with four maize kernels/pot 2–3 days prior to inoculation. The pots contained a mixture of fine river sand and lateritic clay loam soil in equal proportion. The initiation of the primordia of basidiomata was observed in 5–7 days and fully mature fructifications developed after another 5 or 6 days; the process of production thus was completed in about 12 days in an ambient temperature regime of 25 to 30°C. The basidiomata thus produced agreed in all characters with those found on leaf lesions in nature except that they were more robust and had larger pilei and stipes.

The fungus under discussion was first recorded by Petch[2] from Sri Lanka on *Paspalum dilatatum* Poir as *Marasmius paspali* which was transferred to *Marasmiellus* by Singer[3]. The same fungus was found to be associated with an identical leaf disease of maize in Guinea[4] and in Sierra Leone[5].

Inability to determine the taxon at species level by Latterell and Rossi[1] possibly might have been due to non-availability of mature fructifications possessing basidiospores. In the Indian material, mature fructifications (figure 1) were spotted on maize leaves after considerable search and only on occasions following rainy spells in the field. In fact, as indicated earlier, most often no fructifications are seen on the leaf lesions; the etiology of the disease thus remains obscure. The problem was overcome by devising a method to induce basidiomata production at will from cultures on whole barley grains. This has helped in species determination and gaining information on its geographical distribution. It is clear that the pathogen is present not only in Central America but also in Asia and Africa. During a visit to Venezuela in 1977, one of the authors (MMP) observed the occurrence of this disease also in South America.

Latterell and Rossi[1] described the Marasmiellus disease under the Spanish name of 'borde blanco' (white border). The name alluded to the appearance and development of lesions near the margins of leaf blades. In their description of the symptoms, they did not emphasize the banded or zonate appearance of the lesions although they mention the presence of 'black elliptical rings' and 'fine black lines' in the lesions. It is these which delineate the zonate or banded character, a noteworthy aspect of symptomatology.

A comparison of symptoms of the Marasmiellus disease with the Banded leaf and sheath blight, alluded to earlier, will be instructive. In the latter disease the lesions and blotches show alternating purple or tan zones which result in the characteristic banded lesions[6]. In the Marasmiellus disease also a similar zonate or banded aspect of the lesions is apparent. The symptoms of the two diseases are shown juxtaposed in figure 2. On the left can be seen the symptoms of the Rhizoctonia disease (figure 2A) while on the right (figure 2B) those of the Marasmiellus disease are exhibited. An important difference can be visualized. The banding or zonations are oriented perpendicularly to the midrib or the long axis of the leaf in the Marasmiellus disease. Bands radiate more or less horizontally in the Rhizoctonia disease but they do so vertically in the new Marasmiellus disease.

The foregoing discussion suggests the need for a redesignation of the new disease by an additional or alternate common name—*Vertical banded blight* as opposed to *Horizontal banded blight* induced by *R. solani*. 'Borde blanco', though appropriate *per se*, fails to differentiate the disease caused by *Rhizoctonia solani*—a disease quite widespread in India and which occurs intermixed with the new disease. Indeed at one maize experimental station in India, the new disease caused by *Marasmiellus paspali* is confused with that caused by *R. solani*, and notes on incidence are recorded under the heading of the latter disease.

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1. Latterell, F. M. and Rossi, A. E., *Plant Disease*, 1984, **68**, 728.
2. Petch, T., *Trans. Br. Mycol. Soc.*, 1947, **31**, 19.
3. Singer, R., *Sydowia*, 1955, **9**, 367.
4. Kranz, J., *Sydowia*, 1965, **19**, 92.
5. Pegler, D. N., *Kew Bull.*, 1968, **21**, 499.
6. Ahuja, S. C. and Payak, M. M., *Phytoparasitica*, 1982, **10**, 41.

A NEW TECHNIQUE FOR BIOASSAY OF NATURAL PLANT PRODUCT

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UTILIZATION of natural plant products is assuming importance in combating various phytopathogenic

micro-organisms[1-5] due to their obvious advantages over hazardous synthetic pesticides. A simple technique of exposing the test extracts under germicidal UV lamp before mixing (for avoiding contamination) with the media and testing against the pathogen is described in the note.

Fresh leaves of *Aegle marmelos* Corr were washed with 5% ethanol and rinsed in sterile double distilled water thrice before drying in an oven to obtain 1 kg powder. One hundred grams of this powder were extracted (1:5 W/V) in double-distilled sterile water, ethanol (95%) hexane, benzene, chloroform and acetone in Erlenmeyer flasks separately and supernatants were decanted in other beakers. The extraction process continued until almost clear solvents were obtained. These extracts were reduced to 50 ml through rotary type vacuum pump flash evaporator (Toshniwal make). Aqueous extracts were evaporated and reduced to 50 ml on a water bath at 60°C. Sixteen ml were exposed under germicidal UV lamp (254 nm) for 30 min at a height of 25 cm. Another set of aqueous extract (16 ml) was mixed in 120 ml of PDA medium and sterilized at 1.05 kg/cm^2 pressure for 15 min. Two ml of all the other extracts were mixed with 15 ml of previously sterilized and melted PDA medium and poured in 9 cm dia sterilized Petri plates. However, the extracts sterilized along with media (17 ml) were poured directly in Petri plates.

Freshly isolated seven-day old virulent cultures of *Pyricularia oryzae* Cavara and *Drechslera oryzae* Subramanian and Jain and 4-day old culture of *Rhizoctonia solani* Kuhn were seeded separately using a 5 mm dia sterilized cork borer in all the treatments. Appropriate controls were maintained. In all, three replications were maintained and incubated at 25 ± 2°C during 1984 to 1985.

Observations were recorded on the 7th day for *P. oryzae* and *D. oryzae* and 4th day for *R. solani*. Data on radial growth were transformed into angular values and analyzed on computer model HCL 1800.

All the treatments except sterilized aqueous extracts were significantly superior in inhibiting growth thus demonstrating the potential of *A. marmelos* leaves to control rice blast pathogen *in vitro*. The compound appears to be thermolabile because it was inactivitated when autoclaved.

Table 1 *Effect of various extracts of Aegle marmelos leaves on the growth inhibition of three fungal pathogens of rice*

Treatments	Pathogens Radial growth in angular values		
	P. oryzae	*D. oryzae*	*R. solani*
Aqueous extract	26.2^{+++}	45.6^{+++}	64.0^{+++}
Germicidal UV-exposed aqueous extracts	22.9^{-}	41.2^{-}	63.1^{-}
Ethanolic extracts	27.0^{+}	64.3^{+}	60.4^{+}
Hexane fraction	47.3^{++}	48.7^{++}	68.0^{++}
Benzene fraction	14.3^{+}	50.8^{+}	63.7
Chloroform fraction	24.6^{+}	44.7^{+}	64.2
Acetone fraction	27.5^{++}	41.1^{+}	60.2^{+}
Sterilized extraction	90.0^{-}	90.0^{-}	90.0^{-}
Mean	27.1	48.1	63.4
Control	90.0	90.0	90.0
C. D. to compare interactions between treatments and pathogens		5%	1%
	P. oryzae	1.7	2.4
	D. oryzae	2.0	2.8
	R. solani	1.5	2.0

+++ = Severe bacterial contamination (>10 bacterial colonies); ++ = Moderate contamination (<10 bacterial colonies but >5); + = Slight contamination (<5 bacterial colonies); − = No contamination.

Inhibitory activity of *A. marmelos* aqueous leaf extract when exposed under UV was superior over the other treatments. Aqueous extract without either autoclaving or exposing under germicidal UV lamp showed equally good inhibition with those of chloroform, ethanolic and acetone extracts. However, none other than UV exposed aqueous extract treatment was free from bacterial contamination. Obviously bacterial contaminants were sterilized due to UV irradiation treatment. This is important in the present context so as to attribute a single factor (plant extract) as growth inhibitor.

Although, growth of *D. oryzae* and *R. solani* was not effectively inhibited as *P. oryzae*, there was no contamination in UV-exposed aqueous extracts. A similar technique can also be used to study inhibitory efficacy of other plants.

The data on efficacy of UV irradiated *A. marmelos* aqueous extract are given in table 1, where higher inhibition of *P. oryzae* growth free from bacterial contaminants was observed (22.9^{-}) than all the other except in benzene extracted treatment (14.31^{+}). Later treatment could not be considered ideal owing to its inability in preventing bacterial contamination. Development of bacterial colony (+, ++, +++) as recorded in various treatments shown in the table was thought to interfere in the growth of the test pathogen. Hence, in such cases it would be paradoxical to ascertain inhibitory activity due to plant extract, bacterial contaminants or synergistic effect of plant extract + bacterial contaminants. Since UV-irradiated plant extract treatment alone inhibited radial growth of *P. oryzae* free from bacterial contamination, it was selected for further studies.

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1. Allen, E. H., *Phytopathol. Z.*, 1970, **69**, 152.
2. Janardhan, K. K., Ganguly, D., Baruah, J. N. and Rao, P. R., *Curr. Sci.*, 1963, **32**, 227.
3. Tripathi, N. N., Dikshit, A., Tripathi, R. D. and Dixit, S. N., *Int. J. Crude Drug Res.*, 1983, **21**, 71.

CYTOTOXICITY OF METHYLISOCYANATE IN HUMAN LUNG EPITHELIAL CELL LINE (L-132)

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EXPOSURE of methylisocyanate (MIC) at Bhopal in December 1984 affecting large number of human and animal population prompted us to study the toxicity of MIC in a cell culture model. Different cell lines viz Hela, mammalian cell lines and human leukocytes have been used by various workers to study the cytotoxicity of chemical compounds[1–4]. However, to the best of our knowledge no such reports are available pertaining to the *in vitro* toxicity study with MIC. The present study was therefore undertaken to evaluate the toxicity of MIC on human embryonic lung epithelial cell line (L-132).

L-132 cell line was maintained in milk dilution bottles at 37°C using Eagles minimal essential media with non-essential aminoacids and Earle's salts (MEM) enriched with 7% fetal bovine serum and 3% glutamine. When the cell density of 2–3 million cells/bottle was attained they were distributed in 10×6 sets of Leighton tubes (L-tubes) containing coverslips and the tubes were then incubated at 37°C till the complete monolayers were obtained.

MIC was synthesized in the chemistry laboratory of this establishment. Ten-fold dilutions containing 3.0 to 0.0003 mg/ml of MIC were prepared in chilled MEM without serum and the pH was adjusted to 7.2. Media from all L-tubes was drained off and the tubes were rinsed twice with MEM containing no serum. Each dilution of MIC was inoculated in 0.2 ml aliquots on the monolayers and incubated for 1 hr at room temperature (25°C). A set of ten tubes was taken for each dilution. One set of 10 tubes was kept as control. After 1 hr, the tubes were rinsed with MEM without serum. They were then inoculated with serum-free MEM. The tubes were incubated at 37°C for 24–120 hr. Monolayers were observed at 24 hr intervals for cytopathic effects (CPE). Simultaneously cell viability was determined by Erythrocine B dye exclusion test. Monolayers were stained with giemsa and coverslips mounted on glass slides.

A dose-dependent CPE was observed as evident from table 1. At a concentration of 3 mg/ml of MIC, the cells showed feeble shrinkage of cell wall.