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Comparative study of four isolates of *Aspergillus niger* causing collar rot of groundnut

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Four natural isolates namely S_1, S_2, S_3 and S_4 of *Aspergillus niger*, the collar rot and seed rot pathogen of groundnut were isolated from four different agro-climatic zones of West Bengal. All the isolates are pathogenic to groundnut cv. JL-24 though degrees of pathogenecity are at different levels. *Aspergillus niger* population was present in soils of different zones. All the isolates are tolerant to copper oxychloride (Blitox) and dithiocarbamate (Dithane M) fungicides and non tolerant to mercury (Emisan) and carbandazim (Bavistin) among the fungicides tested.

Key Words : Collar rot, Groundnut, *Aspergillus niger*, Isolates, Comparative characters

INTRODUCTION

A major oil seed crop in India like groundnut suffers with seed rotting in storage and collar rotting in the field by a number of mycoflora such as *Aspergillus niger*, *Sclerotium rolfsii* and *Penicillium* spp. of which *Aspergillus niger* is most important. *A. niger* is a facultative parasite, remains in soil and on seeds. Seed infestation can be minimized by seed dressing with different fungicides like Emisan, Blitox, Dithane or Bavistin. But *Aspergillus* for its saprophytic nature it can not be eliminated and thus it would infect groundnut seeds in field resulting into poor seedling stand.

Keeping in view, this investigation was under taken to study the comparative characters of different isolates of *Aspergillus niger*, isolated from soils of four agro-climatic zones of West Bengal.

MATERIALS AND METHODS

Collection and analysis of soil samples

To have natural isolates of *A. niger*, soil samples were collected from four agro-climatic zones of West Bengal namely red lateritic soil of Birbhum, clay soil of Midnapur, sandy loam of Coochbehar and gangetic alluvial soil of Nadia districts. Soil samples were collected from cultivated fields following usual method of soil sampling and different physicochemical properties were analysed.

Physical properties of soil were determined by field method. Among the chemical properties, organic matter content was estimated by the method of Jackson (1967), total nitrogen by modified Kjeldahl method and soil pH determined by using glass Electrode pH meter with the ratio of 1:2.5 soil:water suspension.

Isolation of soil fungi

Aspergillus niger was isolated following dilution plate technique (Clerk, 1965)from different soil samples collected from Birbhum,Nadia, Midnapur and Coochbehar districts. For this purpose potato dextrose agar medium was used. The fungus was identified by cultural characteristics and microscopical studies following Gilman (1967). The fungus, *A. niger* was isolated from different soil samples and was marked S1, S2, S3 and S4 to Birbhum, Nadia, Midnapur and Coochbehar districts, respectively.

Cultural characteristics

All the isolates were grown in Petriplates (10 cm diam) containing potato dextrose agar medium. After inoculation, the Petriplates were incubated for 7 days in B. O. D. incubator at 27°±1°C. The criteria for morphological and microscopical studies were taken into account following Sundas and Raj (1988).

Radial growth

The four isolates of *A. niger* isolated from different soil samples of West Bengal were grown in potato dextrose agar medium. Petriplates (10 cm diameter) were used for this purpose. The plates were inoculated with a disk of freshly prepared culture at the centre and incubated in a B. O. D. incubator at 27 C±1°C for 4 days. Radial growth of different isolates in the Petriplates was measured using millimeter scale. In all the cases three replications were taken.

Data were statistically analysed using factorial experiment performed in randomised block design.

Dry weight of fungal biomass

A disk (5 mm diam.) of pure culture was introduced into 250 ml conical flask containing PD broth. The pH of the medium was adjusted at 6.0. The inoculated flasks were incubated in a B.O.D. incubator at 27°C ± 1°C for 7 days. After 7 days' growth the biomass were harvested and blot dried. The operation was repeated thrice. Previously oven dried (60°C) filter paper, was weighed and the biomass was kept in the filter paper and were dried in low temperature oven at 70°C for 48 hours to ensure total removal of moisture content in it. Dry weight of biomass was taken by subtracting the weight of oven dried blotting paper. Dry weight of biomass was measured in milligram. The data were statistically analysed using completely randomised design.

Pathogenecity test of isolates

For this purpose groundnut cultivar JL-24 was used, collected from Department of Agronomy, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Kalyani.

Inoculation of seeds

The surface sterilized seeds (with 0.1 percent mercuric chloride aquous solution for 2 minutes) cv. JL-24 were mixed up with 5 days' old cultures grown in potato dextrose agar medium in Petriplates. One single plate containing medium and culture of *A. niger* isolate was used for inoculation of 100 groundnut kernels. Of which twenty five inoculated seeds were sown in each earthen pot (25 cm diam.). Three replications were taken and one pot was kept as control (sown with non-inoculated seeds) for comparison. Watering was done regularly. Observation was made from 7th day after germination of seeds and continued upto 14th day. Isolation of pathogen was done from rotten seeds and seedling infection at collar region. The fungus was isolated in pure form in PDA medium for comparison with parental isolates.

Seedling inoculation

In this case, groundnut cv. JL-24 seeds were surface sterilized and sown in earthern flats (25 cm diam.) and were allowed to grow for 7 days. Watering was

done regularly. Twenty five seeds were sown in each flat. After 7 days of germination of seeds, a five day old culture of *Aspergillus niger* grown in sand maize meal medium in conical flask (250 ml) was used for each flat. Inocula were put around the seedlings and covered with soils. The seedlings got infected by the pathogen by 3rd day. The fungus was re-isolated from infected seedlings and compared with parental isolates. Data regarding seed and seedling infection were statistically analysed using randomised design.

Fungicide tolerance test

Four isolates namely S1, S2, S3 and S4 may have some tolerance towards widely used fungicides like Emisan 6 (Methoxy ethyl mercury chloride, manufactured by Excel Industries Limited), Dithane M45 (Zinc + Manganous ethylene bis dithiocarbamate, maufactured by Indofil Chemical Limited), Blitox 50 (Copper Oxychloride, manufactured by Rallis India Ltd.), and Bavistin 50 W.P. (Methyl-2 benzimidazole carbomate, manufactured by BASF India Limited). Concentration used for different fungicides were for Dithane M 45 at 0.2 percent and 0.1 percent, for Blitox 50 at 0.6 percent and 0.4 percent for Bavistin 50 at 0.1 percent and 0.05 percent and for Emisan 6 at 0.1 percent and 0.05 percent.

In each case, three replications were taken. One control plot (without fungicide) was maintained for comparison.

The tolerance test of the isolates to fungicides was done employing poisoned food technique in Czapek's Dox agar medium following Sundas and Raj (1988).

RESULTS AND DISCUSSION

The data in Table 1 revealed that red lateritic Birbhum soil contains low C : N ratio, high organic matter, high organic carbon, acidic in nature. Sandy loam of Coochbehar soil contains high C : N ratio, high organic matter, organic carbon, and acidic in nature. Heavy clay soil of Midnapur district contains high C : N ratio, low organic matter, low organic carbon and neutral soil while Gangetic alluvial of Nadia district contains low C : N ratio, very low organic matter and organic carbon and nearly neutral in nature. All soil samples were collected from cultivated fields.

The data in Table 2 showed that in all the soil samples, irrespective of C : N ratio, organic matter and organic carbon content, acidic to neutral soil reaction, *Aspergillus niger* was present. Among the four different soil samples collected from four different agro-climatic zones of West Bengal, heavy clay soil of Midnapur contains less number of mycoflora than red late ritic soil of Birbhum,

Table 1. Physical and chemical properties of soil samples

Soil Samples	Colour	Texture	Structure	pH	Organic matter	Organic carbon	C:N Ratio
Red lateritic soil(Birbhum)	Brick Red	Loam (Medium)	Prismatic columnar	6.15	1.263	0.733	8.14 : 1
Sandy loam soil (Cooch Behar)	Ashy	Sandy loam (Light)	Highly porous crumb	6.35	1.206	0.700	11 : 1
Clay soil (Midnapur)	Blackish	Clay (Heavy)	Blocky	7.15	1.092	0.634	10.15 : 1
Gangetic Alluvial soil (Nadia)	Dirty white	Light Sandy loam (Medium)	Spherical granular	7.30	0.944	0.548	8.6 : 1

Table 2. Mycoflora isolated from different soil samples

Birbhum	Coochbehar	Midnapur	Nadia
Rhizopus stolonifer	*Aspergillus niger*	*Rhizopus sp.*	*Rhizopus stolonifer*
Acremonium sp.	*A. flavus*	*Aspergillus* sp.	*Aspergillus* sp.
Aspergillus niger	*Aspergillus* sp.	*A. niger*	*A. niger*
A. flavus	*R. stolonifer*	*Alternaria alternata*	*A. flavus*
Aspergillus sp.	*Penicillium* sp.	*Penicillium* sp.	*Verticillium* sp.
Penicillium sp.	*Curvularia* sp.	Unidentified group	*Fusarium* sp.
Verticillium sp.	*Pythium* sp.		*Penicillium* sp.
Fusarium sp.	Unidentified group		*Trichoderma viridi*
Unidentified group			Unidentified group

sandy loam of Coochbehar and gangetis alluvial of Nadia, Results are similar with that of Upadhya and Rai (1979).

It was noted that *Aspergillus niger* isolated from soil samples collected from different agro-climatic zones are pathogenic to groundunt cultivar JL-24 but degrees of pathogenecity are at different levels.

From Table 3, it was noted that isolate S1 (isolated from Birbhum soil) morphologically regular in growth, smooth, thick, light brown colony, grown evenly on the substratum, length of the conidiophore is short. Isolate S2 (isolated from Nadia soil) has also regular growth with smooth, coppery coloured colony, grown sparsely on the substratum, zonation on the substratum is absent. Length of the conidiophore is medium. Isolate S 3 (isolated from Midnapur soil) has regular growth, smooth with slightly dispersed carbon black in colour, grown thickly on the substratum. The length of conidiophore is moderately long and

Isolate S 4 (isolated from Coochbehar soil) has regular in growth, rough, highly dispersed, deep black colour colony, grown thinly on the substratum. The length of the conidiophore is too long.

Table 3. Morphological characters of different isolates of *A. niger*

Characters	Isolates			
	S_1	S_2	S_3	S_4
Growth pattern	Regular	Regular	Regular	Regular
Texture of colony	Smooth thick	Smooth	Smooth & slightly dispersed	Rough & highly dispersed
Colour of Colony	Light brown	Coppery	Carbon black	Deep black
Nature of conidition	Thick and evenly distributed	Sparsely arranged	Thick	Thinly arranged
Zonation	Absent	Absent	Present	Present
Length of Conidiophore	Shortest*	Medium	Medium long	Longest
Colour of the Substratum	Unchanged	Unchanged	Light brownish red	Unchanged

*Length of conidiophore was so long as it was not possible to measure with the help of occular micrometer under general microscope

From Table 4 and Table 5, it was observed that isolate S 2 had maximum redial growth (45.0 mm) in 96 hours and maximum dry matter content of the mycelia (621.0 mg) after 7 days' growth than S1 (36.1 mm) and (589.1mg), S3 (34 3 mm) and (580.0 mg) and S4 (32.3 mm) (565.0 mg). Data revealed that pathogenecity of these isolates may be graded as S2>S1>S3>S4 depending upon their biomass production.

Table 4. Radial growth of different isolates of *A. niger* (average measurement in millimeter scale)

Isolates	Time (h)						
	24	48	72	96	F. value	SEm	C.D.(5%)
S_1	7.3*	18.0	29.5	36 1			
S_2	10.3	21.5	36.8	45.0	S	±0.4736	1.372
S_3	8.7	17.8	28.3	34.3			
S_4	8.7	18.5	29.1	32.3			

*Data are average of 3 replications

Table 5. Biomass production by different isolates of *A. niger* (average weight in milligrams) after 7 days' growth

Isolates	Weight (mg.)	F. Value	S. Em	CD at 5%
S_1	589.0			
S_2	621.0	NS	±45.73	205.66
S_3	580.0			
S_4	565.0			

Pathogenecity test :

From Tables 6 and 7, it is clear that all the isolates of *A. niger* were pathogenic in nature. Degree of pathogenecity was statistically different among the isolates. Different pathogenic isolates isolated from infected seedlings and infected seeds were similar in all respects of cultural and morphological characters of the parental. They may be graded as S2, S1, *S3*, *S4*.

Table 6. Percent seed rotting and seedling infection by 4 isolates of *A. niger* on groundnut cv. *JL-24* (100 seeds sown in flats)

Isolates	Seed infection	*Percent infection to host*	
		Seedling infection	Viable seedlings
S_1	35.0	35.0	50.0
S_2	35.0	32.0	33.0
S_3	25.0	7.0	68.0
S_4	20.0	0.0	80.0
Control	2 0	1.0	97.0

Seed and Seedling inoculation

Table 7. Percent seedling infection by 4 isolates of *A. niger* (data are averages of 5 replication)

Observation on seedling infection	Isolates				
	S_1	S_2	S_3	S_4	Control
Death of seedlings	30.4	40 0	14.2	12.0	3.4
Number of unhealthy seedlings	1.4	1.2	1.8	2.4	0.0
Number of healthy seedlings	68.2	58.8	84 0	85.6	96.6

Tolerance to different fungicides

Four natural isolates of *A. niger* isolated from different soil samples of West Bengal were tested against four different fungicides namely Blitox 50, Dithane M-45, Bavistin 50 WP and Emisan 6 at two different doses.

Table 8. Tolerance to different fungicides of four isolates of *A. niger* (radial growth in millimeter)

Isolate	Fungicides	Time in hours							
		24		48		72		96	
		1	2	1	2	1	2	1	2
S_1	Blitox 50	6.0	4.0	9.0	7.0	10.0	9.5	11.0	10.5
	Dithane M45	—							
	Bavistin 50	—							
	Emisan 6	—							
	Control		8.5		19.0		27.5		35.5
S_2	Blitox 50	3.3	3.0	8.1	5.2	14.1	9 5	12.8	11.0
	Dithane M45	—							
	Bavistin 50	—							
	Emisan 6	—							
	Control	8.5			20.5		32.5		42 0
S_3	Blitox 50	5.0	3.5	9.1	6.5	12.0	10.0	15.0	10.0
	Dithane M45	3.0	—	9.0	—	14.5	—	21.5	—
	Bavistin 50	—							
	Emisan 6	—							
	Control	8.0		17.5		25.5		35.0	
S_4	Blitox 50	5.5	5.0	9.0	7.5	12.0	9.5	15.0	10 0
	Dithane M45	4.0	—	8.5	—	14.0	—	22 5	—
	Bavistin 50	—							
	Emisan 6	—							
	Control	—	9.5		20.0		27.0		32.5

Blitox 1=0.4%, 2=0.6% ; Dithane 1=0.1%, 2=0.2% ; Bavistin 1=0.05%, 2=0.1%
Emizan 1=0.05%, 2=0.1%

Data are averages of 3 replication

From Table 8, it was noted that four isolates of *A. niger* namely S1, S2, S3 and S4, the collar rot and seed rotting pathogen of groundnut cultivar showed different degrees of tolerance towords different fungicides normally used as seed

protectants and for prophylaxis measures against diseases. All those isolates showed no tolerance towards Bavistin and Emisan. Where as isolates had some tolerance to Blitox in both the concentrations (0.4 and 0.6 percent) and Dithane M-45 had only at lower concentration (0.1 percent) in *in vitro* condition when compared with control treatment. From the observations, it may be suggested that groundnut seeds should be treated with mercury fungicide (Emisan) or by carbendazim (Bavistin) before sowing to avoid loss due to seed rot and collar rot. *In vitro* studies i.e. bio assay of fungicides revealed that mercury and carbendazim are toxic to *Aspergillus niger*. The observation is similar with that of Kumar *et al.* (1986), Shekhwat *et al.* (1986) and Sundas and Raj (1988).

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