Bull. Environ. Contam. Toxicol. (1999) 63:560-566

Bulletin of Environmental Contamination and Toxicology

Genotoxic Activity of Vinasse and Its Effect on Fecundity and Longevity of *Drosophila melanogaster*

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Received: 7 September 1999/Accepted: 30 July 1999

Vinasse is a wastewater of alcohol factories. Approximately 9-14 liters of vinasse is obtained for I L of alcohol as a wastewater during alcohol production. It has a high pollution potential. The chemical oxygen demand and biochemical oxygen demand values of this wastewater may be as high as 85 g/L and 70 g/L, respectively. There have been various studies on degradation of vinasse (Ohmomo et al. 1987; Yesilada and Fiskin 1995). It is well know that these types of wastes have negative and toxic effects in soil and plants (Srivastava and Sahai 1987; Algur and Kadioglu 1992). The environmental impact of vinasse is very high, due to its organic and inorganic matter content and its dark color. The dark color of vinasse is caused by high molecular weight compounds (Maestro-Duran et al.1993; Pena Miranda et al. 1996).

Now, to improve our knowledge on the mechanisms of vinasse toxicity, *D. melanogaster* was chosen as the organism of study because of its well-defined genetic and biochemical characteristics. In this context, the effect of vinasse on the fecundity and longevity of this organism was investigated. Also, the genotoxic effect of vinasse and its effect on larval survival ratio was tested. In a previous study, it was found that adult life-span and fecundity depend on both the genetic composition of an organism and various environmental factors (Bozcuk 1981; Yesilada and Bozcuk 1995). The genotoxic effect test was carried out using the wing Somatic Mutation and Recombination Test (SMART) of *D. melanogaster.* SMART is a rapid and inexpensive *in vivo* assay which detects genotoxic agents using somatic cells of a higher eukaryote. In this test, both somatic mutation and mitotic recombination are being screened and test itself is quite sensitive to a wide variety of both direct-acting mutagens and mutagens that require bioactivation (Frei et al. 1992; Graf 1995; Graf and Würgler 1996). This test is based on the principle that, the loss of heterozygosity of suitable marker genes in cells from imaginal disks of larvae can cause the formation of clones of mutant cell colonies. Later on, the mutatic effect is being expressed as spots on the wing of adult flies. Several parameters influencing the frequency and/or the size of the spots on the wings have been studied (Graf 1995).

MATERIALS AND METHODS

The Oregon-R strain of *Drosophila melanogaster* was used as a test strain for the effect of vinasse on fecundity and longevity. The flies were kept at constant

25±1°C on standard cornmeal-agar medium (Bozcuk 1978) and were always kept in dark, except during transfers and counting of eggs. To test the effect of vinasse on fecundity, 1 female and 3 males which were raised on standard medium, were transferred to empty glass culture bottles. Then spoons, containing culture media (0%, 25%, 50% and 75% vinasse), were placed in these culture bottles. These spoons were changed for every 24 hr and the eggs were counted for a period of 10 days. It was stated that in the first 10 days of adult life, the egg production was a good reference for the whole adult life egg-production of this organism (Mc Millan et al. 1970; Yesilada and Bozcuk 1995).

The effect of vinasse on the longevity of *D. melanogaster* was investigated on 10 flies which were initially placed in 2.5x7.5 cm glass vials containing culture media (0%, 25%, 50% and 75% vinasse). The flies used in the experiments were virgins. The experimental populations could be considered as contemporaneous. One hundered flies were used to generate each survival curve. The number of surviving flies were transferred (twice a week) to fresh medium. The statistical analysis of the results were carried out using analysis of variance (ANOVA) and Student's t test.

The genotoxic effect of vinasse was evaluated by somatic mutation and recombination test. We used two stocks of *D. melanogaster* for a cross of mwh males with flr^3/In(3LR)TM3,$ri\ p^p$sep $bx^{34e}e^s$ Ser females. Detailed information on the genetic markers of these stock organisms is given by Lindsly and Zimm (1992). These stocks were orginally obtained from laboratory of Ulrich Graf, Swiss Federal Institute of Technology (ETH) and University of Zurich. Eggs from the cross mentioned above were collected for 8 h and 72±4 hr later the larvae were floated off the food with a 17% NaCl solution. Groups of 50 larvae were transferred to individual glass vials containing food prepared from 0.5 g of Drosophila Instant Medium (Ward's Biology) and 2 mL of the test solution which varried in vinasse content (0%, 25%, 50%, 75% and 100% vinasse). A 5 mM aqueous solution of ethyl methanesulphonate (EMS;Merck) served as a positive control. The adult flies eclosed by the treatment of vials were collected on days IO-12 after egg laying. The number of eclosions was also counted and the survival rate was calculated. Only the wing of trans-heterozygous individuals (mwh flr^+/ mwh^+flr^3) were mounted and scored for the occurrence of spots (i.e. mwh or flr^3 single spots or mwh/flr^3 twin spots). The size of the mutant spots was determined by counting the number of mutant cells in each spot. Three types of spots were evaluated separately. The wing spot data of treated and control series were compared by conditional binominal test (Frei and Würgler 1988). Each statistical test was performed at the 5% significance level. Based on the number of mwh clones, the number of wings analysed, and the number of cells scored in each wing (approx. 24,400) the clone formation frequency per cell cycle and 10^5 cells was calculated (Frei et al. 1992).

RESULTS AND DISCUSSION

Table 1 shows the effect of different concentrations of vinasse on fecundity. The mean daily egg-production per female during the first 10 days of adult life was 7.21 for untreated control group. However, the mean daily egg production decreased to 5.55, 4.58 and 3.61 in 25%, 50% and 75% vinasse treated groups, respectively. These results showed that fecundity decreased with the increasing

concentrations of vinasse. The difference between the control and vinasse treated groups was statistically significant.

Table I. Effect of vinasse on mean daily egg-production of *D.melanogaster*

Vinasse Conc. (%)	Number of f emale	Egg-prod. Per female ± S.E.	S.D	P
0 (control)	17	7.21 ± 0.54	2.22	
25	17	5.55 ± 0.59	2.41	< 0.05
50	16	4.58 ± 0.72	2.86	< 0.01
75	16	3.61 ± 0.66	2.65	< 0.001

S.E.: Standard error of the mean, S.D.:Standard deviation

Life table was constructed for both sexes of the treatment groups. The life span of controls are 77.52 and 83.10 days for males and females, respectively, as shown in Table 2. The difference between the mean life-span of control and 25%, 50% and 75% vinasse treated groups were all significant in favour of control. But there was no difference between the mean life span of control females and 25% vinasse group of females. In addition, there was significant difference between the opposite sexes of all groups and was so between the mean life span of females and males, females having a longer life span than males. In order to illustrate the effect of vinasse on longevity, the survival curves were drawn as shown in Fig. 1 and Fig. 2.

The results of the experiments of the wing spot test was given in Table 3 based on the data from three categories: small single spots (1 or 2 cells in size), large single spots (3 or more cells) and twin spots. Also, the number of total spots

Table 2. Effect of vinasse on mean life-span of *D. melanogaster*

Class no	Vinasse Concn. (%)	Sex	No. of flies	Mean life-span (days) ± S.E.	S.D.	Significant differences between some classes (P<0.05)
1	0(control)	M	100	77.52 ± 1.53	15.30	1-2 1-3
2		F	90	83.10 ± 1.61	15.31	1-5 1-7
3	25	M	100	64.97 ± 1.22	12.17	2-6 2-8
4		F	100	84.35 ± 1.63	16.29	3-4 3-5
5	50	M	100	58.68 ± 1.28	12.81	3-7 4-6
6		F	100	72.15 ± 1.20	11.96	4-8 5-6
7	75	M	100	72.79 ± 1.17	11.70	5-7 6-8
8		F	100	77.87 ±0.94	9.44	7-8

S.E.: Standard error of the mean, S.D.: Standard deviation.

was given in this table. The small single spots and large single spots were assumed to be due to gene mutations, chromosomal deletion, non-disjunction, or

mitotic recombination. The twin spots were assumed to be the products of the mitotic recombination (Graf et al. 1984). In addition, the number of spots with a mwh clone formation frequency per 10^5 cells was presented in this table.

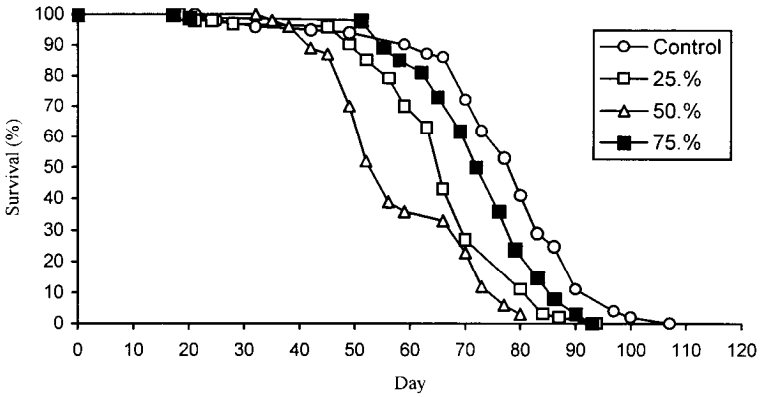

Figure 1. The survival curves for the males of control and vinasse treated groups of *D. melanogaster.*

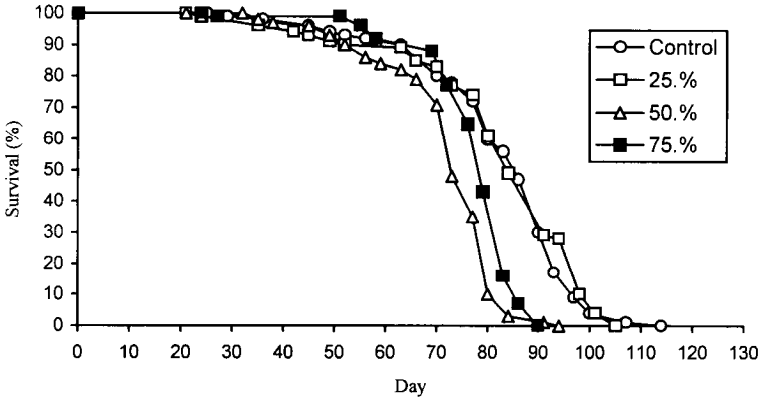

Figure 2. The survival curves for the females of control and vinasse treated groups of *D. melanogaster.*

The frequency of total spots per wing (sp/w) was 0.04 for the negative control and 6.88 for the positive control (5mM EMS). Different concentrations of vinasse gave inconclusive and positive results for all of the spots types and also for the total spots. But no dose-related effects could be observed. The only group which showed positive result for twin spots was 50% vinasse treament group. All the other vinasse treated groups gave inconclusive results for twin spots. While the

Table 3. Summary of results obtained from *Drosophila* wing spot test

			Spots Per wing (no.of spots) and stat. diagn.[a]				
Concn. (%)	Survival ratio (%)	Wings (no.)	Small single spots (l-2 cells) m= 2.0	Large single spots (>2 cells) m=5.0	Twin spots m= 5.0	Total spots m= 2.0	Frequency of clone formation per 10^5 cells[b]
0	89.26	430	0.03 (l5)	0.0l(3)	0.00(1)	0.04(19)	0.2
25	54.49	110	0.16 (18)+	0.02(2)i	0.00(0)i	0.18(20)+	0.4
50	50.43	125	0.09 (11)+	0.01(l)i	0.02(3)+	0.12(l5)+	0.3
75	51.01	229	0.07 (15)i	0.03(7)+	0.02(4)i	0.12(27)+	0.5
l00	46.77	279	0.10 (29)+	0.01(4)i	0.01(4)i	0.13(37)+	0.2
5mM (EMS)	67.88	49	3.06 (150)+	3.02(148)+	0.80(39)+	6.88(337)+	23.8

m = multiplication factor

a Statistical diagnoses according to Frei and Würgler(1988): + = positive; - = negative; i = inconclusive. The conditional binomial test, one-sided, probability levels: $\alpha = \beta = 0.05$

b Frequency of clone formation per 10^5 cells: mwh clones/wing/24400 cells (without size correction).

highest frequency of small single spot was obtained with 25% vinasse, the highest frequency of large single spots was obtained with 75% vinasse. The frequency of clone formation per 10^5 cells higher than 2.0 is indicative of genotoxic activity of the particular treatment (Graf et al. 1994). In the present study, while the clone formation frequency for the negative control was 0.2, it was 23.8 for the EMS positive control. As it is seen from Table 3, this frequency varies between 0.2 and 0.5 for those vinasse treated series. Here, it was suggested that although there were small differences between control and vinasse treated groups, vinasse was not an effective genotoxic agent. All concentration of vinasse (25%, 50%, 75% and 100%) and EMS (5mM) caused a decrease in larval survival (Table 3).

In some previous studies, it was reported that besides other compounds, vinasse contained phenolic compouns and several heavy metals (Yesilada and Fiskin 1995; Garcia et al. 1997). Heavy metals are assumed to be effective agents, reducing the growth rate, viability and the protein synthesis (Waren and McCarrol 1990; Theodore et al. 1991). In this regard, heavy metals and fenolic compounds might have a negative effect on fecundity, larval survival and longevity at higher concentrations of vinasse.

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