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Effect of Different Range of pH on Mycelial Growth and Sporulation of *Botryodiplodia theobromae* and *Colletotrichum gloeosporioides* in vitro at 28°C in Litchi

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Abstract: ***pH has profound impact on uptake of nitrogen, mineral nutrients and on the permeability of cell wall, therefore it plays a very significant role in fungal metabolism. To observe the impact of different pH range on the above two parameters, the culture medium was adjusted at 3.5, 4.5, 5.5, 6.5 and 7.5 pH range. For mycelial growth, dry mycelial weight was carried out. For sporulation, the numbers of spores were counted in hemocytometer. For B. theobromae at temperature 28°C, pH 3.5 had 5 8.4mg of dry mycelial weight (minimum), pH 5.5 had 280.2mg (maximum) and pH 6.5 had 232.6mg and spore density at pH 3.5 was $2x10^4$ml (minimum), pH 5.5 had $17x10^4$ml (maximum), and pH 6.5 had $12x\ 10^4$ml. For C. gloeosporioides at temperature 28°C, pH 3.5 had 68.5mg of dry mycelial weight (minimum), pH 5.5 had 292.4 mg (maximum) and pH 6.5 had 250.2mg; and spore density at pH 3.5 was $4x10^4$ml (minimum), pH 5.5 had $20x10^4$ml (maximum), and pH 6.5 had $16x10^4$ml. Therefore, it may be concluded that pH 5.5 was most suitable for both B. theobromae and C. gloeosporioides with respect to mycelial growth and sporulation. It may be further concluded that rise in pH not always helped in the spore production and mycelial growth. Among the two test organisms, C. gloeosporioides produced maximum mycelial mat and spore density at the same initial pH in comparison to B. theobromae.***

Keywords: *Botryodiplodia theobromae, Colletotrichum gloeosporioides,* Litchi, pH effect, mycelial growth, sporulation

1. Introduction

India is the second largest producer of the litchi fruits, whereas China is considered to be the first. This plant was introduced in India during Buddhist era when the disciples of Buddha from China were visiting India frequently. Litchi cultivation is also reported from Thailand, Japan, New Zealand, Australia, South Africa, West Indies, Brazil and certain part of United States. In India it is grown in the states of Bihar, Tripura, West Bengal, Uttar Pradesh, Punjab and Haryana. Of the total production of litchi in India, 74 percent is contributed by Bihar. Litchi is an important fruit of North Bihar in general and Muzaffarpur in particular. In early days Litchi plantation was not done at commercial scale but nowadays its plantation is being done at commercial scale because the fruits are not limited to the local markets only, rather it is being exposed to other states as well as abroad also. The value of the crops may be judged by the fact that one acre orchard when in fruiting stage is being auctioned at the rate of 75 thousand to one lakh. This cost is due to utilization of Litchi fruits in the preparation of Litchi squash, Litchi wine and other preparation from the pulp. Based on the chemical analysis of the pulp it is now said that it is not a simple fruit rather it bears several medicinal values which includes antioxidants, chemicals that reduces diabetes and risk of strokes.

pH is defined as the logarithm of reciprocal of hydrogen ion concentration denotes the degree of acidity or alkalinity. Acid medium has an excess of positively charged hydrogen ions, whereas alkaline medium has an excess of negatively charged hydroxyl ions. pH has profound impact on uptake of nitrogen, mineral nutrients and on the permeability of the cell wall, therefore, it plays a very significant role in fungal metabolism. Therefore, mycelial growth and sporulation may also be affected if the fungus is cultured in a medium having varying range of pH.

2. Material and Methodology

pH range for satisfactory growth of different fungi vary considerably and the cardinal points may also differ appreciably. We get information that due to variation of pH the general i.e., primary as well as the secondary metabolite production vary at different pH range. The impact of pH on general metabolite is evident by the dry biomass produced by the fungus which is calculated by taking the weight of the dry mycelium after certain period of incubation in different range of pH. Similarly, sporulation is calculated by counting the spore density of the fungus in 1ml of suspension culture.
To observe the impact of different pH range on the above twoparameters. The culture medium was adjusted at 3.5, 4.5, 5.5, 6.5 and 7.5 pH range. After incubation periods of 10 days the mycelial mat was obtained after filtering the culture through Whatman filter paper no.1. The mycelial mat was washed properly with hot water to remove traces of culture medium. This was dried with the help of pre sterilized filter paper and then the mat was transferred carefully on a pre weighed and pre sterilized filter paper. It was again weighed and the weight of wet mycelial mat was obtained by deducting the weight of the filter paper. The mycelial mat was dried in the incubator at 60°C till the constant weight was obtained. In this way the biomass was obtained for the cultures at different pH.

3. Observation and Table Discussion

Table: Data showing effect of different range of pH on mycelial growth and sporulation *Botryodiplodia theobromae*, *Colletotrichum gloeosporioides* in vitro at 28°C

pH of the medium	*B. theobromae*		*C. gloeosporioides*	
	Dry weight of Mycelial in 100ml of culture	Spore density in 1.0ml of suspension culture	Dry weight of Mycelial in 100ml of culture	Spore density in 1.0ml of suspension culture
3.5	058.4	$02x10^4$	068.5	$04x10^4$
4.5	071.8	$08x10^4$	105.8	$11x10^4$
5.5	280.2	$17x10^4$	292.4	$20x10^4$
6.5	232.6	$12x10^4$	250.2	$16x10^4$
7.5	080.5	$06x10^4$	110.6	$10x10^4$

Perusal of the table indicates that when *B. theobromae* was cultured at 5 different pH range i.e. 3.5, 4.5, 5.5, 6.5 and 7.5 that was the initial pH, it is clear that dry mycelial weight in 100ml of culture medium was the lowest at 3.5 initial pH, which was 58.4 mg only. Maximum dry mycelial weightwas noted at the initial pH 5.5 and temperature 28°C which is 280.2mg when the initial pH was raised to 6.5 at the same temperature the dry mycelial weight was 232.6mg for 100ml. of culture. There was further decrease in the dry mycelial weight at the initial pH 7.5. For the calculation of spore density at the various pH range, the mycelial bit with spores were inoculated in the culture medium having initial pH at 3.5, 4.5, 5.5, 6.5 and 7.5. The cultures were incubated at 28°C for 15th day. These culture flasks were taken out and with the help of camel hair brush and sterile distilled water, spores were harvested in a suspension culture. They were then studied for their numbers in 1ml suspension with the help of hemocytometer. For each initial pH experiments were set in triplicate and the data were obtained by taking mean. This was also used for spore density calculation.

From the table it is evident that minimum density of spores was obtained in the culture having 3.5 as an initial pH. Here the spore density was only $2x10^4$ in 1ml suspension culture. Maximum numbers of spores were produced in the culture having initial pH 5.5 which was $17x10^4$ in 1ml of suspension culture. It is further observed that cultures having initial pH lower than i.e. 4.5 or higher than i.e. 6.5 had lower number of spores in 1ml of suspension i.e. $8x10^4$ at 4.5 and $12x10^4$ at 6.5. There was further decrease in the spore density when the initial pH was maintained at 7.5, which was only $6x10^4$. Here it may be noted that there was an increasing tendency in the spore density along with the increase in the initial pH i.e. from 3.5 to 4.5 to 5.5. However when this initial pH was increased further, i.e. 6.5 or 7.5 we get no further increase in the density of the spores rather there is gradual decrease in it as we move above 6.5. Initial pH of culture medium was maintained at 3.5, 4.5, 5.5, 6.5 and 7.5 respectively. Mycelial bit along with the spores of *C. gloeosporioides* were inoculated in the above cultures and the culture flasks were incubated at 28°C. Now 10 days old cultures were taken and filtered through Whatman filter paper no.1. The mycelial mat was washed properly with hot water to remove the traces of culture medium from it. Then it was transferred to a pre weight and sterilized filter paper. Before transfer the mycelia mat was dried with pre sterilized dry filter paper. Now the weight of the mycelial mat was taken along with the filter paper. When the weight of the filter paper was deducted from it the actual mycelial weight was obtained. The mycelial mat was placed in incubator at 60°C and weight was taken till the constant weight was obtained. The experiment was done in triplicate and the averages of the data were tabulated in the table.

For spore density, spores were harvested from 15th day old cultures having different range of initial pH. The spores were harvested with the help of sterile distilled water and camel hair brush. From the above spore suspension 1ml was used for the calculation of spore density. From the table it is apparent that *C. gloeosporioides* produced minimum mycelium mat at the initial pH 3.5, which was 68.5mg only. It may note that at the initial pH i.e. 5.5 there was maximum elaboration of mycelial mat that was 292.4mg. It may further be noted that initial pH below this or above it had lower mycelial growth i.e. 105.8mg at 4.5 initial pH & 250.2mg at 6.5 initial pH. Similarly there was further decrease in the dry mycelial growth at the initial pH 7.5. It is found from the table that when the pH was increased from 3.5 to 5.5, there was an increasing tendency in the dry mycelial weight. However, above this it may be noted that there is gradual decrease in the dry mycelial weight of the fungus. Spores suspension prepared from the 15th day old cultures at different initial pH were used for the counting of the spore density. The data obtained have been placed in the above table. From the table it is clear that the density of spores in 1ml suspension culture was only $4x10^4$ at the initial pH 3.5. However, the highest density i.e. $20x10^4$ was obtained at the initial pH 5.5 of the culture. It may be noted here also that when the initial pH was less than 5.5 i.e. 4.5 or more than 5.5 i.e. 6.5, the spore production rate was reduced. However, from the initial pH 3.5 to 5.5, there was an increasing tendency in spore production but beyond this i.e. at 6.5 and at 7.5 the trend became downward that is declination of the spore production may be noted. Here at 6.5 the spores were $16x10^4$ while at 7.5 it was$10x10^4$.

4. Conclusion

Therefore, finally it may be concluded that 5.5 pH was most suitable for both *B. theobromae* and *C. gloeosporioides* with respect to mycelial growth and spore production which are evident with the dry mycelial weight and spore density at this initial pH. It may be further concluded that rise in pH not always helped in the spore production and mycelial growth that is why we get less dry weight and spore density at higher pH.

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