Table 1 *Per cent mycorrhizal infection in roots (and scale-like leaves) of two cultivars of Canna indica L*

Cultivars	No of segment/leaves Examined	+ ve for VAM infection.	Infection %
Cultivar-1 (green leaved)	108 (108)	81 (76)	75 (70)
Cultivar-2 (brown leaved)	108 (108)	79 (74)	73 (68)

The values within parantheses are for scale-like leaves and those outside parantheses are for roots.

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INTERACTION OF DIFFERENT SEED-BORNE FUNGI OF PEARL MILLET (*PENNISETUM AMERICANUM*) AND ITS EFFECT ON PATULIN PRODUCTION BY *ASPERGILLUS TERREUS*

S. GIRISHAM and S. M. REDDY
Department of Botany, Kakatiya University, Warangal 506 009, India.

THE interaction of different seed-borne fungi of pearl millet (*Pennisetum americanum*) and its effect on patulin production by *Aspergillus terreus* were studied. *Aspergillus parasiticus*, *A. niger*, *Curvularia brachyspora* and *Fusarium solani* were responsible for complete suppression of patulin production by *A. terreus*, while *A. ustus*, *A. deflectus* and *Drechslera rostrata* allowed *A. terreus* to produce patulin only in traces. *Aurobasidium pullulans* and *Trichoderma viride* which acted as strong inhibitors also suppressed patulin production. In general production of patulin by *A. terreus* decreased significantly in the presence of other fungi. Probably this may be the reason for the low incidence of patulin in nature.

Recently studies have been reported[1,2] on varied relation among the seed-borne fungi of maize and sesamum respectively and the possible control of aflatoxin production by some of these biocides. *Rhizopus oligosporus* is reported to inhibit the aflatoxin production *A. flavus* and *A. parasiticus*[3]. Hence, it was considered worthwhile to investigate the nature of interaction between *A. terreus* and spermosphere mycoflora of pearl millet and its impact on patulin production by *A. terreus*.

The relation between different seed-borne fungi of pearl millet (*Penisetum americanum*) and *A. terreus* was established by inoculating the buffered 2% malt extract agar (pH 6.0) with 25 different fungal species by pairing them separately with *A. terreus*. Each pair was inoculated over the agar surface maintaining equidistance to all test pairs. The plates were incubated at 27–29°C for 7 d. At the end of the incubation period the diameter of each fungus and relation between the two fungi was recorded. The relation between the two fungi was categorized into one of the following, as suggested by Johnson and Curl[4]. A—Mutual intermingling of the two organisms; B—Mutual inhibition on contact, the space between the two colonies is small, but clearly marked; C—Mutual inhibition at a distance; D—Inhibition of one organism on contact, the antagonist continues to grow, unchanged or at a reduced rate, through the colony of the inhibited

Table 1 *Interaction of different seed-borne fungi of pearl millet and its effect on patulin production by A. terreus*

Name of the antagonist	Final pH	Type of interaction	Production of patulin (in ppb)
Acremonium terricola	7.1	A	30
Aurobasidium pullulans	7.9	E	18
Aspergillus parasiticus	7.4	D	nil
A. niger	3.0	C	nil
A. deflectus	8.4	D	Traces
A. ustus	7.0	C	Traces
Corynespora cassiicola	7.8	B	18
Curvularia brachyspora	8.2	D	nil
C. ergrostidis	8.2	B	42
C. lunata	8.6	B	24
C. geniculata	8.1	D	42
Drechslera rostrata	7.8	D	Traces
Fusarium solani	6.0	D	nil
Microascus triganospora	5.5	A	35
Myrothecium roridum	7.2	B	nil
Paecilomyces lilacinus	5.0	C	5
P. varioti	7.4	A	11
Penicillium citrinum	7.4	C	60
Phoma sp	7.3	D	70
Syncephalastrum racemosum	7.9	A	42
Thielavia terricola	7.0	A	40
Trichoderma viride	6.8	E	6
Trichothecium roseum	8.2	A	35
Ulocladium botrytis	8.2	D	24
Humicola grisea	9.2	D	60
Control	6.0	—	95

organism; E—Inhibition of organism at a distance, the antagonist continues to grow through the resulting clear zone at an unchanged or reduced rate.

A. terreus was grown along with one of the seed-borne fungi in 50 ml of minimal liquid medium (glucose 40 g, $NaNO_3$ 2 g, KCl 0.520 g, $MgSO_4.7H_2O$ 0.520 g, KH_2PO_4 1.520 g, $FeSO_4$ 0.01 g and distilled water 1 litre pH 6.5) contained in 250 ml Erlenmayer conical flasks. They were harvested after 15 d of incubation at 27–29°C. The patulin was extracted from culture filtrate with equal volume of ethyl acetate and estimated as suggested by Subramanian[5]. The extract was reduced to 1 ml by flash evaporation and separated by TLC. The ethyl acetate extract (0.3 ml) was spotted on TLC and developed in toluene: ethyl acetate: formic acid (5:4:1) and sprayed with phenyl hydrazine hydrochloride solution. The yellow colour spot was eluted with 5 ml of *n*-butanol and the intensity of the colour was read at 540 nm.

Trichoderma viride and *Aurobasidium pullulans* were found to be strong inhibitors of *A. terreus*, while *Microascus triganosporus*, *Paecilomyces varioti*, *Syncephalastrum racemosum*, *Trichothecium roseum*, *Acremonium terricola* and *Thielavia terricola* failed to influence the growth of *A. terreus* (table 1). The rest of the fungi influenced the growth of *A. terreus* to some extent.

The presence of different seed-borne fungi of pearl millet significantly affected the patulin production by *A. terreus*. *Aspergillus parasiticus*, *A. niger*, *Curvularia brachyspora*, *Fusarium solani* and *Myrothecium roridum* completely checked the patulin production by *A. terreus*. *A. terreus* produced patulin in trace amounts in the presence of *A. deflectus*, *A. ustus*, *Drechslera rostrata* and *Trichoderma viride*. *Phoma* sp., *Penicillium citrinum*, *Paecilomyces lilacinus* and *Humicola grisea* failed to affect the production of patulin.

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INHIBITION OF POLYAMINE METABOLISM INDUCES AMOEBIC ENCYSTATION

SANJIV GUPTA, D. K. SRIVASTAVA* and O. P. SHUKLA

Division of Biochemistry, Central Drug Research Institute, Lucknow 226 001, India.

**Department of Biochemistry, College of Basic Sciences and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar 263 145, India.*

ACANTHAMOEBA CULBERTSONI, the pathogenic free-living amoeba causes fatal meningoencephalitis in experimental animals as well as in man[1,2]. This organism has served as a model for differentiation of a unicellular eukaryotic cell[1,3]. This amoeba encysts readily in non-nutrient media containing magnesium chloride in combination with taurine[4], biogenic amines[5], inorganic ions[2] and other organic effectors[6,7]. An intermediary role of cAMP in encystation of this amoeba seems likely[3] and the system is also regulated by catabolite repression[8].

Aliphatic polyamines viz putrescine, spermidine and spermine are ubiquitously distributed in organisms[9,10], and regulate the biosynthesis of RNA/DNA, cell growth, differentiation, and stability of macromolecules and cellular organalles. Selective inhibition of polyamine metabolism of protozoal parasites has been identified as an alternative target for their chemotherapy. Polyamine metabolism is closely regulated during normal growth, differentiation, action of some hormones and altered regulation of polyamine biosynthesis has been observed during neoplastic growth[9,10]. Spermidine and putrescine have been identified as the major aliphatic amines in *A. culbertsoni*[11,12]. The present paper reports the changes in polyamine levels during encystation of this amoeba and the effect of certain inhibitors of polyamine metabolism on amoebic encystment.

An axenic culture of *A. culbertsoni* was grown[13] in a medium containing 2% peptone, 0.5% sodium chloride, 1 mg/100 ml thiamine and 0.5 μg/100 ml cyanocobalamine, pH adjusted[14] to 6.9–7.0. The required volume of the medium was distributed in one litre Erlenmeyer flasks, autoclaved at 15 lbs/in^2 pressure of steam for 20 min, and steamed after 24 hr to kill any germinated spores. Flasks were inoculated with 25 ml of 6-day-old static culture of *A. culbertsoni* having a cell density of 8×10^5 cells/ml, and incubated at 37 ± 2°C with shaking for four days. Amoebae were harvested aseptically by centrifugation (500 g, 10 min), washed and suspended in 0.9% saline. The basal encystment medium (NM) for testing the effect of different compounds contained 15 mM $MgCl_2$ and 86 mM NaCl (pH adjusted to 7.8). The medium (4.5 ml) was dispensed in 25 mm × 150 mm tubes and sterilized. Stock solutions of polyamines and inhibitors were sterilized by filtration through millipore filters (0.22 μm), and incorporated in the basal medium to the requisite concentration. Freshly harvested amoebae (0.5 ml) of desired cell density were inoculated in each tube, and the tubes incubated with shaking at 28 ± 2°C. Encystation was monitored in small aliquots removed from the tubes by counting the trophozoites, intermediate forms (single-walled cysts) and mature cysts (double-walled) using a haemocytometer. Viability of cells was examined by rapid eosine (0.125% w/v in water) staining. Eosine stained dead cells within few seconds while living ones remained unstained up to 15–20 min. All the operations during growth and encystment were conducted under aseptic conditions, and the sterility of media was checked at the end of experiment, by inoculating aliquots on nutrient agar slants.

Polyamines were characterized and quantitated by thin layer chromatography of their Dansyl derivates. Aliquots were removed at different incubation periods, cells counted using haemocytometer, sedimented by centrifugation and homogenized with 4% perchloric acid. The polyamines in the supernatant were converted to their Dansyl derivatives by the method of Fleischer and Russel[15] and separated on silica gel plates by ascending chromatography serially in following three solvents (a) benzene, cyclohexane, methanol (127:23:1.9 v/v) (b) cyclohexane, ethyl acetate (1:1 v/v) (c) cyclohexane; ethylacetate (3:2 v/v) as described earlier[16]. The spots were visualized by their fluorescence in UV light, scraped and suspended in 3 ml benzene, shaken in vortex mixer and centrifuged at 2000 rpm for 15 min. Fluorescence was measured in the supernatants using a spectrofluorimeter (Aminco