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Physiological Studies of *Drechslera* State of *Trichometasphaeria holmii* Causing Leaf Spot/Blight of *Heliconia*

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Heliconia **is generally known as "wild plantain" or "lobster's claw". *Heliconia*s are grown for cut flower and landscape plants. Its brilliant colour, exotic form, long straight peduncles and excellent post harvest characteristics make it an outstanding flower for the florist's trade. The leaf spot/ blight disease was observed in severe form on the *Heliconia orthotricha* var. she and *Drechslera* state of *Trichometasphaeria holmii* (Luttrell) Subramanian and Jain, was observed to be constantly associated with the disease. Under physiological studies eight synthetic and semi-synthetic media tested, Czapeck's (Dox) agar, potato dextrose agar and Elliot's agar were found to be the best solid media and potato dextrose broth, Richards' broth, Czapeck's broth, glucose asparagines broth and potato carrot sucrose broth were found the best liquid media for the growth and sporulation of the pathogen. The fungus grew and sporulated optimum under pH, ranging from 5.5 to 6.0. Out of nine nitrogenous sources tried sodium nitrate, ammonium phosphate, asparagines, ammonium sulphate and ammonium chloride proved to be the best for the growth and sporulation of the pathogen.**

Key words: *Heliconia*, *Drechslera* state of *Trichometasphaeria holmii* , Leaf spot/blight, Synthetic and Semi-Synthetic Media, pH, Nitrogen Sources

*Heliconia*s are gaining importance and became popular among the florists and plant lovers almost round the world due to their diversity in both colour and form, and have good potential as commercial cut flower. Under the Indian sub-tropical climatic conditions, *Heliconia* perform satisfactorily in partial shade generally in ground planting (Goel, 2004)[1]. The tropical, humid and heavy rainfall region of South Gujarat and medium black soils are suitable for cultivation of *Heliconia*. Out of various factors responsible for successful growing of *Heliconia*, disease management is one of the most important factors and as the crop is newly introduce in India, not much research work is done. The leaf spot/ blight disease was observed in severe form on the *Heliconia orthotricha* var. she and *Drechslera* state of *Trichometasphaeria holmii* (Luttrell) Subramanian and Jain, was observed to be constantly associated with the disease. Looking to the disease severity, the

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present investigation was carried out to study and generate more scientific information on this newly reported disease for the first time from Gujarat and India as well.

MATERIAL AND METHODS

Synthetic and Semi-synthetic Media

Solid media

The agar agar based sterilized media (Table – 1) were poured aseptically into 90 mm diameter pre-sterilized (oven temperature 180°C for 20 minutes) Petri plates @ 20ml plate^{-1}. After solidification, 5 mm diameter culture block of 8 days old pure culture of *Drechslera* state of *Trichometasphaeria holmii* (Luttrell) Subramanian and Jain., was placed in the centre of the Petri plates cut with the help of sterilized cork borer and transferred with sterilized needle. Three repetitions per each treatment were kept for recording observation on colony diameter and cultural haracters of the fungus. The Petri plates were incubated at 27 $\pm$ 2°C temperature in B.O.D incubator. The radial growth was daily measured till one of the Petri plate was covered with the fungal mycelium. The data thus, obtained were subjected to statistical analysis.

Broth / Liquid media

The liquid media were prepared by keeping their composition similar, except no addition of agar agar (Table – 1). Hundred ml of these broths were poured into 250 ml volume of conical flasks and were plugged with non-absorbent cotton, repeating four times. These flasks were autoclaved at 1.2 kg cm^{-2} pressure for 20 minutes. A pure culture block of 5 mm diameter was cut and placed aseptically in the medium as mentioned in solid media. After 15 days of incubation, mycelial mats were harvested on previously weighed; oven dried Whatman's filter paper No. 42 from three repetitions in each case. The filter papers with mycelial mats were dried in an oven at 60°C till constant weight was obtained and dry weight of the mycelium was recorded by deducting the weight of filter paper. The spore count was recorded from fourth repetition at the end of incubation period by transferring the whole of the fungal growth to beaker containing 50 ml sterilized distilled water, stirred thoroughly and filtered through muslin cloth. A drop of suspension was examined under microscope. The numbers of conidia per low power magnification (100 X) microscopic field were recorded from four randomly selected microscopic fields in each case and average was calculated.

pH

The best growth and sporulation of the fungus was obtained on PDA medium therefore, it was used as standard medium for all the remaining physiological studies. PDA medium was prepared excluding agar agar from its composition to get it in liquid form, in sets of eight different pH levels (Table – 3), ranging from 4.0 to 8.0. Hundred ml of PDA broths were poured into 250 ml volume of conical flasks and were plugged with non-absorbent cotton, repeating four times. The pH was adjusted by addition of 0.1 N NaOH or 0.1 N HCl with the help of a pH Tester 30 (Mfd by EUTECH instruments). Remaining procedures remains the same as mentioned in case of liquid media tested the data were subjected to statistical analysis.

Nitrogen Sources

Hundred ml of sterilized liquid Richard's medium was poured in to 250 ml conical flasks. Potassium nitrate (KNO_3) in the basal medium was replaced by various inorganic and organic sources of nitrogen *viz.,* urea (46% N), potassium nitrate (13% N), sodium nitrate (16.5 % N), calcium nitrate (14% N), ammonium chloride (26 % N), ammonium sulphate (20.5% N), ammonium phosphate (42 % N), diammonium hydrogen phosphate (28 % N) and asparagines (20% N) (Table - 4). Nitrogen sources were added singly to furnish 1.38gm of nitrogen per liter of basal medium. The basal medium without nitrogen source served as control. Each treatment was replicated four times. Remaining procedures remains the same as mentioned in case of liquid media tested and the data were subjected to statistical analysis.

RESULTS AND DISCUSSION

Media

Eight different media including synthetic and semi-synthetic media in solid and liquid state were used to test the suitable media for the growth and sporulation of *Drechslera* state of *Trichometasphaeria holmii* (Luttrell) Subramanian and Jain,. The results on the growth and

sporulation are presented in Table - 1. The colony and cultural characters of the fungus were recorded in different solid media are presented in Table - 2.

Solid media

The results revealed that among all solid media tested; the mycelial growth of the pathogen was significantly higher on Czapeck's (Dox) agar (88.50 mm) followed by potato dextrose agar (86.50 mm) which was at par with Elliot's agar (85.00 mm) which in turn was at par with Richards' agar (84.33 mm) and Glucose asparagines agar (83.90 mm). The least mycelial growth was observed in Host leaf

Table 1. Effect of different solid and liquid media on growth and sporulation of *Drechslera* state of *Trichometasphaeria holmii* (Luttrell) Subramanian and Jain, *in vitro*

S. No.	Name of Medium	Solid media Av. colony diameter of pathogen (mm)	Liquid media Av. dry mycelial weight (mg)		No. of conidia/low power microscope (100 x magnification)
1.	Elliot's	85.00	(2.86)*	730.33**	+
2.	Host leaf extract #	79.66	(2.87)	708.66	++
3.	Glucose asparagines	83.90	(3.07)	1206.66	+++
4.	Potato dextrose	86.50	(3.33)	2173.33	++++
5.	Asthana and Hawker's	74.50	(2.57)	385.66	+
6.	Potato carrot sucrose	65.00	(3.02)	1133.33	+++
7.	Czapeck's (Dox)	88.50	(3.12)	1336	+
8.	Richards'	84.33	(3.30)	2013	++
	S.Em. ±	0.7038	0.0635		
	C.D. at 5 %	2.1099	0.1903		
	C.V. %	1.51	3.54		

#(*Heliconia* leaves extract, Agar- agar and Distilled water)
* Figures indicate logarithmic transformed values
** Figures indicate original values
Sporulation = No. of conidia/low power microscope (100 x magnification)
+ = Poor (below 5)
++ = Moderate (6-15)
+++ = Good (16-30)
++++ = Excellent (above 30)

Table 2: Colony / cultural characteristics of *Drechslera* state of *Trichometasphaeria holmii* growing on different solid media

S. No.	Media	Cultural characters
1	Elliot's agar	Fast growing, circular with smooth margin, zonation present, dark brown to black colony.
2	Host leaf extract agar	Moderate growth with slight zonation.
3	Glucose asparagines agar	Fast growing, circular with wavy margin, slight zonation, black colony.
4	Potato dextrose agar	Fast growing, circular with smooth margin, zonation present, grey to black colony.
5	Asthana and Hawker's agar	Slow growing, circular with smooth margin, grey colony.
6	Potato carrot sucrose agar	Slow growing, circular with zonation, black colony.
7	Czapeck's (Dox) agar	Fast growing, circular, flat growth with smooth margin, without zonation, dark brown colony.
8	Richards' agar	White, fast growing, circular, flat colony with no zonation.

extract agar (79.66 mm), Asthana and Hawker's agar (74.50 mm) and very poor on Potato carrot sucrose agar (65.00 mm).

Broth / Liquid media

In the liquid media (Table – 1), potato dextrose broth supported significantly superior growth (2173.33 mg) which was statistically at par with Richards' broth (2013 mg)which in turn at par with Czapeck's (Dox) broth (1336 mg) which in turn at par with Glucose asparagines broth (1206.66 mg) and Potato carrot broth (1133.33 mg). While Elliot's broth (730.33 mg), Host leaf extract (708.66 mg) and Asthana and Hawker's broth (385.66 mg) yielded poor mycelial dry weight. Regarding sporulation, the fungus produced excellent sporulation on potato dextrose broth while good sporulation was observed in potato carrot sucrose broth and Glucose asparagines whereas Richards' broth and Host leaf extract broth produced moderate sporulation while the fungus *Drechslera* state of *Trichometasphaeria holmii* produced poor sporulation on Elliot's broth, Czapeck's (Dox) broth and Asthana and Hawker's broth medium. The dry mycelial weight and spore count after incubation period were recorded in liquid medium.

pH

From eight different pH regimes tested (Table – 3)**,** the fungus grew and sporulated in wide pH range from 4.0 to 8.0 in liquid medium. The dry mycelial weight was significantly higher at pH 5.5 (1445 mg) which was at par with pH 6.0 (1064.66), pH 4 (944.33 mg) and pH 5 (867.66 mg) which in turn was at par with pH 6.5 (625 mg) followed by pH 7.0 (616.33 mg). The significantly least growth of the fungus was recorded at pH 7.5 (244.66 mg) and pH 8 (171.66 mg). Regarding sporulation, the fungus produced excellent sporulation on pH 5.5 and pH 6.0 , while in pH 4 and pH 5 produced good sporulation and moderate sporulation was observed at pH 6.5 and pH 7 whereas the fungus *Drechslera* state of *Trichometasphaeria holmii* produce poor sporulation at pH 7.5 and pH 8.

Nitrogen Sources

Nine different nitrogenous sources (Table – 4) were tested in liquid Richards' medium to know their effect on the growth and sporulation of the pathogen. It is observed from the results that among the nine nitrogenous sources tested, sodium nitrate was found to be significantly best,

Table 3. Effect of different pH regimes on growth and sporulation of *Drechslera* form of *Trichometasphaeria holmii in vitro*

S. No.	pH	Liquid medium (after 15 days) Av. dry weight of mycelium (mg)		No. of conidia/low power microscope (100 x magnification)
1	4.0	(2.96)*	944.33**	+++
2	5.0	(2.93)	867.66	+++
3	5.5	(3.14)	1445	++++
4	6.0	(3.01)	1064.66	++++
5	6.5	(2.80)	625	++
6	7.0	(2.78)	616.33	++
7	7.5	(2.38)	244.66	+
8	8.0	(2.23)	171.66	+
	S.Em. ±	0.0771		
	C.D. at 5 %	0.2311		
	C.V. %	4.81		

* Figures indicate logarithmic transformed values
** Figures indicate original values
Sporulation = No. of conidia/low power microscope (100 x magnification)
\+ = Poor (below 5)
++ = Moderate (6-15)
+++ = Good (16-30)
++++ = Excellent (above 30)

giving maximum growth of the fungus (4083.33 mg) which was statistically at par with ammonium phosphate (3712.66 mg) and asparagines (3350 mg) which was in turn at par with ammonium sulphate (2676.33 mg) and ammonium chloride (2489.66 mg). Significantly low mycelial dry weight was obtained in potassium nitrate (1994.33 mg) which was in turn at par with calcium nitrate (1680 mg) and diammonium hydrogen phosphate (1483.66 mg) which was in turn at par with urea (1180.33 mg).

Regarding sporulation, the fungus produced excellent sporulation on ammonium phosphate and sodium nitrate while good sporulation was observed in ammonium sulphate and asparagines. Sporulation was found moderate in ammonium chloride and potassium nitrate, sporulation was found poor in urea, diammonium hydrogen phosphate, calcium nitrate and control *i.e*., Richards' agar medium (without nitrogen).

Table 4. Effect of different nitrogen sources on growth and sporulation of *Drechslera* state of *Trichometasphaeria holmii in vitro*

S. No.	Name of nitrogen source	Liquid medium (after 15 days) Av. dry weight of mycelium (mg)		No. of conidia/low power microscope (100 x magnification)
1	Diammonium hydrogen phosphate	(3.16)*	1483.66**	+
2	Ammonium phosphate	(3.56)	3712.66	++++
3	Ammonium chloride	(3.39)	2489.66	++
4	Ammonium sulphate	(3.42)	2676.333	+++
5	Calcium nitrate	(3.22)	1680	+
6	Sodium nitrate	(3.60)	4083.33	++++
7	Asparagine	(3.52)	3350	+++
8	Urea	(3.06)	1180.33	+
9	Potassium nitrate	(3.29)	1994.33	++
10	Control	(2.46)	293.66	+
	S.Em. $\pm$	0.0464		
	C.D. at 5 %	0.1369		
	C.V. %	2.46		

* Figures indicate logarithmic transformed values
** Figures indicate original values
Sporulation = No. of conidia/low power microscope (100 x magnification)
\+ = Poor (below 5)
++ = Moderate (6-15)
+++ = Good (16-30)
++++ = Excellent (above 30)

CONCLUSION

Considering the overall performance of different solid media Czapeck's (Dox) agar, potato dextrose agar and Elliot's agar supported maximum growth of *Drechslera* state of *Trichometasphaeria holmii ,* while in liquid media, potato dextrose broth, Richards' broth, Czapeck's (Dox) broth , Glucose asparagines broth and potato carrot sucrose broth were found to support maximum growth and sporulation of the pathogen. The present results are in confirmation with Misra & Mishra (1969)[2], Somal (1974)[3] and Lande & Utikar (1978)[4].

Excellent growth and sporulation of *Drechslera* sp. was recorded at pH 4.0 to 6.0, which was in conformity with Gupta *et al*.(1978b)[5] observed that the best growth and sporulation of *Drechslera rostrata* (Drechsler) Rich. and Fr., was observed at 5.8 pH. Looking to the effect of different pH regimes on the growth and sporulation, pH 5.5 to 6.0 proved very effective indicating that the fungus preferred acidic to near neutral medium for the growth and sporulation as compared to alkaline medium.

Thus sodium nitrate, ammonium phosphate, asparagines and ammonium sulphate

were found to be the best sources for the growth and sporulation of the *Drechslera* state of *Trichometasphaeria holmii*. It is very clear that diammonium hydrogen phosphate, calcium nitrate and urea did not support the growth and sporulation of the pathogen. The present investigation was found in harmony with earlier findings of Dutt & Bedi (1974)[6] and Pande & Verma (1992)[7].

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