

## EFFECTS OF TREATMENT WITH ZINC SULFATE AND COPPER ACETATE ON A BACK-MUTATED *DROSOPHILA MELANOGASTER* POPULATION

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**Abstract:** Our study is focused on monitoring and comparing the prolificity of *Drosophila melanogaster* (grown on culture medium enriched with three different concentrations of zinc sulfate and respectively copper acetate) for three successive generations and those sex ratio. The occurrence of new mutations, the differences between wild type and mutants mutability, but also the differences of effects depending on concentrations of tested solutions was monitored.

### INTRODUCTION

Zinc and copper are oligominerals of exceptional biologic importance. In increased amounts, copper and zinc salts can be poisonous to higher organisms, leading to metabolic disorders, with serious consequence on health. Copper deficiency can often produce anemia-like symptoms. Zinc deficiency depressed growth, delayed sexual maturation, eye and skin lesions, impaired appetite, depresses immunity. The aim of this work was to characterize two populations of *Drosophila melanogaster*: a natural population, from Iași, and a mutant (Ebony) experimental population, regarding the prolificity, the eclosion duration and the sex ratio in three generations, under the influence of consanguinization and of treatment with different concentrations of zinc sulfate and respectively copper acetate. Different effects of the actions of some chemical substances on *Drosophila melanogaster* individuals, were showed by Ciobotari and Băra (2005), and also by Costan and Băra, (2008).

### MATERIAL AND METHODS

The individuals of *Drosophila melanogaster* were grown in laboratory on standard medium (control variants) and on medium enriched with following concentrations: 0,001%; 0,005%; 0,0025% of zinc sulfate and respectively 0,001%; 0,005%; 0,0025% copper acetate solutions. There were monitored three successive generations.

In the case of the first generation were used: 1 vial with standard medium for control, and one vial for each experimental variant (represented by a specific zinc sulfate and respectively copper acetate solution concentration).

For the next two treatment generations, two vials were used for each experimental variant.

In each vial were placed two *Drosophila melanogaster* females and two males, belonging to back mutant line, resulting from the cross between Ebony mutant line and natural population from Iași. It is known that Ebony mutant line is very instable, reaching a high level of back mutations.

The flies were observed for few days, to notice if females were fertilised, and then set free by opening the vial. After about one week it was checked adults occurrence (first generation), every day at the same time.

### RESULTS AND DISCUSSIONS

The obtained results pointed out some effects induced by mentioned compounds, depending on their concentration and on duration of treatment.

In the case of our experiment, it was observed that for 0,001% zinc sulfate solution concentration, the average number of individuals were 482 per vial, for 0,0025% concentration, there were 431 flies per vial, and for 0,005% concentration the average number was 450 per vial. In the case of control variant, the average number was 423 individuals per vial. It could be noticed the stimulating effect of zinc sulfate on the prolificity of the flies in the first generation, especially for the 0,001% solution concentration, when the number of eclosed individuals increased with 12,24% comparing to control. In the case of 0,0025% solution concentration, the number of individuals increased with 1,85% comparing with control, and for 0,005% solution concentration, with 6% comparing with control.

In the second generation, the number of individuals decreased with 9,46% comparing with controle in the case of treatment with 0,001% solution, and increased with 22,54% for the 0,0025 solution concentration, and with 11,08% for the 0,005% treatment concentration.

In the third generation, the number of individuals decreased with 6,53% comparing with controle variant in the case of treatment with 0,001% solution concentration, and increased with 29,30%, in the case of treatment with 0,0025% concentration, and only with 16,56% in the case of treatment with zinc sulfate solution with 0,005% concentration. It could be noticed the stimulating effect of zinc sulfate on the prolificity of the flies for the 0,0025% solution concentration (Fig. 1.).

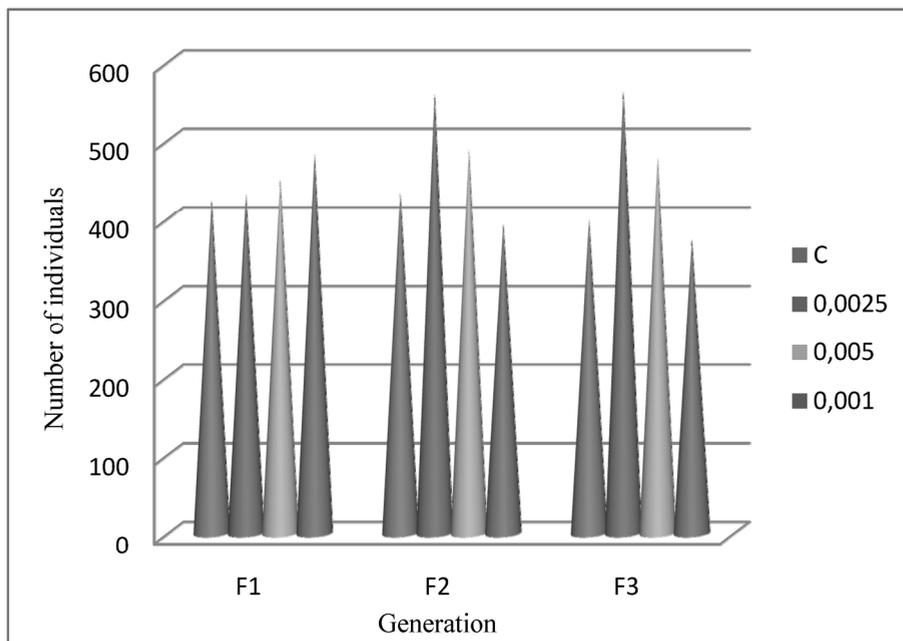


Fig. 1. Number of individuals obtained in three successive generations, after treating with different concentrations of zinc sulfate solutions

By comparing the number of eclosed individuals per vial, for each concentration of zinc sulfate solution in the three generations, it could be noticed that it decreased from generation to generation for zinc sulfate solution 0,001%, but for the other two concentrations (0,0025% and 0,005%) increased (Fig. 2). So, this chemical substance, in all concentrations, had in the first generation benefic effects on individuals prolificity, similar to the results obtained by *Ciobotari and Băra*, (2005), after treatment with with lead acetate, K<sub>2</sub>MnO<sub>4</sub>, caffeine, urea, phenol, nicotine, and E vitamin, administered in different concentrations on *Drosophila melanogaster* individuals, from natural populations. The benefic effect is preserved in the further generations just for the higher concentrations, maybe because the chemical is accumulated from medium inside the cell and the persistence in the next generation depends on cells needs for this substance.

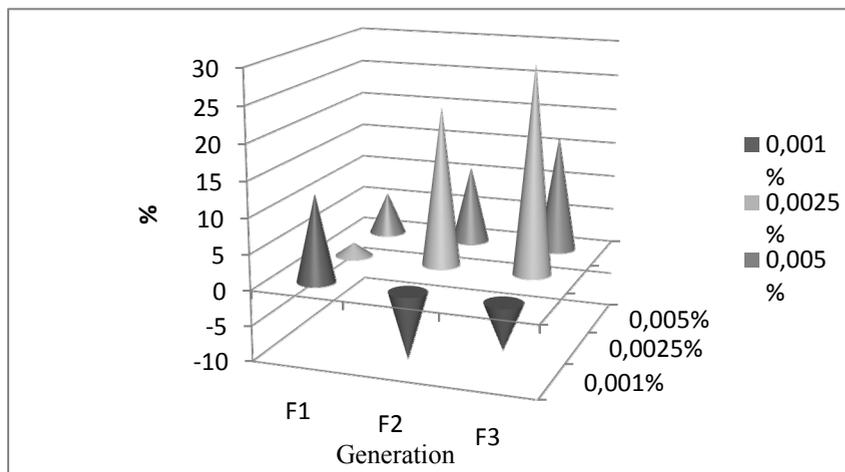


Fig.2. Percent of individuals per vial, during three successive generations after treating with different concentrations of zinc sulfate solutions

By treating with copper acetate, in the first generation, the number of individuals decrease comparing with controle with 7,56% for 0,001% solution concentration, with 34,51% for 0,0025% concentration and with 20,33% for 0,005% concentration. It could be noticed the inhibitory effect of treatment on the prolificity of *Drosophila melanogaster*, especially in the case of treatment with 0,0025% copper acetate solution concentration.

In the second generation, the number of individuals decrease comparing with controle with 39,95% for 0,001% solution concentration, with 52,65% for 0,0025% concentration and with 40,31% for 0,005% concentration. It could be noticed the inhibitory effect of treatment on the prolificity of *Drosophila melanogaster*, especially in the case of treatment with 0,0025% copper acetate solution concentration. The inhibitory effect of treatment was again stronger for 0,0025% concentration of copper acetate solution.

In the third generation, the number of individuals decrease comparing with controle with 40,45% for 0,001% solution concentration, with 54,27% for 0,0025% concentration and with 51,50% for 0,005% concentration. If we compare the percentages of individuals hatched per tube, for each concentration of copper acetate, over the three generations, there is a sharp decrease in the number of individuals in each generation treatment, the strongest inhibitory effect was again for 0,0025% concentration of copper acetate solution.

Regarding sex ratio, it could be noticed that, in the first generation after treatment with zinc sulphate (Fig. 5), 0,001%, were obtained 215 females and 267 males per vial, for 0,005% concentration 210 females and 240 males per vial, and for 0,0025%, 191 females and 240 males. For the controle, there were 208 females and 206 males per vial. There is a positive influence of zinc sulfate on male hatching, in the first generation. In the second generation, for zinc sulfate 0,001%, the average number per vial was 227 for females, and 182 for males. For 0,005% zinc sulfate solution: 253 females and 234 males. For 0,0025% zinc sulfate solution concentration, 307 females and 257 males. In the third generation, for 0,001% solution

concentration, the average number per vial was 202 females and 220 males. For 0,005% solution: 259 females and 218 males, and for 0,0025% solution, 314 females and 254 males. (Fig. 5).

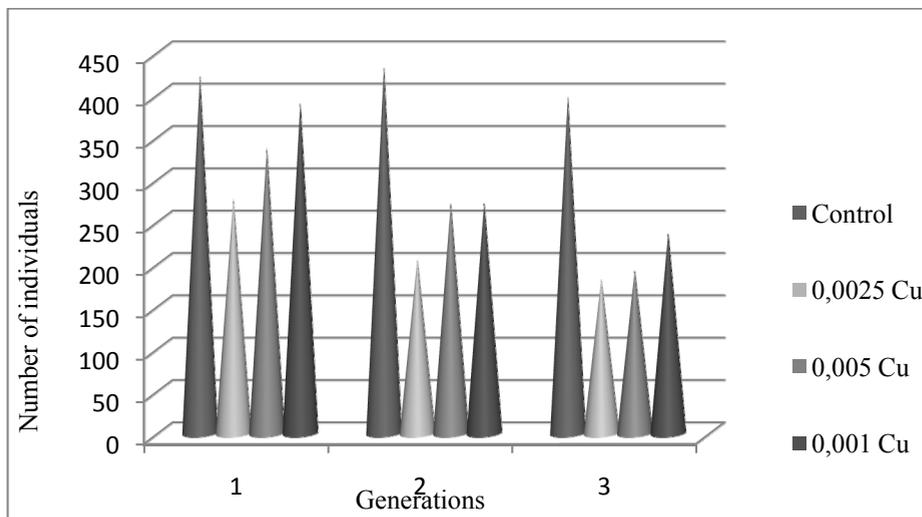


Fig.3. Comparison between the number of individuals of *Drosophila melanogaster*, obtained over three generations treated with three solutions of different concentrations of copper acetate, relative to controle.

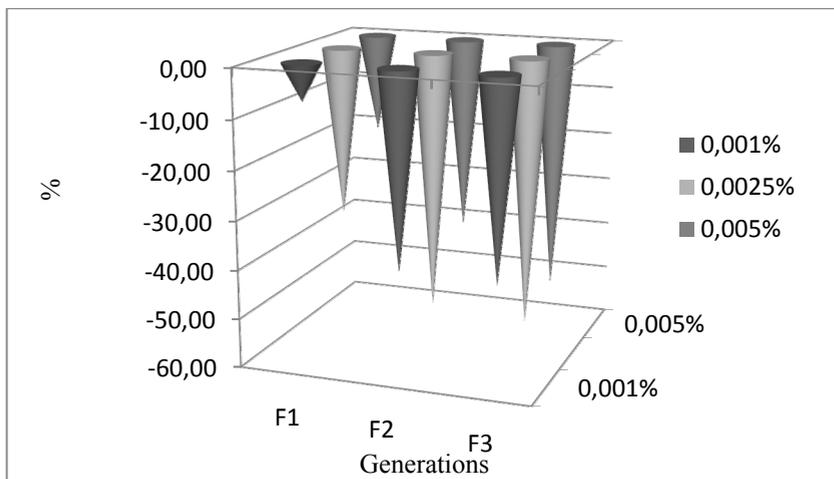


Fig.4. Comparison of percentages of numbers of individuals hatched per tube, over the three generations of *Drosophila melanogaster* treated with copper acetate solution.

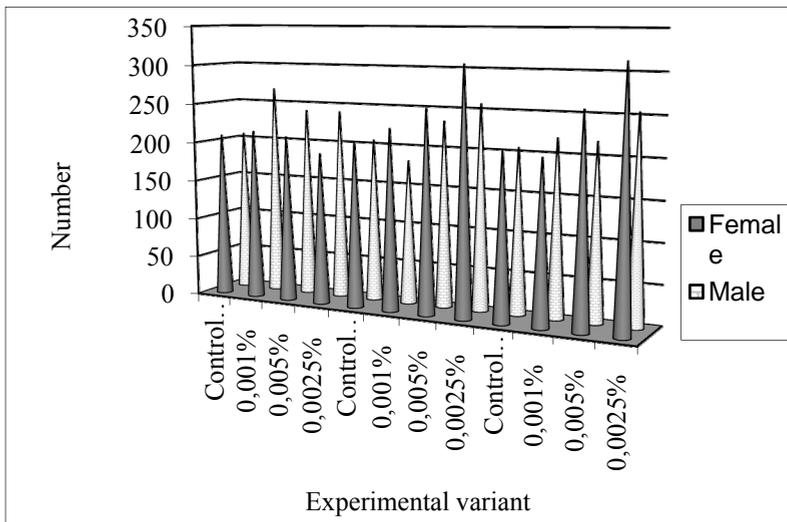


Fig.5. Comparison between the number of individuals, separated by sex, under treatment with zinc sulphate

For samples treated with copper acetate, the first generation recorded a number of 205 females and 106 males for 0,001% solution, 177 females and 160 males for 0,005% concentration, and for 0,0025%: 147 females and 130 males

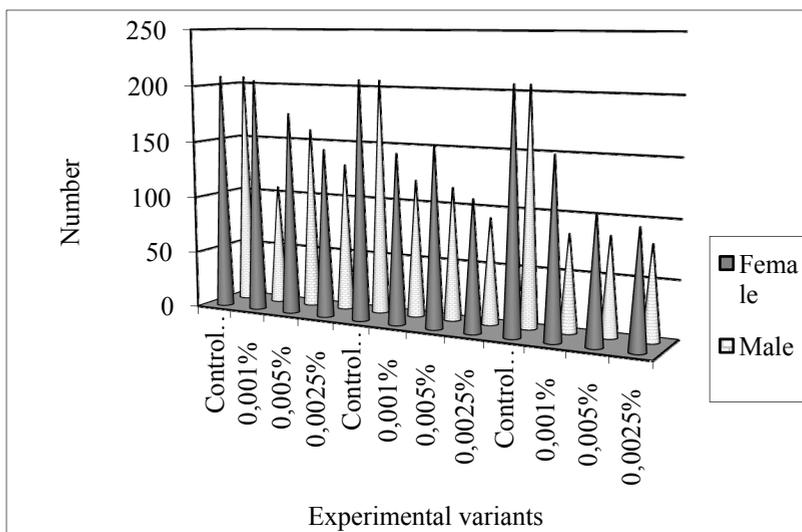


Fig.6. Comparison between the number of individuals, separated by sex, under treatment with copper acetate.

In F2, 147 females and 120 males for 0,001% solution, 156 females and 116 males for 0,005% concentration, and for 0,0025%: 113 females and 92 males. In F3, 153 females and 84 males for 0,001% solution, 108 females and 85 males for 0,005% concentration, and for 0,0025%: 100 females and 81 males (Fig. 6).

Regarding controle, ratio between number of individuals belonging to the wild type and mutant remains similar: 50,83%, Ebony, and 49,17% wild type from the total number of individuals. In the case of treatment with zinc sulfate, ratio between Ebony and wild type, differs from controle. Increase to 86,43% for wild type and decrease to 13,57 for Ebony, in the first generation. In F2, there are 88,73% wild type individuals, and 11,27% mutant, and in F3 75, 65% wild type comparing to 24,35% mutant (Fig 7).

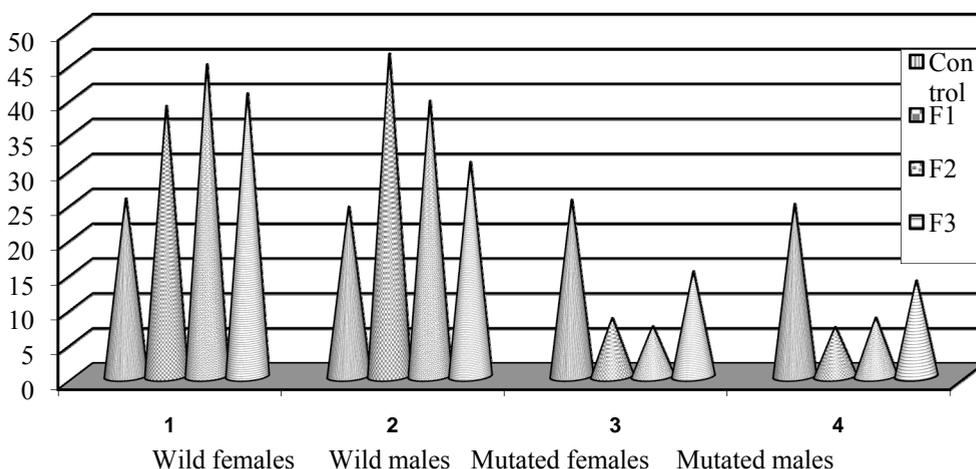


Fig.7. The dynamic of wild type and Ebony *Drosophila melanogaster* female and male individuals, after treating with zinc sulfate.

By treating with copper acetate, in F1 was obtained a rate of 82,67% wild type individuals, comparing to 17,32% Ebony. Similar percentage was obtained in F2 and F3. This result shows that copper acetate has an inhibitory or lethal effect on Ebony type individuals. We concluded that the concentration values which had stimulating or inhibiting effects are specific for each used substances, as shown also by *Ciobotari and Băra* (2005) or *Costan and Băra* (2008).

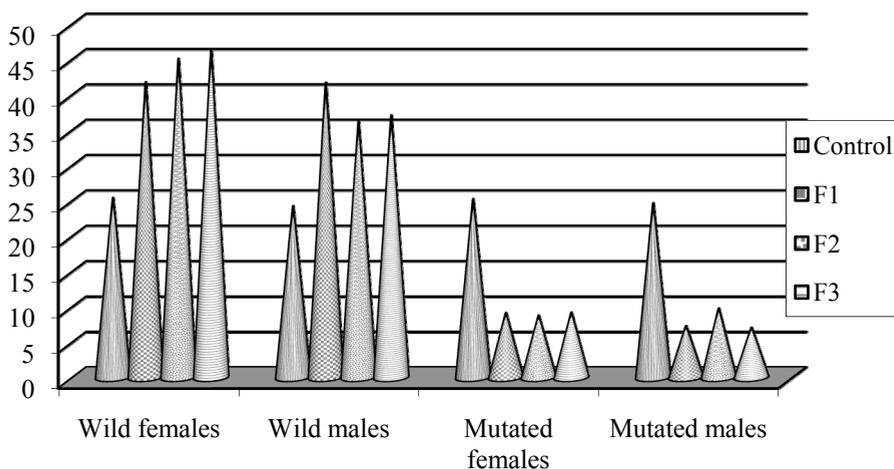


Fig.8. The dynamic of wild type and Ebony *Drosophila melanogaster* female and male individuals, after treating with copper acetate.

Regarding aberrations type, could be noticed only those concerning wings: curly wings, curved wings, vestigial or reduced wings, deformed wings, for both wild type or Ebony individuals. In F1, the percent of new occurred mutations is low (1,2-1,6% for wild type and 4,5-8,3% for mutant type after treating with zinc sulfate, and 0,8-2,65% for wild type and 5,3-7,6% for Ebony after treating with copper acetate), in F2, F3 it could be noticed an increase of mutants percentage. If for the control the period of occurrence of adults, is 7 days, in the case of treatment with both substances, the first generation required 8 days, and for the next two generations the period is spread over ten days. This proves that both substances slow down the processes of maturation of *Drosophila melanogaster*, more pronounced in F2 and F3 generations.

### CONCLUSIONS

The two substances, zinc sulfate and copper acetate acts differently on individuals of *Drosophila melanogaster*.

Zinc sulfate has a benefic effect on *Drosophila melanogaster* prolificity, effect that increases with each generation for 0,005% and 0,0025% solution concentration.

Contrariwise, for 0,001% zinc sulfate, it could be noticed an inhibitory effect.

Copper acetate has a pronounced inhibitory effect from the first generation, an effect that is amplified in F2 and then F3 to all three concentrations applied.

Both substances have inhibitory effect on mutant type Ebony.

Aberration frequency increased when treatment with copper acetate with decreasing number of individuals.

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