Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(5): 1421-1424
Received: 02-07-2020
Accepted: 21-08-2020

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Effect of *Urocystis agropyri* and different inoculum levels of *Heterodera avenae* on chlorophyll content and growth of wheat

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Abstract
A pot experiment was done for studying the effect *U. agropyri* and different inoculum levels of *Heterodera avenae* on wheat cultivar HD 2967. The experiment was conducted under screen house conditions in Department of Nematology, CCS HAU, Hisar during Nov. 2018 – April 2019. SPAD chlorophyll content was measured 30, 40, 50 and 60 days after sowing, while plant height and plant weight at crop maturity. Maximum reduction in plant height and plant weight was observed at highest inoculum level (15 eggs and juveniles/ g soil) of nematode. SPAD chlorophyll content increased from 30-60 days after sowing and was maximum at 60 days after sowing. Per cent reduction in chlorophyll increased from 30-60 days after sowing and maximum reduction was observed at 60 days after sowing in 15 eggs and juveniles/g soil of *Heterodera avenae* and *Urocystis agropyri.*

Keywords: SPAD, *U. agropyri*, chlorophyll, *H. avenae*, wheat

Introduction
Wheat is main staple food of millions of Indians, particularly in the northern and north western parts of the country. It is self-pollinated crop that belongs to family Poaceae. Geological record suggests that wheat was first cultivated in the regions of the Fertile Crescent around 9600 BC. India is the fourth largest producer of wheat in the world after Russia, USA and China.

The cereal cyst nematode, *H. avenae*, is widely distributed in the States of Rajasthan, Haryana and Punjab and is confined to few locations in Jammu & Kashmir, Himachal Pradesh, Uttar Pradesh and Delhi (Mathur *et al.*, 1986) [16]. *H. avenae* cause "Molya" disease in wheat and barley. Nematode parasitism alters host physiological process like photosynthesis and water absorption capacity that ultimately affect growth and productivity of the host plant (Melakeberhan *et al.*, 1987) [17].

Flag smut incited by *Urocystis agropyri* has the potential to cause substantial reduction in yield and quality of wheat production. It was first documented from South Australia and later on from Chile, China, Egypt, India, Japan, Mexico, Pakistan, South Africa, and USA (Toor *et al.*, 2013 and Beniwal 1992) [20, 2] documented 23–65% yield losses from flag smut infection in nine commercial wheat cultivars.

Moreover, reduction in tillering, plant height, ear head length, and 1,000 grainweight were recorded 19–37%.

(Hassan *et al.* 2012) [11] Found that simultaneous inoculation of *H. avenae* and *Fusarium culmorum* had an additive effect on yield losses due to the two pathogens. One month prior inoculation of *H. avenae* caused grain yield reduction to a tune of 43.8 per cent that exceeded the sum of individual losses caused by the nematode and fungus. Sufficient work has been done on *Heterodera avenae* and *Urocystis agropyri* but there is no work on their combined effect. This experiment was conducted to study on their combined effect on wheat.

Materials and Methods
The experiment was conducted under screen house conditions in Department of Nematology, CCS HAU, Hisar during Nov. 2018-April 2019. The cysts of *H. avenae* were obtained from bulk soil samples collected from naturally infested wheat fields. These samples were processed through Cobb's decanting and sieving method (Cobb, 1918) [5] for collection of eggs. These cysts were hand picked, crushed under the microscope and used as inoculums @ 5, 10 and 15 eggs and J2/g soil. The flag smut inoculum was obtained from department of Plant Pathology, CCSHAU, Hisar. The material was ground in grinder to make the fine powder to release the 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. Observation on chlorophyll was 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. There were four replications. The design of experiments was CRD (Completely Randomized Design) and data were analyzed by using OPSTAT software available on CCSHAU website www.hau.ac.in.

Results

Data on the effect of *Heterodera avenae* and *Urocystis agropyri* on plant growth parameters in wheat at different inoculum levels of nematode and constant inoculum of fungus are given in table 1. There was significant difference in plant height in all the treatments except fungus alone. Plant height of fungus alone was found statistically at par with control. Similarly, there was no significant difference among the nematode alone (71.9) and simultaneous inoculation of nematode and fungus (69.0). Minimum plant height (56.9 cm) was recorded in highest inoculation of nematode (15 eggs and juveniles) and fungus followed (59.6cm) by 15 eggs and juveniles and maximum height (78.1cm) was found in control. Maximum plant weight (4.62 g) was in the control followed by (3.78 g) fungus alone. Minimum (1.49 g) plant weight was in when 15 eggs and J_2 present and fungus present together.

The data in table 2 indicated that chlorophyll content increase from 30 to 60 days in all the treatments. Among all treatments maximum chlorophyll (42.17) was observed in un-inoculated control followed by *U. agropyri* (38.84) alone. Maximum per cent reduction (41.17%) when nematode (15 eggs and juveniles) with fungus present simultanelusly followed by nematode (15 eggs and juveniles) and (33.18%) nematode (10 eggs and J_2) and *U. agropyri* simultaneous. Minimum per cent reduction was found in *U. agropyri* (7.90%). All treatments were found significant with respect to control. Maximum (39.18) chlorophyll was found in control followed by (37.15) *U. agropyri* alone at 30DAS. Same trend was observed 40, 50 and 60 DAS. As nematode inoculums increases with fungus chlorophyll content and growth of wheat decreases.

Maximum nematode population was obtained in the highest inoculum level i.e. 15 eggs and J_2/g soil.

Table 1: Effect of *Heterodera avenae* and *Urocystis agropyri* alone and in combination on plant growth parameters in different inoculum levels

Treatments	Plant height (cm)	Plant weight (g)
Urocystis agropyri	75.4	3.78
H. avenae (5 eggs and J_2/g soil)	71.9	2.08
H. avenae (5 eggs and J_2/g soil) +*U. agropyri*	69.0	1.95
H. avenae (10 eggs and J_2/g soil)	65.7	1.89
H. avenae (10 eggs and J_2/g soil) + *U. agropyri*	62.3	1.79
H. avenae (15 eggs and J_2/g soil)	59.6	1.69
H. avenae (15 eggs and J_2/g soil) +*U. agropyri*	56.9	1.49
Un inoculated control	78.1	4.62
C.D. at 5%	4.41	1.68

Table 2: Effect of *Heterodera avenae* and *Urocystis agropyri* alone and in combination on SPAD chlorophyll content 30, 40, 50 and 60 days after sowing

Treatments	Chlorophyll content									
	30DAS	% Reduction over control	40 DAS	% Reduction over control	50DAS	% Reduction over control	60DAS	% Reduction over control	Mean	% Reduction over control
Urocystis agropyri	37.15	5.17	38.25	7.27	39.35	8.86	40.63	9.87	38.85	7.87
H. avenae (5 eggs and J_2/g soil)	33.45	14.61	34.53	16.30	35.58	17.60	36.43	19.19	35.00	17.00
H. avenae (5 eggs and J_2/g soil) +*U. agropyri*	30.58	21.95	31.05	24.73	32.20	25.42	33.18	26.38	31.75	24.71
H. avenae (10 eggs and J_2/g soil)	27.88	28.84	28.90	29.94	29.10	32.60	29.32	34.96	28.80	31.71
H.avenae (10 eggs and J_2/g soil) +*U. agropyri*	27.18	30.63	27.78	32.67	28.40	34.22	29.12	35.40	28.12	33.32
H. avenae (15 eggs and J_2/g soil)	26.10	33.38	26.98	34.61	27.58	36.13	28.13	37.60	27.20	35.50
H. avenae (15 eggs and J_2/g soil) +*U. agropyri*	23.55	39.89	24.33	41.03	25.33	41.34	26.05	42.21	24.82	41.14
Un inoculated control	39.18		41.25		43.18		45.08		42.17	
Mean	30.63		31.63		32.59		33.49			
C. D. at 5%	Days = 0.41, Treatments = 0.58, Days × Treatments = 1.17									

Discussion

In this experiment, with increase in inoculum level of nematode corresponding reduction in plant height, plant weight and chlorophyll content was obtained. Minimum plant height was recorded where 15 eggs and juveniles of *H. avenae* and fungus were inoculated simultaneously, followed by 15 eggs and juveniles of *H. avenae* alone. Maximum height was found in uninoculated control. Maximum plant weight was observed in uninoculated control followed by fungus alone. Minimum plant weight was in 15 eggs and J_2 of *H. avenae* and fungus simultaneous inoculation. Khalequzzaman (2003) [13] also reported correspondingly lower plant growth of soybean, nodulation and yield per plant from lower to higher levels of inoculation ranging from 4-10 egg masses of *M. javanica* with 0.025-0.1 per cent w/w of *S. rolfsii*. Galling incidence was negatively correlated with plant growth, nodulation and yield. Hassan *et al.* (2012) [11] found that on durum wheat cv. Sham 3, grain yield reduction caused by *H. avenae* and *Fusarium culmorum* alone was 12.3 and 25.5 per cent, respectively. The simultaneous inoculation of *H. avenae* and *F. culmorum* resulted in 38.4 per cent reduction, indicating an additive effect of yield losses due to the two pathogens. Siddiqui and Mahmood (1992) [19] while studying the effect of inoculum levels of *H. cajani* (250, 500,

1000, 2000 and 4000 J_2/ g soil) on pigeonpea in a glasshouse test, found that pigeonpea growth and rhizobia nodulation progressively decreased as the inoculum level increased. Significant reduction in plant growth occurred with an inoculum of 500 or more J_2 of *H. cajani*.

In present study, there was no effect of inoculum level of *H. avenae* on germination of wheat. Dharamveer (1998) [7] also reported that per cent seed germination was not affected significantly by any level of inoculum of fungus as compared to control.

Total chlorophyll content increased from 30 to 60 days in all the treatments. Among all treatments maximum chlorophyll was in uninoculated control followed by *U. agropyri* alone. In all the observations of chlorophyll, maximum reduction was found when 15 eggs and J_2 of *H. avenae* inoculated simultaneously with fungus followed by 15 eggs and J_2 of *H. avenae* alone and 10 eggs and J_2 of *H. avenae* and *U. agropyri* simultaneously. Minimum reduction was found in *U. agropyri* alone. Similar results were obtained by Melakeberhan *et al.* (1987) [17] who found that photosynthesis significantly decreased when *Phaseolus vulgaris* was inoculated with increasing levels (0, 2000, 4000 or 8000 J_2) of *Meloidogyne incognita*. Nehra *et al*. (2001) [18] found that total chlorophyll content and net photosynthetic rate in barley decreased with increasing inoculum levels of *H. avenae* in diseased plants over their healthy control.

Lobato *et al.* (2010) [15] studied the effect of *Colletotrichum lindemuthianum* on photosynthetic pigments in *Phaseolus vulgaris* plants. They found higher chlorophyll reduction with increase in number of days. Akai and Fukutomi (1964) [1] also observed decrease in chlorophyll contents of diseased leaf tissues of rice due to *Sclerophthora macrospora*. Garg and Mandhar (1975) [10], Bhatia and Thakur (1991) [3] and Kumar (1994) [14] also recorded the decreased level of total chlorophyll content in pearl millet leaves infected with *Sclerospora graminicola*, which is in agreement with the present studies.

In present work, with the increased inoculum level, there was increase in cyst population and maximum nematode population was obtained in the highest inoculum level i.e. 15 eggs and J_2/g soil. Final cyst population decreased when fungus was present with nematode. Minimum cyst population at lowest inoculum level i.e. 5 eggs and J_2/g soil and fungus. Gao *et al.* (2006) [8] found that *H. glycines* and *F. solani* f. sp. *glycines* reduced the growth of soybeans. They found that reproduction of *H. glycines* was suppressed by high inoculum levels but not by low levels of fungus. Carter (1975) [4] observed that when 2500 or 5000 *M. incognita* larvae per plant were combined with *R. solani*, seedling disease severity increased over that caused by *R. solani* alone. Disease severity increased with the highest level of *R. solani* inoculum either alone or when combined with *M. incognita*. Garber *et al*. (1979) [9] found that galling was directly proportional to the initial population level of *M. incoginta* in cotton. Fusarium wilt symptoms occurred without nematodes with 77000 fungus propagules or more per gram of soil while, with 50 J_2, 650 propagules were able to cause wilt disease. Cook (1975) [75] found that less fecund, fewer and smaller cysts were produced by *Heterodera avenae* in presence of *Gaeumannomyces graminis* in barley roots. Ketudat (1968) [12] also had similar observations while studying the effect of soil borne fungi on *Globodera rostochiensis* in tomato. The reason for the low nematode population in presence of fungus may be changes in physiology and biochemistry of host plant brought by fungus which are unfavorable for nematode.

Conclusion

It is concluded from the present study that increases in inoculum level of *Heterodera avenae* with *Urocystis agropyri* simultaneously reduced chlorophyll content and plant growth parameter.

Acknowledgement

Authors are thankful to Dr. R. S. Beniwal (Department of Plant Pathology), CCSHAU Hisar for providing fungus culture and Dr. Renu Munjal (Deparment of Botany and Plant Physiology) CCSHAU Hisar, for providing the SPAD meter.

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