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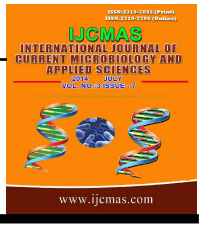

Original Research Article

Mutagenicity of fungicide Mancozeb by *Salmonella* Reverse Mutation assay

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A B S T R A C T

Mancozeb, a fungicide of class dithiocarbomates, was studied for its mutagenic potential. Ames *Salmonella* mutagenicity assay was used in which mancozeb at different concentrations was checked for its ability to induce reverse mutation at histidine locus in three strains of *Salmonella typhimurium* (TA98,TA100 and TA102) in the presence and in absence of metabolic activating system (S9) containing mammalian microsomal enzymes. Based on Cytotoxicity test 0.002 and 0.001 mg of mancozeb/plate was selected as the highest concentration to be tested in the mutagenicity test in the absence and in the presence of metabolic activation, respectively. In case of mean, number of revertant colonies at 0.002 mg plate^{-1} in absence of metabolic activation for TA98 was 12, TA100 was 92 and TA102 was 226 which were below positive control and number of revertants at 0.001mg plate^{-1} in presence of metabolic activation for TA98 was 12, TA100 was 105 and TA102 was 282 which were also less as compared to positive control. Thus, mancozeb up to concentration of 0.002 mg plate-1 in absence of metabolic activation and 0.001 mg plate-1 in presence of metabolic activation (5% v/v S9 mix), was non mutagenic to all three strains of *Salmonella typhimurium* when tested under specific conditions. There was no significant increase in number of revertant colonies accomplished that mancozeb with this concentration can be considered as non mutagenic.

Keywords

Mancozeb, Auxotrophic, *Salmonella typhimurium*, Mutagenicity, Ames test

Introduction

Mancozeb is marketed by the trade names Dithane, Maneb, Nemispot, and Manzane. The fungicide mancozeb is one group of pesticides known as ethylene bisdithiocarbomate (EBDCs) registered for use on a variety of vegetable, fruit, nut and grain crops. (Hayes *et al*., 1990). Different doses of mancozeb were checked for its effect on *Alternaria* leaf blight of potatoes and were proved to be effective and also ecofriendly (Gondal *et al*., 2012). It acts a as fungicide by inhibiting enzyme activity in fungi by forming a complex with metal-containing enzymes including those involved in production of adenosine triphosphate (ATP). However, it is listed as a cancer-causing chemical by California's Office of Environmental Health Hazard

Assessment (OEHHA) under proposition 65 dated July 26, 2013. (http:// oehha. ca.gov/ prop65/prop65_list/Newlist.html) Thus, it can be considered as multipotent carcinogenic agent **(**Belpoggi *etal*., 2002). It is reported to alter structural and functional changes in thyroid of rats and also affect level of glycogen, proteins and lipids in testis, liver and kidney (Kackar *et al*., 1997; Ksheerasagar *et al*., 2010; and Axelstad *et al*., 2011). Mancozeb induces genotoxicity and apoptosis in cultured human lymphocytes (CHLs). The Studies conducted on micronuclei and chromosome abbration in blood of healthy donors were found to induce apoptosis in CHLs (Srivastava *et al*., 2012).

Ames Salmonella Test is used for assaying mutagenicity. It is simple, inexpensive and extremely sensitive test which makes use of a rat or human liver homogenate for carcinogen activation (thus supplying mammalian metabolism) and a set of *Salmonella* histidine mutants for mutagen detection. It is an initial screening method for detecting carcinogenicity of chemicals (Mortelmans *et al*., 2000). Muhammed *et al*. (2003) reported that mancozeb had some effect on the hematological parameters of rainbow trout. The RBC/WBC level may be increased due to decrease in WBC count during the study.

Investigations regarding its degradation in tomatoes homogenate were studied to prevent the risk of it and its metabolites exposure to diet (Certel *et al*., 2011). It also interferes with carbon and nitrogen assimilation and thus in carbon and nitrogen cycle and may further lead to toxicity in inbuilt soil microorganisms (Cernohlavkova *et al*., 2008). Therefore, the aim of the present study was to figure out non toxic concentration of mancozeb and to evaluate mancozeb for its ability to induce reverse mutations at the histidine locus in three strains of *Salmonella typhimurium* (TA98, TA100 and TA1537), in the presence and absence of an exogenous metabolic activation system (S9) containing mammalian microsomal enzymes

Materials and Methods

Salmonella typhimurium strains (TA98, TA100 and TA102) were procured from Toxicology Department, Jai Research Foundation. The strains were checked for histidine dependency, biotin dependency, histidine/biotin dependency, Rfa mutation, ampicillin and tetracycline resistance. Rat liver S9 fractions were prepared from Phenobarbital-treated rats. Plate incorporation assay was done according to the standard protocols (Mortelmans e*t al*., 2000) Cytotoxicity test was performed to select treatment concentration for mutagenicity test. Mancozeb was dissolved in DMSO (dimethyl sulfoxide) and diluted prior to treatment according to test concentration. A liver S9 mixture was added at the concentration of 0.5 ml per plate.

Toxicity of mancozeb was determined by plating 100 µl of diluted test substance with 100 µl overnight grown standard bacterial cultures and top agar enriched with 0.5mM histidine /biotin. A positive control 2-Nitrofluorene (7.5 µg plate-1) for TA98, Sodium azide (5 µg/plate) for TA100, Mitomycin-C (0.5 µg plate-1) for TA102 and positive control devoid of 2-aminoanthracene for all the three strains in absence of metabolic activation was used. Along with it positive Control 2-Aminoanthracene (10 µg plate-1) for TA102 and (5 µg plate-1) for TA98 and TA100 in presence of metabolic activation was used to check mutagenicity of the strains. A dose response increase in number of revertants over the positive control value was taken as the affirmation for mutagenicity.

Results and Discussion

Cytotoxic testpartial background bacterial lawn inhibition, was observed at the concentration of 0.00098 and 0.03907 mg/plate in the absence and presence of metabolic activation (5% v/v S9 mix), respectively. Reduction in the number of revertant colonies was observed at the concentration of 0.0009765625 and 0.001953125 mg plate-1 in the absence and presence of metabolic activation (5% v/v S9 mix), respectively. Hence, 0.002 and 0.001 mg of mancozeb/plate was selected as the highest concentration.

In case of mean number of revertant colonies obtained in the absence of metabolic activation at 0.002 mg plate-1 for TA98 was 12, TA100 was 92 and TA102 was 226 and in case of presence of metabolic activation at 0.001mg plate-1 the number of revertants obtained with TA98 was 12, TA100 was 105 and TA102 was 282 which are below positive control. So the mean number of revertant colonies obtained were far less as compared to positive control. Thus, none of the plate incorporation assays and linear regression analysis tester strains used showed significant increase in revertant numbers (Table 1 and 2).

It was observed that at 0.002 and 0.001 mg of mancozeb/plate in the presence and absence of metabolic activation, none of the tester strain showed toxic effect and no significant increase in the number of revertants was observed when compared with positive control. Thus, the true mutagenic potential of mancozeb may be masked by its toxic effect on the tester strain used. Further modification of Ames test may provide more sensitive assessment for mutagenic activity. Also significant inhibition in chromosome aberration was found with mancozeb at 250 mg kg-1 b.wt. in Swiss albino mice and no significant effect in growth and reproduction in *E.fetida* was found with mancozeb at recommended dose (8mg kg-1) (Tripathi *et al*., 2011; Vermeule *et al*., 2001), which may suggest that concentration taken in Experiment shows non mutagenicity. A similar class of fungicide, maneb did not show double increase in revertants in micronucleus and chromosome aberration test and was proved to be non-mutagenic. While another fungicide, thiram in Ames test, proved lethal at higher concentration to micro-organisms (Andres *et al*., 1999).

So it increase in number of revertants with increase in dose concentration. However, with evaluation of toxic effect of mancozeb at concentration of 500 mg kg-1 body weight. in rats showed toxicity in testis in males and in female mice show gonaldal toxicity which in case of female may be due to hormonal reaction or its own toxicity (Joshi *et al*., 2005; Baligar *et al*., 2001). This correlation suggests that it may lead toxicity with high dosage level . and also toxicity varies with respect to specificity in rats, human and other animals.

It is evident from the present study that mancozeb exhibited weak mutagenic response at tested dosage and if the similar dose seems to be having fungicidal activity we can use mancozeb at this concentration as a fungicide. Further studies with different metabolic activation system may give a better understanding of the genotoxic potential of mancozeb, as it is still used in India.

Table.1 Mean count of His+ revertant colonies in negative control (NC) and positive control (PC) and treatment plates in the absence of metabolic activation

Mancozeb Concentration (mg plate^{-1})	HIS^+ revertant colonies/plate(Absence of metabolic activation)					
	TA98		**TA100**		**TA102**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
NC(DW)	26.00	±1.41	132.00	±12.73	222.50	±3.54
0.0000625	21.50	±4.95	81.50	±12.02	232.00	±7.07
0.000125	22.50	±3.54	77.50	±0.71	243.00	±11.31
0.00025	18.50	±4.95	102.50	±13.44	236.00	±1.41
0.0005	23.50	±0.71	100.50	±14.85	231.00	±14.14
0.001	*20.00*	*±0.00*	115.00	±26.87	267.50	±33.23
0.002	12.00	±4.24	92.00	±8.49	226.00	±11.31
PC	495.00	±42.45	1263.50	±232.64	372.00	±46.67
PC-2Aa	-	-	143.5	±14.85		

SD = Standard Deviation, NC = Negative Control, PC = Positive Control, DW = Distilled Water, - = Not applicable

Table.2 Mean count of His+ revertant colonies in negative, vehicle and positive controls and treatment plates in the presence of metabolic activation (Trial I)

Mancozeb Concentration (mg plate^{-1})	HIS^+ revertant colonies/plate(Presence of metabolic activation)					
	TA98		**TA100**		**TA102**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
0.00003125	21.00	±7.07	107.00	±7.07	271.50	±20.51
0.0000625	23.50	±0.71	94.50	±26.16	222.00	±14.14
0.000125	20.50	±4.95	85.50	±2.12	250.50	±33.23
0.00025	21.50	±0.71	106.50	±14.85	280.00	±5.66
0.0005	13.50	±0.71	89.00	±9.90	256.50	±3.54
0.001	*12.00*	*±5.66*	105.50	±3.54	282.00	±15.56
PC	795.00	±43.84	1225.50	±14.85	1526.50	±71.42

SD = Standard Deviation, NC = Negative Control, PC = Positive Control, DW = Distilled Water

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