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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; SP-10(12): 1304-1307

www.thepharmajournal.com
Received: 11-10-2021
Accepted: 18-11-2021

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Variability in cultural and morphological characters of different isolates of *Helminthosporium sativum*

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Abstract
Leaf blight symptom collected from different location on various wheat cultivars showed remarkable variation. Symptoms incited by *Helminthosporium sativum* on wheat cultivars varied from light yellow to light orange. The maximum colony diameter was recorded in case of isolate H_5 (66.56mm) and minimum in case of H_3 (48.38mm).Regarding dry mycelial weight maximum dry weight was recorded in isolate H_4 (160.60 mg.) and minimum in H_3 (128.25mg.). The maximum number of spores were recorded in isolate H_5 (45.33000/ml.) and minimum in H_3. The purpose of this study to see the variations among the isolates, collected from different locations.

Keywords: cultural, morphological characters, *Helminthosporium sativum*

Introduction
Wheat is one of the most cereal crop grown worldwide and liking staples of nearly 2.5 billion of world population. India is the second largest producer of wheat worldwide (29.58mh and global area 14% and production 99.70mt.) reported by Sharma and Sendhil (2015 and 2016) [8, 9]. Leaf blight of wheat caused by *Helminthosporium sativum* is a serious problem for wheat growers. The leaf blight, which used to pose a serious problem in the eastern regions of the country under warm and humid weather conditions, have become far and wide in the country. A number of pathogens causing leaf spot, blight and blotch on wheat crop has been reported in India. Among them *H. sativum* Pammel, King and Bakke is more important than *Alternaria triticina* (Singh *et al.* 1992) [10]. Variability in cultural characteristics was observed in morphology and growth rate. However, there was no relationship between morphological variability and virulence amongst isolates (Oliveira *et al.*1998) [6]. Mitra (1931) [5] described the variations in the growth features of two strain of *Bipolaris sorokiniana* obtained from wheat and barley on four types of media.

Materials and Methods
The leaves collected from infected wheat plant show blight symptoms from CRC, GBPUA&T, Pantnagar and from NDUA&T, Faizabad, Uttar Pradesh. Samples were done on Potato Dextrose Agar (PDA) medium. The pathogen was grown on PDA medium. The composition of PDA was as: Peeled potato- 200 gram, Dextrose 20gram, Agar-agar - 20gram and Distilled Water - 1000ml. The blighted spots were cut into small pieces of half a centimeter in size. These pieces were sterilized with mercuricm chloride solution (1:1000) for one minute followed by 2-3 washing with sterilized distilled water. The pieces were later dried using sterilized blotters and plated on the PDA on sterilized plastic Petri dishes (90 mm diameter) @ 5 pieces per plate at equal distance.
These petridishes were incubated at 25±1 ^{0}C in BOD incubator. After 48 hours of incubation, the growing mycelium from the margins of the apparently distinct colonies were sub cultured on fresh PDA slant.

Purification and Maintenance of the Culture: The culture of the fungus isolates were purified by Single spore isolation technique and maintained on PDA at low temperature (5±1 ^{0}C) in the refrigerator. To keep the culture bible, sub culturing was done at an internal of 15-20 days (Duveiller and Altamirano, 2000) [4]. The re-culture on as per need.

Result and Discussion

Cultural characters

Growth on PDA

H. sativum isolates were grown on PDA for six days and growth was recorded on 3rd and 6th days of inoculation. The growth of isolates after 3rd and 6th days of inoculation was 39.69 and 74.99 mm, respectively and these were significantly different (Table-1). Comparison among individual isolates of H. sativum indicated that isolates H_5 had highest growth of 66.56 mm and was significantly superior over others. It was followed by isolate H_4 (60.33 mm), H_2 (57.33 mm) and H. (54.16 mm). The lowest radial growth was recorded in isolate H_3 (48.38 mm) which was significantly lower than all the other isolates. Isolates H_3, H_4 and H_5 showed significant differences among themselves however, H, and H2 were statistically at par.

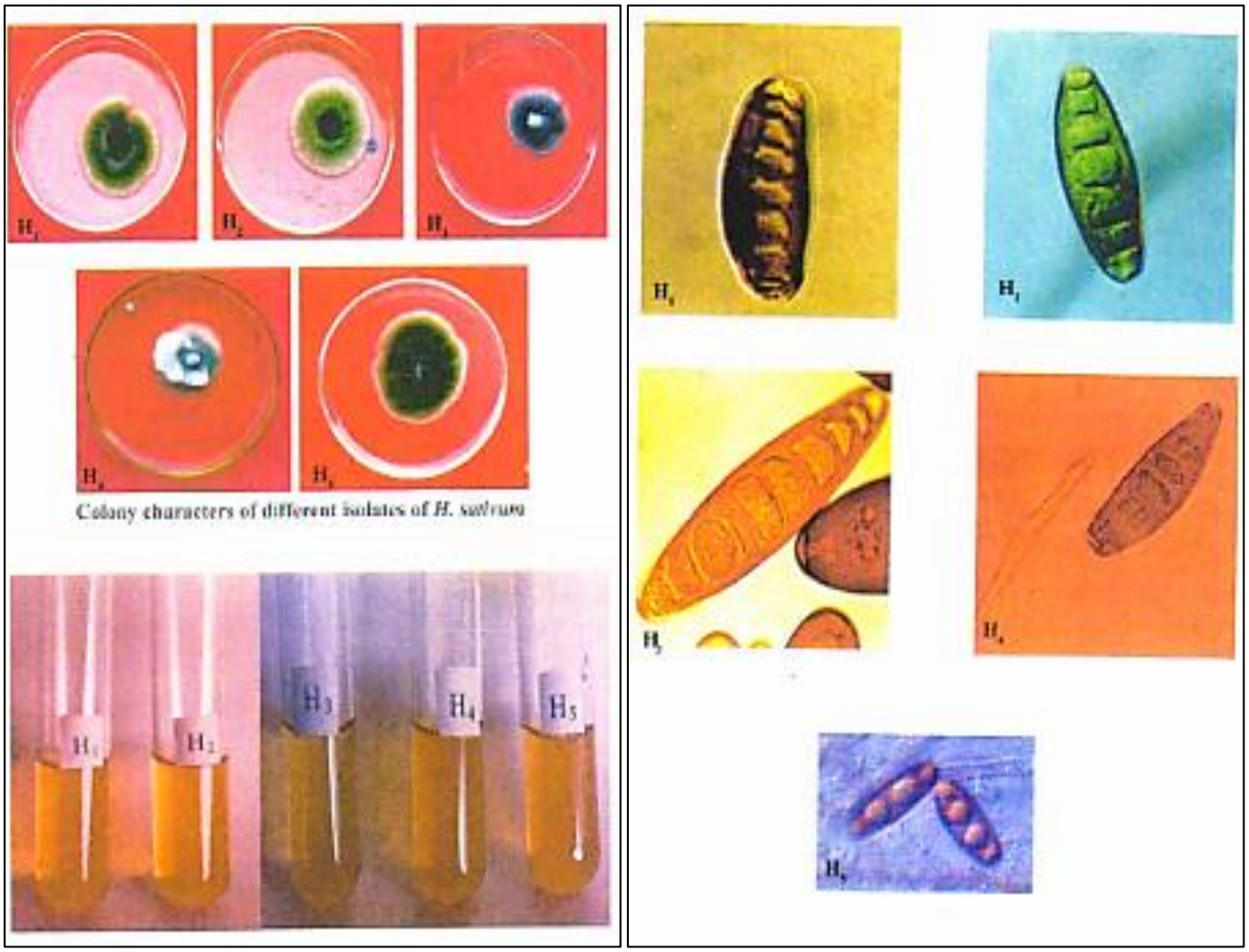

Fig 1: Variability in colony character and conidia of different isolates of *Helminthosporium sativum*

Table 1: Colony diameter of different isolates of *Helminthosporium sativum*

Colony diameter (mm)* after			
Isolates	**3 day**	**6 days**	**Mean**
H_1	35.00	73.33	54.16
H_2	44.33	70.33	57.33
H_3	30.11	66.66	48.38
H_4	40.66	80.00	60.33
H_5	48.33	84.00	66.56
Mean	39.69	74.99	57.34

Cd_1 at 5% 3.26 (interval), Cd_2 at 5% 1.16 (Isolates), Cd_3 at 5% 7.30 (D x I) CV = 7.47

Growth in liquid medium

Isolates were grown in liquid medium (PD broth) and dry mycelial weight was recorded after 8,16 and 24 days of inoculation. Average mycelial dry weight at different intervals showed that the growth of isolates increased with the time, recording 111.98, 142.12 and 171.50 mg, respectively. These values were statistically significant from each other. The results are given in Table 2.

Table 2: Mycelial dry weight of different isolates of *H. sativum* in liquid medium

Dry weight (mg)				
Isolates	8 day	16 days	24 Days	Mean
H_1	100.63	172.35	159.76	144.25
H_2	103.75	133.73	163.03	133.50
H_3	135.05	111.64	138.06	128.25
H_4	129.49	161.84	190.46	160.60
H_5	90.98	131.03	206.20	142.74
Mean	111.98	142.12	171.50	141.87

CD_1 (Days intervals) at 5% 2.37, CD_2 (Isolates) at 5% 3.06 CD_3 (DXI) at 5% 5.31
CV 8.24
*Mean of three replications.

Dry weight of mycelium ranged from 160.60 mg in isolate H_4 to 128.25 mg in H_3. Isolate H_4 had significantly higher mycelial dry weight than all the other isolates which was followed by H_1 and H_5 giving mycelial dry weight yield of 144.25 and 142.74 mg, respectively. Isolates H_1, H_2 and H_3 with mycelial dry weight of 144.25, 133.50 and 128.25 mg,

respectively had significant differences among themselves whereas isolate H_1 (144.25) and H_5 (142.74) were at par. The isolate H_2 H_3 and H_4 showed significant differences among themselves.

The growth of isolates recorded at first observation i.e. after 8 days of inoculation indicated that the highest dry weight was in isolate H_3 (135.05 mg) and the lowest (90.98mg) in isolate H_5. All the isolates had significantly different growth except isolate1 and H_2 which were at par.

At the second observation i.e. after 16 days of inoculation the highest mycelial dry weight of 172.35 mg was recorded in isolate H_1 and the lowest (111.64 mg) in isolate H_3. All the isolates had statistically significant differences among themselves except H_2 and H_5 which were statistically at par.

Third observation was taken after 24 days of inoculation, the isolates showing significant differences among themselves except H (159.76 mg) and H_2 (163.03 mg). Isolate H_1 was found to record highest (206.20mg) mycelial yield followed by the isolate H_4 (190.46 mg). The lowest (138.06mg) mycelial dry weight was recorded by H_3 isolate.

Sporulation

Isolates were grown on PDA and number of spores were counted after 5 and 10 days of inoculation. The observations are presented in Table 3.

The average sporulation of the isolates on 5th and 10th day of inoculation was found to be $29x10^3$ and $49.53\ x10^3$ spores/ml which were significantly different.

Table 3: Sporulation of Different isolates of *H. sativum* on PDA

Number of spores (000/ml)* after			
Isolates	5 day	10 days	Mean
H_1	31.66	43.33	37.50
H_2	26.33	50.66	38.50
H_3	23.33	50.00	36.66
H_4	29.00	47.66	38.33
H_5	34.55	56.00	45.33
Mean	29.00	49.53	39.26

CD at 5% 2.36 (interval), CD_2 at 5% 3.73 (isolates), CD_3 at 5% 5.28 (D XI) CV 7.90

* Mean of three replications

Comparison among isolates indicated that H_5 produced maximum spores ($45.33x10^3$ spores/ml) which were significantly superior to all the other isolates. Number of spore produced by the isolates H_1, H_2, H_3 and H_5 were statistically at par.

Interaction between isolates and interval showed that at the first observation i.e. 5 days after inoculation, the highest number of spores ($34.55\ 10^3$) was produced by isolate H_5 which was significantly superior over that of all the other isolates. Isolates H_3, H_4 and H_1 had significant differences among themselves whereas H_2 and H_3 were statistically at par.

At the time of second observation, the highest number of spores (56.00×10^3) was found in isolate H_5 and this was also significantly superior over others. The lowest number of spores (43.33×10^3) was produced by isolate H_1 which was at par with H_4 (47.66×10^3). However, isolate H_4 was at par with isolates H_3 and H_2 as well. Bidari and Govindu (1976) [2] also reported to marked differences in sporulation of the pathogen. Valim *et al.* (1997) [11] also studied the variation in cultural characters that is growth rate, colour, presence of sectors and textures among ten wheat isolates of *Bipolaris sorokiniana.*

Morphological characters

Growth rate of the pathogen based on colony diameter was medium (H_1, H_2 H_3 and H_4) to fast (H_5) with growth ranging between 39.69 to 74.99 mm. Colony shape was irregular (H_1 and H_5) to circular (H_2, H_3 and H_4) with smooth margin (H_1 and H_5) to rough margin (H_1, H_2, and H_3) without zonation, texture was thick-fluffy in H_1 and thick to thick dense in all the isolates other than H_1 Light yellow pigment produced by H_1 H_2 and H4, Pale yellow by H_3 and light orange by isolate H_5. The colour of colony was blackish gray (H_1 and H_2), shooty black (H_3 and H_5) and grayish black (H_4). Conidia cylindrical in isolates H_5 to ellipsoid straight to curved in H_1, H_2, H_3 and H_4 measuring 45.62 x 56.15 - 17.70 x 19.90 µm (Table 4 and Plate A, B and C). The similar observation were also recorded by Chauhan *et al.* (2017) [3] and Mitra (1931) [5].

Table 4: Comparative morphological characters of different isolates of *H. sativum* of PDA

Characters	**H_1**	**H_2**	**H_3**	**H_4**	**H_5**
Growth Characters	Medium	Medium	Medium	Medium	Fast
Shape	Irregular	Circular	Circular	Circular	Irregular
Margin	Scattered	Wavy	Smooth	Wavy	Smooth
Colour	Blackish Gray	Blackish Gray	Shooty black	Blackish Black	Shooty black
Texture	Thick Fluffy	Thick Dense	Thick dense	Thick Dense	Thick
Pigmentation	Light Yellow	Light Yellow	Pale Yellow	Light Yellow	Light Orange
Conidial Size	45-62 × 17.967µm	46.98 × 17.72 µm	56.10 × 19.89 µm	52.30 × 19.25 µm	25.13 × 8.62 µm
Conidial shape	Ellipsoid	Ellipsoid	Ellipsoid Straight	Ellipsoid Curved	Cylindrical

The present study, variations in cultural characters agreement with the finding of Pascual and Raymundo (1995) [7] where cultural characters were exhibited by 20 isolates grown on three media *viz*. PDA, V-8 juice agar and wheat extract agar. Such variations in morphological characters were also reported by Oliveira *et al.* (1998) [6]. This experiments was resembles with findings of Ahmed *et al*. (1997) [1] in colour variation of isolates.

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