

TABLE 1

Effect of media on the sporulation of *Helminthosporium turcicum* incitant of leaf blight of maize

Medium containing extracts of	Number of spores ($\times 10^4$ spores/ml) of <i>H. turcicum</i>	
	Dharwar isolate	Hyderabad isolate
Maize	3.5	1.6
Sorghum	6.6	1.0
Barley	1.2	1.2
Wheat	0.8	0.6
Maize leaf	25.6	19.6
PDA (Check)	4.3	2.8

S.E. medium 0.46; S.E. isolate 0.165

C.D. medium at 1% 1.358; C.D. isolate at 1% 0.504

increasing the sporulation more than six times in Dharwar isolate and nearly ten times in Hyderabad isolate, over the conventional general purpose medium, Potato Dextrose Agar.

The results of this investigation lead us to the enormous possibility of inducing sporulation in defiant non-sporulating fungal pathogens on host extract media. These also lead us to the possible use of sporulation character to index the reaction of host cultivars to pathogens.

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INCREASED SUSCEPTIBILITY OF THIOACETAMIDE INJECTED MICE TO BACILLUS CALMETTE GUERIN

REETA SRIVASTAVA AND S. K. GUPTA
Division of Microbiology, Central Drug Research Institute, Lucknow 226 001, India.

BACILLUS Calmette Guerin (BCG) is normally avirulent strain in both man and animals. Alteration of its avirulence to virulence has been attempted in thioacetamide treated mice.

CDRI Swiss mice (36) weighing $ca 24 \pm 2$ g were divided into a thioacetamide treated group of 20 animals and an untreated control group of 16 animals. Thioacetamide was given 250 mg/kg subcutaneously and then both the groups were challenged intravenously with *M. bovis* BCG 3 mg. (moist weight) per mouse. Ten days after the challenge the thioacetamide treated group was given a further dose of 100 mg/kg thioacetamide subcutaneously. On the 40th day of infection, two mice from each of the two groups were killed with chloroform and portions of the spleens and livers were collected in sterile 1 inch petri dish for performing viable counts. The lung, portions of the liver and spleen were put in Zenker's fixative and 5 μ sections were cut and stained with haematoxylin eosin.

The mortality was observed daily in both groups of animals. The day of death of mice of each group was recorded and the mice were autopsied and the lung, liver, spleen were examined. Smears from the above mentioned organs were smeared on microscopic slides, fixed by heat and stained with Ziehl Neelson's stain for detection of acid fast bacilli.

Table 1 shows that all the mice of the thioacetamide treated group died with a mean survival time of 36.1 ± 0.76 days. The last mouse died on the 59th day of BCG injection. Whereas all but one mice of the

TABLE 1

Virulence of *M. bovis* BCG in Thioacetamide treated mice

	No. of mice	
	20	16
Thioacetamide treatment (I.S.C. injection)	250 mg/kg at 0 days infection plus 100 mg/kg 10 days after infection	Nil
No. of specific deaths	18	1
Mean survival time in days \pm S.E.	36.1 ± 0.76	> 81 days
No of survivors (Not taken)	0/18	13/14
Percentage survival	0	92.8
No. of c.f.u. per gm of tissue		
Liver	9×10^9	1.1×10^6
Spleen	3.6×10^8	3.6×10^5

control group were alive on the 81st day of infection. On this day the experiment was terminated and the thirteen mice alive were autopsied, for seeing the presence of tuberculosis lesion in the lung, liver and spleen. Minimal to no disease was seen in the lung, liver and spleen of these animals. On the 40th day of infection, 2 livers and 2 spleens of the thioacetamide treated and control group were homogenized in 0.05% Tween 80 in 0.85% saline and were diluted from $10^{-1} \times 10^{-10}$ and aliquots smeared onto tubes of Lowenstein Jensen's media. Two tubes were used per dilution. The number of colony forming units (c.f.u.) in the livers of the thioacetamide treated mice were 9×10^9 whereas the control livers showed 1.1×10^6 c.f.u. units. The number of c.f.u. in the spleens of the thioacetamide group was 3.6×10^8 whereas the control group showed a c.f.u. of 3.6×10^5 .

Histopathologically on the 40th day no specific disease was seen in the lung, liver, spleen of the control group whereas in the thioacetamide group tubercular disease was present mostly in the liver and spleen. The liver showed epitheloid cell tubercles in between large tracts of necrotic liver (figure 1).

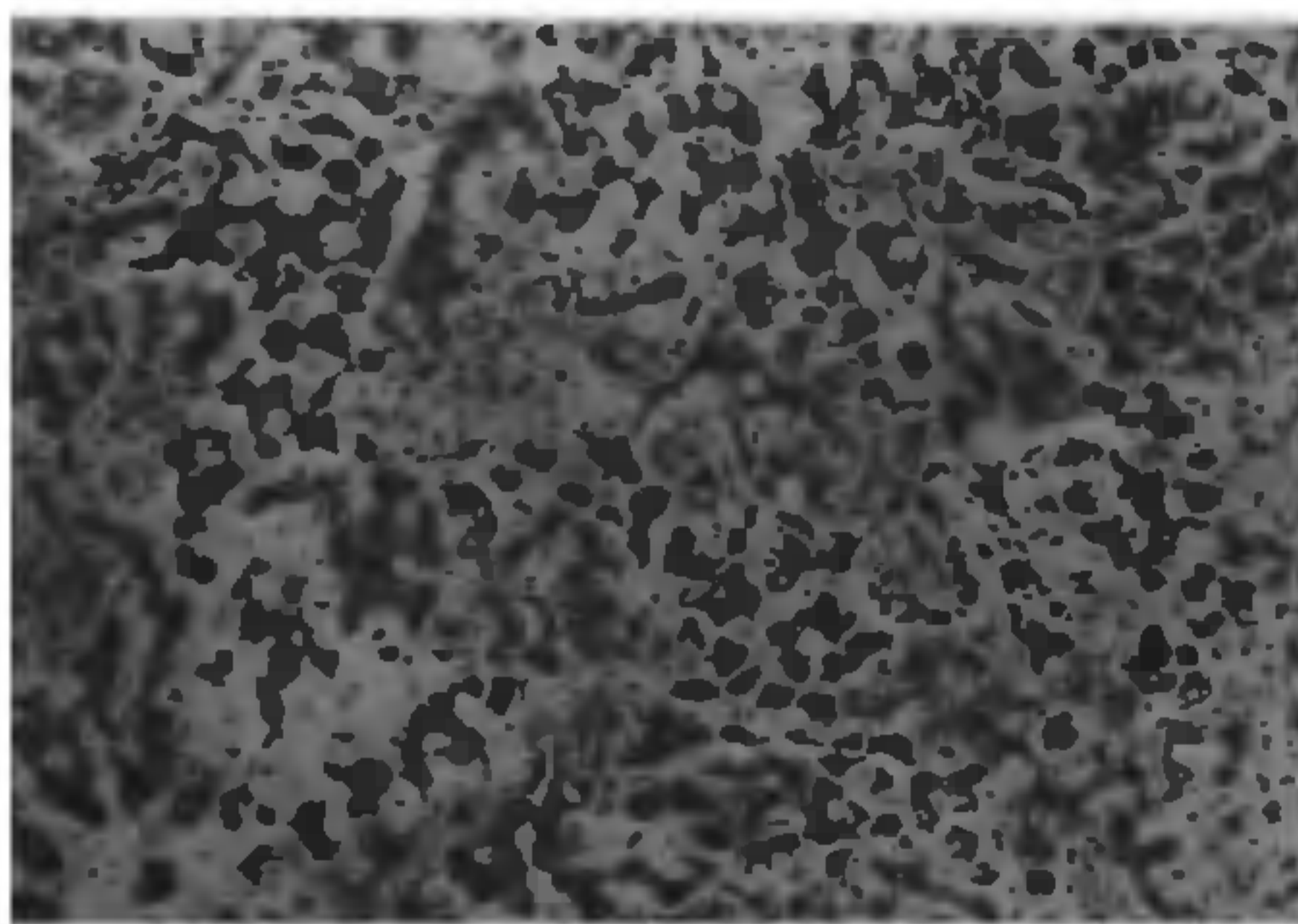


Figure 1. Thioacetamide damaged liver of mice showing large tracts of epitheloid cell tubercles scattered all over the liver parenchyma on the 40th day of infection, H & E $\times 45$.

From the data it, appears that liver damage due to thioacetamide increases the growth rate of *M. bovis* strain BCG so that it behaves like a moderately virulent strain. Similar findings with attenuated *Mycobacterium avium* have been obtained in rabbits treated with carbontetrachloride injection¹.

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I. Singh, N. B., Mathur, I. S., Gupta, H. P., Srivastava, A. and Gupta, S. K. J., *Med. Microbiol.*, 1980, 13, 319.

INDUCED REGENERATION IN STEM EXPLANTS OF *ACACIA NILOTICA*

INDU MATHUR AND N. CHANDRA
Department of Botany, University of Rajasthan,
Jaipur 302 004, India.

ACACIA nilotica, a thorny tree is of great economic importance for its timber and tannin producing bark. The present report describes induced regeneration of shoots and root and polarity in regeneration under *in vitro* condition in segments of stem comprising a portion of the internode and a higher node.

Young stem segments each having a portion of internode and a node (*ca* 10–20 mm) were taken from young twigs of *A. nilotica* growing in the campus of the University. The leaf bases and axillary buds were removed by a sharp scalpel from these segments. The segments were washed with distilled water, surface sterilized with 0.1% (w/v) mercuric chloride solution for 5 min. After a thorough washing with sterilized distilled water, the segments were implanted aseptically on Murashige and Skoog medium (MS)¹ supplemented with various growth substances and their combinations. The pH of the medium was 5.8 before autoclaving at 1.06 kg cm⁻² pressure for 20 min. The cultures were incubated under continuous diffused light (*ca* 1000 lux) at $26 \pm 2^\circ$ C.

Stem segments when implanted on different combinations of kinetin and an auxin on MS medium produced a creamish yellow, friable and slow-growing callus which turned brown presumably due to the accumulation of phenolic compounds. In many cases the brown or purplish brown substance leached out into the medium. Addition to the medium of PVP (Poly vinyl pyrrolidone) up to 500 mg/l could not prevent formation of phenolic substance in the callus.

When stem segments were cultured on MS medium with IAA (0.5–1 mg/l), a few shoot buds developed from the nodal portion and roots emerged from the opposite pole in 10–15 days of incubation (figures 1–3). The shoot buds that differentiate at the nodal region arise *de novo* (the leaf base and its axillaries were chopped off before implantation). This is in contrast to most of the earlier reports where a cytokinin was found essential for the differentiation of shoot buds². However, other explants like leaf, cotyledon, etc did not show differentiation on any of the combinations of hormones tried, although callus formation was observed. The number of shoot buds induced per explant varied from 1 to 4.

Although, polarity in regeneration was recorded as early as 1878 by Vochting³ in twigs of Willow (*Salix*), kept under moist conditions in different orientations this type of polar regeneration in *A. nilotica* in