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Effect of nitrogen sources on the growth of different species of *Curvularia, Fusarium, Phoma* and *Botryodiplodia*

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Abstract

Fruits and vegetables are eaten by man usually by uncooked methods so that several pathogenic organisms find their way into the human body. Fungal diseases play an important role in destruction of fruits and vegetables. The estimates reveal that losses due to plant diseases in India are over 200 crores of rupees annually. During the study greater frequency of Deuteromycetes was observed. The members of these species of *Curvularia, Fusarium, Phoma* and *Botryodiplodia* were the most dominant species among them. These fungi exhibit certain degree of specificity in their choice of food, but nitrogen substances are the most important substances required by them with regard to their vegetative and reproductive growth. The present studies deal with understanding the effect of thirteen nitrogen sources on species of *Curvularia, Fusarium, Phoma* and *Botryodiplodia*.

Keywords: *Curvularia, Fusarium, Phoma, Botryodiplodia* , nitrogen sources.

INTRODUCTION

Cereals, pulses, vegetables and fruits are the essential requirements of our diet. Among these some vegetables and fruits plays an important role in our normal health. The daily minimum consumption should be about 280gm per head. The disease factor alone is deterrent.[1, 3, 4] More attention needs to be given by Indian plant pathologists to the studies on diseases in the field, at harvest, in transit, in storage, and in the market places as a basis for developing suitable control measures.[12]

Four species of *Curvularia* viz. *Curvularia lunata, Curvularia senegalensis, Curvularia clavata, Curvularia prasadii*; three species of *Fusarium* viz. *Fusarium equiseti, Fusarium moniliforme, Fusarium oxysporum*; two species of *Phoma* viz. *Phoma nebulosa, Phoma vulgaris* and *Botryodiplodia theobromae* were selected for Physiological studies.[20]

MATERIAL AND METHODS

Collection of Material and Isolation of Fungi

Extensive survey of diseases caused to fruits and vegetables in Amravati region was carried out and samples were taken from different localities of this district.[9,10,11]For sampling purpose specification of localities were made on the basis of ecological and geographical variation found in Amravati district. The infected hosts were collected out of which species of *Curvularia, Fusarium*, *Phoma* and *Botryodiplodia* were selected for the physiological behavior.[18, 19]

The diseased fruits and vegetables were collected separately in polythene bags and symptoms on different hosts were recorded. Completely rotten fruits and vegetables were avoided for isolations as they contained mostly secondary infections. Collections were made in different seasons at different places on same hosts to know the difference in developing stages of symptoms of a fungus. Slides were prepared by scrapping the fruiting bodies, produced in infected regions. Isolations were made by cutting small fragments of diseased spot along with healthy region. The diseased tissues were surface sterilized with 90% alcohol and transferred aseptically to Asthana and Hawker's medium 'A' (5g glucose, 3.5g KNO3, 1.75gKH2PO4, 0.75g MgSO4. 7H2O and 15g agar agar.)[2] The slants were completely sterilized to avoid the secondary and bacterial infection. Inoculation was carried out in sterilized inoculation chamber at the temperature 27^{0}C. ($\pm$ 20 C) After 2 or 3 days of inoculation the mycelium coming out of the diseased tissue was picked up and transferred to another fresh slant. Morphological and cultural characters of the organisms were carefully recorded. Identification of isolates were made from stock cultures present in mycological laboratory of Brijlal Biyani Science College, which were previously identified from Common wealth Mycological Institute, Kew, Surrey, England.[8]

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Physiological Studies

1.Growth
2.Dry Mycelial Weights

Effect of Nitrogen Sources-[16]

Four species of Curvularia [17]viz *Curvularia lunata, Curvularia senegalensis, Curvularia clavata, Curvularia prasadii* [5], three species of Fusarium [13,14,25] viz. *Fusarium equiseti, Fusarium moniliforme, Fusarium oxysporum,* two species of Phoma viz, *Phoma nebulosa, Phoma vulgaris* and *Botryodiplodia theobromae*[21,22] were selected for physiological studies.[15,23,24,26]

Dry mycelial weight, PH and sporulation [6]were studied during the physiological experiments. Asthana and Hawkers Medium 'A' in liquid state was used as a basal medium (Glucose 5.0g, KH2PO4

1.75g, MgSO4. 7H2O 0.75g, KNO3 3.5g and distilled water 1litre)[7]. Potassium nitrate from the basal medium was substituted by thirteen different nitrogen compounds respectively. There quantities were so adjusted as to furnish 485 mg nitrogen per liter. Pure chemical lied by B.D.H Apex or Sarabhai Mark were used for this purpose. 25ml of liquid sterilized medium was used for observing dry mycelial weight and the degree of sporulation.25 ml sterilized liquid medium was poured in conical flasks (corning) of 250ml and conical flasks along with medium were allowed to sterilized at 15 lbs of autoclave for 15minutes. 1ml of spore suspension was incubated in each flask. Incubation was carried out in inoculation chamber under UV lamp in order to avoid contamination. Spore suspension was prepared by crushing the lumps of culture medium in sterilized water by glass rod having rubber pad. Average number of spores per ml was recorded. All possible precautions were taken to avoid any type of contamination during incubation. Liquid medium from conical flasks was filtered by known weighted Whatman filter paper No.1 after the interval of every 4, 8 and 12 days of incubation. Filtrations were made on sterilized funnels and filter papers were dried in oven at 40^0 to 50^0 for 30 to 50 minutes so that filter paper becomes completely dry. Again the weight of filter paper was taken with the help of chemical balance. Difference between two weights (W2- W1) was considered as dry mycelial weight.

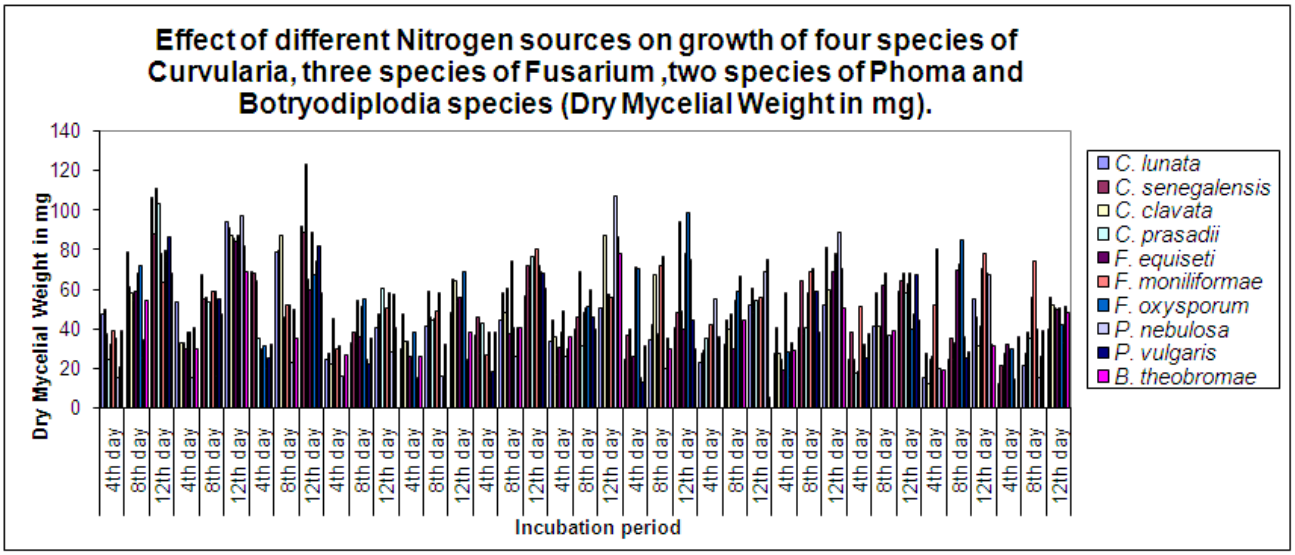
RESULTS AND DISCUSSIONS

Effect of thirteen nitrogen sources on four species of *Curvularia*, three species of *Fusarium*, two species of *Phoma* and *Botryodiplodia theobromi* is tabulated in table no.1 and graph no.1.

Table 1.Showing the effect of nitrogen sources on the growth of different species of *Curvularia, Fusarium, Phoma and Botryodiplodia*

S.No.	Nitrogen Source	Incubation Period	*C. lunata*	*C. senegalensis*	*C. clavata*	*C. prasadii*	*F. equiseti*	*F. moniliformae*	*F. oxysporum*	*P. nebulosa*	*P. vulgaris*	*B. theobromae*
1.	Potassium nitrate	4th day	47.3	49.6	37.7	24.2	32.1	39.1	35.2	15.2	20.3	39.1
		8th day	78.9	61.1	58.2	54	59.2	68.2	72.1	25.2	34.2	54.2
		12th day	106.3	88	111.2	103.1	77.8	63.3	79.2	74.1	86.2	68.1
2.	Sodium nitrate	4th day	53.2	23.6	33.2	32.6	29.6	38.2	38.2	15.2	40.4	30.2
		8th day	67.6	55.1	55.7	53.8	52.1	59.2	59.2	15.9	55.1	47.6
		12th day	94.4	90.9	86.9	85.7	83.9	87.2	81.2	96.8	82.2	69.1
3.	Ammonium nitrate	4th day	69.2	68.2	64.2	35.2	23.2	30.1	31	20.2	25	32.2
		8th day	79.1	79.4	87.2	46.2	33.1	52.2	52.1	23.2	50.1	35.1
		12th day	92	88.4	123.2	64.8	60	89.1	67.2	74.3	82.1	58.2
4.	Ammonium sulphate	4th day	24.4	27.7	22.3	45.2	22.8	30.2	31.2	16.1	15.2	27
		8th day	33.2	38.1	33.2	54	36.1	51	55.2	24.1	22.1	35.2
		12th day	40.2	47.5	44.2	60.7	40.1	50.2	58.1	28.2	57.2	40.2
5.	Urea	4th day	30.2	47.1	33.8	33.8	25.8	24.1	38.1	15	14.2	26.1
		8th day	41.6	59.2	46.1	44.1	45.1	49.2	58.2	16.2	15.2	32.1
		12th day	48.1	65	64.2	53.4	56.2	56.1	69.1	24.1	20.9	38.2
6.	DL-Alanine	4th day	36.4	45.6	27.3	42.6	24.9	26.8	38.2	15.1	18.2	38.2
		8th day	44.7	58.1	48.2	60.3	37.2	74.2	40.2	26.2	40.2	40.2
		12th day	56.6	72.1	60.2	76.4	50.2	80.1	72.1	69.2	68.2	60.2
7.	L-Asparagine	4th day	33.5	44.2	36.3	17.2	30.4	37.9	48.9	26	30	35.9
		8th day	39.7	46.2	68.5	31.2	48.2	50.2	51.2	59.9	46.2	40.1
		12th day	50.2	50.3	87.3	57	57.2	56.2	56.2	107.2	86.2	78.2
8.	Glycine	4th day	24.2	37.1	40.1	24.2	25.8	71.2	70.1	15.2	13.2	31.1
		8th day	34.7	42.1	67.4	37.4	32.1	72.1	76.2	20.2	35.1	30.2
		12th day	40.6	48.2	94.2	49.1	40.1	78.2	99	75.2	44.1	30.2
9.	DL-Leucine	4th day	22.6	27.2	29.2	35	28.2	42.2	29.2	55	15.2	36.2
		8th day	32.1	44.2	40	47.2	30.2	54.2	59.2	66.2	25.1	44.1
		12th day	52.1	60.1	48.1	54.2	50.3	56.1	52.1	69.1	75	5
10.	L-Cystine	4th day	27.4	40.7	27.2	24.1	19.2	58.2	28.2	15.2	33.2	29.1
		8th day	40.2	64.3	37.2	40.2	58.2	69.2	70.2	59.2	59.2	38.2
		12th day	52.1	80.9	59.7	55.3	68.9	78.2	71.9	89.1	70.1	50.2
11.	DL-Valine	4th day	24.2	38.2	24.2	17.4	18.2	51.2	16.2	32.1	25	37.2
		8th day	41.5	58.2	41.2	24.8	62.1	68.1	30.2	36.9	36.2	39.2
		12th day	59.2	64.1	68.2	58.4	62.8	68.2	40.1	47.2	67.2	44.2
12.	Arginine	4th day	15.2	27.9	12.3	24.1	26.2	52.1	80.1	20.1	17.1	19.2
		8th day	24.8	35.3	24.8	33	69.8	72.9	85.3	36.2	25	28.4
		12th day	55.2	46	31.5	41.2	70.2	77.8	68.1	67.1	32.1	31.1
13.	L-Tyrosine	4th day	12.3	21.2	20.1	27.2	32.1	29.2	30.2	14.2	1`7.2	35.8
		8th day	21.2	27.3	37.9	35.2	56.2	74.2	40.1	15.2	26	39.2
		12th day	40.1	56.2	52.2	50.2	50.1	50.2	42.1	33.1	51.2	48.1

Graph 1. Showing the effect of nitrogen sources on the growth of different species of *Curvularia, Fusarium, Phoma and Botryodiplodia*



It reveals from above table and figures that relatively good growth of species of Curvularia Fusarium, Phoma and Botryodiplodia in sodium nitrate, potassium nitrate and ammonium nitrate where as ammonium sulphate and urea induced moderate growth and there was no consistency regarding the growth of present fungi on amino acids.

Amongst the nitrates, potassium nitrate supported excellent growth of species of *curvularia lunata, C.clavata, C.prasadii,* good in *C.senegalensis*, *P.vulgaris*, moderate in *F.equiseti*, *F.oxysposodium* nitrate and ammonium nitrate showed good to moderate growth in *C.lunata, C.Senegalensis, C.prasadii, F.equiseti, F.moniliforme, F.oxysporium, P.nebulosa, P.vulgaris, B.theobromae* except *C.clavata* which showed excellent growth in ammonium nitrate.

Ammonium sulphate induced moderate to poor growth of most of the species of *Curvularia, Fusarium, Phoma* and *Botryodiplodia, C.Prasadii F.moniliforme, F.oxysporum, P.Vulgaris* showed moderate growth while *C. lunata, C.senegalensis, C.clavata, F.equiseti, P.nebulosa, B.theobromae* showed poor growth.

Amongst the organic nitrogen sources, urea showed moderate to poor growth in most of the species moderate growth in C.senegalensis,C.clavata, C.prasadii, F.equiseti, F.moniliformae, F.oxysporum and poor growth was observed in *C.lunata, P.nebulosa, P.vulgaris, B.theobromae.*

DL-alanine induced good growth in *C.senegalensis C.clavata, C.prasadii F.moniliformae, F.oxysporum, P.nebulosa, P.vulgaris, B.theobromae* while moderate in *C.lunata, F.equiseti.*

L-asparagine was observed as an excellent growth in *P.neblosa* good in *C.clavata, P.vulgaris , B.theobromae* and moderate in *C.lunata C.senegalensis , C.prasadii , F.moniliforme , F.oxysporum.* Glycine showed good growth in, *C.clavata , F.moniliforme F.oxysporum , P.nebulosa,poor in C.lunata, C.senegalensis , C.prasadii, F.equiseti , P.vulgaris, B.theobromae.*

DL- leucine and L-cystine induced good to moderate growth of different species of *Curvularia Fusarium, Phoma* and Botryodiplodia. DL-valine acted as moderate to poor source for the growth of majority of the species. In *C.lunata, C.senegalensis, C.clavata, C.prasadii, F.equiseti, F.moniliforme, P.vulgaris* it showed moderate growth while in *F.oxysporum, P.nebulosa, B.theobromae* it showed poor growth.

Moderate to poor mycelial growth was observed on ariginine in majority of the species. It induces moderate growth *C.lunata, C.equiseti, F.oxysporum, F.moniliforme, P.Nebulosa* and poor growth of *C.Senegalensis, C. clavata, C. Prasadii, P.vulgaris, B.theobromae.* L.Tyrosine induced moderate to poor growth in majority of the species. In *C.senegalensis, C. Clavata, C.prasadii, F.equiseti, F.Moniliforme, P.vulgaris* and poor in *C.lunata, F.oxysporum, P.nebulosa, B.theobromae.*

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