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EFFECT OF ENVIRONMENTAL FACTORS, DIFFERENT FORMS OF CARBON, INORGANIC AND ORGANIC FORM OF NITROGENOUS COMPOUNDS ON MYCELIAL GROWTH OF *Alternaria alternata* ISOLATED FROM INFECTED FRUIT OF BOTTLE GOURD (*Lagenaria siceraria* (Molina)st.)

ARCHITA PRAKASH[a1] AND PREETI PRASAD[b]

[a] Department of Botany ,Bhoot Nath Degree College, Baghan, West-Champaran, Bihar, India
Former Head, Department of Botany, Plant Pathology Laboratory, B.R. Ambedkar Bihar University, Muzaffarpur, Bihar, India

ABSTRACT

***Alternaria alternata* was isolated from the infected fruit of bottle gourd (*Lagenaria siceraria*) and pure culture was maintained in the laboratory. *In vitro* impact of different environmental factors, sources of carbon, inorganic and organic nitrogenous compound, was observed on mycelial growth, as determined by the radial growth on solid culture medium, while the dry mycelial weight in the same culture broth. The study was undertaken to assess mycelial growth of the fungus under different environmental conditions such as pH & temperature as well as under different nutritional conditions such as carbon and nitrogen sources. Ten different pH and seven different temperatures were used in which the fungus was cultured. Likewise, nine different carbon sources and six inorganic and eight organic sources of nitrogen compounds were tested. Among the pH, 6.5was found more favorable where the dry mycelial weight was the maximum, 695.75 mg/100ml of culture, while the radial growth was 84.66 mm, followed by 6.0 pH, where the dry mycelial weight was 672.56 and radial growth 72.85. At both pH 3.0 and 8.0, both the dry mycelial weight and the radial growth were the lowest. Highest dry mycelial weight, 688.72 mg/100 ml and radial growth 78.48 were noted at 28^0C, followed by 626.28 mg/100 ml, dry mycelial weight and 68.34 mm radial growth at 32^0C. Among the carbon sources tested, glucose promoted maximum dry mycelial weight (680.18 mg/100 ml), while the radial growth was 82.32 mm, followed by fructose, 672.84 mg/100 ml dry mycelial weight and 76.28 mm radial growth. These values were significantly superior over all the carbon sucrose evaluated followed by sucrose (628.88 mg/100 ml) and 72.56 mm as control. Among the nitrogen sources alanine induced maximum dry mycelial weight (682.32 mg/100 ml) and 82.38 mm radial growth, followed by potassium nitrate (678.24 mg/100 ml) and 78.15 mm radial growth. Minimum dry mycelial weight 422.38 and radial growth 46.26 mm were obtained in case of p-amino benzoic acid. Among the inorganic sources Aluminum nitrate promoted the minimum (538.18 mg/100ml) and 60.72 radial growths.**

KEYWORDS: Bottle gourd, Radial growth, Dry mycelial weight, In-organic nitrogen sources, p-aminobenzoic acid, *Alternaria alternata*

Bottle gourd is an important vegetable for the common people. Both winter and summer crops are being grown at commercial scale. However, summer crops are being damaged by different pathogens. This causes heavy loss to the farmers. From the infected fruits *Alternaria alternata* was isolated. Experiments were performed to observe the impact of different pH, and temperatures, various carbon and inorganic and organic sources of nitrogenous sources *in vitro*. From the survey of literature it was found that different workers have done experiments to evaluate the effects of above parameters on different fungal pathogen, as well as on *Alternaria* species too. Some of them may be mentioned here. Lilly and Barnnet (1951), Bais *et al.,* (1970) on *Curvularia pallescens*, Hasiza (1970) with *Alternaria* spp., Verma (1970), on three pathogenic fungi, Reddy (1971) on Species of *Helminthosporium*; Goyal, (1977) on *Alternaria tenuis*; Mathur and Sarboy (1977) with *A. alternata*; Mahapatra *et al.,* (1977); with *A. sesame*; Rawla and Tondon (1977); Gomawat and Ghos (1980); Bharti *et al.,* (2007); in case of *Metarhizium* spp., Prathibha *et al.,* (2008); Pose *et al.,* (2009); Bhale (2010); Thawre *et al.,* (2010); Dange (2012); Mishra and Mishra (2012); Mehta *et al.,* (2012); Bist (2013); Devi *et al.,* (2014); Dhal *et al.,* (2014); Itoo *et al.,* (2014); Koley and Mahapatra (2015); Hashan *et al.,* (2015); Shilpa *et al.,* (2015); Gawai and Mangnalikar (2018). To determine the most readily utilizable source of carbon, nitrogen and most suitable pH, and temperature by the fungus, *in vitro*, the present study was undertaken.

MATERIALS AND METHODS

In the present study Richard's Medium was used for the study and different parameters as mentioned above were tested.

[1]Corresponding author

Different Ranges of pH

The pH of the medium was adjusted between 3.0 to 8.0 using 0.1NHCl and 0.1 N NaOH, with the help of digital pH meter. Here for the determination of dry mycelial weights as an indicator of growth, at different pH, liquid medium was used. After adjustment of the pH, each culture flask was inoculated with 8 mm culture disc, taken from actively growing peripheral region of the fungus in Petri Plates. The culture disc was taken with the help of pre-sterilized Cork-borer under aseptic condition. The flasks were incubated at 28^0C in the shaker incubator and mycelial mats were harvested from 10 days old culture.

Similarly, the culture flasks having liquid culture medium were inoculated as described above. However, here the flasks were cultured at 16^0C, 20^0C, 24^0C, 28^0C, 32^0C, 36^0C and 40^0C separately in the shaker incubator. Harvesting was done after 10 days of incubation.

Carbon Sources

To determine the most readily utilizable source of carbon, by the test organism, *Alternaria alternata* the original carbon source of Richard's medium was replaced by other form of carbon. Quantity of each carbon composition was determined on the basis of their molecular weight so as to provide equivalent amount of carbon as that of sucrose present in the basal medium. The carbon sources used were glucose, galactose, mannose, fructose, xylulose, mannitol, lactose, maltose, raffinose, and sucrose was used as control. 30 ml of each medium was taken in 100 ml flask. After sterilization inoculation was done as described above.

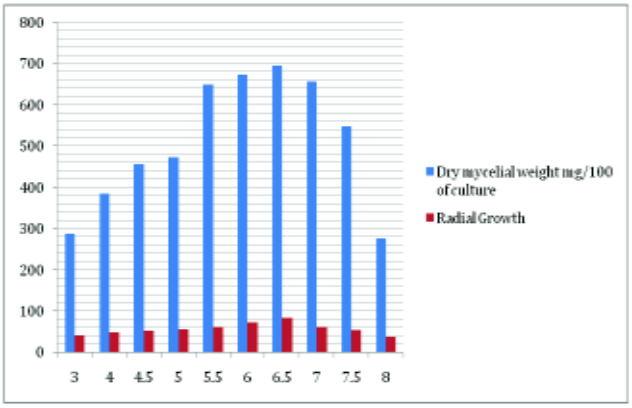
Nitrogen Sources

Potassium nitrate in the basal medium was replaced by equal amount of different inorganic organic sources of nitrogen. This was done by calculating the amount of nitrogen in each compound. Here also Richard's agar medium was used for radial growth while the broth for dry mycelial weight. From the broth the mycelial mat was collected on pre-sterilized and weighed, Whatman filter paper No.-42.

The mycelial mat was washed with hot water with the help of jet pipette to remove traces of culture medium. Mycelial mat from 250 ml of cultures were collected together and placed in hot air incubator adjusted at 50^0C. Weight was taken and it was again dried. This was repeated till the constant weight was obtained. Each experiment was performed in triplicate and each time 15 culture flasks were used. The mean of the data was tabulated in the form of tables for analysis and discussion. They are represented by the graph from 1 to 5.

RESULTS

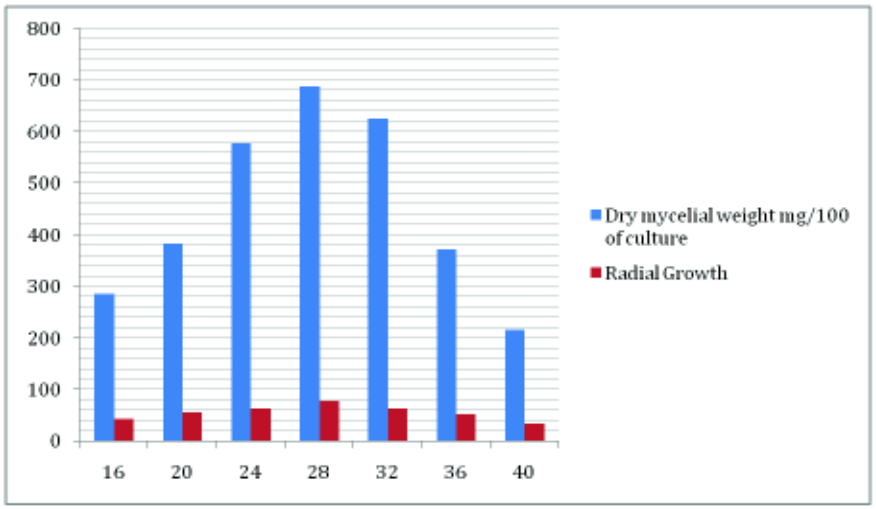
From the graph 1 it was clear that highest dry mycelial weight 695.75 mg/100 ml of culture was obtained at pH 6.5, followed by 672.56 mg at pH 6.0. It was further noted that the test organism that was *Alternaria alternata* could grow in wide range of pH i.e., from 3.0 to 8.0 in above experiments. However, the rate of growth was influenced by the lowest as well as the highest pH that was 3.0 and 8.0, respectively. Due to this at this pH the poor growth of the fungus was recorded. The radial growth in the solid medium also followed the similar pattern as the maximum diameter 84.66 mm was at pH 6.5, followed by 72.85 mm at pH 6.0. Here also lowest radial growth 38.60 mm was noted in case of culture having 8.0 pH.



Graph 1: Impact of different range of pH on mycelial growth of *Alternaria alternata* as indicated by dry mycelial weight and radial growth.

The effect of different temperature levels viz., 16, 20, 24, 28, 32, 36 and 40^0C was studied to observe their impact on mycelial growth the results depicted in graph 2 revealed that fungus could grow at all the temperature levels as mentioned above. However, maximum dry mycelial weight 688.72 mg/100 ml of culture and the maximum radial growth on Richard's solid medium was 78.48 mm at 28^0C. This was followed by dry mycelial weight at 34^0C (626.28) and radial growth 68.34 mm. Lowest dry mycelial weight 216.74 mg/100 ml of culture

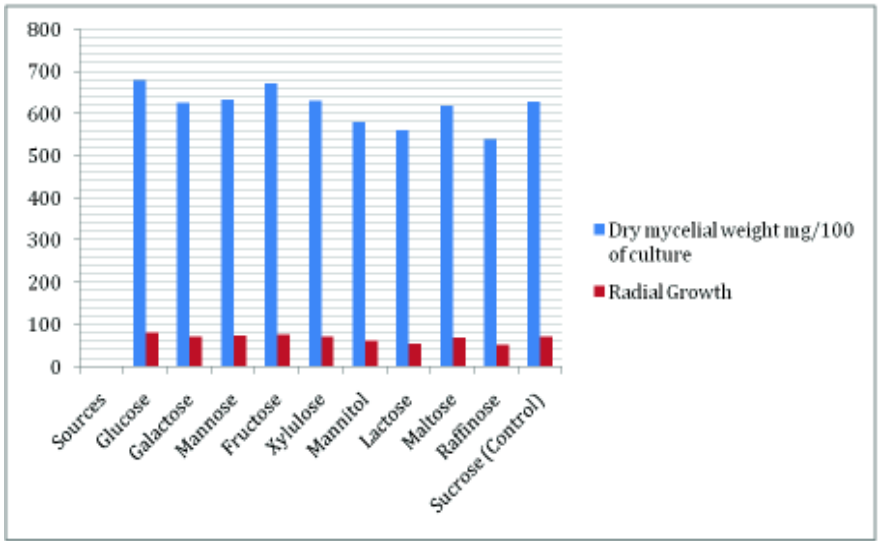
and 34.42 mg radial growth was obtained at 40^0C at which the cultures were incubated.



Graph 2: Impact of different temperature ranges on mycelial growth of *Alternaria alternata* as indicated by dry mycelial weight and radial growth.

Carbon Source Utilization

Culture characteristics viz., mycelial dry weight and radial growth of *A. alternata* were studied *in vitro* using nine carbon sources, where as sucrose was used as control. All sources of carbon tested exhibited growth of the test fungus but with different rate of growth. The results, graph 3 revealed that of the nine carbon sources tested, glucose was found most suitable that induced maximum dry mycelial weight 680.18 mg/100 ml of culture and maximum radial growth 82.34 mm.

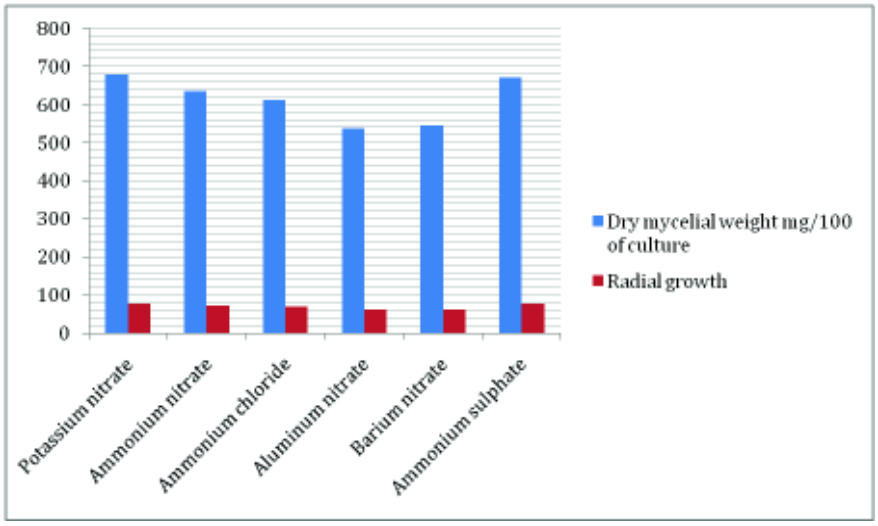


Graph 3: Impact of different Carbon Sources on mycelial growth of *Alternaria alternata* as indicated by dry mycelial weight and radial growth.

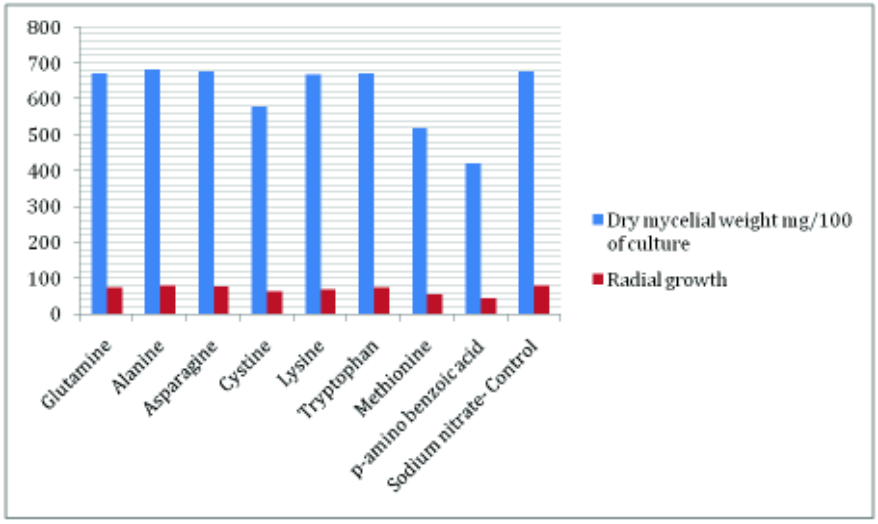
This was followed by fructose 672.84 mg/100 ml of culture and 76.28 mm radial growth, and mannose 638.64 mg/100 ml dry mycelial weight and 73.52 mm radial growth. Raffinose was found less suitable source of carbon where the dry mycelial weight was 540.28 mg/100 ml of culture medium and 52.36 mm radial growth.

Effect of Nitrogen Sources

Dry mycelial weights and radial growth of *A. alternata* were studied *in vitro* using six inorganic sources of nitrogen and eight organic sources of nitrogen. From the graph 4&5 it may be noted that all the sources of nitrogen induced growth of the test pathogen *in vitro*, however, there were difference in the quantum of the growth. Among the inorganic sources of nitrogen, potassium nitrate induced maximum mycelial growth, as is supported by the dry mycelial weight 678.24 mg/100 ml of culture and the radial growth that is 78.15 mm. This was followed by ammonium sulphate that was 670.84 mg/100 ml of culture and 76.22 mm radial growth. Here aluminium nitrate was less suitable where dry mycelial weight was 538.18 mg and the radial growth 60.72 mm. Among the organic sources of nitrogen Alanine was most favourable as here the dry mycelial weight was 682.32 mg/100 ml of culture and the radial growth was 82.38 mm. This was followed by asparagines, 676.52 mg/100 ml dry mycelial weight and 77.68 mm radial growth. This was followed by tryptophan (672.12 mg/100 ml) and (73.24 mm) radial growth. Poor dry mycelial weight (422.38 mg/100 ml) and (46.28 mm radial growth) were noted in the medium containing p. amino benzoic acid as the source of nitrogen.



Graph 4: Impact of different Inorganic Nitrogen Sources on mycelial growth of *Alternaria alternata* as indicated by dry mycelial weight and radial growth.



Graph 5: Impact of different Organic Nitrogen Sources on mycelial growth of *Alternaria alternata* as indicated by dry mycelial weight and radial growth.

DISCUSSION

In the present study, effects of different ranges of pH and temperatures, different source so carbon and nitrogen were observed *in vitro* for mycelial growth of *Alternaria alternata*. Here it was observed that the pathogen could grow in different ranges of pH and temperatures but the quantum of growth rate was not equal. This clearly revealed that best pH for better growth was 6.5, while the most suitable temperature was 28^0C. Present findings are in agreement with that of the findings of Sami and Hegedorn (1970) in case of *Alternaria* spp., Kumara and Rawal (2008) in *Colletotrichum gloeosporioides*; Hubballi *et al.,* (2010) in *Alternaria alternata*, Deshmukh *et al.,* (2012); in *Colletotrichum gloeosporioides*.

Devi *et al.,* (2014) in *Alternaria helianthi*; Prasher *et al.,* (2014) in *Arthimium* spp., Taware *et al.,* (2014) in *Alternaria carthami*; and Chaudhary *et al.,* (2017) in *A. alternata*.

In the present study, among the carbon sources, glucose was found most suitable for maximum mycelial growth of *Alternaria alternata in vitro.* Similarly, among the inorganic sources of nitrogen compound potassium nitrate promoted maximum mycelial growth while among the organic nitrogen sources alanine was most promising source of nitrogen with respect to mycelial growth of *Alternaria alternata in vitro*. Among the inorganic source of nitrogen Aluminium nitrate, and among the organic, p-amino benzoic acid were the poor choice of the pest pathogen used in the present study. Present findings corroborate with the findings of Bais (1970); Goyal (1977); Bharti *et al.,* (2007); Kumar *et al.,* (2008); Thaware *et al.,* (2010) in case of *Alternaria alternata*, Dange (2012); Bist (2013); Dhale *et al.,* (2014); Itoo *et al.,* (2014); *Ectomycorrhizae*, Hashan *et al.,* (2015) in *Aspergillus carbonaris*; Koley & Mahapatra (2015); Kushwaha (2015) *Colletotrichum capsici*, Shilpa *et al.,* (2015); Fusarium sp and Sinha *et al.,* (2015) in *Alternaria solani*.

CONCLUSION

Findings of the present work as well detailed literature survey, showed that fungal pathogens are capable to grow in diverse conditions of pH, temperature, and carbon and nitrogen sources. Here it was observed that although the pathogen can grow in different range of pH and temperatures but the maximum growth was favoured at a particular pH and temperature. This was also true for the carbon and nitrogen sources also. Here also particular carbon and nitrogen induced maximum growth, than others inspite of the facts that all conditions & the pathogen was similar. This reveals adaptive capability of the pathogen for their survival in nature.

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