

Original Research Article

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Effect of Various Carbon and Nitrogen Sources on *Aspergillus niger* a 'Phosphorous' Solubilizing Organism

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ABSTRACT

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Three species of *Aspergillus* (*A. niger*, *A. flavus*, *A. terreus*) were found to be effective in phosphorous solubilization in order to utilize these species for large scale production of bioformulation, the basic studies on effect of various carbon and nitrogen sources on growth and sporulation was done. Richard's broth was used as basal medium to study the influence of various carbon and nitrogen sources. Among four carbon and three nitrogen sources tested for growth of *A. niger*, *A. flavus*, *A. terreus* maltose was most effective carbon source and calcium nitrate was found to be most effective nitrogen source for growth and sporulation of all *Aspergillus* spp.

Introduction

Phosphorus is one of the major essential nutrient for plant growth it is among the macro element required as a plant nutrient second only to nitrogen. It helps for growth and health of plant. Phosphorus helps in photosynthesis, nutrient and energy transfer. It affects plant structure at acellular level. It is very important for quality and higher production of crops. Therefore, it is required in relatively high amount. The total phosphorus concentration in agricultural crops generally varies from 0.1% to 0.5%.

A greater part of soil phosphorus is present in the form of insoluble phosphate and can not be utilized by plants (Vassileva *et al.*, 1998). To increase the availability of phosphorus for plants, large amount of phosphatic fertilizers are being applied to soil.

But a large proportion of phosphatic fertilizers after their application are quickly transformed to the insoluble forms (Omar, 1998). Therefore very little percentage of applied phosphorus is available to plants, making continuous application necessary (AbdAlla, 1994).

Many soil fungi and bacteria are known to solubilize inorganic phosphates (Asea *et al.*, 1988 Illmer and Schimmer, 1992). Phosphate solubilizing microorganism (PSMs) play an important role in supplementing phosphorous to plants allowing sustainable use of phosphatic fertilizers. Several mechanisms like lowering of PH by acid production, ion chelation, and exchange reaction in the growth environment have been reported to play a role in phosphate solubilization by PSMs (Goldstein 1986, Halder *et al.*, 1991, Abd Alla 1994, Goldstein 1986 and Whitelaw, 2000). Among PSMs, fungi perform better phosphate solubilization in acidic soil conditions (Hmed and Jha 1968).

Aspergillus niger is a versatile phosphate solubilizer and is abundantly present in different soil type (Gaur 1990). It solubilize eight insoluble metal compounds *viz.* Al_2O_3 , $Al_2(PO_4)_2$, $CaCO_3$, $Ca_3(PO_4)_2$, $Mn(PO_4)$, ZnO and $Zn_3(PO_4)_2$. *Aspergillus* lowers the soil PH and brings about dissolution of immobile form of soil phosphate. Some organic acid produced by it may chelate calcium, aluminium ferrous and magnesium further increasing phosphorous availability.

Thombre (2009) studied the phosphate solubilization ability of some fungi isolated from the rhizosphere of coconut. Out of that six *Aspergillus* spp. Were studied for the phosphorus solubilization ability *in vitro*. *A.niger*, *A. flavus* and *A.terreus* (44.98, 40.47, 23.01% respectively) where found to be efficient phosphate solubilizers. This paper describes effect of Carbon and Nitrogen sources on *Aspergillus niger* strain effective for Phosphorus solubilization.

Materials and Methods

Cultures- Fungal cultures which are found to be good phosphate solubilizer were obtained from Biofertilizer Production center,

Department of plant pathology. These fungal spp. are *Aspergillus niger*, *A. vericolor* and *A. sydowii*. Potato dextrose agar (PDA) was used for maintaining of the cultures during the course of investigation.

Effect of different carbon sources on the growth and sporulation of *Aspergillus* spp

In order to study the influence of various carbon sources Richard's broth was selected as basal medium. Sucrose in basal medium was substituted by other carbon sources. The quantity of each carbon source was calculated on the basis of equivalent weight substituting the amount of carbon present in 50gm of sucrose/liter of basal medium.

Fifty ml broth was dispensed in 100 ml flask, plugged and autoclaved at 15lbs/sq inch, for 15 minutes. Three replications were maintained for growth and degree of sporulation. Each flask was inoculated with 5mm diameter disk of 8 days old culture of *Aspergillus* spp. the flasks were incubated at room temperature (27 ± 1) for 10 days.

The weight of filter paper was taken after drying at $60^{\circ}C$ till constant weight is obtained after repeated drying. After 8 days mycelial mat was harvested at previously weighed whatman's no.1 filter paper. The filtrate was collected for determination of final pH.

The filter papers along with mycelial mats were dried at $60^{\circ}C-70^{\circ}C$ for 48 hours. After drying these filter papers were taken out from the oven. The weight of dried filter paper with mycelial mat was calculated by deducting weight of dry filter paper from dry filter paper with mycelial mat. Sporulation was counted by using haemocytometer by taking 0.1ml suspension on it under microscope. 12 readings from each replicate were taken hence 24 readings were taken for counting sporulation.

Effect of different nitrogen sources on the growth and sporulation on *Aspergillus* spp

In order to study the influence of various nitrogen sources Richard's broth was selected as a basal medium. Potassium nitrate in a basal medium was substituted by other nitrogen source. The quantity of each nitrogen source was calculated on the basis of equivalent weight substituting the amount of nitrogen present in 10gm of KNO₃/liter of the basal medium.

Results and Discussion

Effect of different carbon sources on growth and sporulation of *Aspergillus* spp

In present investigations the test fungus was grown on four different carbon sources. Richard's medium is used as basal medium. The data regarding growth and sporulation of the *A.niger*, *A.flavus*, *A.terreus* with different carbon sources are given in table 3 and 4 respectively.

Effect of carbon sources

The data presented in table revealed that, among four carbon sources tested for growth of *A.niger*, *A. flavus*, *A. terreus* maltose was most effective carbon source where mean of mycelial growth irrespective of *Aspergillus* spp. was 602.56mg and it was followed by starch where mycelial growth irrespective of *Aspergillus* spp was 563.78mg. Mannitol ranked 3rd in supporting the mycelial growth of *Aspergillus* spp. while glucose was found to be poor supporter for mycelial growth of *Aspergillus* spp.

Effect of *Aspergillus* spp

The perusal table 10 reveals that significantly maximum mycelial growth was reported by the *A. niger* (484.67 mg). and it was followed

by *A. flavus* (412.42 mg). Whereas the growth of *A. terreus* was significantly low (378.42 mg).

Interaction of *Aspergillus* spp. and carbon sources

Highest mycelial growth was obtained when maltose was used as a carbon source for *A. niger*. The growth was 650.00mg which was significantly highest than rest of the interactions. Lowest mycelial growth was obtained when glucose was used as carbon source for *A. terreus* where mycelial growth was 55.33mg.

Effect of carbon sources

The data presented in the table four revealed that, among four carbon sources tested for sporulation of *A.niger*, *A.flavus*, *A.terreus* starch was most effective carbon source where mean sporulation irrespective of *Aspergillus* spp. was $58.15 \times 10^6 \text{ ml}^{-1}$ and it was followed by mannitol where mean sporulation irrespective of *Aspergillus* spp. was $47.00 \times 10^6 \text{ ml}^{-1}$. Maltose ranked third in supporting sporulation of *Aspergillus* spp. while glucose was found to be poor supporter for sporulation of *Aspergillus* spp.

Effect of *Aspergillus* spp

The perusal table four reveals that significantly maximum sporulation was reported by *A. niger* ($72.75 \times 10^6 \text{ ml}^{-1}$) and it was followed by *A. flavus* ($32.97 \times 10^6 \text{ ml}^{-1}$). Whereas the sporulation of *A. terreus* was significantly low and on at par with *A. flavus*.

Interaction of *Aspergillus* spp. and carbon sources

Highest sporulation is obtained when mannitol was used as a carbon source for *A. niger*. The sporulation was $90.33 \times 10^6 \text{ ml}^{-1}$

which was significantly highest than rest of the interactions. Lowest sporulation was obtained when glucose was used as carbon source for *A. terreus* where sporulation was $13.33 \times 10^6 \text{ ml}^{-1}$.

Effect of different nitrogen sources on growth and sporulation of *Aspergillus* spp

In the present investigation, the test fungi were grown using three different nitrogen sources. Richards medium was used as abasal medium. The data regarding growth and sporulation of *A. niger*, *A. flavus* and *A. terreus* are presented in table 5 and 6 respectively.

Effect of nitrogen sources

It is clear from the table 5 that highest growth was obtained when CaNO_3 was used as a nitrogen source irrespective of the *Aspergillus* spp. grown. The mean mycelial growth in this treatment was 455.56 mg. it was followed by Ammonium sulphate which recorded 427.89 mg irrespective of *Aspergillus* spp. Urea was found to be poor Nitrogen source for mycelial growth of *Aspergillus* spp. as it recorded least mycelial growth (123.00 mg).

Effect of *Aspergillus* spp

It is revealed that, *Aspergillus niger* was superior in mycelial growth as a maximum growth (351.44 mg) was obtained irrespective of nitrogen sources used. It was followed by *Aspergillus flavus* and *Aspergillus terreus* where mycelial growth was 328.67 mg and 326.33 mg and was at par.

Interaction of *Aspergillus* spp. and nitrogen sources

Their interaction effect of *Aspergillus* spp. on Nitrogen sources depicted in table 5 revealed that the interaction between *A. terreus* and

CaNO_3 was highly significant as it recorded the maximum mycelial growth (460 mg.) it was followed by interaction between *Aspergillus flavus* and CaNO_3 which recorded the growth to the tune of 455 mg. the interaction between *A. terreus* and Urea was highly undesirable as a growth was 88 mg.

Effect of nitrogen sources

It is clear from the table 6 that highest sporulation was obtained when CaNO_3 was used as a nitrogen source. Source irrespective of the *Aspergillus* spp. grown. The mean sporulation in this treatment was $54.87 \times 10^6 \text{ ml}^{-1}$. It was followed by Ammonium sulphate which recorded $30.49 \times 10^6 \text{ ml}^{-1}$ Conidia per ml irrespective of *Aspergillus* spp. urea was found to be poor nitrogen source for sporulation of *Aspergillus* spp. as it recorded least sporulation ($12.43 \times 10^6 \text{ ml}^{-1}$).

Effect of *Aspergillus* spp

It is revealed that, *A. niger* was superior in conidial production as a maximum sporulation ($69.83 \times 10^6 \text{ ml}^{-1}$). Was obtained irrespective of nitrogen sources used. It was followed by *Aspergillus flavus* and *A. terreus* where sporulation was $14.36 \times 10^6 \text{ ml}^{-1}$. Respectively.

Interaction of *Aspergillus* spp. and nitrogen sources

The interaction effect of the *Aspergillus* spp. and nitrogen sources depicted in Table 6 revealed that the interaction between *A. niger* and CaNO_3 was highly significant as it recorded the maximum sporulation ($118.72 \times 10^6 \text{ ml}^{-1}$). It was followed by the interaction between *A. niger* and Ammonium sulphate which recorded the sporulation to the tune of $156.05 \times 10^6 \text{ ml}^{-1}$. The interaction between *A.*

terreus and Urea was highly undesirable as no sporulation was observed when incubated for one week.

Effect of carbon

Out of four carbon sources tested for growth of *A. niger*, *A. flavus* and *A. terreus*, maltose was most effective carbon source where mean of mycelial growth irrespective of *Aspergillus* spp. was 602.56 mg and it was followed by starch where mean mycelial growth irrespective of *Aspergillus* spp. was 563.78 mg. mannitol ranked third in supporting the mycelial growth of *Aspergillus* spp. this result were accordance with those reported by Agnihotri (1964) who showed that peptone in combination with mannitol and starch supported maximum growth of *Aspergillus* spp. similarly Steinberg and bowling (1939) reported that mannitol and starch in combination with peptone and urea supported good growth of *Aspergillus* spp. a combination of starch with Ammonium sulphate produce better growth.

Glucose was found to be poor supporter for mycelial growth of *Aspergillus* spp. which was almost contradictory to the findings of Cerezina *et al.*, (1998) where he found that glucose and fructose are most favorable for growth of phosphate solubilizing *Aspergillus* spp.

The interaction effect between carbon sources and *Aspergillus* spp. revealed that maximum mycelial growth was obtained when maltose was used as a carbon source for *A. niger*. The growth was 650.00 which was significantly highest than rest of the interactions. Lowest mycelial growth was obtained when glucose was used as a carbon source for *Aspergillus terreus* and *A. flavus* where mycelial growth was 55.33 mg.

Out of four carbon sources tested for sporulation *A. niger*, *A. flavus*, *A. terreus* starch was most effective carbon source where mean sporulation irrespective of *Aspergillus* spp. $58.15 \times 10^6 \text{ ml}^{-1}$. And it was followed by mannitol where mean sporulation irrespective of *Aspergillus* spp. was $47.00 \times 10^6 \text{ ml}^{-1}$. Maltose ranked third in supporting sporulation of *Aspergillus* spp. while glucose was found to be poor supporter for sporulation of *Aspergillus* spp. highest sporulation was obtained when mannitol was used as a carbon source for *Aspergillus niger*. The sporulation was $90.33 \times 10^6 \text{ ml}^{-1}$. Which was significantly highest than rest of the interaction. Lowest sporulation was obtained when glucose was used as a carbon source for *Aspergillus terreus* where sporulation was $13.33 \times 10^6 \text{ ml}^{-1}$.

Effect of nitrogen

Maximum growth was obtained when CaNO_3 was used as nitrogen source irrespective of *Aspergillus* spp. grown the mean mycelial growth was 455.56 mg. it was followed by ammonium sulphate which recorded 427.89 mg. irrespective of *Aspergillus* spp. this result were in accordance with those reported by Josephine *et al.*, (1998). Where he found that maximum growth of *Aspergillus niger* was obtained when nitrate source of nitrogen was used. Similarly Barraso *et al.*, (2006). Reported that ammonium nitrate and sodium nitrate was good for growth of *Aspergillus niger*. Narsian and patel (2000). Recorded that ammonium sulphate was a best nitrogen source for solubilization of rock phosphate and for growth followed by urea. Urea was found to be poor nitrogen source for mycelial growth of *Aspergillus* spp. as it recorded least mycelial growth (123.00 mg) interaction between *A. terreus* and CaNO_3 recorded maximum mycelial growth (460 mg). it was followed by interaction between *A. flavus* and CaNO_3 .

Table.1 Following carbon sources were used in the present study

Sr. No.	Carbon sources	Quantity
1.	Glucose	85.33gm
2.	Mannitol	87.12gm
3.	Starch	76.44gm
4.	Maltose	50gm

Table.2 Following nitrogen sources were used in the present study

Sr. No.	Nitrogen sources	Quantity
1.	Ammonium sulphate	7gm
2.	Calcium nitrate tetrahydrate	12.20gm
3.	Urea	7.46gm

Table.3 Effect of carbon sources on growth of *Aspergillus* spp. (weight in mg)

Carbon sources	<i>A. niger</i> *	<i>A. flavus</i> *	<i>A. terreus</i> *	Mean
Maltose	650.00	580.67	577.00	602.56
Mannitol	590.33	453.00	381.33	474.89
Glucose	67.67	55.33	55.33	59.44
Starch	630.67	560.67	500.00	563.78
Mean	484.67	412.42	378.42	
	S.E.m.±			C.D.
Carbon sources	0.52			2.07
<i>Aspergillus</i> spp.	0.45			1.80
Carbon sources × <i>Aspergillus</i> spp.	0.91			3.59

*Means of three replications

Table.4 Effect of carbon sources on sporulation of *Aspergillus* spp. (weights in mg)

Carbon sources	<i>A. niger</i> *	<i>A. flavus</i> *	<i>A. terreus</i> *	Mean
Maltos	71.89	27.22	32.78	43.96
Mannitol	90.33	23.11	27.56	47.00
Glucose	49.55	35.22	13.33	32.70
Starch	79.22	46.33	48.89	58.15
Mean	72.75	32.97	30.64	
	S.E.m ±			C.D.
Carbon sources	0.57			2.25
<i>Aspergillus</i> spp.	0.49			1.94
Carbon sources x <i>Aspergillus</i> spp.	0.98			3.89

*Means of three replications

Table.5 Growth in nitrogen sources (weight in mg)

Nitrogen sources	<i>A.niger</i> *	<i>A.flavus</i> *	<i>A. terreus</i> *	Mean
CaNO3	451.67	455.00	460.00	455.56
Urea	181.33	99.67	88.00	123.00
Ammonium sulphate	421.33	431.33	431.00	427.89
Mean	351.44	328.67	326.33	
	S.E.M±			C.D.
Nitrogen sources	0.52			2.11
<i>Aspergillus</i> spp	0.52			2.11
Nitrogen sources X <i>Aspergillus</i> spp.	0.90			3.65

*Means of three replications

Table.6 Sporulation Nitrogen Sources

Nitrogen Sources	<i>A. niger</i> *	<i>A. flavus</i> *	<i>A. terreus</i> *	Mean
CaNO3	118.72	29.55	16.31	54.87
Urea	34.73	2.55	0.00	12.43
Ammonium sulphate	56.05	8.67	26.75	30.49
Mean	69.83	13.59	14.36	
	S.E. M±			C.D.
Nitrogen Sources	0.48			1.94
<i>Aspergillus</i> spp.	0.48			1.94
Nitrogen Sources x <i>Aspergillus</i> spp.	0.82			3.35

*Means of three replications

The interaction between *A. terreus* and urea was highly undesirable as growth was 88 mg. maximum sporulation ($54.87 \times 10^6 \text{ ml}^{-1}$). It was obtained when CaNO₃ was used as nitrogen source irrespective of *Aspergillus* spp. urea was found to be poor nitrogen source for sporulation of *Aspergillus* spp. as it recorded least sporulation ($12.43 \times 10^6 \text{ ml}^{-1}$). In present study the interaction between *Aspergillus niger* and CaNO₃ was highly significant as it recorded the maximum sporulation ($118.72 \times 10^6 \text{ ml}^{-1}$). It was followed by the interaction between *Aspergillus niger* and ammonium sulphate which recorded the sporulation to the tune of $56.05 \times 10^6 \text{ ml}^{-1}$. The interaction between *A.terreus* and urea was highly undesirable as no sporulation was

observed when incubated for one week. Maltose was found to be good carbon source for growth of all *Aspergillus* spp under study (602.56mg).It was followed by starch with (563.78mg) mycelial growth.

It also supported maximum sporulation of all *Aspergillus* spp. Calcium nitrate was found to be good nitrogen source for growth and sporulation of all *Aspergillus* spp. (455.56 mg and $54.87 \times 10^6 \text{ ml}^{-1}$) respectively. So it is conformed that nitrate source of nitrogen is good for growth of all *Aspergillus* spp. urea was found to be poor source of growth of all *Aspergillus* spp. when urea is used as nitrogen source no sporulation was obtained for *A.flavus*.

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