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# Effect of herbicide Butachlor on the earthworm *Eutyphoeus waltoni* Michaelsen

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**ABSTRACT**

*The lethal effect of herbicide butachlor (2-chloro 2,6diethyl N, butoxymethyl acetanilide) on the earthworm Eutyphoeus waltoni was evaluated in different combination of feed materials under the laboratory conditions. Eutyphoeus waltoni were exposed to different concentrations of butachlor (0.3, 0.6, 0.9, 1.2 mg/kg) in feed material i.e. buffalo dung, wheat straw and gram bran and soils for time range from 24 to 240h.The maximum toxicity was observed in the Sandy soil 24, 48, 72, 96, 120, and 240h with* $LC_{50}$ *values ( 95% of confidence limits) estimated by probit analysis were 0.952 (0.689 to 2.071), 0.652 (0.486 to 0.852), 0.543 (0.346 to 0.724), 0.449 (0.246 to 0.602), 0.388 (0.127 to 0.533) and 0.287 (0.056 to 0.432), respectively. There were dose and time dependent increase in the mortality rate due to exposure to the herbicide. Maximum toxicity was observed in the sandy soil whereas, minimum in combination of buffalo dung with gram bran.*

***Keywords:*** *Toxicity, Herbicides, Butachlor, Earthworm, Soil, Buffalo dung, Agro-wastes.*

## INTRODUCTION

Earthworms are represent a major component of the soil and play an important role in improving the soil structure and soil fertility[1]. The large amounts of organic wastes are produced in intensive agriculture. Disposal of these large quantities of animal dung and agro-wastes are serious problems, if not properly managed. Many animal wastes cause serious odour and pollution problems[2]. The earthworms act as bioindicator of soil toxicity and play an important role in ingest large quantity of decomposed litter, manure and other organic matter deposited on soil helping to convert it into rich topsoil[3,4].

Use of herbicides or chemical fertilizer in the agricultural fields were caused deleterious effect of water reservoir[5].The need to produce more food for ever increasing world population especially in the developing economics requires extensive use of agrochemical which effect non- target soil fauna population[6]. Herbicide have adverse effect on the growth, reproduction and survival of earthworms[7-9]. According to Riepert *et al.*,[10] the acute earthworm test is part of basic test set but reproduction test is considered ecologically more relevant. The earthworm *Eutyphoeus waltoni* have been found abundantly in the agricultural field of eastern Uttar Pradesh in India[11]. Earthworms have been used as model animals to the study the effect of agrochemicals on the soil fauna[12,13].

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The herbicide and pesticides have adverse effect and their histopathological effects[13-16].

The combination of animal dung with different agro wastes are the best suitable feed material for better growth and development of earthworm *Eisenia fetida*[17]. The combination of buffalo dung with wheat straw and gram bran resulted in maximum biomass, weight and length[4, 18]. Singh and Singh[19] reported that the sub-lethal exposure of tertiary combination of buffalo dung with wheat straw and gram bran have more potency to increase the tolerance power of earthworm *Eutyphoeus waltoni*. Mortality has been the most frequently used parameter to evaluate the chemical toxicity in earthworms[20-22]. Moreover, studies have shown that earthworm's skin is a significant route of contaminant uptake[23] and thus investigation of earthworm biomarkers in the ecological risk assessment can be helpful[24]. It is postulated, however, that survival is less sensitive from an ecotoxicological point of view[25]. The acute mortality tests would not provide the most sensitive risk estimate for earthworms in the majority (95%) of cases[26]. Amorim *et al.,*[27] tested with herbicide Phenmedipham and found reproduction to be a more sensitive endpoint than mortality in *Enchytraeus albidus*and *Enchytraeus luxuriosus*. It is suggested that the chronic test, aiming at sub-lethal effects, is more sensitive and is a more realistic approach for the prediction of environmental effects because in the field, the exposure concentrations of pesticides are usually quite low[15].

The aim of present study was to determine the toxicity of butachlor herbicide on the earthworm *Eutyphoeus waltoni* in different combination of feed material of buffalo dung with agro-wastes in laboratory conditions.

## MATERIALS AND METHODS

### Collection of the earthworm:

The cultured earthworm *Eutyphoeus waltoni* were in the experiment.

### Collectionof animal dung and agro-wastes:

The buffalo dung, wheat straw and gram bran were collected from different part of Gorakhpur districtin U.P., India.

### Herbicide:

Commercially available herbicides butachlor (2-chloro 2,6 diethyl N, butoxymethyl acetanilide) was paucharsed from Aristo biotech and life science Pvt. Ltd., E-26, G.I.D.C. Manjusar, savli,Distt. Vadodara-391755 , Gujrat (India) and used in the experiment.

### Determination of $LC_{50}$ :

Toxicity experiment was performed by the method of Agarwal and Singh[28]. Twenty adult earthworms were kept in vermibed of two kg feed material. The each vermibed were exposed to different conc. of herbicides (Table-1). Six vermibed were set up for each dose of herbicide. The vermibed without any treatment were used as control. Mortality was recorded at different exposure periods like that 24, 48, 72, 96, 120, 240h. Lethal concentration ($LC_{50}$) value, its upper and lower confidence limits (UCL and LCL) and slope value were calculated according to the method of POLO computer programmers of Russel *et al.,*[29].

### Statistical Analysis:

All the investigations were replicated at least six times. Product momentum correlation coefficient was determined between exposure time and different values of $LC_{50}$. Analysis of variance were used to analyze the significant difference between $LC_{50}$ of different combination and exposure time[30].

## RESULTS

Laboratory toxicity was evaluated for different concentration of butachlor against earthworm *E.waltoni* in different combination of buffalo dung with agro-wastes as feed materials. No mortality was recorded in control groups throughout the study period. The toxicity of butachlor was both time and dose dependent against earthworm *E.waltoni* in all exposures (Table-2). There was a negative significant product momentum coefficient ($p< - 0.05$) was observed between exposure time and different value of $LC_{50}$ of butachlor (Table- 2). The order of 24h exposure toxicity of butachlor is Sandy soil>Clay soil>Loamy soil >BD > BD+Ws >BD+Ws+GB>BD+Gb. The slope value given in the Table-2 were steep and separate estimation of $LC_{50}$ of different combination was found to be based on the each of the six replicates was found to be within the 95% confidence limits of $LC_{50}$. The t- ratio is the greater than 1.96 and heterogeneity less than 1. There was a significant co- relation coefficient (r) between all exposure time and $LC_{50}$ value of butachlor was obtained (Table-2). Analysis of variance (ANOVA) was used to analysed the significant difference between $LC_{50}$ of different combination of feed material and exposure period (Table-2).The toxicity of butachlor against *E.waltoni* was higher in the sandy soil in all exposure period of 24h to 240h. Whereas, minimum in the buffalo dung+Gram bran as feed material (Table 2 & 3; Fig - 1).Plot curve shows that maximum and minimum percent mortality response in the combination of BD+Gb and Sandy soil in 24h and 240h exposure.( Plot curve 1,2, 3 and 4 ).

**Table 1: Concentration used for toxicity determination against earthworm (*Eutyphoeuswaltoni)***

| Name | Combination | Concentration (mg/kg) |
|---|---|---|
| Butachlor | BD | 0.3, 0.6, 0.9, 1.2 |
| | BD+Ws | 0.3, 0.6, 0.9, 1.2 |
| | BD+Gb | 0.3, 0.6, 0.9, 1.2 |
| | BD+ws+Gb | 0.3, 0.6, 0.9, 1.2 |
| | Loamy soil | 0.3, 0.6, 0.9, 1.2 |
| | Clay soil | 0.3, 0.6, 0.9, 1.2 |
| | Sandy soil | 0.3, 0.6, 0.9, 1.2 |

**Table : 2 Toxicity of herbicide Butachlor against earthworm *Eutyphoeuswaltoni* in different feed materials**

| Periods | combinations | $LC_{50}$ | Lower limits | Upper limits | Slope value | t- ratio | Herterogeniety |
|---|---|---|---|---|---|---|---|
| 24h | BD | 1.044 | 1.075 | 7.117 | 2.567 | 2.829 | 0.28 |
| | BD+Ws | 1.053 | 0.747 | 3.166 | 1.954 | 2.783 | 0.01 |
| | BD+Gb | 1.533 | 1.011 | 4.596 | 2.606 | 2.966 | 0.06 |
| | BD+Ws+Gb | 1.397 | 0.793 | 1.964 | 2.531 | 3.320 | 0.16 |
| | Loamy soil | 0.982 | 0.717 | 2.128 | 2.131 | 3.015 | 0.08 |
| | Clay soil | 0.977 | 0.807 | 1.322 | 3.868 | 4.083 | 0.76 |
| | Sandy soil | 0.952 | 0.689 | 2.071 | 1.069 | 2.956 | 0.05 |
| 48h | BD | 0.805 | 0.631 | 1.106 | 2.923 | 3.963 | 0.84 |
| | BD+Ws | 0.849 | 0.603 | 1.628 | 2.008 | 2.956 | 0.12 |
| | BD+Gb | 0.925 | 0.690 | 1.657 | 2.312 | 3.243 | 0.23 |
| | BD+Ws+Gb | 0.895 | 0.750 | 1.126 | 4.258 | 4.413 | 0.83 |
| | Loamy soil | 0.722 | 0.491 | 1.155 | 2.034 | 3.045 | 0.61 |
| | Clay soil | 0.659 | 0.470 | 0.888 | 3.525 | 4.648 | 0.54 |
| | Sandy soil | 0.652 | 0.486 | 0.852 | 2.769 | 3.975 | 0.24 |
| 72h | BD | 0.615 | 0.360 | 0.923 | 1.917 | 2.922 | 0.20 |
| | BD+Ws | 0.632 | 0.465 | 0.825 | 2.755 | 3.935 | 0.07 |
| | BD+Gb | 0.645 | 0.430 | 0.923 | 2.175 | 3.249 | 0.99 |
| | BD+Ws+Gb | 0.643 | 0.516 | 0.784 | 3.783 | 4.862 | 0.87 |
| | Loamy soil | 0.569 | 0.320 | 0.816 | 1.980 | 3.013 | 0.02 |
| | Clay soil | 0.555 | 0.335 | 0.763 | 2.174 | 3.268 | 0.99 |
| | Sandy soil | 0.543 | 0.346 | 0.724 | 2.379 | 3.524 | 0.99 |
| 96h | BD | 0.507 | 0.348 | 0.647 | 2.843 | 4.061 | 0.53 |
| | BD+Ws | 0.524 | 0.392 | 0.647 | 3.399 | 4.602 | 0.84 |
| | BD+Gb | 0.532 | 0.309 | 0.730 | 2.153 | 3.241 | 0.99 |
| | BD+Ws+Gb | 0.528 | 0.342 | 0.696 | 2.488 | 3.661 | 0.27 |
| | Loamy soil | 0.479 | 0.301 | 0.625 | 2.585 | 3.759 | 0.74 |
| | Clay soil | 0.437 | 0.281 | 0.559 | 2.940 | 4.119 | 0.12 |
| | Sandy soil | 0.449 | 0.246 | 0.602 | 2.341 | 3.451 | 0.98 |
| 120h | BD | 0.403 | 0.262 | 0.512 | 3.186 | 4.269 | 0.07 |
| | BD+Ws | 0.415 | 0.286 | 0.518 | 3.468 | 4.534 | 0.16 |
| | BD+Gb | 0.479 | 0.210 | 0.683 | 2.585 | 3.759 | 0.74 |
| | BD+Ws+Gb | 0.432 | 0.227 | 0.582 | 2.330 | 3.426 | 0.58 |
| | Loamy soil | 0.407 | 0.180 | 0.560 | 2.181 | 3.225 | 0.99 |
| | Clay soil | 0.403 | 0.262 | 0.512 | 3.186 | 4.269 | 0.07 |
| | Sandy soil | 0.388 | 0.127 | 0.533 | 1.962 | 2.933 | 0.99 |
| 240h | BD | 0.333 | 0.138 | 0.462 | 2.438 | 3.397 | 0.61 |
| | BD+Ws | 0.366 | 0.109 | 0.533 | 1.951 | 2.908 | 0.99 |
| | BD+Gb | 0.444 | 0.186 | 0.622 | 1.984 | 3.000 | 0.99 |
| | BD+Ws+Gb | 0.370 | 0.177 | 0.496 | 2.501 | 3.531 | 0.99 |
| | Loamy soil | 0.311 | 0.068 | 0.463 | 1.988 | 2.895 | 0.09 |
| | Clay soil | 0.301 | 0.085 | 0.438 | 2.203 | 3.085 | 0.80 |
| | Sandy soil | 0.287 | 0.056 | 0.432 | 2.040 | 2.891 | 0.59 |

Each set of experiment was replicates six times. Product momentum correlation showed that there was significant negative coefficient ($p<0.05$) was observed between exposure time and different value of $LC_{50}$ of Butachlor. In all cases t-ratio is greater than 1.96, heterogeneity factor is less than 1.0.

**Summary of computation of analysis of variance (ANOVA) of the data of Table 2**

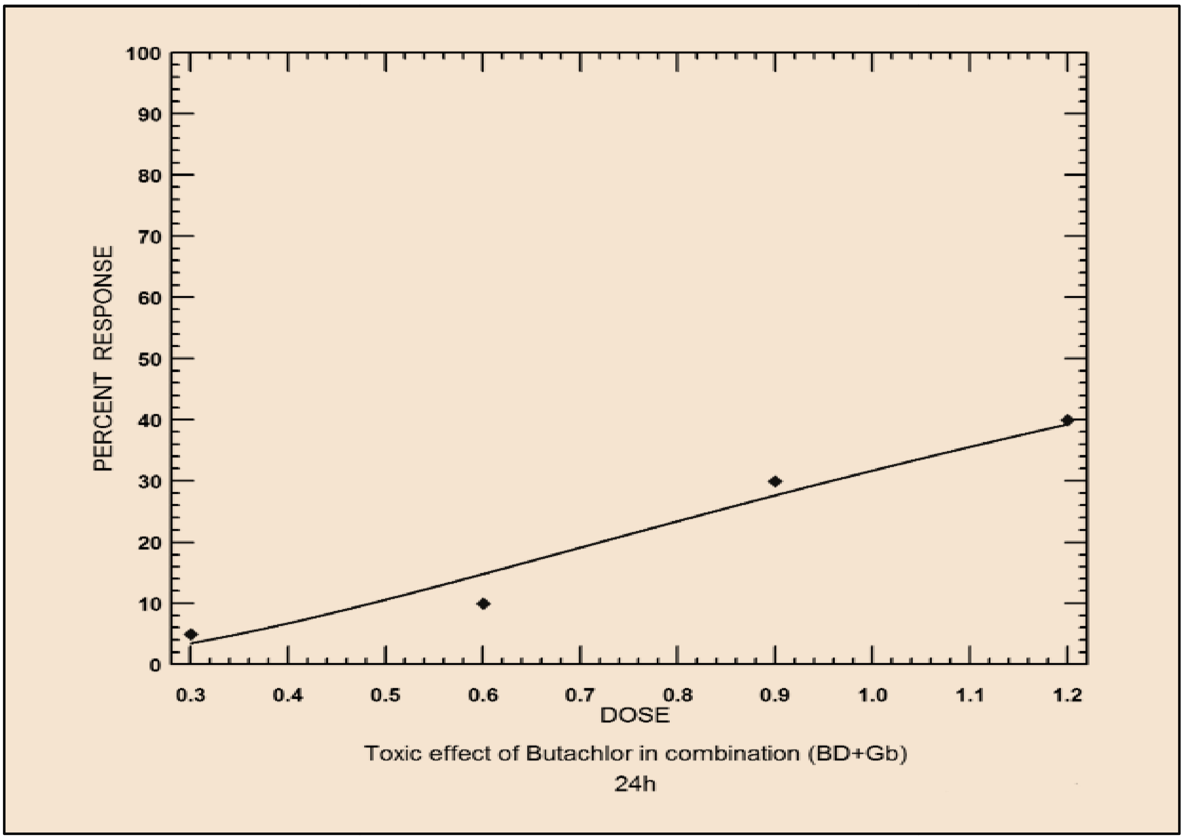
| **Source of variation** | **Toxicity of different combination of wastes** | | | | | |
|---|---|---|---|---|---|---|
| **Source of variation** | **D.F.** | **S.S.** | **Variance** | **F-ratio** | **P<0.01** | **P<0.05** |
| Between treatment | 5 | 0.234 | 0.0469 | **6.8** | 5.6 | 3.3 |
| Between Time | 6 | 2.971 | 0.4951 | **71.9** | 7.5 | 4.1 |
| Error | 30 | 0.207 | 0.0069 | | | |
| Total | 41 | 3.412 | | | | |

**Table:3 Lethal concentrations of herbicide Butachlor against earthworm Eutyphoeuswaltoni in different feed materials**

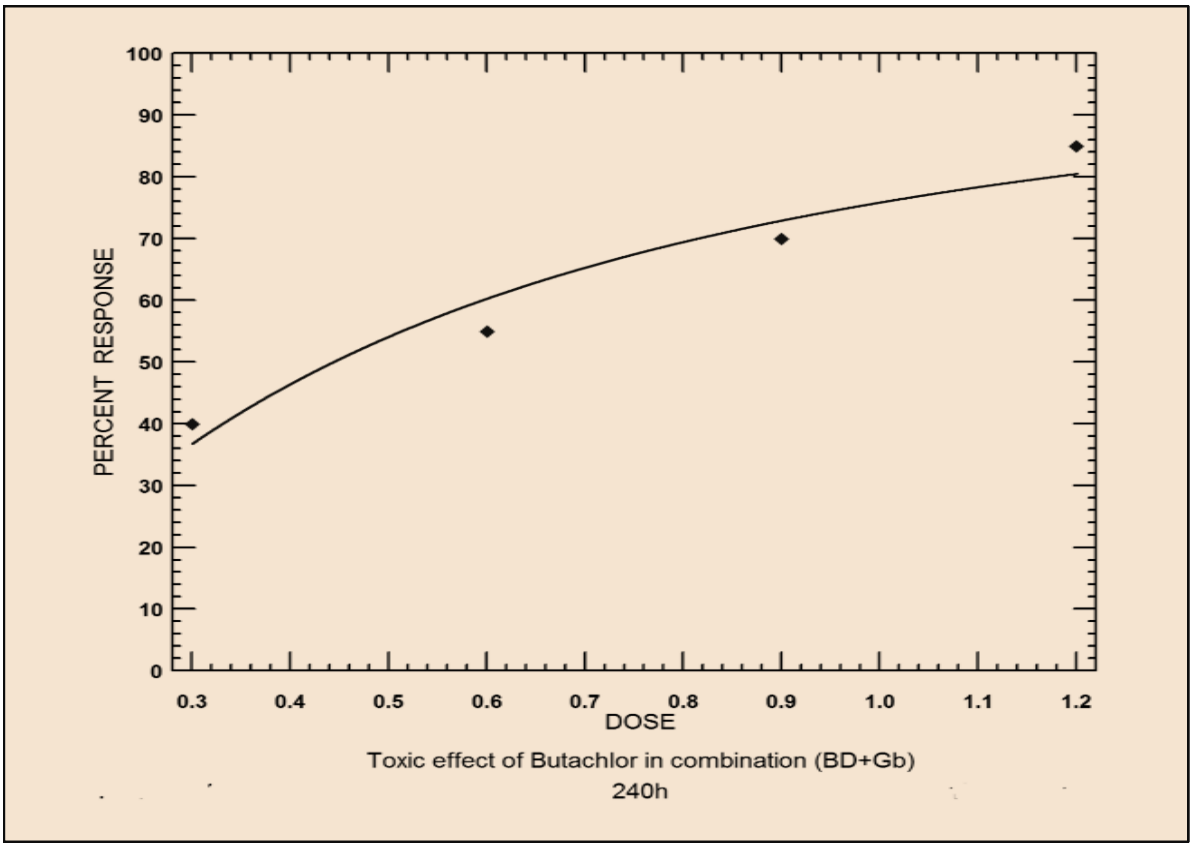
| **Periods** | **Combinations** | **$LC_{10}$** | **$LC_{50}$** | **$LC_{90}$** |
|---|---|---|---|---|
| 24h | BD | 0.325 | 1.044 | 3.349 |
| | BD+Ws | 0.233 | 1.053 | 4.769 |
| | BD+Gb | 0.486 | 1.533 | 4.841 |
| | BD+Ws+Gb | 0.450 | 1.397 | 3.336 |
| | Loamy soil | 0.246 | 0.982 | 3.921 |
| | Clay Soil | 0.430 | 0.977 | 2.096 |
| | Sandy Soil | 0.229 | 0.952 | 3.962 |
| 48h | BD | 0.425 | 0.805 | 2.209 |
| | BD+Ws | 0.195 | 0.849 | 3.690 |
| | BD+Gb | 0.258 | 0.925 | 3.315 |
| | BD+Ws+Gb | 0.448 | 0.895 | 1.790 |
| | Loamy soil | 0.169 | 0.722 | 3.081 |
| | Clay Soil | 0.285 | 0.659 | 1.522 |
| | Sandy Soil | 0.227 | 0.652 | 1.874 |
| 72h | BD | 0.132 | 0.615 | 2.867 |
| | BD+Ws | 0.217 | 0.632 | 1.846 |
| | BD+Gb | 0.162 | 0.645 | 2.505 |
| | BD+Ws+Gb | 0.295 | 0.643 | 1.403 |
| | Loamy soil | 0.128 | 0.569 | 2.528 |
| | Clay Soil | 0.143 | 0.555 | 1.018 |
| | Sandy Soil | 0.157 | 0.543 | 1.876 |
| 96h | BD | 0.180 | 0.507 | 1.501 |
| | BD+Ws | 0.220 | 0.524 | 1.248 |
| | BD+Gb | 0.135 | 0.532 | 1.248 |
| | BD+Ws+Gb | 0.161 | 0.528 | 1.730 |
| | Loamy soil | 0.153 | 0.479 | 1.501 |
| | Clay Soil | 0.127 | 0.449 | 1.565 |
| | Sandy Soil | 0.160 | 0.438 | 0.961 |
| 120h | BD | 0.160 | 0.403 | 1.018 |
| | BD+Ws | 0.139 | 0.415 | 0.971 |
| | BD+Gb | 0.100 | 0.444 | 1.965 |
| | BD+Ws+Gb | 0.122 | 0.432 | 1.535 |
| | Loamy soil | 0.105 | 0.407 | 1.573 |
| | Clay Soil | 0.160 | 0.403 | 1.018 |
| | Sandy Soil | 0.086 | 0.388 | 1.746 |
| 240h | BD | 0.099 | 0.333 | 1.119 |
| | BD+Ws | 0.113 | 0.366 | 1.192 |
| | BD+Gb | 0.100 | 0.444 | 1.965 |
| | BD+Ws+Gb | 0.082 | 0.370 | 1.681 |
| | Loamy soil | 0.071 | 0.311 | 1.372 |
| | Clay Soil | 0.079 | 0.301 | 1.149 |
| | Sandy Soil | 0.067 | 0.287 | 1.217 |

In all cases t-ratio is greater than 1.96, heterogeneity factor is less than 1.0 and g-values is less than 0.5 at all probability level.

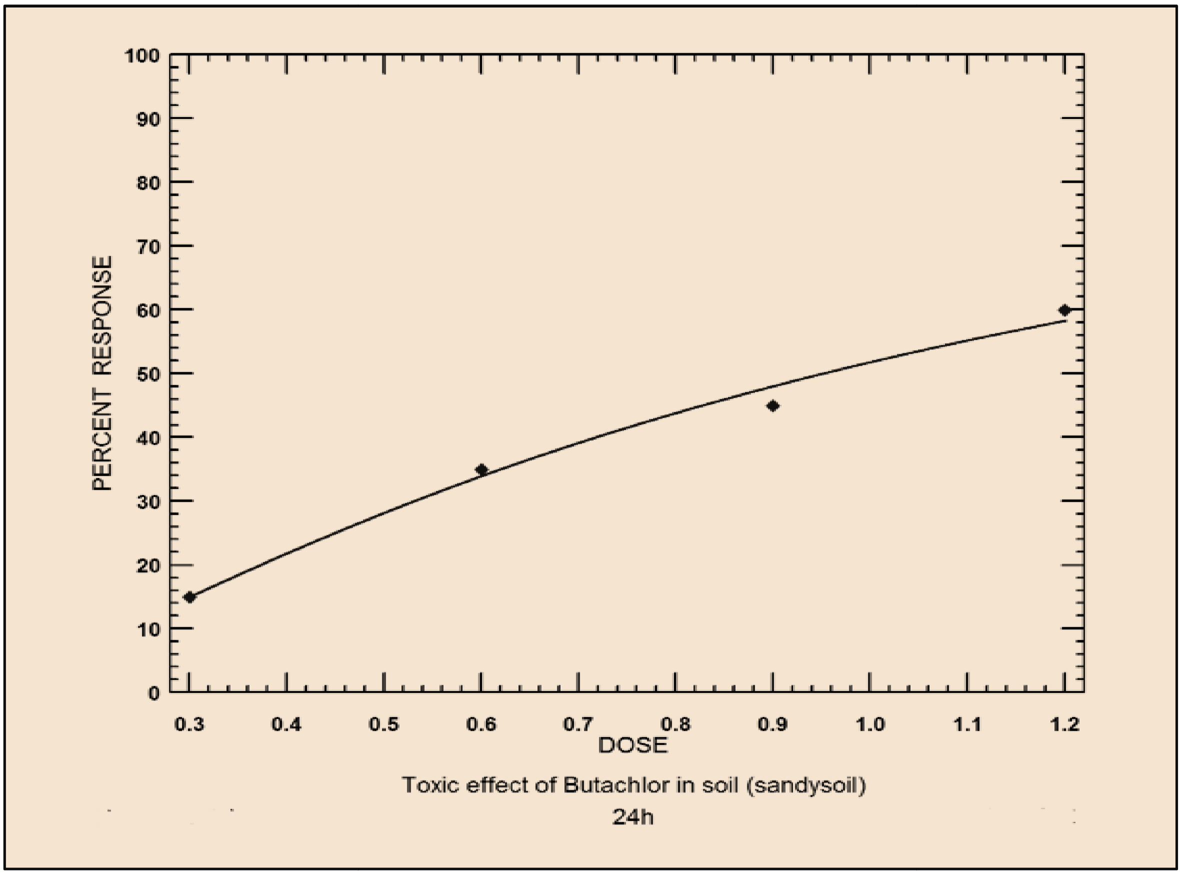
 



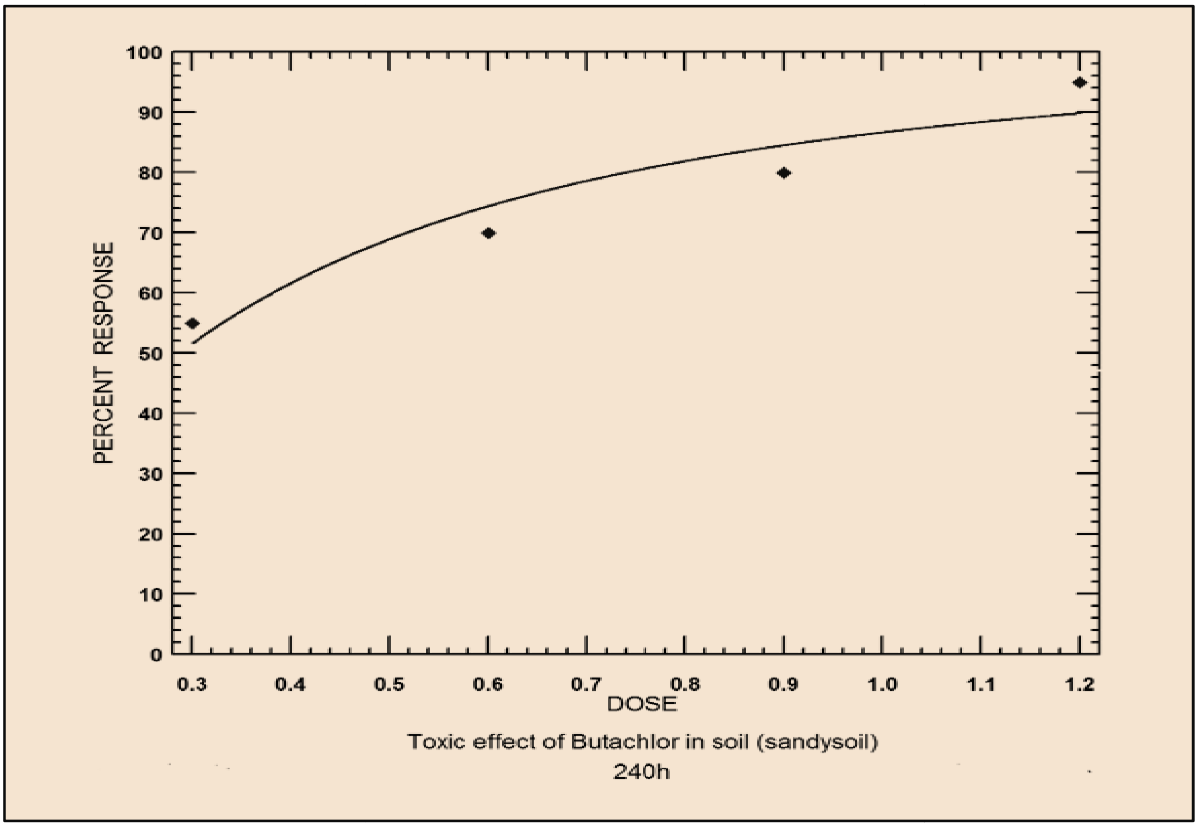

**Plot Curve – 1: The percent response of 24h exposure of Butachlor against earthworm *Eutyphoeus waltoni***




**Plot Curve - 2 : The percent response of 240h exposure of Butachlor against earthworm *Eutyphoeus waltoni***




**Plot Curve -3: The percent response of 24 h exposure of Butachlor against earthworm *Eutyphoeus waltoni***




**Plot Curve - 4 : The percent response of 240 h exposure of Butachlor against earthworm *Eutyphoeus waltoni***

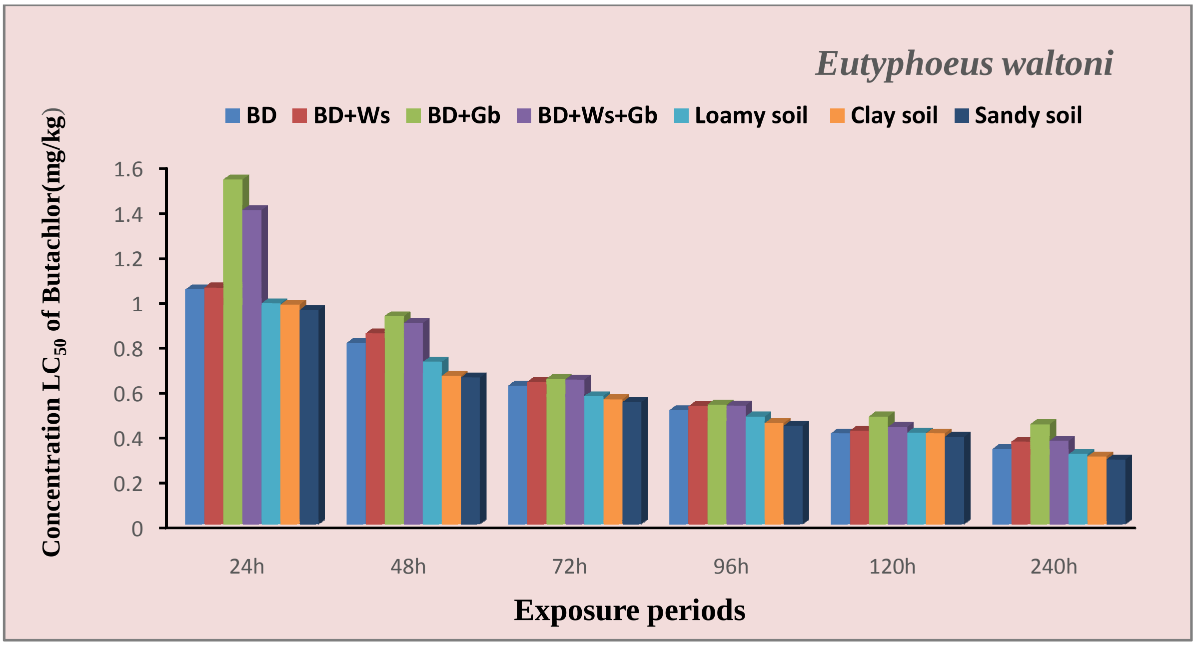



**Fig. 1 Effect of herbicide Butachlor in different feed materials on earthworm *Eutyphoeus waltoni* in different exposure periods**

## DISCUSSION

Toxicity of Butachlor $LC_{50}$ value obtained in this study for the earthworm *E. waltoni*. This study mainly focused on of toxicity of herbicide butachlor to earthworm *Eutyphoeus waltoni*. Use of earthworms in ecotoxicological studies is common and a large database on pesticides effect on earthworms exist [26] to field effects [31-33]. It is evident from the result that the observed toxicity of butachlor was time and dose dependent against earthworm *E.waltoni*. The herbicide acetochlor caused adverse effect on the sperm number and DNA of *Eisenia fetida*[34]. Singh and Singh[35] reported that the toxicity of 2,4-D against earthworm *E.waltoni* was higher in the sandy soil at 24h upto 240h exposure periods.

Butachlor has also been reported to be carcinogenic and can adversely disrupt the reproductive process and affect the thyroid and sex steroid hormones in Zerbra fish[36-37]. Gobi *et al.*,[38] were found the glandular cell enlargement and vaccualization in the intestine of the earthworm *perionyx sansibaricus* exposed to sub lethal concentration of herbicide butachlor. According to the Stephenson[39] recovery could be brought by the chloragogen cells.The result clearly indicate that current observation on butachlor toxicity support the conclusion that *E.waltoni* is sensitive to the herbicide and their mortality rate is dose and time dependent. The significance of different combination and exposure time in assessing the hazards of the herbicide butachlor to earthworm *Eutyphoeus waltoni.* Agricultural use of butachlor in the environment must be restricted to avoid the sever risk associate with the use of the herbicide butachlor.

The repeated and discriminiate use of herbicides, careless handling accidental spillage or discharge of untreated effluents into agricultural fields has harmful effects on the earthworm *E.waltoni* and other terrestrial organism. Acute and chronic toxicity tests are widely used to evaluate the toxicity of chemicals on non- target organisms [40]. The abundance and activity of earthworm in arable lands depends strongly on management practices; therefore, earthworms can act as potential bioindicators of land use practices[41]. The toxicity of pesticides to soil organisms depends on the compound bioavailability, which is affected by the physicochemical properties of the compound and the soil, and by the uptake routes of exposed organisms. Therefore, ecotoxicity studies can benefit from using experimental designs that for local exposure condition in the field[42].Vermicastings have led to significant increases in the yields of several crops, with significant reductions in pesticide use and almost zero chemical fertilizer inputs[43]. Lin *et al.*,[44] reported that increase in sunlight enhanced photo degradation of butachlor in water and that the half life of the herbicide in non- filtered river water was shorter than filtered samples.

The toxicity of butachlor against earthworm *Eutyphoeus waltoni* was higher in sandy soil have less organic content and other nutritional components than other combination of agro wastes. The combinations of buffalo dung with gram bran have minimum toxicity on butachlor against *Eutyphoeus waltoni* because it is possible that this combination have rich amount of organic nutrients which tolerate the toxicity of butachlor. There was no mortality recorded after240h exposure period in all the treatment, which may be due to the development of tolerance power against butachlor in earthworms.

## CONCLUSION

It is evident from the result that the toxicity of butachlor has more toxic effect against earthworm *Eutyphoeus waltoni*.The use of different combination of buffalo dung and agro wastes in the agricultural fields are the suitable feed material for earthworms which provides better nourishment to tolerate the toxic effect of the herbicides.

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## REFERENCES

1. Bartlett, M.D., Briones, M.J.I., Neilson, R., Schmidt, O., Spurgeon, D., Creamer, R.E., A critical review of current methods in earthworm ecology: from individuals to populations. *Eur. J. Soil Biol.* **46:** 67–73 (2010).
2. Gupta, P.K., Vermicomposting for sustainable agriculture. Bharat Printing Press, Jodhpur, India, pp 11-14 (2005).
3. Reinecke, S.A. and Reinecke, A.J., Lysosomal response of earthworm coelomocytes induced by longterm experimental exposure to heavymetals. *Pedobiologia*, **43(6):** 585-593 (1999).
4. Chauhan, H.K. and Singh, K., Effect of binary combinations of buffalo, cow and goat dung with different agro wastes on reproduction and development of earthworm *Eiseniafoetida*. World J Zoo, **l7(1):** 23-29 (2012).
5. Meena, D., Organic farming: Scope and importance. *Agrobios Newsletter* **6(4):** 14 (2007).
6. Stanley, O.N., Nwanyoluaru, O.R.N., Joy, O.A., Edwin, O., Toxicity and histopathological effect of atrazine (herbicide) on the earthworm Nsukkadrilusmbae under laboratory conditions. *Animal Res Int,* **7(3):** 1287-1293 (2010).
7. Reinecke, S.A. and Reinecke, A.J., Effect of the fungicide copper oxychloride on the growth and reproduction of *Eiseniafoetida* (Oligocheata). *Ecotoxi Environ Saf,* **46:** 108-116 (2000).
8. Zhou, S.P., Duan, C.P., Fu, H., Chen, Y.H., Wang, X.H.and Yu, Z.E., Toxicity assessment for chlorpyrifos contaminated soil with three different earthworm test methods. *J Environ Sci,* **19(7):** 854-858 (2007).
9. Correia, F.V. and Moreira, J.C., Effects of Glyphosate and 2,4-D on earthworm (*Eisenia foetida*) in laboratory tests. *Bull Environ ContamiToxicol,* **85:** 264–268 (2010).
10. Riepert, F., Rombke, J.and Moser, T., 2009. Ecotoxicological Characterization of Waste Springer New York NY USA.
11. Singh, K. and Kumar, Y., 2014. Earthworm diversity and ecology. Gene-Tech Books, New Delhi.
12. Cock, A.G., Critchley, B.R.V., Perfect, J.J. and Yeadon, E., Effect of cultivation and DDT on earthworm activity in aforest soil in the subhumidtropics. *J App. Ecol,* **17:** 21-29 (1980).
13. Gobi, M., Suman, J.,Ganesan, S.V., Sublethal toxicity of the herbicide butachlor on the earthworm Perionyxsansibaricus and its histological changes. *J Soil Sediment,* **5(2):** 62-86 (2004).
14. Lydy, M.J., Linck, S.L., Assessing the impact of triazine herbicides on organophosphate insecticide toxicity to the earthworm *Eiseniafoetida*. *Arch Environ Contam Toxicol,* **45(3):** 343-349 (2003).
15. Rombke, J., Garcia, M.V., Scheffczyk, L., Effect of the fungicide Benomyl on earthworm in laboratory tests under tropical conditions. *Arch Environ Contam Toxicol,* **53(4):** 590-598 (2007).
16. Mosieh, Y.Y., Assessing the toxicity of the herbicide isoproturon on *Aporrectodeacaliginosa* (Oligocheata)and its fate in soil ecosystem. *Environ Toxicol,* **24(3):** 396-403 (2009).

17. Chauhan, H.K. and Singh, K., Effect of tertiary combinations of animal dung with agrowastes on the growth and development of earthworm *Eisenia fetida* during organic waste management. *Int J Rec Org Waste Agri,* **2:** 11 (2013).
18. Nath, G., Singh, K., Singh, D.K., Effect of different combinations of animal dung, and agro/kitchen wastes ongrowth and development of earthworm *Eisenia foetida*. *Aust J Basic Appl Sci,* **3(4):** 3672-3676 (2009).
19. Singh, V., Singh, K., Effects of sub-lethal exposure of LC50 of 2,4-D on the reproduction and development of earthworm *Eutyphoeuswaltoni*Michaelsen(Oligochaeta:Octochaetidae). *Research Journal of Science and Technology* **(4):** 203-210 (2014).
20. Van Gestel, C.A.M. and Van Dis, W.A., The influence of soil characteristics on the toxicity of four chemicals to the earthworm *Eisenia fetida* Andrei (Oligochaeta). *BiolFerti Soil* **6(3):** 262-265 (1988).
21. Van Gestel, C.A.M., Van Dis, W.A., Van Breemen, E.M. and Sparenburg, P.M., Development of a standardized reproduction toxicity test with the earthworm species *Eisenia fetida* Andrei using copper, pentachlorophenol, and 2,4-dichloroaniline. *Ecotoxi EnvironSaf,* **18(3):** 305-312 (1989).
22. Robidoux, P.Y., Hawari, J., Thiboutot, S., Ampleman, G., Sunahara, G.I., Acute toxicity of 2,4,6-trinitrotoluene inearthworm (*Eiseniaandrei*). Ecotoxicol Environ Saf, **44(3):** 11-321 (1999).
23. Lord, K.A., Briggs, G.G., Neale, M.C. and Manlove, R., Uptake of pesticides from water and soil by earthworms. *Pesticide Science,* **11(4):** 401-408 (1980).
24. Sanchez-Hernandez, J.C., Earthworm biomarkers in ecological risk assessment. Rev Environ Conta Toxico1, **88:** 85-126 (2006).
25. Moriarty, F., Ecotoxicology: The Study of Pollutants in Ecosystems Academic Press London UK.
26. Frampton, G.K., Jansch, S., Scott-Fordsmand, J.J., Rombke,, J. and Van den, Brink P.J., Effects of pesticides on soil invertebrates in laboratory studies: a review and analysis using species sensitivity distributions. *Environ Toxicolm Chem,* **25(9):** 2480-2489 (2006).
27. Amorim, M.J.B., Rombke, J. And Soares, A.M.V.M., Avoidance behaviour of *Enchytraeusalbidus*: effects of Benomyl, Carbendazim, phenmedipham and different soil types. *Chemosphere,* **59(4):** 501-510 (2005).
28. Agarwal, R.A., Singh, D.K., Harmful gastropods and their control. *Acta Hydro Chim Hydrobio.,* **116:** 113-138 (1988).
29. Russel, R.M., Robertsion, J.L.andSavin, N.E., Polo: a new computer programme for profit analysis. *Bul Ent SocAmer,* **23:** 209-213 (1977).
30. Sokal, R.R. and Rohlf, F.J., 1973. Introduction of biostatistics. W.H. Freeman and Co. San Francisco.
31. Forster, B., Garcia, M., Francimari, O., Rombke, J., Effects of carbendazim and lambda- cyhalothrin on soil invertebrates and leaf litter decomposition in semi- field and tests under tropical condition (Amazonia, Brazil). *Eur J Soil Biol.* **42:** 171-179 (2006).
32. Reinecke, S.A., Reinecke, A.J., The impact of organophosphate pesticides in orchards onearthworms in the Western Cape, South Africa. *Ecotoxicol EnvironSaf,* **66:** 244–251 (2007).
33. Casabe, N., Piola, L., Fuchs, J., Oneto, M. L., Pamparato, L., Basack, S., Gimenez, R., Massaro, R., Papa, J. C., Kesten, E., Ecotoxicological assessment of the effects of glyphosate and chlorpyrifos in an Argentine soya field. *J. Soil Sediments,* **7:** 232-239 (2007).
34. Xiao, N., Jling, B., Ge, E., Liu, L., The fate of herbicide acetochlor and its toxicity to Eiseniafoetida underlaboratory conditions. *Chemosphere,* **62(8):** 1366-1373 (2006).
35. Singh, V., Singh, K., Toxic effect of herbicide 2,4-D on the earthworm *Eutyphoeuswaltoni* Michaelsen. *Environ. Process* (2015); DOI 10.1007/s40710-015-0057-7.
36. Ou, Y., Chung, P., Chang, Y., Ngo, F., Hsu, K., Chen, F., Butachlor, a suspected carcinogen, alters growth and transformation characteristics of mouse liver cells. *Chem Res Toxicol,* **13:** 132-1325 (2000).
37. Chang, J., Liu, S., Zhou, S., Wang, M., Zhu, G., Effects of butachlor on reproduction and hormone levels in adult zebrafish (Daniorerio).Exp.Toxico.Pathol.doi:2011; 10.1016/j.etp.08.007.
38. Gobi, M., Suman, Janardhanan., and Ganesan, S. Vijayalakshmi., 2005. Sublethal toxicity of the herbicide butachlor on the earthworm Perionyxsansibaricus and its histological changes. *Journal of soils and sediments* **2:** 82-86 (2011).

39. Stephenson, J., 1930. The oligochaeta.Oxford : Clarendon press.
40. Santos, L.H.M.L.M., Araujo, A.N., Fachini, A., Pena, A., Deleure-matos, C., Montenegro, M.C.B.S.M., Ecotogicological aspects related to the presence of pharmaceuticals in the aquatic environment. *J Hazard Meter,* **175:** 45-95 (2010).
41. Suthar, S., Earthworm communities a bioindicator of arable land management practices: a case study in semiarid region of india. *Ecological Indicators* **9:** 588-594 (2009).
42. Filser, J., Koehler, H., Ruf, A., Rombke, J., Prinzing, A., Schaefer, M., Ecological theory meets soil ecotoxicology: challenge and chance. *Basic and applied ecology,* **9:** 346-355 (2008).
43. Dash, M.C., Senapathi, B.K., National Seminar on organic wastes utilize, vermin comp. part-13 proceedings, pp: 157-177 (1986).
44. Lin, Y.J., Lin, C., Yeh, K.J., Lee, A., Photo degradation of the herbicides butachlor and ronstar using natural sunlight and diethylamine. *Bull Environ ContamToxicol.* **64:**780-785 (2000).