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RESEARCH ARTICLE

PROS AND CONS OF *P. FLORIDA* CULTIVATION FOR MANAGING WASTE OF HANDMADE PAPER AND CARDBOARD INDUSTRIES

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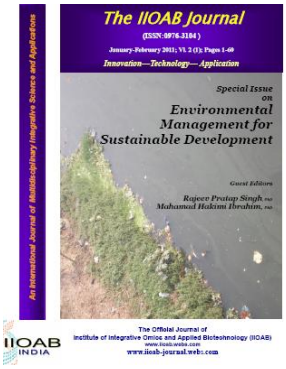
ABSTRACT



The possibility of utilizing handmade paper and cardboard industrial sludges in the production of edible mushrooms involves risk of introducing toxic substances into the human food chain. Therefore, in the present study, genotoxic assessment of P. florida (Pleurotus florida) cultivated on these industrial sludges and their combination with wheat straw was done by Ames test using Salmonella typhimurium TA 98 and TA100. Interestingly, P. florida carpophores, cultivated on wheat straw did not show either frameshift or basepair mutagenicity as revealed by mutagenicity ratio (<2) and mean number of revertants which was found to be 81.3 and 93.4 revertants per plate in the absence of S9 mix. However, this number was found to be increased to 112.1 and 226.3 revertants with S9 mix. P. florida cultivated on waste and its combination showed increase in number of revertants (123.4-170.1 revertants with TA 100 and 79.5-84.1 revertants with TA 98) in the absence of S9 mix over control. Further, increase in number of revertants (229.0-247.3 with TA 100 and 100.3-129.1 with TA 98) was observed on adding S9 mix with both strains S. typhymurium but still mutagenicity ratio was found to be below 2. Hence, these mushrooms were not found to be genotoxic. This mushroom cultivation technique, will not only provide proteinaceous food but also help in reducing industrial wastes. Besides, these can serve as very good source of income for the poor workers working in these industries that can collect the waste from the industries and use it for P. florida cultivation.

Key words: ***handmade paper industries; cardboard industries waste; sludge; waste management; genotoxicity, mutagenicity***

[I] INTRODUCTION

Waste management services are becoming increasingly important because quantities of wastes continue to raise inspite of waste prevention and cleaner production efforts. Handmade paper and cardboard industries of Sanganer (Jaipur, India) are using colored and white cotton, linen rags and waste paper for making handmade paper and cardboard respectively. These handmade paper and cardboard industries generate colored sludge and brown sludge respectively which accumulates in the vicinity of industries or nearby drains. Non-recycled cellulosic and lignocellulosic pulp fibers are difficult to manage. This industrial waste management is often not only an issue of disposal, but a question of utilizing it for generating wealth from waste. Mushroom cultivation on lignocellulosic and cellulosic industrial wastes, agricultural wastes, agro-industrial sludges are thought to be good techniques to manage waste. It not only removes wastes from the environment but also provide a very good source of protein. This can be very good approach for dealing solid waste especially in developing countries where there is problem of protein deficiency.

Hence, handmade paper and cardboard industrial wastes were used for mushroom cultivation in the previous study [1]. However, it is necessary to analyze the safety aspects of mushroom cultivation before recommending it for consumption especially when industrial waste is used as substrate because mushrooms are well known for their ability to bioaccumulate the toxic substances in their carpophores [2; 3]. In the present study, pros and cons of mushroom cultivation on handmade paper (HMPI) and cardboard industries (CI) waste was analyzed. The

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present study was thus planned to assess the genotoxic potential of mushroom *P. florida* cultivated on handmade paper and cardboard industrial waste. This will help us not only in generating extra source of income but also providing an attractive way of managing industrial wastes.

[II] MATERIALS AND METHODS

P. florida spawn was purchased from National Research Centre for Mushroom, Chambaghat, Solan. This spawn was used for cultivating mushroom on handmade paper and cardboard industrial waste and their combination with wheat straw (WS) [1].

After harvesting of fruiting bodies (carpophores), aqueous extract of mushroom fruiting bodies was prepared by taking one gram of basidiocarp in homogenizer tube and adding 20ml of sterile distilled water. These samples were homogenized for 10min and raw aqueous extract of mushroom basidiocarps was collected in the tube by filtering the sample using simple filter paper.

Genotoxicity of this aqueous extract were analyzed by *Salmonella*/Microsome reversion assay or Ames assay using *Salmonella typhimurium* TA 98 and TA 100 in the presence and absence of S9 mix [4, 5, 6]. In this study, five dose levels of individual samples (2µl, 5µl, 10 µl, 50µl and 100µl) were tested. The revertant colonies were clearly visible in a uniform background lawn of auxotrophic bacteria revertants appeared on plate were counted for each dose level. Positive controls used for TA 98 and TA 100 were 2- Nitrofluorine (1 µg / plate: 104 revertants) and Sodium azide (1 µg / plate: 594 revertants) respectively. Sterile distilled water was used as negative control.

Analysis of data was done using mutagenicity ratio as described by Mortelmans and Zeiger, 2000 [6]. 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). The following values of spontaneous revertants were obtained for the two strains: Revertant / plate: without metabolic activation TA 98 (28), TA 100 (146); with metabolic activation, slightly higher values were obtained: TA 98 (43), TA 100 (251). Genotoxicity ratio of 2.0 (two fold) or more is regarded as a significant indication of genotoxicity and the compound is considered significantly mutagenic [6]. Experiment was repeated five times and data was pooled and also analyzed statistically by one way ANOVA only at specific dose level i.e. 100 µl.

[III] RESULTS AND DISCUSSION

Results of mutagenicity analysis of *P. florida*, cultivated on industrial sludges and their combination with wheat straw are depicted in Table 1. In this study, aqueous extract of *P. florida* , cultivated on 100% Wheat straw (WS), produced 93 and 81 revertants/ 100 µl of sample with *S. typhimurium* strain TA 100 and TA 98 respectively in the absence of S9 mix. These numbers of revertants were found to increase (TA 100: 226 revertants and TA 98:112 revertants/100 µl of sample) on adding S9 fraction of mouse liver. However, in this case mutagenicity ratios were found to be below 2 indicating that revertants appeared on the plate is mainly spontaneous revertants. Hence, carpophores cultivated on wheat straw were found to be non-mutagenic. In this investigation, these carpophores were used as control for comparing the mutagenicity of *P. florida* cultivated on industrial sludge.

When handmade paper industrial waste alone was used for mushroom cultivation then number of revertants (123 revertants/100µl of sample) was found to increase over control (93 revertants/100µl of sample) with strain TA 100 in the absence of S9 mix. These revertants were found to increase (238 revertants/100µl of sample) on adding hepatic fraction of mouse liver but still aqueous extract cannot be considered as mutagenic due to having mutagenicity ratio below 2. Similarly, aqueous extract of *P. florida* cultivated on 100% HMPI waste did not show frameshift mutagenicity with strain TA 98, in the absence (80 revertants/100µl of sample) and presence (118 revertants/100µl of sample) of S9 mix as revealed by their mutagenicity ratio (<2).

Similarly, on using the waste of cardboard industries alone for mushroom cultivation, aqueous extract of mushroom was showing 170 and 247 revertants/100µl of sample with strain TA 100 in the presence and absence of S9 mix. Again, mutagenicity ratio was found to be below 2 which lead us to conclude that the revertants are spontaneous revertants. However, with strain TA 98, 84 revertants and 129 revertants/ 100µl of sample were found to be appeared on the plate. This case is considered as inconclusive because of having mutagenicity ratio is equal to 2.

In the present investigation, mushroom was also cultivated on combination of wheat straw and handmade paper and cardboard industrial sludge. When 50% WS was mixed with 50%HMPI waste, number of revertants (TA 100: 123 revertants and TA 98: 80 revertants/100µl of sample) was found to increase non-significantly over control but decrease over 100% HMPI waste. Addition of S9 mix was found to increase mutagenicity (TA 100: 238 revertants and TA 98: 118 revertants/100µl of sample) but still mutagenicity ratio was found to be below 2. Hence, mushroom cannot be considered as genotoxic.

On using the waste of 50% CI + 50% WS for mushroom cultivation, aqueous extract of mushroom showed 166 revertants and 79 revertants/ 100µl of sample with strain TA 100 and TA 98 respectively. On adding metabolic activation, these revertants increased to 240 and 122 revertants/ 100µl of sample. Again, mutagenicity ratio was found to be below 2 and differing non-significantly from control and 100% HMPI.

Most of the colored industrial wastes were found to possess mutagenicity. The possibility of utilizing these colored industrial sludges in the production of edible mushrooms implies the risk of bringing mutagenic substances into human food chain. In this study, amazingly, it was found that *P. florida* cultivated on colored handmade paper and cardboard industrial sludge and its combination was found to be non-genotoxic inspite of having colored industrial waste. This may be possibly due to some anti-genotoxic capacity of *P. florida* which has been reported for other mushroom species also including *Pleurotus* [7, 8, 9]. This study shows that *P. florida* cultivated on handmade paper and

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cardboard industrial waste may be safe for consumption due to having no genotoxicity.

[V] CONCLUSION

Mushroom cultivation is usually rural practice which takes advantage of cheap labour instead of large investment. Labourers and workers working in these industries can collect waste from these industries in spare time and use it for *P. florida* cultivation. Besides reducing the waste, this technology will also provide extra earning to them as Jaipur is center of attraction for tourist who are fond of mushroom and its recepies.

Table: 1. Mutagenicity of aqueous extract of *Pleurotus florida,* cultivated on sludge of handmade paper and cardboard industries alone and its combination with wheat straw

S. No	Substrate used	Dose (in µl)	Raw mushroom extract			
			TA100		TA98	
			S9-ve	S9 +ve	S9-ve	S9 +ve
1	100% WS	2	25.2 ± 2.4 (-)	66.7 ± 5.1(-)	12.1 ± 3.1 (-)	15.1 ± 1.2 (-)
		5	42.1 ± 0.09(-)	91.1 ± 8.6(-)	17.5 ± 4.5 (-)	28 ± 3.2 (-)
		10	65.6 ± 4.8(-)	134.0 ± 12.1(-)	39.8 ± 11.2 (-)	57.0 ± 21.5(-)
		50	88.3 ± 5.4(-)	186.1 ± 13.4 (-)	64.4 ± 15.2 (-)	84.3 ± 18.9 (-)
		100	93.4 ± 3.2(-)	226.3 ± 14.5 (-)	81.3 ± 27.9 (-)	112.1 ± 21.2 (-)
2	100% HMPI waste	2	33.4 ± 1.1(-)	65.3 ± 5.6 (-)	10.2 ± 0.02 (-)	15.3 ± 3.4 (-)
		5	56.3 ± 3.1(-)	103.4 ± 4.5 (-)	24.0 ± 1.09 (-)	32.5 ± 8.9 (-)
		10	87.8 ± 4.5 (-)	146.2 ± 13.1 (-)	46.2 ± 6.5 (-)	55.7 ± 15.6 (-)
		50	98.2 ± 3.8 (-)	182.0 ± 13.2 (-)	68.8 ± 10.8 (-)	75.5 ± 16.8 (-)
		100	123.4 ± 4.2 (-)	238.2 ± 20.8(-)	80.4 ± 14.9 (-)	118.4 ± 23.5 (-)
3	50% WS + 50% HMPI waste	2	28.3 ± 4.5 (-)	58.3 ± 5.6 (-)	9.6 ± 3.4 (-)	11.1 ± 3.6 (-)
		5	45.5 ± 4.4 (-)	87.1 ± 6.8 (-)	20.2 ± 4.5 (-)	23.4 ± 2.5 (-)
		10	77.1 ± 8.6(-)	140.1 ± 13.8(-)	45.2 ± 6.7 (-)	58.1 ± 7.8 (-)
		50	91.3 ± 7.8 (-)	173.4 ± 14.5 (-)	67.1 ± 10.1 (-)	76.8 ± 12.1 (-)
		100	117.3 ± 15.9 (-)	229.0 ± 30.1 (-)	77.4 ± 21.2 (-)	100.3 ± 30.9 (-)
4	100% CI waste	2	42.4 ± 5.6 (-)	50.8 ± 5.6 (-)	16.5 ± 4.5 (-)	20.0 ± 2 (-)
		5	81.3 ± 6.7(-)	97.3 ± 7.6 (-)	27.3 ± 7.9 (-)	43.3 ± 4.1 (-)
		10	110.4 ± 17.8 (-)	116.9 ± 12.1 (-)	40.2 ± 11.3 (-)	66.6 ± 17.8 (-)
		50	146.8 ± 16.6 (-)	195.3 ± 23.4(-)	68.3 ± 13.6 (-)	97.7 ± 14.6 (-)
		100	170.1 ± 23.3(-)	247.3 ± 30.4 (-)	84.1 ± 15.1 (i)	129.1 ± 11.9 (i)
5	50% WS + 50% CI waste	2	35.4 ± 5.4(-)	48.3 ± 4.6(-)	10.1 ± 1.01 (-)	12.5 ± 3.6 (-)
		5	84.2 ± 5.6 (-)	90.1 ± 5.4 (-)	22.3 ± 10.4 (-)	25.4 ± 4.5 (-)
		10	103.4 ± 11.5 (-)	103.4 ± 10.2 (-)	43.3 ± 17.6 (-)	59.5 ± 6.3 (-)
		50	125.8 ± 12.7 (-)	180.5 ± 24.4 (-)	62.5 ± 21.5 (-)	86.3 ± 12.4 (-)
		100	166.1 ± 30.1(-)	240.5 ± 35.1 (-)	79.5 ± 12.8 (-)	122.6 ± 14.8 (-)

Symbols: (-): Mutagenicity ratio below 2 (non-mutagenic); (i): Mutagenicity ratio inconclusive; Numerals are showing mean number of revertants ± Standard deviation; WS-Wheat Straw; HMPI-handmade paper Industries; CI-Cardboard Industries

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