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Combined Effect of Heavy Metal Pollution on Oviposition Rhythm of *Drosophila Melanogaster*

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ABSTRACT:

The egg laying rhythm is complex phenomenon. It is regulated both by external and internal environment in insects. Circadian timer governs egg laying rhythm it is shown that the period of the rhythm remains more or less unchanged with change in temperature and nutrition with in permissible range. Heavy metals have been considered as one of the key environmental toxicants with a wide range of health effects on humans. The bulk of information available today is mainly focused on the single toxin studies with only few studies enumerating the synergistic, additive or protective effects of these heavy metals. *Drosophila* has great potential as a model system for studying toxic effects because they have metallothione similar to those of mammals. In current study, *Drosophila* was used as a model to investigate the effects of two prominent heavy metals- Lead (Pb) and Zinc (Zn) with respect to the oviposition rhythm of the *D. melanogaster*. Flies were treated with different doses of the lead, Zinc and zinc+lead (Lt1 & Lt2: Zt1 and Zt2; St1 and St2). Subsequently, number of eggs produced/ female in four hour interval (7AM, 11AM, 3PM, 11PM, 7PM, 3AM) was recorded and subjected to chi-square periodogram and ANOVA. Our results show that developmental exposure of sub lethal concentrations of lead acetate and zinc chloride provokes disturbances development of the flies, hence reduced in the offsprings. Whereas timing of the egg laying period is not affected. Physiology of insect reproduction is highly protected by the external environment

KEYWORDS:(*Drosophila melanogaster*, Lead acetate, Zinc chloride, oviposition rhythm, Circadian rhythm)

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INTRODUCTION:

Heavy metals are the known toxicants used in many industries; some metals act as trace elements needed in small amounts for eg, Zinc, lead, copper, iron etc., when it is expose to beyond limit the cells metallothione to counteract with the excess metals. Due to industrialization and other reasons the number of toxic elements and its exposure has become mandatory, researchin this field focused predominantly in only few toxicants viz., arsenic (As), mercury (Hg), lead (Pb), and cadmium (Cd). Part of this "preference" reflects an elevated toxicity and a high risk of exposure for these four elements, which place them at the top of the priority list of hazardous substances of the Agency for Toxic Substances and Disease registry (ATSDR, 2016). Recently, several natural and anthropogenic sources of exposure to several toxic elements have been identified in various regions around the globe. However, conventional toxicology testing (basic experimental toxicology and risk assessment evaluation) does not account for this complexity. In the real world any living organism (plant or animal) is exposed to a mixture of substances, some potentially more toxic than others synergistic effect much more severe than the single metal[1]. Long term exposure to such toxicants may have an effect on their fitness and other health hazards. Excess accumulation of copper zinc forms a major causative group for cognitive, neurological, nutritional and physiological implications[2.] *Drosophila* has great potential as a model system for studying toxic effects because*Drosophila* has a lot of similarities with human beings in cell signaling pathways as well as some protein-coding genes[3].The egg laying rhythm is complex phenomenon involving at least two separate physiological processes, vitellogenesis and egg retention[4,5.] Egg laying is continues to be rhythmic even in continuous light (24h LL), temperature compensated and nutrition compensated[6,7,8].Few studies have clearly demonstrated the fitness advantages of circadian response compared to the direct response to environmental stimuli. Hence, present work is undertaken to understand the whether oviposition rhythm is affectedby the heavy metals such as zinc, lead and its synergistic effect.

MATERIALS AND METHODS:

Oviposition rhythm

To analyze oviposition rhythm virgin males and females were collected from the experimental stocks which were aged for five days. A pair of male and female flies were transferred to vials(2.5×9.5cm) containing 3ml of wheat cream agar medium mixed with small amount of charcoal (for counting of eggs).

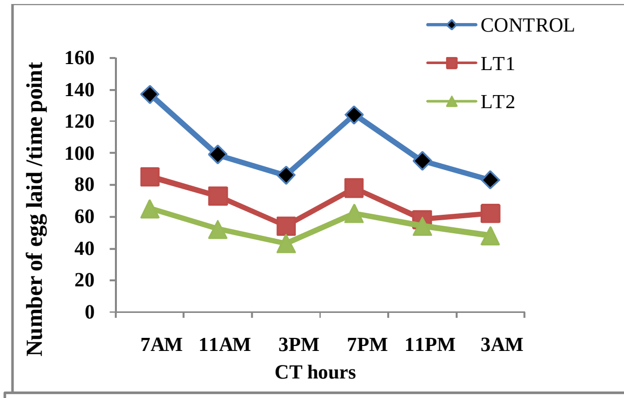
For the concentration we followed the method of Stafny and Shakunthala 2015. Two concentration groups were chosen on the basis of pilot experiment. It is abbreviated as LT1 and

LT2 for lead and ZT1 and ZT2 for Zinc chloride and ST1(LT1+ZT1) and ST2 (LT2+ZT2) for Lead+Zinc mixture.25 replicates for each concentration were maintained for each group. For control group heavy metal concentration free media were used. Pair of twenty five day old aged virgin male and female flies was transferred in to separate vials containing different concentration media. Every 4 hours the flies were transferred into vials with mediacontianing same concentrations for each group. The number of eggs laid for every 4 hour intervals i.e. 7AM, 11AM, 3PM, 11PM, 7PM, 3AM, in 24 hours was counted. This procedure was carried for 7days[9].

RESULTS AND DISCUSSION:

Fig.1 reveals that the oviposition rhythm in control and treated groups. The peak of oviposition observed in all the groups at 7Am and 7Pm. The periodicity is approximately 24 hours. Fig.2 the number of eggs laid at each time point by treated group is reduced compared to control. It is significant with F value 58.060, df 2. Percentage of eggs in control group is more compared to Lead acetate treated group. Eggs laid in LT2 group is the least when compared to LT1 and it is significant ($P<0.001$). Dose dependent reduction is observed. Fig.3 Similarly Zinc chloride treated group showed same result as Lead acetate treated ones. The egg laying rhythm is not affected by all the three groups. Peak of egg laying is at 7Am and 7Pm. Further the number of eggs laid at each time point was significant varied in different groups. Fig.4depict oviposition rhythm of Zinc chloride treated flies. When compared to control the eggs laid by Zinc chloride treated flies are significantly reduced. The ZT1 treated group showed better performance compared to ZT2. Comparison between the mean values is highly significant with F value 81.475, df 2, ($P<0.001$). Fig.5 divulges that the combined effect of two heavy metals on oviposition rhythm. These graphs further confirm that the oviposition rhythm is not affected by the heavy metal combined effect.Fig.6 Similar to their individual effect the number of eggs laid at each time point tremendously reduced by the joint effect and it is significant with F value 142.07, df 2 ,($P<0.001$).Of the three treatment groups used, the combined effect is maximum. Egg laying by the female is much affected compared to individual effect of zinc chloride or Lead acetate. Interestingly, the egg laying rhythm is not altered by the heavy metals used. The chi-square periodogram revealed that the period length of the entire group is ≈24h.

Heavy metals have become mandatory to many industry their exposure to human has become obvious. In the real world any living organism (both plant and animals) is exposed to mixture of substances, some potentially more toxic than others[1].Synergistic effect is seen on its oviposition preference and longevity[10, 11]. Long term exposure to such heavy metals may have severe effect on its fitness and other health hazards[12]. Many studies have also showed that excess accumulation of copper zinc forms a major causative group for cognitive, neurological, nutritional and physiological implications in humans[2]The endogenous control of daily rhythm is generally thought to be the fundamental adaptation of organisms anticipating the temporal organization of environment[13].Circadian timer governs egg-laying rhythm it is necessary to show that the period of the rhythm remains more or less unchanged with increase/decrease in temperature and nutrition within physiologically permissible range. In the present investigation, the timing of the egg laying is not affected by the heavy metal pollution. Though the immediate environment play a very important role on the eclosion, locomotor rhythm and courtship behaviour, timing of egg laying rhythm is not affected by heavy metal pollution. The physiology of reproduction in insects is highly protected by the external environment. Responses to metals are dose dependant. Increase in the concentration of metals decrease the egg production of the flies. As suggested by Bonneton[14]one of the reasons can be because higher concentrations of metals can interfere with the necessary enzymes for hormone production. Dhir and Dhand[15]have already shown that chronic exposure to lead on female rats affected their reproductive performance with sustained poor fertility. Early reports by Sutron and Nelson[15] showed that 0.5- 1% Zinc caused reduction in growth, anemia and poor reproduction in rats.Fouad et al[17], have revealed that heavymetals damage the intestine and reproductive tissues, histopathological study on the German cockroach have revealed that the alimentary canal, gonads and the abdominal tissue showed evident signs of damage. Further, they opine that these signs suggested irreversible metabolic alterations and disorders in the mitotic and meiotic pathways. This suggests that the reduction in the fecundity, fertility and viability is due to damage in the reproductive tissue. In the present investigation, though there is no effect on the oviposition rhythm the number of eggs produced by the lead, zinc and synergistic groups are reduced compared to the control group. Probably, reduction in the egg production is cause of tissue damage hence affecting the overall fitness of the treated groups. Reduction of egg laying ability is dose dependent manner, in LT2, ZT2 groups showed more compared to LT1 and ZT1 respectively. Further, the groups which contain both metal reduced the egg laying ability compared to all other groups.



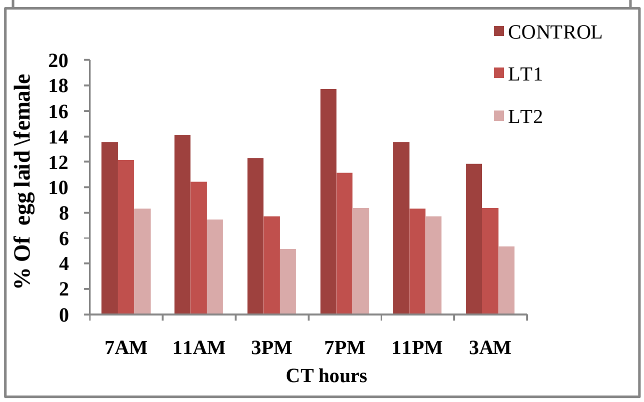
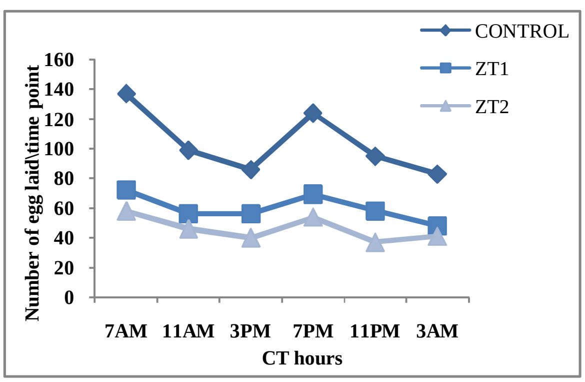


Fig.1: Oviposition rhythm of *D. melanogaster* showing the number of eggs laid in control and lead acetate treated groups. Fig.2: Oviposition rhythm *of D. melanogaster* showing percentage of eggs laid in control and lead acetate treated groups.



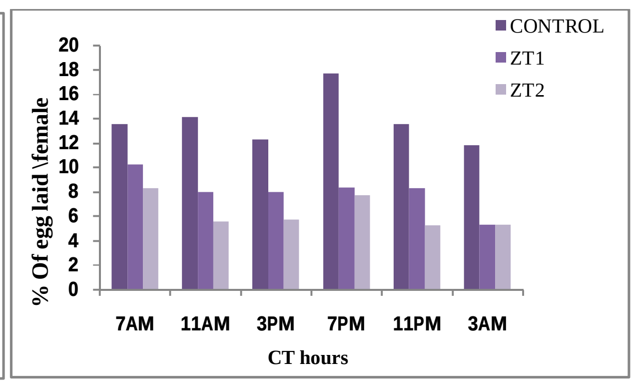
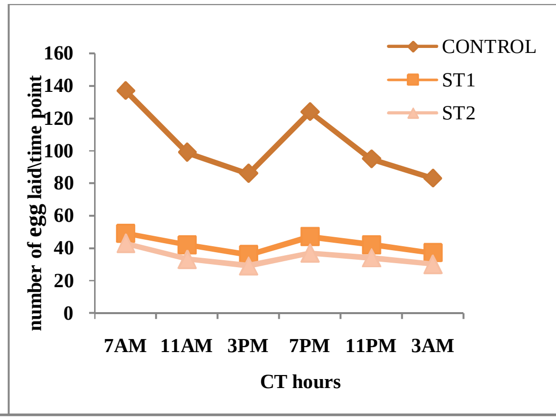


Fig.3 : Oviposition rhythm of *D. melanogaster* showing the number of eggs laid in control and zinc chloride treated groups. Fig.4 : Oviposition rhythm *of D. melanogaster* showing percentage of eggs laid in control and zinc chloride treated groups



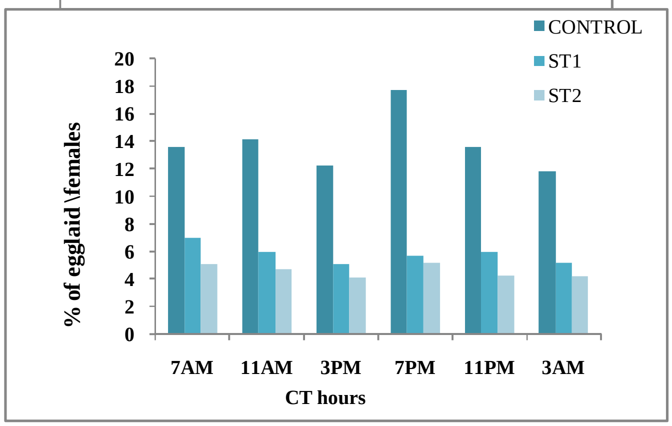


Fig.5 : Oviposition rhythm of *D. melanogaster* showing the number of eggs laid in control and combined treatment groups. Fig.6 : Oviposition rhythm of *D. melanogaster* showing the percentage of eggs laid in control and combined treatment groups.

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