TABLE 1

Content of histidinol phosphate in copper toxicity and its reversal by manganese in N. crassa

Type	*Mycelia grown in presence of*		Histidinol phosphate (μg/100 mg wet wt)
	Cu^{2+} (mM)	Mn^{2+} (mM)	
Control	Nil	Nil	145.2 (100)
Toxic	1.57	Nil	216.7 (149)
Reversal	1.57	3.6	146.0 (100)

Figures indicated in parantheses are percentage values. The values are the average of three separate experiments.

accumulation of histidinol phosphate is higher by 49% in copper toxicity and no such accumulation occurred under conditions of reversal of copper toxicity by supplemented manganese.

The present study thus reveals histidinol phosphate as a significant component of the accumulated imidazoles in copper toxicity of the mould. Histidinol phosphate accounted for 30% of the total accumulated imidazoles. While the accumulation of other related imidazoles cannot be ruled out, the accumulation of histidinol phosphate appears to correlate with our earlier observation of a decreased activity of histidinol phosphate phosphatase under conditions of copper toxicity. The reduction in the amount of histidinol phosphate to that of control values, under conditions of manganese reversal, further corroborates our earlier observation that manganese is able to reverse copper toxicity not by limiting the copper uptake but by a specific mechanism, probably by relieving the inhibition of histidinol phosphate phosphatase and promoting the biosynthesis of histidine.

In *Salmonella typhimurium* manganese has been identified as a necessary metal ion for the activity of the bifunctional enzyme imidazole glycerol phosphate dehydrogenase-histidnol phosphate phosphatase and the activity of the enzyme was also shown to be inhibited by copper[6]. The present observation thus supports the view that the mechanism of copper toxicity apparently involves the inhibition of histidine biosynthesis.

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A SIMPLE METHOD TO INDUCE SPORULATION IN *HELMINTHOSPORIUM TURCICUM* INCITANT OF LEAF BLIGHT OF MAIZE

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LOSS of capacity to sporulate through repeated culturing is one of the difficulties encountered by plant pathologists, while handling axenic cultures of plant pathogens. This poses an acute problem, especially when the pathogen in question is required to be introduced in the form of spores into the suscept.

A common method used to induce sporulation is by exposing the cultures to alternating cycle of 12 hr exposure to near ultraviolet with a radiation peak at 365 nm and extending approximately from 310 nm to 410 nm and 12 hr darkness[1-4]. The incitant of maize leaf blight *Helminthosporium turcicum* sporulates well on the lesions and loses this capacity after a few transfers in axenic culture. A simple method is suggested here to induce sporulation in *H. turcicum*.

The fungus was grown on different media containing extracts of grains of maize, sorghum, barley, wheat and extract of leaves of highly blight susceptible maize cultivar. The final concentration of the extract in the medium was 2%. In addition to the extract the medium contained 2% dextrose also. The media were solidified by using 2% agar agar. Potato Dextrose Agar served as the check.

Two isolates of the fungus (one obtained from Dharwar and the other locally isolated) were used. The isolates were inoculated on media in petri dishes from 6 day old cultures grown on Potato Dextrose Agar. The inoculated petri dishes were incubated at 25° C for 15 days. Each treatment was replicated three times. The sporulation was estimated using a haemocytometer. The data were analysed following a split plot design and are presented in table 1. The highest number of spores were observed on the medium containing maize leaf extract (25.6×10^4 spores/ml)

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 *H. turcicum*	
	Dharwar isolate	Hyderbad 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

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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 ± 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 5μ 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 ± 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