at 30, 40, 50 and 60 days after sowing and plant growth parameters (plant height, plant weight) at crop maturity. Wheat sown in last week of November shown maximum reduction in plant height and weight where fungus and nematode inoculated simultaneously. SPAD chlorophyll content was increased from 30-60 days after sowing and maximum at 60 days after sowing. Chlorophyll per cent reduction increased from 30-60 days after sowing and maximum reduction was observed at 60 days after sowing (23.11%) in nematode and fungus inoculated simultaneously. Chlorophyll reduction was maximum in last week of November (24.73%) followed by second week of December (22.66%) and first week of November (21.92%). Disease incidence was same in all the treatment where *U. agropyri* was inoculated. Nematode population was maximum in wheat sown in last week of November.

Keywords: Chlorophyll, *Heterodera avenae*, SPAD, *Urocystis agropyri*, Wheat

Introduction
Wheat is extensively grown under irrigated and non irrigated conditions around the world due to its adaptability to wide climatic and geographic conditions as well as dietary traditions [6]. Uttar Pradesh, Punjab and Haryana are the three prominent wheat producing states of India popularly known as the 'Granary of India'Several abiotic and biotic agents are limiting factors to both wheat and barley cultivation, which adversely affect its yield parameters such as plant height, straw yield, weight of 1000 kernels and grain yield [5].
Plant-parasitic nematodes damage a great number of crops worldwide and are major pathogens in tropical regions [12] Cyst nematodes are the most studied plant parasitic nematodes on wheat [11, 13]. known for causing 'Molya' disease in wheat. Yield losses caused by cereal.
cyst nematode could go up to 90% in severely infested fields [14].
Urocystis agropyri causes 39-78% loss in Rajasthan [1].
Wheat is grown from early November to last week of December in north plains of India. *H. avenae* and *U. agropyri* are of common occurrence in light soils. Nematode and disease infections affect the plant physiology. Reduced chlorophyll content and increased protein and sugar content were observed in the plants due to *Meloidogyne incognita* and *Fusarium oxysporum* [10]. Total chlorophyll, carotenoid , chlorophyll fluorescence, photosynthetic rate and stomatal conductance is affected adversely by *H. avenae* [16].
The objective of the study is to know the effect of sowing times on chlorophyll content of wheat.

Materials and methods
The experiment was conducted under screen house conditions in Department of Nematology, CCS HAU, Hisar during Nov. 2018 – April 2019 to find out the effect of nematode and fungus different at sowing dates. The flag smut inoculum was obtained from department of Plant Pathology, CCSHAU, Hisar. The plant material of flag smut was ground in laboratory grinder to fine powder for release of spores. The seeds of wheat cultivar HD 2967 were treated with this powder @ 20g/kg seed. The seeds were kept in Petri plate with inoculum spread over it, and shaken vigorously to coat the teliospores on the seeds. Samples of *Heterodera avenae*

were processed through Cobb's decanting and sieving method [4]. The inoculum of *H. avenae* @ 5 eggs and J_2/g soil were inoculated in steam sterilized soil 10 days after sowing of wheat. There were four treatments i.e. *H. avenae*, *U. agropyri*, *H. avenae* + *U. agropyri* and un inoculated control, with four Replications. There were three sowing times *i.e*. first week of November, last week of November and second week of December. Observations on chlorophyll were taken measured 30, 40, 50 and 60 days after sowing by using SPAD (Soil Plant Analysis Development) and plant growth parameters were recorded at crop maturity.

Statistical Method: The design of experiments was CRD (Completely Randomized Design) and data were analyzed by OPSTAT software of CCSHAU, Hisar.

Results and Discussion

The effect of plant growth parameters and chlorophyll content of wheat due to *Heterodera avenae* and *Urocystis agropyri* was found to be significantly (p<0.05) higher in last week of November sown plants as compared to the plant sown in first week of November and second week of December plant growth parameters and chlorophyll content were more in first week of November sown plants as compared to other sowing dates. Most appropriate time for wheat planting with respect to plant weight and chlorophyll content was first week of November.

In present study, total chlorophyll content was maximum in first week of November followed by last week of November and second week of December. Maximum reduction was observed in last week of November in nematode and fungus simultaneous inoculated plants. Minimum reduction of chlorophyll was observed in fungus alone.

In the crop sown, in second week of December, chlorophyll content was found less than other dates of sowing.

After 30 days of sowing chlorophyll content in all the treatments decreased over control. Overall reduction in all sowing date was highest when both pathogens present simultaneous followed by *H. avenae* (14.85%) alone. Maximum reduction (23.82%) was found in simultaneous inoculation of nematode and fungus in last week of November followed by second week of December (20.26%) and in first week of November (17.79%). Among all the sowing dates minimum chlorophyll reduction was recorded in *U. agropyri* alone (8.30 %) (Table 1).

Table 1: Effect of *Heterodera avenae* and *Urocystis agropyri* alone and in combination on SPAD chlorophyll content of wheat 30 days after sowing

Treatments	Total chlorophyll							
	First week Of Nov.	% Reduction over Control	Last week of Nov.	% Reduction over Control	Second week of Dec.	% Reduction over control	Mean	% Reduction over control
Heterodera avenae	33.75	13.63	31.68	16.86	32.43	14.11	32.62	14.85
Urocystis agropyri	35.60	8.89	33.48	12.14	36.33	3.77	35.13	8.30
H. avenae + *U. agropyri*	32.13	17.79	29.03	23.82	30.10	20.26	30.42	20.60
Un inoculated control	39.08		38.10		37.75		38.31	
Mean	35.14		33.07		34.15			
C. D. at 5%	Sowing Time = 0.57, Treatments = 0.66, sowing time × Treatments = 1.15							

After 40 days of sowing chlorophyll content in all the treatments decreased as compared to control. Overall reduction in all sowing times highest reduction (21.98 %) when both pathogens present simultaneous followed by *H. avenae* (16.51%) alone. Maximum reduction was found in simultaneous inoculation of nematode and fungus on last week of November (24.08%), followed by second week of December (21.59 %) and in first week of November (20.36 %). Among all the sowing dates minimum chlorophyll reduction was recorded in *U. agropyri* alone (9.62 %) (Table 2).

Table 2: Effect of *Heterodera avenae* and *Urocystis agropyri* alone and in combination on SPAD chlorophyll content of wheat 40 days after sowing

Treatments	Total chlorophyll							
	First week of Nov.	% Reduction over control	Last week of Nov.	% Reduction Over control	Second week of Dec.	% Reduction over Control	Mean	% Reduction over control
Heterodera avenae	34.63	16.06	32.60	16.94	32.48	16.52	33.23	16.51
Urocystis agropyri	36.63	11.21	34.13	13.06	37.15	4.50	35.97	9.62
H. avenae + *U. agropyri*	32.85	20.36	29.80	24.08	30.50	21.59	31.05	21.98
Un inoculated control	41.25		39.25		38.90		39.80	
Mean	36.34		33.94		34.76			
C. D. at 5%	Sowing Time = 0.51 Treatments = 0.60 Sowing time × Treatments = 1.02							

After 50 days of sowing total chlorophyll content observed at three dates of sowing was significantly different among all the sowing dates. Overall in all treatments highest chlorophyll was recorded in control (41.23) followed by *U. agropyri* (36.86) alone. Maximum reduction in total chlorophyll (22.58%) was recorded in *H. avenae* + *U. agropyri* inoculated plants in all three sowing dates followed by (17.83%) in *H. avenae* alone. Among sowing dates maximum reduction (24.23%) in last week of November sowing followed by second week of December (22.45%) and first week of November when *H. avenae* and *U. agropyri* present together. Minimum reduction in chlorophyll (10.60 %) observed in *U. agropyri* in all sowing dates. (Table 3).

Table 3: Effect of *Heterodera avenae* and *Urocystis agropyri* alone and in combination on SPAD chlorophyll content of wheat 50 days after sowing

Treatments	Total chlorophyll							
	First week of Nov.	% Reduction over Control	Last week of Nov.	% Reduction over Control	Second week of Dec.	% Reduction over Control	Mean	% Reduction over control
Heterodera avenae	35.33	16.78	33.18	19.62	33.15	17.07	33.88	17.83
Urocystis agropyri	37.60	11.43	35.30	14.48	37.68	5.75	36.86	10.60
H. avenae + *U. agropyri*	33.48	21.14	31.28	24.23	31.00	22.45	31.92	22.58
Un inoculated control	42.45		41.28		39.98		41.23	
Mean	37.21		35.26		35.45			
C. D. at 5%	Sowing Time = 0.49, Treatments = 0.57, Sowing time × Treatments = 1.08							

After 60 days of sowing total chlorophyll content observed at three dates of sowing was significantly different among all the sowing dates. Overall in all treatments highest chlorophyll was recorded in control (42.24) followed by *U. agropyri* (37.31) alone. Maximum total chlorophyll per cent reduction in *H. avenae* + *U. agropyri* (23.11%) inoculated plants in all three sowing dates followed by (18.80%) in *H. avenae* alone. Among sowing dates maximum reduction in last week of November (24.73%) sowing followed by last week of December (23.11%) and first week of November when *H. avenae* and *U. agropyri* present together. Minimum per cent reduction in chlorophyll was observed in *U. agropyri* in all sowing dates. (Table 4).

Table 4: Effect of *Heterodera avenae* and *Urocystis agropyri* alone and in combination on SPAD chlorophyll content of wheat 60 days after sowing

Treatments	Total chlorophyll							
	First week of Nov.	% Reduction over control	Last week of Nov.	% Reduction over Control	Second week of Dec.	% Reduction over control	Mean	% Reduction over control
Heterodera avenae	36.30	17.31	33.65	20.73	32.95	18.39	34.30	18.80
Urocystis agropyri	38.48	12.36	36.18	14.78	37.28	7.68	37.31	11.67
H. avenae + *U. agropyri*	34.28	21.92	31.95	24.73	31.23	22.66	32.48	23.11
Un inoculated control	43.90		42.45		40.38		42.24	
Mean	38.24		36.06		35.46			
C. D. at 5%	Sowing Time = 0.44, Treatments = 0.51, Sowing time × Treatments = 0.89							

Total chlorophyll content was found to decrease with age in the rusted (*Puccinia arachidis*) leaves of groundnut, whereas, the reverse is true in case of healthy leaves [15]. Chlorophyll was reduced in infected leaves of pearl millet following infection of *Sclerospora graminicola* which is in agreement with the present studies [7]. Nematode and fungus synergistically affect the chlorophyll content of wheat, both of pathogens affect the magnesium absorption and it is the main component of chlorophyll

Plant height significantly decreased in all the three sowing times as compared to their respective control. Overall maximum plant height was found in un-inoculated followed by *U. agropyri*, height (77.3 cm) was recorded on *H. avenae* (73.2 cm). Between different sowing time minimum plant height (68.5cm) was recorded when *H. avenae* and *U. agropyri* were inoculated simultaneously followed by in *H. avenae* in last week of November sown plants. Among the three sowing times, maximum plant second week of December followed by (74.4 cm) first week of November in control (Table 5). Due to both of pathogen growth of wheat plant affect drasitically.

Table 5: Effect of *Heterodera avenae* and *Urocystis agropyri* alone and in combination on height of wheat in different sowing times

Treatments	Plant height (cm)			
	First week of November	Last week of November	Second week of December	Mean
Heterodera avenae	75.0	71.5	73.1	73.2
Urocystis agropyri	75.1	72.6	74.7	74.1
H. avenae + *U. agropyri*	71.5	68.5	69.3	69.7
Un inoculated control	76.2	75.8	77.3	76.4
Mean	74.4	72.1	76.4	
C. D. at 5%	Sowing time = 1.60 , Treatments = 1.84 , Sowing time × Treatments = NS			

Irrespective of date of sowing maximum plant weight was recorded in control (5.51 g) followed by *U. agropyri* (4.89 g) and *H. avenae* (4.25 g). In first week of November and second week of December weight of all the treatment was significantly ($P< 0.05$) different from each other. In last week of November sowing date simultaneous inoculation of *H. avenae* and *U. agropyri* (3.37 g) was statistically ($P< 0.05$) at par with *H. avenae* (3.42 g) (Table 6).

Table 6: Effect of *Heterodera avenae* and *Urocystis agropyri* alone and in combination on weight of wheat in different sowing times

Treatments	Plant weight (g)			
	First week of November	Last week of November	Second week of December	Mean
Heterodera avenae	5.25	3.42	4.08	4.25
Urocystis agropyri	5.79	4.54	4.35	4.89
H. avenae + *U. agropyri*	4.59	3.37	3.52	3.82

Un inoculated control	6.35	5.46	4.73	5.51
Mean	5.49	4.20	4.17	
C. D. at 5%	Sowing time = 0.07, Treatments = 0.08, Sowing time × Treatments = 0.15			

Except simultaneous inoculation of nematode and fungus on last week of November and second week of December, number of cyst differed significantly ($P< 0.05$) in all the three sowing dates. Maximum cyst population (115) was found when nematode was inoculated alone followed by in second week of December (95). Reproduction and damage potential of *H. glycines* in resistant and susceptible cultivars of soybean harvest densities of *H. glycines* were lower for late-planted than for early-planted susceptible soybeans [17]. Highest infestation of *M. graminicola* in wheat crop, which was sown in second week of October followed by 7.67 and 3.17 galls/plant in first and second week of November sowing [9].

In presence of fungus, population of nematode decreased which may be due to certain metabolites released by fungus or changes in plant by fungus which may be responsible for population reduction [3]. Maximum cyst population of *H. avenae* was recorded in timely sown crop than late or early sown crop [8]. Higher larval content of *H. avenae* from roots of wheat plants sown on 15th November as compared to early and late sown plants [2].

Conclusion

It is concluded from the present study that *Heterodera avenae* and *Urocystis agropyri* simultaneously reduced chlorophyll and plant growth parameter and maximum reduction was observed second week of November sown wheat at 60 days after sowing.

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