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Studies on Physiology of *Helminthosporium setariae*, the Causal Agent of Leaf Spot Disease of *Setaria italica* – II. Carbon Nutrition

by

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Rangaswami and Pandurangan (1962) observed the occurrence of *Helminthosporium setariae* on Tenai *(Setaria italica)* in Madras State for the first time. The outbreak of the disease in severe form in 1967 in and around Coimbatore, Tamil Nadu made it necessary to take up the studies on the pathogen. In an earlier communication, Vidhyasekaran *et al.* (1969) have reported the N nutrition of the fungus. The present paper reports the effect of various carbon sources on growth and sporulation of the fungus.

Material and methods: To assess the efficacy of various carbon sources to induce growth and sporulation of the fungus, Czapek'z medium was used as the basal medium and sucrose was replaced by 3% of various carbon sources. All the media were adjusted to pH 7.0 and autoclaved at 10 lbs. pressure for 15 minutes. Single spores from plain agar medinm were inoculated to avoid any carry-over nutrients. After 10, 20 and 30 days growth, mycelial weight

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was recorded. The spore concentration was assessed in 20 days old culture by using a haemocytometer. The effect of pH on the growth and sporulation of the fungus was studied by adjusting the pH of the Czapeks medium from 1 to 11 with Beckman's glass electrode pH meter with 0.1 N NaoH or 0.1 N HCl.

To study the utility of organic acids at different pH levels, the Czapek's media with the organic acids, oxalic, succinic, citric and formic acids were adjusted to 9, 7, 5 and 3 pH and the fungus was inoculated. Final pH of the media, mycelial weight and sporulation were assessed after 20 days of growth.

Results: Four monosaccharides, D-xylose, rhamnose, glucose and galactose, 3 disaccharides, maltose, sucrose and lactose, 3 polysaccharides, starch, dextin and cellulose, a sugar alcohol, mannitol and 5 organic acids, succinic, oxalic, citric, formic and tannic acids were tested for their efficacy to induce growth. The data obtained are presented in Table 1.

TABLE 1. *Utilisation of various carbon sources by H. setariae*

Sl. No.	Carbon sources	Mycelial weight in mg			pH		
		10 days growth	20 days growth	30 days growth	After 10 days	After 20 days	After 30 days
1.	D-xylose	784	2180	682	7.3	7.3	7.0
2.	Glucose	532	1895	1104	7.3	7.3	7.3
3.	Galactose	371	1905	666	6.7	7.3	7.3
4.	Maltose	591	2592	678	7.3	7.3	7.3
5.	Sucrose	625	1617	960	7.3	7.3	7.3
6.	Lactose	333	967	782	7.3	7.3	7.3
7.	Rhamnose	138	180	296	6.7	6.7	6.7
8.	Starch	209	206	1519	6.4	6.7	7.3
9.	Dextrin	539	1318	1942	7.3	7.3	7.5
10.	Cellulose	38	506	683	6.7	6.7	7.0
11.	Mannitol	247	285	273	6.4	6.7	6.5
12.	Tannic acid	175	167	177	5.2	5.8	4.0
13.	Succinic acid	76	412	294	8.5	9.7	11.0
14.	Oxalic acid	137	234	183	8.5	10.0	11.0
15.	Citric acid	109	235	141	7.3	8.8	9.5
16.	Formic acid	288	409	102	7.3	8.8	8.5
17.	Control	68	122	119	6.7	6.7	6.5

Interaction : Significant at 1% level

10 days — 1, 5, 4, 9, 2, 3, 6, 16, 11, 8, 12, 7, 14, 15, 13, 17, 10

20 days — 4, 1, 3, 2, 5, 9, 6, 10, 13, 16, 11, 15, 14, 8, 7, 12, 17

30 days — 9, 8, 2, 5, 6, 10, 1, 4, 3, 7, 13, 11, 14, 12, 15, 17, 16

The maximum growth was obtained after 20 days of incubation in almost all the carbon sources except in polysaccharides viz , starch, dextrin and cellulose. Maltose and D-xylose were found to be the best carbon sources to yield maximum mycelial weight on the 20th day. But on the 30th day dextrin and starch produced maximum growth. Rhamnose, a pentose and tannic acid were found to be poor carbon sources. It is of interest to note that organic acids produced more growth than starch on 20th day. The organic acids utilization shifted the pH of the media towards alkaline range. In case of succinic and oxalic acids the pH raised even upto 11.

The various carbon sources tested for their efficacy to induce growth were also tested for their ability to produce sporulation. The spore concentration was found out after 20 days of incubation and the data are presented in Table 2.

TABLE 2. *Efficacy of various carbon sources to induce sporulation in H. setariae*

Sl. No.	Carbon sources	Spores in lakhs in the whole of the mycelial mat
1.	D-xylose	262.5
2.	Glucose	187.5
3.	Galactose	100.3
4.	Maltose	112.5
5.	Sucrose	412.3
6.	Lactose	737.5
7.	Rhamnose	25.0
8.	Starch	212.5
9.	Dextrin	175.5
10.	Cellulose	200.0
11.	Mannitol	50.0
12.	Tannic acid	0.0
13.	Succinic acid	50.0
14.	Oxalic acid	0.0
15.	Citric acid	12.5
16.	Formic acid	12.5
17.	Control	0.0

Significant at 1% level

Conclusion : 6, 5, 1, 8, 10, 2, 9, 4, 3, 11, 13, 15, 16, 12, 14, 17

Lactose and sucrose were found to be the best carbon sources to induce sporulation. The polysaccharides, starch, cellulose and dextrin also produced some good sporulation. Organic acids were found to be poor sources for sporulation. Since the organic acids shifted the pH towards alkaline range the poor growth and sporulation in those sources may be only due to the pH effect. To assess the affect of pH on growth and sporulation of the fungus, the fungus was grown in the media adjusted to 1 to 11 pH. The results are presented in Table 3.

TABLE 3. *Effect of pH on the growth and sporulation of H. setariae*

Sl. No.	Initial pH	Final pH	Mycelial weight in mg	Spores in lakhs in the whole of the mycelial mat
1.	1.0	1.4	146.5	0.0
2.	2.0	3.0	426.5	13.8
3.	3.0	3.8	1093.5	225.5
4.	4.0	5.8	1262.5	206.0
5.	5.0	6.7	1287.5	297.0
6.	6.0	7.0	1333.5	600.5
7.	7.0	7.0	1182.5	306.5
8.	8.0	7.9	641.5	380.8
9.	9.0	7.9	645.5	367.5
10.	10.0	7.8	585.0	370.0
11.	11.0	8.4	610.5	371.0

Interaction : Significant at 1% level

Mycelial weight : 6, 5, 4, 7, 3, 9, 8, 11, 10, 2, 1

Sporulation : 6, 8, 11, 10, 9, 7, 5, 3, 4, 2, 1

Maximum growth and sporulation were obtained at pH 6. Both pH 1 and 2 were found to be inhibitory to the fungal growth and sporulation. Lower pH 7, 5, 4 and 3 supported fairly good vegetative growth when higher pH 8, 9, 10 and 11 induced appreciable sporulation.

The results indicated that by controlling the pH of the medium the utility of organic acids may be increased. Hence an experiment was set up in which initial pH of the media with the four organic acids, viz., succinic, oxalic, citric and formic acids were adjusted to 3, 5, 7 and 9. The basal medium without any carbon source was also adjusted to the different pH and kept as control. The final pH of the media, mycelial weight and spore concentration were tested after 20 days of inoculation. The results are presented in Table 4.

TABLE 4. *Utilization of organic acids in the media at different pH levels by H. setariae*

Sl. No.	Organic acid	Initial pH of the medium	Final pH of the medium	Mycelial weight in mg	Spores in lakhs in the whole of the mycelial mat
1.	Oxalic acid	9.0	9.0	147.5	0.0
		7.0	8.5	172.5	0.0
		5.0	7.0	192.0	0.0
		3.0	5.0	240.5	0.0
2.	Succinic acid	9.0	10.0	205.5	79.3
		7.0	9.5	299.0	60.3
		5.0	6.0	755.0	32.3
		3.0	3.4	356.6	20.8
3.	Citric acid	9.0	9.0	172.0	51.8
		7.0	9.0	289.5	44.3
		5.0	6.0	434.5	20.0
		3.0	3.1	340.5	7.0
4.	Formic acid	9.0	9.0	172.5	30.3
		7.0	8.0	497.5	16.0
		5.0	6.5	692.5	11.3
		3.0	4.0	516.5	4.8
5.	Control	9.0	9.0	43.0	0.0
		7.0	7.5	28.0	0.0
		5.0	6.0	51.0	0.0
		3.0	4.0	46.0	0.0

Interaction : Significant at 1% level

Oxalic acid : pH 3, 5, 7, 9 — Citric acid : pH 5, 7, 3, 9

Succinic acid : pH 5, 7, 3, 9 — Formic acid : pH 5, 7, 3, 9

In succinic, formic and citric acids, more growth of the fungus was obtained if tee initial pH of the media was adjusted to 5.0 in which media the pH during the growth period increased only upto 6.0 or 6.5. In oxalic acid some good growth was obtained only when the initial pH. sporulation in all the organic acids media was poor.

Discussion: Maltose and D-xylose were found to support the maximum growth on 20th day of incubation. Maltose and D-xylose were found to be the best carbon sources for many fungi (Cochrane, 1958). But on the 30th day when these sources showed reduction in mycelial weight due ro autolysis, the

polysaccharides, particularly dextrin and starch, showed maximum growth. The delayed use of the polysaccharide may be due to the adaptive enzyme like amylase produced by the fungus to breakdown the polysaccharides into simple sugars (Christensen and Daly, 1951). Satarch and dextrin, which gave maximum growth, were usually broken down by the enzyme, amylase, to maltose which was incidenty found to be the best carbon source for 20 days growth.

Lactose and sucrose induced the maximum sporulation. Lactose was found to increase sporulation in *Lophotrichus ampullus* (Sushikumar and Grover, 1967). Sucrose was found to be easily converted to glucose-1-phosphate than glucose itself (Doudroff, 1945) and hence sucrose was often found to be better carbon source for sporulation (Misra and Mukherjee, 1962). Cellulose, which was found to support lesser growth induced more sporulation than glucose, maltose and galactose. Cellulose, being a slowly available compound for most fungi, was found to be the best carbon source for sporulation in many cases (Cochrane, 1958).

Organic acid utilization increased the pH. Increase in pH in organic acid media has been reported by Leonian and Lilly (1940). The pH played an important role in the growth and sporulation of *H.setariae*. pH 3 to 7 were found to be favourable for growth when pH 5 to 11 were found to support good sporulation. Acid range was often favourable for growth when alkaline range was found to be favourable for sporulation (Robbins and Schmitt, 1945 and Lilly and Barnett, 1947). If the initial pH of the media was adjusted to 5.0, succinic, formic and citric acids were found to be good carbon sources for the induction of growth. Though succinic and citric acids were found to be utilized by many fungi (*Brian et al.*, 1946), the utilization of formic acid is somewhat specific to *H. setariae*. So far, formic acid has been reported to be toxic to the fungi (Cochrane, 1958). Oxalic acid is not utilized by *H. setariae*. All the organic acids tested were found to be poor in inducing sporulation although the pH of the medium was controlled.

Summary: Sixteen carbon sources were tested for their efficacy to induce growth and sporulation of *Helminthosporium setariae* Saw, the causal organism of the Tenai leaf spot disease. Maltose and D-xylose produced the maximum growth when lactose and sucrose induced more sporulation. Organic acids shifted the pH towards alkaline range and it was observed that pH had important role in growth and sporulation of the fungus. If the initial pH of the medium was adjusted to 5.0, succinic, citric and formic acids were utilized well by *H. setariae*. Formic acid, which was reported to be toxic to the fungi was found to be utilized by the fungus. All the organic acids tested were found to be unable to induce sporulation, irrespective of the pH of the medium.

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