land/houses, because of the availability of naturally fertile, irrigated soil (when the water recedes after rain), which produces maximum of profit with minimum of labour. Moreover, because of the dam, the general water level in wells, streams and its tributaries was also raised, thereby increasing the breeding potential of vectors manifold. People have made their huts on the top of the hill, even though the dam reservoir is just a few feet away from their huts during rainy season and the whole area becomes highly unhygienic and infested with snakes, crabs, spiders, leeches, etc. To control malaria in these villages, special efforts are required such as, surveillance should be tightened throughout the year and prompt single dose treatment with fansidar/pyralfin (sulfadoxine and pyrimethamine) should be given. For this purpose, rapid and accurate new diagnostic tools such as the antigen detection dipstick/ICT may be used[14,15]. Further, health education should be given top priority so that the community may understand the importance of prompt diagnosis and treatment in prevention of deaths, human suffering and spread of transmission. Villages should be sprayed with an effective insecticide such as deltamethrin or insecticide-impregnated curtains should be given, as experience had shown that in tribal villages, where insecticide-treated bed nets were not effective because of socio-economic and cultural factors[16], insecticide-treated curtains were quite effective (Singh, personal communication). People should also be encouraged to use personal protection methods such as skin repellent or neem oil on exposed body parts during outdoor sleeping[17]. A more lasting solution of the problem is to shift these villages to some other area away from the dam reservoir.

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Evaluation of mycobacillin formulation for the control of rice blast disease

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Field trials on the control of blast disease of rice indicate that the disease was controlled to the extent of 96% by mycobacillin formulation as against 90%, 72%, 72%, 76% and 48% by Hinosan, Agrozim, Fuji, Topsin, S-1901 and mycobacillin respectively. Efficacy of mycobacillin is thus greatly enhanced by formulation.

MYCOBACILLIN, an antifungal tridecapeptide, was isolated from the culture filtrate of *Bacillus subtilis* B_3 (ref. 1). Since its isolation, it has been of much interest in our laboratory for a number of studies including those on chemistry[1–3], biosynthesis *in vivo* and *in vitro*[4,5], genetic analysis of mycobacillin pathway[6], mode of action of the antibiotic including its use as a membrane probe in release and uptake[7–9], and finally its physiological role in the sporulation of the producer organism[10–12]. We observed that mycobacillin has only a limited use as a drug against dermatophytes[13]. The compound, however, appears to be very effective against plant pathogens particularly *Pyricularia oryzae* (Cav.). The minimum inhibitory concentration (MIC) of the antibiotic against *P. oryzae* (Cav.) is 10 µg/ml (ref. 14). The brown leaf spot disease of rice caused by *Drechslera oryzae* Van Breda de Haan (Syn. *Helminthosporium oryzae*) has already been reported to be controlled to the extent of 55% by foliar spray in the laboratory scale experiments[15]. Subsequent experiments showed that mycobacillin is active only topically[16]. Hence, a formulation was developed according to the method of Bhattacharya *et al.*[17] with the adjuvants (% by g/v), glycerol 1, tween-20 1, methyl cellosolve 1 and indole-3-acetic acid

Table 1. Analysis of the effect of mycobacillin, mycobacillin formulation and other fungicides on the control of rice blast disease

Antibiotic or fungicides	Active ingredients	Concentration	Per cent disease index ± SEM Control	Per cent disease index ± SEM Experimental	Per cent disease control ± SEM
Mycobacillin	Mycobacillin	250 µg/ml	22.86 ± 0.52	11.9 ± 0.36	47.93 ± 1.02
Mycobacillin formulation	Mycobacillin	250 µg/ml	37.43 ± 1.99	1.52 ± 0.16	96.89 ± 0.48
Hinosan 50% EC	0.Ethyl-s-s-diphenyl-dithiophosphate	0.1%	28.76 ± 3.03	2.57 ± 0.22	90.38 ± 1.47
Fuji	1.3. Dithiolan-2-yldine malonate	0.1%	29.52 ± 4.50	7.05 ± 0.98	72.54 ± 5.31
Agrozim	Methyl-2-benzimidazole carbamate	0.1%	24.28 ± 2.08	6.39 ± 0.42	72.07 ± 3.93
Topsin	Thiophanate methyl 70% W.P.	0.1%	21.33 ± 0.98	5.06 ± 0.18	75.89 ± 1.81
S-1901	4 Chloro-3-methyl benzothiazolone	3 g/m^2	22.09 ± 0.55	8.96 ± 0.62	59.56 ± 2.92

Average of five replications/30 plants/replication, $p < 0.001$.
Healthy and infected leaves were divided into eight scales from zero to seven depending upon the size of the lesions:
Per cent disease index (PDI) was calculated as follows:

$$\text{PDI} = \frac{\text{Total disease rating of all examined leaves} \times 100}{\text{Total number of leaves examined} \times 7}$$

$$\text{Per cent disease control (PDC)} = \frac{\text{PDI}_{\text{control}} - \text{PDI}_{\text{treated}}}{\text{PDI}_{\text{control}}}$$

(50 µg/ml). This formulation exerts its antifungal action *in vivo*[17]. This gave us an opportunity to evaluate the formulation of mycobacillin against blast disease of rice caused by *P. oryzae* (Cav.). In this communication, we describe experiments performed to evaluate the formulation of mycobacillin for controlling systemic infection of blast disease of rice. Since we could not induce the disease experimentally on rice plants (data not shown), experimental trials were undertaken in Kalimpong, West Bengal, India, where the disease is endemic.

Other fungicides used for comparison were Hinosan (Bayer India Ltd.), Agrozim (Gujarat Agro Industries Corporation Ltd.), Fuji, Topsin (Motilal Pesticides India Pvt. Ltd.), and S-1901 (Rallis India Ltd.). Mycobacillin was obtained from the culture filtrate of a strain of *Bacillus subtilis* B_3. The rest of the fungicides, which were commercially available, were obtained through the courtesy of B. D. Sharma, Mycologist, Govt. of West Bengal, India. The other chemicals used were of reagent grade and were purchased from Indian chemical companies. Rice cultivar 'Kalomashino' was used for field evaluation. The cultivated rice plants developed blast disease as indicated by spindle-shaped spots with ashy centers by way of natural infection by *P. oryzae* (Cav.). All the fungicides except S-1901 were applied in the form of aqueous solution at a concentration of 0.1%. S-1901 was applied (3 g/m^2) in the form of powder between the furrows and thereafter the soil was stirred up vigorously. Immediately on the onset of infection, the plants were sprayed repeatedly to run off level with the help of a chromatographic sprayer. The disease index was calculated by a modification of the method used by Chattopadhyay and Bose[15]. The observed data of the above experiments were statistically analysed following the method of Richterich[18] using the Student's *t* test.

The results of field evaluation experiments are shown in Table 1. Mycobacillin alone at a concentration of 250 µg/ml controlled naturally-infected rice blast disease to the extent of 47.93 ± 1.02. Since the SEM of per cent disease control (PDC) for only mycobacillin treatment was 1.02 as against the SEM values of the disease indices 0.36 and 0.52 of the treated and untreated groups respectively, the mycobacillin treatment for disease control appeared to be very specific if not effective. This observation of the specific effect and high potency was further extrapolated by the fact that when mycobacillin formulation was used, the PDC value was of greater magnitude (95.89 ± 0.48) with lower individual variation (with low SEM, 0.48). The potency of mycobacillin formulation was found to be even higher than the most frequently used and active fungicide Hinosan, so far as the lowering of disease index was concerned (for mycobacillin formulation PDC was 95.89 ± 0.48 compared to that of Hinosan, 90.38 ± 1.47, $p < 0.001$, as in the table). Similar statistical calculations for other fungicides tested revealed that they were less active in the lowering of disease index values than mycobacillin formulation and Hinosan.

Thus, mycobacillin formulation was the most efficient among the fungicides tested in the present investigation in controlling the blast disease of rice.

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Influence of superoxide dismutase on chromosome aberrations induced by bleomycin alone or in combination of reduced glutathione

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In this study, an attempt has been made to see the influence of superoxide dismutase (SOD) on the DNA damaging effect of bleomycin (BLM) alone or in combination of reduced glutathione (GSH), which potentiates BLM action by reducing oxidized BLM. Present results in human peripheral lymphocytes demonstrate that SOD reduces the clastogenic action of BLM alone or in combination with GSH, indicating involvement of superoxide radicals in induction of chromosome aberrations. SOD treatment before GSH addition to BLM-treated cells shows better protective effect and this could be due to scavenging of superoxide radicals by SOD which would otherwise involve in conversion of Fe(III) to Fe(II) with increased GSH or cysteine level inside the cells.

BLEOMYCINS (BLM) are glycopeptide antibiotics used in cancer chemotherapy. The antitumour activity of BLM has been attributed to its ability to cause DNA damage[1,2]. It induces chromosome aberrations (CAs) at all stages of cell cycle[3] and comparable with those induced by X-irradiation[4,5]. The mechanism whereby BLM exerts cytotoxicity is believed to involve the activation of molecular oxygen to produce toxic oxygen metabolites such as superoxide anion (O_2^-) (ref. 6). Superoxide dismutase (SOD), an enzyme that scavenges O_2^-, has been reported to reduce the DNA degrading activity of BLM[7,8] and its genotoxic effect in CHO cells[9]. However, no data are available on the protective effect of SOD against the clastogenic action of BLM with respect to CAs induction. Therefore, in the present investigation we tested SOD as a protector against CAs produced by BLM alone or in combination with reduced gluthione (GSH). GSH is a well-known radioprotector constitutively present in the cell and plays an important role in cellular radioresistance[10–12]. Tumour cells cultured *in vitro*, in particular those of human origin, were shown to contain extremely high levels of GSH[13,14], and these were made resistant to some anticancer drugs, e.g. melphalan, *cis*-platin and adriamycin[15,16]. Therefore, it is of interest to see the effects of cellular GSH level on the cytotoxicities of chemicals like BLM. An attempt has been made to increase the level of endogenous GSH by treating GSH and GSH-ester exogenously. It has been demonstrated[17,18] that increased GSH potentiated the clastogenic action of BLM in muntjac and human lymphocytes *in vitro*. This potentiation was attributed to GSH acting as a reducing agent in reactivating oxidized BLM. Recently it has been shown that depletion of endogenous GSH by buthionine sulfoximine treatment reduced the clastogenic action of BLM[18] in human lymphocytes. Thus in order to see the influence of SOD on CAs induction by BLM alone or in combination with GSH, we have carried out this study and the results show that superoxide radical is a major factor in inducing CAs by BLM with or without GSH.

Heparinized peripheral blood from 4 healthy male donors were used immediately after venipuncture. These samples were cultured in RPMI 1640 medium (Gibco, USA) supplemented with 10% heat-inactivated foetal calf serum (Biological Industries Ltd., Israel) following phytohaemagglutinin (PHA; Gibco, USA) stimulation and incubated at 37°C. To obtain differential sister chromatid staining, 5 $\mu g\ ml^{-1}$ 5-bromodeoxyuridine (BrdU; Sigma, USA) was added to the cultures at the time of initiation. Cells were harvested at 48 h and colcemid (0.1 $\mu g\ ml^{-1}$) was added 2 h prior to that.

Commercially available bleomycin (Bleocin; Nippon Kayaku, Japan) was hydrated with sterilized triple-distilled water, from which a fresh working solution of 100 $\mu g\ ml^{-1}$ was prepared by addition of RPMI 1640 (pH 6.8) just prior to each use, and given to cells to make a final concentration of 20, 40 and 60 $\mu g\ ml^{-1}$. Freshly prepared SOD (Sigma, USA) 40 $\mu g\ ml^{-1}$ was added 30 min before or after treating the blood with BLM, which will be referred to in test as a pre- or a post-treatment. In separate sets of experiments, GSH