

PATHOGENICITY OF FUNGICIDE TOLERANT ISOLATES OF *ASPERGILLUS NIGER* VAN TEIGHEM ON GROUNDNUT (*ARACHIS HYPOGAEA* L.) SEEDLINGS

BY

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An isolate of *Aspergillus niger* adapted to 3 fungicides namely Blitox 50 (6000 ppm), Dithane M₄₅ (6000 ppm) and Emisan_G (450 ppm) inoculated with ground nut seedlings (var J.L. 24) to evaluate the pathogenicity. It revealed that fungicide adapted isolates had lesser pathogenicity than parental one with regards to root and shoot length and total mortality. Pre-emergence and post emergence collar rot of ground nut seedlings were also found in lower percentage.

INTRODUCTION

Fungicide resistance by plant pathogenic fungi has mainly been studied in the laboratory and comparatively little information is available as to its significance in the field. There are, however, a few reports of resistant strains of fungi being developed in fields where a particular fungicide has been used continuously for several years. Resistance of *Physalospora obtusa* in apple orchards to copper fungicides (Taylor, 1953), of *Phytophthora infestans* in potato fields (Horsfall, 1956) to Bordeaux mixture, and of *Venturia inaequalis* in apple orchards to Dodine (Szkolnik and Gilpatric, 1970) are some examples of development of fungicide tolerant strains in the field due to continuous application of a particular fungicide.

Studies were, therefore, undertaken to know the pathogenicity of an isolate of *Aspergillus niger*, collar rot pathogen of groundnut adapted to three fungicides with respect to seedling emergence, collar rot development, root and shoot growth of ground nut seedlings.

MATERIALS AND METHODS

Pure culture of *Aspergillus niger* was obtained from single hyphal tip isolation on PDA medium and incubated at $27^{\circ}\text{C} \pm 1^{\circ}\text{C}$ in a B.O.D. incubator for 8 days. The pathogen was trained in Blitox₅₀ and Dithane M₄₅ starting from 50 ppm to 6000 ppm and Emisan₆ from 20 ppm to 450 ppm by repeated transfers in increased concentrations of fungicides amended PDA medium.

Three fungicides used in the present study, were Dathane M₄₅ (Zinc + Manganous ethylene bis-dithiocarbamate (75%) of Indofil Chemicals Ltd., Blitox₅₀ (50% Copper oxychloride) of Rallis India Ltd., and Emisan₆ (Methoxy ethyl Mercury chloride) of Excel Industries Ltd.

Groundnut seeds of spreading type variety J.L.24 was used as test host. Seeds were collected from Department of Agronomy, Faculty of Agriculture, B.C.K.V., Kalyani.

For studying the pathogenicity, the isolates of test fungus *A. niger* showing adaptability to the highest doses of different fungicides were used. The fungicide free isolate (parental) was used as control.

Freshly collected seeds of groundnut variety J.L.24 washed with tap water and finally with sterile distilled water. Thirty seeds were mixed with the 8-days-old culture of fungicide adapted isolate for each treatment. Seeds and the fungal cultures were thoroughly mixed in such a way so as to have uniform inoculum in each case. Parental isolate was used as control. For this purpose, freshly collected seeds were inoculated with 6000 ppm of Blitox₅₀, 6000 ppm of Dithane M₄₅ and 450 ppm of Emisan₆ adapted isolates of *A. niger*. The inoculated seeds were sown in the sterilized sand in 30 cm earthen pots with 3 replications in each case. The seeds were sown at 2.0-2.5 cm below the sand surface. Sand was sterilized at 20 lbs pressure for 20 minutes in autoclave. Pots were then kept in full sunshine. Watering was done regularly to maintain the proper moisture level for germination and plant growth. After 10 days, 20 days and 30 days from the date of sowing, germination of seeds, emergence of seedlings and plant growth were observed. The length of roots and shoots were measured in centimeter scale.

RESULTS AND DISCUSSIONS

Pathogenicity of fungicide tolerant isolates of *A. niger* on groundnut seedlings was studied.

From Table 1, it was observed that the highest number of seedling emergence was noticed when the seeds were inoculated with Emisan₆ tolerant isolate of the fungus (94.44 percent) while in other fungicide tolerant isolates the emergence of seedlings were 90.00 percent in case of Dithane M₄₅ and 87.77 percent in case

Table 1. Pathogenicity of fungicide adopted isolates and parental isolates (Data are averages of 3 replications)

Treatments	Percentage of			
	Emergence	Pre-emergence mortality	Post emergence mortality	Total mortality
Parental isolate (Control)	77.77 (61.82)	22.22 (28.11)	7.77 (16.11)	29.99 (33.15)
Dithaine M ₄₅ (6000 ppm)	90.00 (71.57)	10.00 (18.43)	4.44 (12.11)	14.44 (22.20)
Emisan ₆ (450 ppm)	94.44 (76.31)	5.55 (13.56)	3.33 (10.47)	8.88 (17.26)
Blitox (6000 ppm)	87.77 (69.47)	12.22 (20.44)	5.55 (13.56)	17.77 (24.88)

Figures in the () brackets are Angular Transformed values.

of Blitox₅₀. In case of seeds inoculated with fungicide untreated isolate, it showed lowest percent of seedlings emergence (77.77 percent). From the observation it is clear that fungicide tolerant isolates have less pathogenicity as compared to fungicide untreated (parental) isolate of *Aspergillus niger*. Similar trend was found in total mortality percentage. In case of Emisan₆ tolerant isolate the total mortality of seedlings was noted less (8.88 percent) as compared to fungicide untreated culture inoculated seeds (29.99 percent). In case of Dithane M₄₅ tolerant isolate and Blitox₅₀ tolerant isolate, the total mortality were 14.44 and 17.77 percent respectively. From the table, it was also clear that Emisan₆ tolerant isolate showed less pathogenicity than that of Dithane M₄₅ tolerant isolates. It was also noted that pre-emergence damping off was highest and maximum mortality was observed at the early stage of the seedling growth. Similar results were obtained by Horsfall (1956), Jones and Walker (1976), and Siddaramaiah *et al.* (1981).

The experiment was designed to evaluate the effect of fungicide tolerant isolates of *A. niger* on different ages of seedlings over control. The data regarding root and shoot lengths were statistically analysed using "Split plot design" and the interactions between root and shoot of groundnut seedlings were taken as, criteria for the evaluation of effect of fungicide tolerant isolates, and the data are presented in Table 2.

From the data (Table 2) it was observed that the length of roots were significantly shorter (9.06) in case of seedlings arose out of the seeds inoculated with control (parental) culture in comparison to fungicide tolerant isolates of *A. niger*. Interaction between age of seedlings and the pathogenicity of fungicide tolerant isolates were insignificant on root growth. Thus it may be concluded that the

Table 2. Effect of fungicides tolerant isolates of *A. niger* on root and shoot growth of groundnut seedlings and their interactions (data are averages of 3 replications and of ten random sampling)

Treatments	Observations (days after emergence)							
	Root length (cm)				Shoot length (cm)			
	10	20	30	Mean(F)	10	20	30	Mean(F)
Control (Parantal)	6.02	10.20	10.96	9.06	19.06	24.93	27.97	23.98
Dithane M ₄₅ (6000ppm)	6.13	10.73	12.12	9.66	17.79	29.32	31.43	26.18
Emisan ₆ (450 ppm)	7.81	13.3	14.74	11.96	17.03	28.89	31.65	28.85
Blitox ₅₀ (6000 ppm)	6.27	11.00	12.33	9.86	17.33	28.93	30.64	25.63
Mean (d)	6.55	11.31	12.53	10.13	17.80	28.02	30.42	25.41
	(d)	(F)	(d x F)	(d)	(F)	(d x F)		
C.D. at 5%	0.6656	0.5825	N.S.	C.D at 5%	1.4686	0.9449	1.6711	
N.S. Non Significant.								

adapted isolates had lesser virulence as the seedlings attained maturity. It was also the same in case of fungicide free isolate of *A. niger*

In case of shoot growth, it was observed that the length of the shoot was longest when the seeds were inoculated with Dithane M₄₅ isolates (26.18) while in other cases it was 25.03 (Blitox₅₀ adapted isolate) and 25.85 (Emisan₆ adapted isolate) while in case of fungicide free isolate (parental) it was 23.98. It revealed that fungicide tolerant isolates had less virulence than that of fungicide free isolate (parental) of *A. niger*.

Interaction between ages of the seedlings and shoot lengths was found to be significant but fungicide adapted isolates were found to be less virulent when the plants attained maturity. Similar results were obtained by Dublsh and Pande(1976).

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