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Effect of Different Carbon and Nitrogen Compounds on the Growth and Sporulation of *Curvularia clavata*

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야자나무 枯調病菌의 生長과 胞子形成에 대한 탄소 및 질소의 효과

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ABSTRACT: The effect of different carbon and nitrogen compounds on the growth and sporulation of *Curvularia clavata* Alcorn (Herberium No. IMI264075) has been studied. All the carbon sources tried were well utilized by the pathogen though glucose, and sucrose supported the best growth while glucose, maltose and sucrose the sporulation of the fungus. Of the nine nitrogen compounds, L-glutamic acid supported the best growth while aspartic acid and L-glutamic acid the sporulation of the fungus. Growth and sporulation were generally better with organic than inorganic nitrogen sources. Ammonium sulphate was the best inorganic source. A sudden drop of pH value of the culture media after 4 days of incubation did not favour good growth of the fungus.

KEYWORDS: *Curvularia clavata* Alcorn, Carbon compounds, Nitrogen compounds

Carbon and nitrogen play a very significant role in the nutrition of fungi. Carbon has been known to be one of the most essential element required by the fungi. Fungi may utilize one source of carbon better than others. Literature also reveals that all sources of nitrogen are not equally good for the growth of the fungi. Robins (1937) and Steinberg (1950) have classified fungi according to their ability to utilize different nitrogen sources.

During the last four decades, a number of workers Agarwal (1958); Bais, Singh and Singh (1969); Panwar (1972); Purohit (1972), Kumar and Arya (1985) and Leslie (1986) have studied the role of nutrients on the growth as well as sporulation of the fungi. Despite these interesting papers, literature presents vague and contradictory data which give much scope to do research on these problems. The present investigation deals with the effect of different carbon and nitrogen sources on the growth and sporulation of *Curvularia clavata* join the causal organism of the seedling blight disease of the oil palm. The pH of the culture solution was also determined at the end of each incubation period in order to determine the possible role of pH on the efficiency of a particular source during the incubation period.

Materials and Methods

The fungus was isolated from infected leaves of the oil palm seedlings affected by the blight disease in a nursery of the Nigerian Institute for Oil Palm Research (NIFOR) near Benin City. Isolation of the fungus was effected by plating infected leaf segment on potato dextrose agar (PDA) and carried in stock cultures on PDA slants. In general, cultural methods outlined by Lilly and Barnett (1951, 1953) were used as far as were practical. A liquid culture technique using 150 m*l* Erlenmeyer flasks was employed in the *in vitro* nutritional studies. Each flask contained 50 m*l* of a glucose-asparagine-

inorganic salt medium similar to that used by Hale and Roane (1961) with the acidity usually adjusted to pH 6 and sterilized at 121°C. 1.1 kg cm^2 for 15 min. Inoculation was done with mycelial discs (0.4 cm in diameter), cut with No. 2 (4 mm diameter) sterilized cork borer from the margin of a 4-day old colony growing on PDA medium in petri dishes. The inoculated flasks were incubated at room temperature (26-27°C) for varying periods, depending on the nature of the experiment. Growth of the fungus was measured by filtering the mycelium on previously dried and weighed filter papers. These were then dried for 2 days to constant weight and the difference in weight was taken as the weight (dry wt.) of the mycelium. Spore count was determined by the use of a haemacytometer.

Results

Studies with carbon sources

When the fungus was cultured on media into which was incorporated each of the C-sources in equimolar carbon concentration at the concentration used in the basal medium, the presence of glucose and sucrose yielded maximum dry weight of fungus mycelium (Table I).

Table I. Dry mycelial weight (mg) and sporulation of *C. clavata* grown in Hale and Roane's liquid medium supplemented with different equimolar carbon sources

Carbon source	Mean dry mycelial weight (mg)	Mean sporulation $\times 10^4$/ml solution*
Glucose	367.00 a	2.00* a
Sucrose	365.00 a	1.95 a
Maltose	345.00 b	1.94 a
Galactose	292.00 c	1.86 b
Fructose	291.00 c	1.84 b
Mannitol	237.00 d	1.61 c
Lactose	227.00 e	1.73 b
Control	27.00 f	0.71 d

1. * Figures are mean conidia counts at 10^4 per ml solution transformed to $\sqrt{x + 0.5}$.
2. Means followed by some letters in the same column are not statistically different (5% level).
3. Data are averages of 5 flasks.

Growth was good in maltose, moderately good in galactose and fructose, rather poor in lactose and mannitol and very poor in the control medium which had no carbon source.

The presence of glucose, sucrose and maltose supported the heaviest sporulation. Sporulaion was moderately good in galactose, fructose and lactose and again very poor in control medium which no carbon source.

In addition to varying the kind of sugar, the concentrations of glucose and sucrose were varied from 2% to 20%. Growth of *Curvularia clavata* increased as the concentration of the carbon was increased up to the highest concentration used (Fig. 1). The results also show that the yields (dry mycelial wt.) of the fungus were

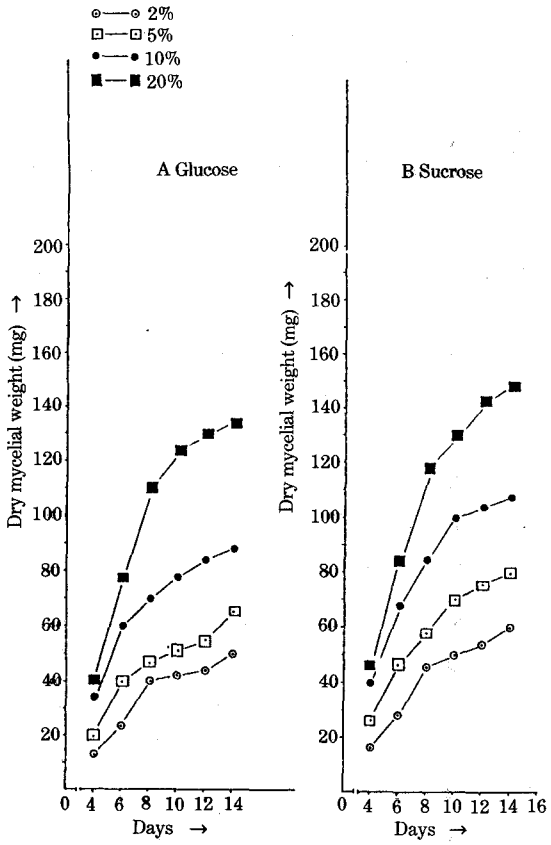

Fig. 1. Dry mycelial weight of *Curvularia clavata* in liquid media.
A. On basal medium with various concentrations of glucose
B. On basal medium with various concentrations of sucrose

correspondingly higher with sucrose than with glucose.

Studies with nitrogen sources

The basal medium contained 0.427 g/*l* of nitrogen supplied as 2g /*l* of asparagine. It was observed that among the nitrogen sources tested, the presence of L-glutamic acid and aspartic acid gave the best dry weight of *C. clavata* (Table II). Growth was however significantly better in L-glutamic acid than aspartic acid. Fairly good growth occurred in the inorganic salts of which ammonium sulphate gave the best result.

L-glutamic acid again supported the heaviest sporulation of the fungus. There was however no significant difference between sporulation in L-glutamic acid and aspartic acid. Of the different nitrogen sources tested with the exception of ammonium sulphate, organic nitrogen in general supported heavier sporulation than the inorganic sources. Sporulation was very poor in the medium that had no nitrogen.

In addition to varying the kind of nitrogen sources, the concentration of asparagine was also varied from 0-3 g/*l* in the basal medium. Growth of the fungus by the 8th day was highest with each concentration used (Fig. 2). As the concentration was increased, there was a corresponding increase in dry weight of the fungus up till 2 g/*l* beyond which there was a fall. A concentration of 2 g/*l* was found to be the most suitable for the growth of the fungus while the organism did very poorly in the medium that had no asparagine.

Relation of nitrogen source to pH

On the basal medium, when initial pH was adjusted to 6.0, pH decreased in all the sources but two in the first few days after inoculation to pH 5 or lower and later increased to pH 7 or even higher by the 11th day (Table III). L-glutamic acid, aspartic acid and L-aspara-

Table II. Dry mycelial weight (mg) and sporulation of *Curvularia clavata* in liquid medium supplemented with different equimolar nitrogen sources

Nitrogen source	Drechslera hawaiiensis	
	Mean dry mycellial weight (mg)	Mean sporulation $\times 10^4$/ml solution**
L-Asparagine	240 d	6.04 b
Sodium nitrate	100 h	3.93 e
Glycine	150 f	5.52 c
Ammonium nitrate	130 g	4.30 d
Calcium nitrate	150 f	3.24 f
Aspartic acid	260 b	6.37 a
Potassium nitrate	160	3.81 e
L-Glutamic acid	330 a	6.52 a
Ammonium sulphate	250 c	5.34 c
Control	60 i	0.71 g

** Figures are mean sporulation at 10^4 per ml transformed to $\sqrt{x + 0.5}$.

Means followed by some letters in the same column are not statistically different.

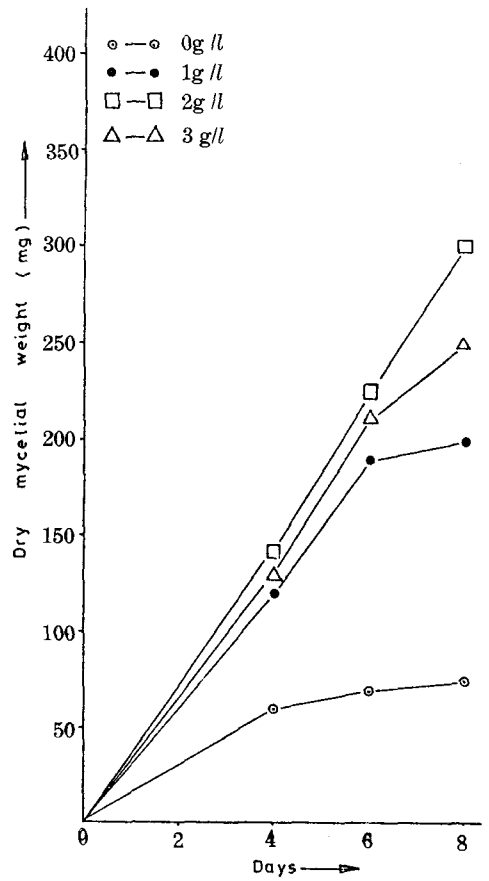

Fig. 2. Growth of *Curvularia clavata* in liquid medium with varying concentrations of asparagine.

Table III. Weight of mycelium of *Curvularia clavata* produced on basal medium with initial pH adjusted to pH 6

Nitrogen source (equimolar amounts)	4 days		7 days		11 days	
	Dry wt. (mg)	Final pH	Dry wt. (mg)	Final pH	Dry wt. (mg)	Final pH
L-Asparagine	150	5.8	240	6.4	230	8.4
Sodium nitrate	60	3.8	100	4.5	230	7.4
Glycine	100	4.8	150	5.5	230	5.8
Ammonium nitrate	75	2.6	130	3.4	150	5.6
Calcium nitrate	110	5.4	150	6.3	220	7.7
Aspartic acid	165	6.3	260	8.0	300	8.3
Potassium nitrate	70	3.9	160	7.1	240	7.8
L-Glutamic acid	205	6.2	330	8.4	320	8.4
Ammonium sulphate	130	5.6	250	8.1	260	8.1
No nitrogen added	30	5.5	60	5.8	60	6.3

* Data average of 3 flasks.

gine which supported very good growth after 4 days of incubation were found to experience minor pH changes from 6.0 to 6.2, 6.3 and 5.8, respectively as against sources like sodium nitrate, potassium nitrate and ammonium nitrate which supported poor growth had drastic fall in pH from 6.0 to 3.8, 3.9 and 2.6 respectively. On the 11th day there was a decrease in the yield (dry mycelial wt.) of the fungus in the flasks that had L-glutamic acid and L-asparagine as compared to the yield on day 7.

Discussion

Glucose was the best among hexoses in supporting the growth and sporulation of the fungus. This could be due to the fact that glucose is biologically the most important sugar and is utilised for growth by virtually all cultivable fungi. Sucrose and maltose were also particularly good in supporting the growth and sporulation of the pathogen. Large quantities of these carbohydrates have been extracted from the oil palm (Anon., 1969) thus providing a favourable nutritional condition for the development of the pathogen. The rather poor growth of the fungus when mannitol was the sole carbon source was in accordance with the earlier observations of many other fungi (Hawker, 1939; Sethi and Munjal, 1963). Lilly and Barnett (1951a) concluded that most fungi appear to utilize the corresponding sugars with greater facility than the sugar alcohols. The very poor growth and lack of sporulation recorded for the fungus in the medium with no carbon compound (control) indicate the importance of carbon in the nutrition of the fungus. Infact almost half of the dry weight of fungus cells consist of carbon. The protoplasm, enzymes, the cell wall and reserve nutrients stored within the cells are compound of carbon (Lilly and Barnett, 1951).

Increased carbon concentrations of glucose and sucrose in the basal medium gave increased dry weight yields of the fungus up to the highest concentration used in the study. Similar results were obtained by Hale and Roane (1961), for *Helminthosporium carbonum*. The results also show that the yield for the fungus was correspondingly higher with sucrose than with glucose, probably because the sugars were not added in equimolar concentrations with respect to carbon.

The fungus was capable of utilising all the nitrogen sources such as nitrate nitrogen, ammonium nitrogen and organic nitrogen investigated for growth and sporulation. This pathogen could be said to be able to reduce nitrogen to the oxidation level of ammonia since it was

capable of utilising nitrate nitrogen (NO_3-) Lilly and Barnett (1951). Growth and sporulation were generally better with the organic than in the inorganic nitrogen sources. Hale and Roane (1961) obtained a similar result for *Helminthosporium carbonum*. L-glutamic acid and aspartic acid which supported good growth and sporulation in the fungus are found free in many plants and are thus available to the fungus in nature (Lilly and Barnett, 1951). The higher yields of mycelia on glutamic and aspartic acids suggest also that these compounds might be utilized as added carbon sources as well as nitrogen sources. This might also be true for asparagine and could account for the increased yield as the asparagine concentration was increased (Fig. 2). The rather poor growth obtained from sodium nitrate, potassium nitrate and ammonium nitrate could be due to the drastic drop of the pH in the first four days after inoculation. This fact is strengthened because earlier preliminary studies revealed that pH as low as 3 did not favour good growth and sporulation. Hale and Roane (1961); J.F. Leslie (1986) who worked on *Helminthosporium carbonum* and *Gibberella zeae* respectively obtained similar results.

The best inorganic source for the fungus was ammonium sulphate. This is of interest because poor growth condition of the oil palm are usually corrected by the application of sulphate of ammonia (Anon, 1960). The use of this fertilizer to obtain adequate growth of the host plant will therefore encourage the growth and sporulation of the organism.

The very poor growth and sporulation obtained in the medium where there was no nitrogen shows the importance of this element in the nutrition of the pathogen. This essential element nitrogen is used by fungi for functional as well as structural purposes. The decrease in the mycelia observed in some media containing nitrogen sources 11 days after inoculation. Table III could be attributed to toxic substances released into the medium as a result of the metabolic activities of the fungus. This toxic substances could lead to the disintegration of the hyphae and hence a loss in dry weight of the mycelia. It could also be due to some endogenous enzymes probably produced in the medium by the fungus which might have led to the digesting of the hyphae, a phenomenon of common occurrence in fungi referred to as autolysis.

A study of the data presented on Table III showed an upswing during the growth of the organism. In most of the cases, the pH at the end of the incubation period indicated alkaline reaction. This change in pH of the culture solution sometimes gives a clue to the metabolism of the organism. J.F. Leslie (1986) obtained a similar result with *Gibberella zeae*. The drop in pH following utilization of ammonium ion in such salts as ammonium nitrate, ammonium sulphate is a phenomenon known to be widespread among fungi and is attributed to the preferential absorption of the cation (Foster, 1949; Lilly and Barnett, 1951; Macmillan, 1956 and Cochrane, 1958).

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