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Mutagenicity assessment of textile dyes from Sanganer (Rajasthan)

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Abstract: *Sanganer town, district Jaipur (Rajasthan, India) is famous worldwide for its hand block dyeing and textile printing industries. These industries use a variety of chemicals and dyes during processing and finishing of raw materials Most of the textile dyes used by these industries have not been evaluated for their impact on health and the environment. The workers in these industries are exposed to such dyes with no control over the length and frequency of exposure. Further, untreated and sometimes even treated effluents from these industries are released into surface waters of Amani Shah drainage or through the drainage systems, seep into the ground water and adjoining water bodies. Since many textile dyes are known carcinogens and mutagens, a complete evaluation of the safety of these dyes in the human environment must include an evaluation of their genotoxicity or mutagenicity. A total of 12 textile dyes from Sanganer were tested for their mutagenicity, by Ames Salmonella reversion assay using strain TA 100 of Salmonella typhimurium.. Only 1 dye, Red 12 B showed absence of mutagenic activity. The remaining 11 dyes were all positively mutagenic.*

Key words: *Textile dyes, Mutagenicity, Ames test*

Introduction

Dye production in India is estimated to be around 64,000 tonnes, which is about 6.6% of the world production. There are around 700 varieties of dyes and dye intermediates produced in India, mainly direct dyes, acid dyes, reactive dyes and pigments. Most of these dyes have not been characterized regarding their chemical nature, purity, possible toxicity or their impact on health and the environment. Yet, they are widely used by textile, leather, paint and even the food industry. The textile industry in India alone consumes up to 80% of the total dyestuffs produced.

In Rajasthan state particularly, textile mills represent an important economic sector. Sanganer is famous for dyeing and printing of colorful dresses, bed sheets, curtains, dress material and variety of other textiles. Bulk of the textile products of these industries is exported. It is located about 15 km south of Jaipur, the State capital that has a population of more than two million people. The total area of Sanganer is about 635.5 Sq. km out of which, 12.9 Sq. km comprises the urban area. Most of the textile industries of Sanganer are concentrated in this urban area. There are estimated to be around 500 block and screenprinting units in Sanganer.

Of all dyes produced across the world, 11% goes out as effluents, 2% from manufacturing and as much as 9% from coloring. The effluents from dyeing and textile industries contain chemicals with intense colors and the release of these effluents to receiving streams may be objectionable for various aesthetic reasons. Besides a number of dyes used by these textile industries are not degradable. Further, these colored dye wastes contain compounds that are difficult to treat biologically due to their resistance against biodegradation. The dye effluents may contain some components or moieties that could be toxic, carcinogenic or mutagenic to aquatic life (Suzuki *et al*., 2001). Central Pollution Control Board has listed the dye and dye intermediates industry as one of the heavily polluting industries (CPCB, 1990). They are thus, a potent hazard to the natural sources like soil, water, flora, fauna, livestock and human population.

Ecological and toxicological problems due to the discharge of wastewaters from Sanganer textile industries in local drainage (Amani Shah Ka Nallah) have been one of the most important water pollution problems in this area. Studies have clearly indicated that the industrial effluents, which are directly discharged into the Amani Shah Ka Nallah, drainage contain highly mutagenic compounds. These compounds are also contaminating the surface and even underground water, thereby, making it unfit for irrigation and drinking (Mathur *et al.,* 2005). High concentrations of heavy metals like Cu, Cd, Zn, Pb, Ni, etc. have also been reported in this area (Khan, 1995).

Dyes and heavy metals have been considered to be the possible source of genotoxic activity in dyeing and textile effluents. Several dyes have been investigated and found to be carcinogenic (Prival *et al.,* 1984). Since large quantities of dyes are used, such pollution due to dyes may occur on a significant scale. Assessment of genotoxicity of dyes is therefore of utmost importance. Most of the dyes, being openly sold in Sanganer

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markets have no information regarding their chemical nature, purity or possible mutagenicity or toxicity. This study was thus planned to investigate the mutagenic potential of the dyes available in markets of Sanganer.

Materials and Methods

Collection of samples: Dyes (12) were obtained at random basis from local Sanganer market. They had no information regarding chemical constituents, purity or hazardous nature. Dye solutions were made by dissolving 1 g dry powder of dye in 100 ml of warm, sterile, distilled water. All the dyes used were water soluble. Five concentrations (2 µl, 5 µl, 10 µl, 50 µl and 100 µl) of these dyes were tested

Ames mutagenicity test: The *Salmonella* reversion assay was conducted using the plate incorporation procedure described by Ames *et al.* (1975) and revised by Maron and Ames (1983). The dye samples were tested with TA 100 strain of *S. typhimurium*, which was obtained from microbial type culture collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTech), Chandigarh (India).

Samples were tested on duplicate plates in two independent experiments. Five dose levels of individual samples were tested. Positive control used for TA 100 was sodium azide (CAS Number: 26628-22-8): 5 µg / plate: 2969 revertants. Sterile distilled water was used as negative control. Fresh solutions of the reference mutagen were prepared immediately before the beginning of each experiment. The revertant colonies were clearly visible in a uniform background lawn of auxotrophic bacteria. The tester strain was maintained and stored according to the standard methods (Mortelmans and Zeiger, 2000). The strain was regularly checked for genetic markers. All reagents used were of analytical grade, supplied by Himedia Laboratories Limited (India) and Sigma Aldrich.

Data analysis: The most common method of evaluation of data from the *Salmonella* assay is the "two fold rule" according to which doubling of spontaneous reversion rate at one or two test chemical concentrations constitutes a positive response (Cariello, 1996; Mortelmans and Zeiger, 2000). This rule specifies that if a test compound doubles or more than doubles, the mean spontaneous mutation frequency obtained on the day of testing, then the compound is considered significantly mutagenic. Using this procedure the following criteria were used to interpret results.

Positive: A compound is considered a mutagen if it produces a reproducible, dose related increase in the number of revertant colonies in one or more strains of *Salmonella typhimurium*. A compound is considered a weak mutagen if it produces a reproducible dose related increase in the number of revertant colonies in one or more strains but the number of revertants is not double the background number of colonies.

Negative: A compound is considered a nonmutagen if no dose related increase in the number of revertant colonies is observed in at least two independent experiments.

Inconclusive: If a compound cannot be identified clearly as a mutagen or a nonmutagen, the results are classified as inconclusive (e.g. if there is one elevated count).

For this analysis the dose related increase in the number of revertant colonies were observed for the test compounds and mutagenicity ratios were calculated. Mutagenicity ratio is the ratio of average induced revertants on test plates (spontaneous revertants plus induced revertants) to average spontaneous revertants on negative control plates (spontaneous revertants). Mutagenicity ratio of 2.0 or more is regarded as a significant indication of mutagenicity.

Results and Discussion

Many of the dyes used by textile industries are known carcinogens (ICPEMC *i.e.* International Commission for Protection against Environmental Mutagens and Carcinogens,1982) and teratogens (Beck, 1983). Yoshida and Miyakawa (1973) reported that occupational exposure to benzidine dyes might have possibly resulted in bladder cancer amongst kimono painters in Japan. Triple primary cancers involving kidney, urinary bladder and liver in a dye worker have also been reported (Morikawa *et al*., 1997).

Table - 1: Mutagenicity ratio of dyes (1g/100ml) with strain TA 100 of *S. typhimurium*.

Dye	Concentration of dyes (µl)				
	2	5	10	50	100
Red 12 B	-	-	-	1.3	1.8
Navy blue	1.1	1.5	4.0	5.3	7.6
Turqish blue	5.0	6.1	6.2	7.1	8.1
Grey	0.8	3.9	4.8	8.7	12.7
Solar brown	1.6	2.3	3.0	7.0	13.5
Golden top	4.2	5.2	5.7	11.2	14.2
Black	4.9	10.8	18.5	39.6	94.5
Parrot green	3.4	54.4	80.9	98.6	120.6
Sky blue	61.9	74.2	92.4	73.7	7.4
Khaki	7.4	16.5	19.6	37.1	14.6
Lemon top	7.5	10.7	25.3	51.9	14.4
Moongia green	1.4	2.5	4.3	14.3	10.6

Dyes are introduced into the environment through industrial effluents of these industries. Excessive and indiscriminate use of dyestuffs has thus become increasingly a subject of concern. Therefore a complete evaluation of the safety of these dyes in the human environment must include an evaluation of their genotoxicity or mutagenicity. A total of 12 dyes were tested for mutagenicity, using strain TA 100 of *Salmonella typhimurium*.

The mutagenicity ratios of the dyes are shown in Table 1. As observed from the Table, only 1 dye Red 12 B had mutagenicity ratio of less than 2.0, showing absence of mutagenicity. The remaining 11 dyes were all positively mutagenic with mutagenicity ratios of more than 2.0.

Amongst the eleven dyes tested in the present study, navy blue and turqish blue had lesser mutagenic activity (1000-1200 induced revertants, per 100 µl of dye) (Fig. 1). The dose response curves of 3 dyes i.e. grey, solar brown and golden top,

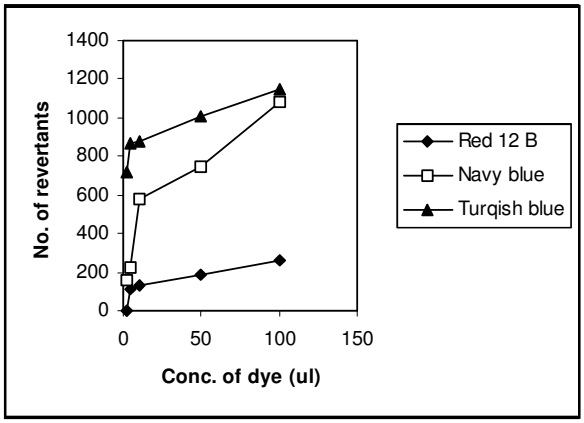

Fig. 1: Dose response curve of dyes with strain TA 100 of *Salmonella typhimurium*
(1) Red 12B, (2) Navy blue, (3) Turqish blue

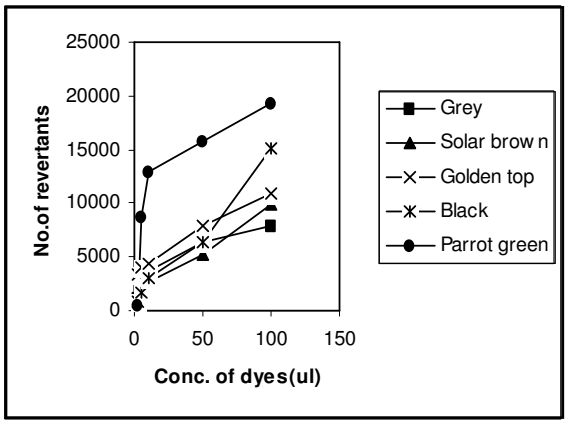

Fig. 2: Dose response curve of dyes with strain TA 100 of *Salmonella typhimurium*
(1) Grey, (2) Solar brown, (3) Golden top
(4) Black, (5) Parrot green

are shown in Fig. 2. Compared to the previously mentioned dyes (navy blue and turqish blue), this group of dyes (grey, solar brown and golden top) showed higher number of revertants, of the order, 3000-10000 induced revertants, per 100µl of dye solution. However, the strongest mutagenic activity was seen with black and parrot green (4000-20,000 induced revertants, per 100µl of dye) (Fig. 2).

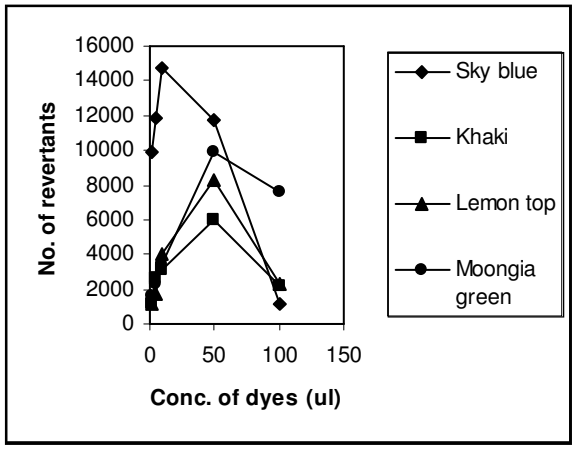

Fig. 3: Dose response curve of dyes with strain TA 100 of *Salmonella typhimurium*
(1) Sky blue, (2) Khaki, (3) Lemon top, (4) Moongia green

These observations are in accordance with several studies that report mutagenicity of a number of dyes like direct black 38, acid red 26, etc. (Garner and Nutman, 1977; Venturini and Tamaro, 1979). Unlimited and uncontrolled use of such dyes can lead to grave consequences in terms of human health and ecological balance.

The presence of impurities in the commercially available dyes has been reported to contribute to the mutagenicity of these dyes (Prival *et al.*, 1984). As the dyes under investigation were not purified, the impurities present in them could have also been responsible for the high mutagenic activity of the dyes. Nevertheless, the fact still remains that highly mutagenic compounds are being used in textile dyeing and printing industries of Sanganer.

Further, four of the tested dyes i.e. sky blue, khaki, lemon top and moongia green were so toxic that they inhibited the growth of bacteria, at higher dose levels. The results of this study clearly indicate that most of the locally used dyes are highly mutagenic, and therefore should be used with great caution.

Since innumerable dyes are available in local markets, chemical analysis of each and every dye is not possible because of the time and cost involved. However, Ames test can easily and quickly assess mutagenic potential of these dyes. Besides, the dyes can be compared on the basis of their mutagenic potencies. Thus, this assay should be used as a regular monitoring tool for assessing the dyes.

Results from genetic bioassays are relevant to human health because the toxicological target is DNA, which exists in all cellular life forms. Thus, it can be extrapolated that compounds shown to be reactive with DNA in one species have the potential to produce similar effects in other species.

In general, perturbations of genetic material are deleterious to the organisms and can lead to severe and irreversible health consequences.

Therefore, indiscriminate use of synthetic chemical dyes should be restricted or the workers while handling these dyes should at least take proper precautions. They should be replaced by vegetable dyes, which are ecofriendly. Besides, before using the dyes at large scale, their mutagenic potential should be assessed by biological assays like Ames test. This bioassay can be used as an initial screening test to analyze various dyes and dye containing effluents, which are causing major damage to the aquatic environment.

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