ARTICLE

EFFECT OF SUBLETHAL EXPOSURE OF HERBICIDES ON THE BIOCHEMICAL PARAMETERS OF EARTHWORM *EUTYPHOEUS WALTONI* MICHAELSEN [OLIGOCHAETA: OCTOCHAETIDAE]

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ABSTRACT

The earthworms play an important role in recycling of organic wastes and maintain fertility of agricultural fields. Herbicides are the plant growth hormones and generally used for removal of unwanted weeds. The aim of the present study was to observe the effect of sub-lethal exposure of [40 and 80%] of LC50 24 h and 240 h of herbicides 2,4-D and Butachlor on the biochemical parameters [Proteins, Amino acid, DNA, RNA, Acid/Alkaline phosphatase and AChE activity] of gonads of earthworm of Eutyphoeus waltoni in feed materials of buffalo dung with agro-wastes. Effect of sub lethal 40% and 80% of LC50of feed material of buffalo dung with agro-wastes of different exposure periods on the biochemical estimation on of earthworm Eutyphoeus waltoni. The maximum reduction [in per cent change] in Protein, Amino acid, DNA, RNA and inhibition in alkaline/ Acid and AChE activity was observed in the treatment of 80% of 240h LC50 of butachlor [14.74%], [35.29%], [29.16%], [26.12%], [28.27%], [33.04%], [17.28%] respectively. There was significant [P< 0.05, t- test] difference between control and 40% and 80% of LC50 of butachlor and 2,4-D. After withdrawal of experiment for 7 days there was no recovery was observed.

KEY WORDS
Herbicides. Toxicity. sub-lethal exposure. Eutyphoeus Waltoni. biochemical parameters

INTRODUCTION

Abundant uses of chemical fertilizers, pesticides and herbicides have made our soil sick and problematic. The long-term use of chemical fertilizers and herbicides has lead to imbalance of our natural ecosystems. The use of chemical herbicides and fertilizers in the agricultural field found to cause adverse effect on soil organisms [1,2].The need to produce more food for the ever increasing world population especially in the developing economics requires extensive use of agrochemicals such as pesticides and herbicides but they have adverse effects on non-target soil organism like the earthworm [3]. Herbicides are commonly known as weed killers to kill unwanted plants. Selective herbicides kill specific targets, while leaving the desired crop relatively unharmed. Some of these act by interfering with the growth of the weed and are often synthetic mimics of natural plant hormones [4].

The 2,4-D [2,4 dichlorophenoxy acetic acid] is a herbicide and a plant growth regulator [5]. Although research into chemical herbicides began in the early 20th century. The first modern herbicide, 2,4-D, was first discovered and synthesized by W. G. Templeman at imperial chemical industries. The chemical; composition of 2,4-D is 2,4-D Acid technical 59% w/w, dimethyl amine 30% w/w, Seqvesting agent [lignin sulphonate] 1% w/w, Diventacqua sufficient 10% w/w. 2,4-D is an herbicide as well as a plant growth regulator [5]. The chemical name of 2,4-D [2, 4-dichlorophenoxyacetic acid] was given by International Union of Pure and Applied Chemistry [IUPAC] which include esters, acids, and several salts [6]. The herbicide 2,4-D and butachlor have toxic effect against earthworm *Eutyphoeus waltoni* in feed materials of buffalo dung with agro-wastes as well as soil alone [7,8].

Butachlor [2-chloro-2', 6'-diethyl-N-[butoxymethyl] acetanilide] is used for the control of undesirable grasses and broadleaf weeds in transplanted paddy and barley fields. Butachlor was the first rice herbicide to be introduced in India. The application of huge amount of herbicides, affect not only target plants but also other non-targeted ones. The pathway of attack can arise from intentional direct consumption, improper application resulting in the herbicide coming into direct contact with people. Under some conditions, certain herbicides can be transported via leaching or surface runoff to contaminate groundwater or distant surface water sources. The use of herbicides has adverse effect on the survival of earthworms as well as its growth and reproduction [9-13].

Earthworms are considered as one of the most important biotic components of the soil because their role in mineralization and breakdown of organic matter which results formation of soil structure and humus soul for maintains the soil fertility. Earthworms are one of the best bio-indicator of pesticides contaminated soil. Due to high concentration of organo-chemicals at upper surface layer of soil, reduced earthworms activities in their habitat [14, 15]. Earthworms have the high biomass of terrestrial invertebrates which play an important role in structuring and increasing the nutrient content of the soil. Therefore, they can be suitable bioindicators of chemical contamination of the soil in terrestrial ecosystems providing an early warning of deterioration in soil quality [16, 17]. The suitability of earthworms as bioindicators in soil toxicity is high due to the fact that they ingest large quantity of the decomposed litter, manure, and other organic matter deposited on soil [18, 19]. Earthworms are often used as test organism because of their important function as decomposer and their sensitive reaction towards environmental influence. In terrestrial ecosystem, earthworms are so important experimental organism for ecotoxicity because their high growth and reproduction rate.

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The aim of the present study was to observe the effect of sub-lethal exposure of [40% and 80%] of LC_{50} 24 h and 240 h of herbicides 2,4-D and Butachlor on the biochemical parameters [Proteins, Amino acid, DNA, RNA, Acid/Alkaline phosphatase and AChE activity] of gonads of earthworm of *Eutyphoeus waltoni* in feed materials of buffalo dung with agro-wastes.

MATERIALS AND METHODS

Collection and culture of earthworm *Eutyphoeus waltoni*

The earthworm *Eutyphoeus waltoni* was collected from agricultural fields of Gorakhpur District. of Uttar Pradesh, India and was reared in the Vermibiotechnology Laboratory, Department of Zoology, Deen Dayal Upadhyaya, Gorakhpur University, Gorakhpur, UP. INDIA at room temperature. Adult earthworms of same age group were used in the experiments.

Collection of biological wastes

The feeding materials [buffalo dung , wheat straw and gram bran] for the earthworm *Eutyphoeus waltoni* were collected from different parts of Gorakhpur district in U.P., India.

Herbicides

Commercially available herbicides butachlor [2-chloro 2,6 diethyl N, butoxymethyl acetanilide] was purchased from Aristo biotech and life science Pvt. Ltd., E-26, G.I.D.C. Manjusar, savli,Distt.Vadodara-391755 , Gujrat [India] and 2,4-D [2'-4' dicholorophynoxy acetic acid] was purchased by Earth Care Pvt. Ltd. Kolkatta [India], used in the experiment at different concentrationsat laboratory conditions.

For biochemical estimation of earthworm *Eutyphoeus waltoni,* the experiment was conducted on cemented earth surface. One kg of different combinations of animal dung, agro wastes in different ratio were kept on 30 × 30× 10 cm in bed form at room temperature in dark. The vermicomposting beds were turned over manually every 24 hours for 10 days in order to eliminated volatile substances. After treatment 40% and 80% of LC_{50} of 2,4-D and butachlor of feed materials of all exposure periods, ten adult earthworms were incubated in each vermibed for the observation of biochemical effects e.g. Proteins, Amino acid, DNA, RNA, Acid/Alkaline phosphatase, and AChE on earthworms by 2,4-D and Butachlor herbicides

Biochemical Estimation

Protein levels in the gonads of earthworm *Eutyphoeus waltoni* were estimated according to lowery et al. [20]. Animals were dissected, their gonads were removed, weighed and homogenates [1mg/ml, w/v] were prepared in 10% TCA. Values have been expressed as µg protein/mg gonads.

Estimation of total free amino acids in gonads of *Eutyphoeus waltoni* were made according to the method of Spies [21]. The gonads were homogenized in 96% ethanol [10:1 w/v] in an electrical homogenizer for 5 min and centrifuged at 8000 g for 20 min.

Estimation of nucleic acid [DNA and RNA] in gonads of *Eutyphoeus waltoni* were estimated according to Schneider [22] by use of diphenylamine and orcinol reagents, respectively. Homogenates [1mg/ml, w/v] were prepared in 5% TCA at 90˚C, centrifuged at 5000 g for 20 min and supernatants were used for estimations.

Activity of acid and alkaline phosphatase in the gonads was determined according to Andersch and Szcypinski [23] as modified by Bergmeyer [24] and Singh and Agarwal [25] by use of p- nitrophenyl phosphate as substrate. Homogenates of gonads [2% w/v] were prepared in ice cold 0.9% Nacl solution and centrifuges at 5000 g [37'C] for 20 min. The supernatants were used as enzyme source.

Acetylecholinesterase activity was measured according to the method of Ellman et al. [26]. Acetylecholinesterase hydrolysis into the product choline and acetate. The method is based on the principle that free 'SH' groups released from the thioesters of choline. The AChE hydrolyze the dye DTNB to form a yellow product.

The gonads of earthworm *Eutyphoeus waltoni* were removed from the body, and homogenized in 1.0 ml of 0.1 M phosphate buffer cold pH 8.0 [50mg/ml] for 5 min. in ice bath. Therefore, centrifuge the homogenate at 1000g for 30 min. in a refrigerated centrifuge [0-4˚C]. Protein estimated was done by the method of Lowry et al. [20].

In withdrawal experiments the earthworms were transferred from sub lethal exposure of 24h and 240h LC_{50} of 2,4-D and butachlor in to the fresh vermibed. The fresh vermibed was changed daily up to 7 days, thereafter biochemical parameters were estimated.

Statistical analysis

The data have been expressed as mean ± SE of six replicates. Student's t-test was applied between control and treated groups to locate significant [$p < 0.05$] variations. Product momentum correlation coefficient was determined between exposure time and different value of LC [27]. Slope values, confidence limits, t-ratio, heterogeneity factors and g-values for different toxicity study were calculated by the method of Robertson et al. [28].

RESULTS

Table 1: Effect of sub lethal exposure (40% and 80% of 24 h LC_{50}) of 2,4-D and butachlor on the protein, amino acid, DNA and RNA level (µg/mg) in the gonads of *Eutyphoeus waltoni.*

Parameters	Sub lethal exposure of 24 h LC_{50}	Control	2,4-D	Butachlor
Protein	40%	96.18 ± 1.06 (100)	70.16±1.40*(72.94)	53.87±1.46*(56.09)
	80%		45.33±1.52*(47.13)	32.83±1.37*(34.13)
Amino acid	40%	38.33±1.30 (100)	31.66±1.04*(82.59)	24.68±1.81*(64.38)
	80%		25.50±1.12*(66.52)	17.43±1.29*(45.47)
DNA	40%	110.85±2.16 (100)	98.15±1.13*(88.54)	92.66±1.33*(83.59)
	80%		65.50±1.21*(59.08)	54.83±1.57*(49.46)
RNA	40%	58.68±1.15 (100)	47.35±1.19*(80.69)	38.31±1.14*(65.28)
	80%		37.18±1.42*(63.36)	21.33±1.22*(36.34)

Each value is mean ± SE of 6 replicates. Values in parentheses are per cent change with control taken as 100%. * Significant ($p<0.05$, t- test) difference between control and treated groups

Table 2: Effect of sub lethal exposure (40% and 80% of 240 h LC_{50}) of 2,4-D and butachlor on the protein, amino acid, DNA and RNA level (µg/mg) in the gonads of *Eutyphoeus waltoni*

Parameters	Sub lethal exposure of 24 h LC_{50}	Control	2,4-D	Butachlor
Protein	40%	96.18 ± 1.06 (100)	31.33±1.76*(32.57)	24.22±1.10*(25.18)
	80%		20.66±1.35*(21.48)	14.48±1.07*(14.74)
Amino acid	40%	38.33±1.30 (100)	19.05±1.03*(49.69)	17.15±1.18*(44.74)
	80%		15.21±1.04*(39.68)	13.53±1.11*(35.29)
DNA	40%	110.85±2.16 (100)	75.23±1.19*(67.86)	69.16±1.79*(62.39)
	80%		36.66±1.02*(33.07)	32.33±1.22*(29.16)
RNA	40%	58.68±1.15 (100)	31.86±1.13*(54.29)	28.18±1.37*(37.79)
	80%		18.73±1.24*(31.91)	15.33±1.08*(26.12)

Each value is mean ± SE of 6 replicates. Values in parentheses are per cent change with control taken as 100%. * Significant ($p<0.05$, t- test) difference between control and treated groups

Table 3: Effect of sub lethal exposure (40% and 80% of 24 h LC_{50}) of 2,4-D and butachlor on the acid, alkaline phosphatase (µm/mg protein/30 min) and AChE activity (µm 'SH' hydrolyzed/min/mg protein) in the gonads of *Eutyphoeus waltoni*

Parameters	Sub lethal exposure of 24 h LC_{50}	Control	2,4-D	Butachlor
Alkaline Phosphatase	40%	4.74 ± 0.69 (100)	3.68±0.55*(77.63)	3.36±0.15*(70.88)
	80%		2.86± 0.47*(60.33)	2.51±0.06*(52.95)
Acid phosphatase	40%	3.42±0.50 (100)	2.89±0.20*(84.50)	2.56±0.10*(74.85)
	80%		2.27±0.27*(66.37)	2.11±0.24*(61.69)
AChE	40%	0.081±0.003 (100)	0.056±0.002*(69.13)	0.048±0.002*(59.25)
	80%		0.038±0.005*(46.91)	0.032±0.003*(49.46)

Each value is mean ± SE of 6 replicates. Values in parentheses are per cent change with control taken as 100%. *Significant ($p<0.05$, t- test) difference between control and treated groups

Table 4: Effect of sub lethal exposure (40% and 80% of 240 h LC_{50}) of 2,4-D and butachlor on the acid/alkaline phosphatase (µm/mg protein/30 min) and AChE activity (µm'SH' hydrolyzed /min/mg protein) in the gonads of *Eutyphoeus waltoni.*

Parameters	Sublethal exposure of 24 h LC_{50}	Control	2,4-D	Butachlor
Alkaline Phosphatase	40%	4.74 ± 0.69 (100)	2.61±0.18*(55.06)	2.42±0.05*(51.05)
	80%		1.84±0.07*(38.81)	1.34±0.06*(28.27)
Acid phosphatase	40%	3.42±0.50 (100)	1.98±0.31*(57.89)	1.25±0.04*(36.54)
	80%		1.39± 0.23*(40.64)	1.13±0.05*(33.04)
AChE	40%	0.081±0.003 (100)	0.035±0.004*(43.20)	0.018±0.003*(22.22)
	80%		0.021±0.002*(25.92)	0.014±0.002*(17.28)

Each value is mean ± SE of 6 replicates. Values in parentheses are per cent change with control taken as 100%. * Significant ($p<0.05$, t- test) difference between control and treated groups

Table 5: The correlation coefficient (r-values) between different biochemical parameters of data Table 19-20.

Bio chemical parameters	DNA	RNA	Protein	Amino acids	Alkaline phos-phatase	Acid phos-phatase	AChE
DNA	1.00	0.915	0.852	0.768	0.681	0.498	0.633
RNA		1.000	0.888	0.892	0.681	0.607	0.724
Protein			1.000	0.883	0.573	0.527	0.667
Amino acids				1.000	0.595	0.579	0.643
Alkaline phosphatase					1.000	0.641	0.711
Acid phosphatase						1.000	0.711
AChE							1.000

All values are correlation coefficient (r-values) at significant 0.01 level, ns=not significant.

There was significant reduction in the level of biochemical parameters [protein, total free amino acid, nucleic acid and inhibition in acid/ alkaline phosphatase and AChE activity] in the gonads earthworms *Eutyphoeus waltoni* after sub-lethal exposure of [40% and 80%] of LC_{50} 24 h and 240 h of herbicides 2,4-D and Butachlor. Table 1 shows that there was significant dose dependent decrease in protein level in the gonads of *Eutyphoeus waltoni* exposed to 40% and 80% of 24 h LC_{50} of 2,4-D and butachlor. Maximum reduction [43% of control] in protein level of gonads was observed in the earthworms exposed to 80% of 24 h LC_{50} of butachlor [Fig. 1]. [Fig. 2] shows that treatment of 40% and 80% of 240h of LC_{50} of 2,4 D and butachlor caused a significant reduction in protein level in the gonads of *Eutyphoeus waltoni*. Maximum reduction [14.74%] in protein level in the gonads of *Eutyphoeus waltoni* was observed after the treatment of 80% of 240h LC_{50} butachlor [Table 2].

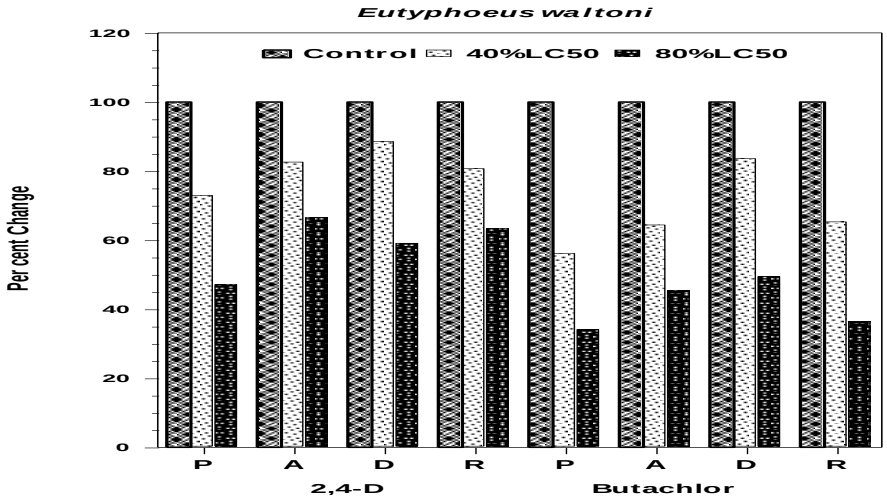

Fig. 1: Effect of sublethal exposure (40% and 80% of 24h LC_{50}) of 2,4-D and butachlor on the per cent change in protein, amino acids, DNA, RNA level in the gonad of earthworm *Eutyphoeus waltoni*. P= Protein, A= Amino acid, D= DNA, R= RNA

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[Fig. 1] shows that treatment of 40% and 80% of 24h LC_{50} of 2,4-D and butachlor cause a significant dose dependent reduction. Reduction in total amino acid level [42.42% of control] was observed in the gonads of earthworm exposed to 80% of 24h LC_{50} of butachlor [Table 1]. [Fig. 2] shows that treatment of 40% and 80% of 240h LC_{50} of 2,4-D and butachlor cause significant reduction in the total free amino acid level in gonads of *Eutyphoeus waltoni*. Maximum reduction in total free amino acid level [35% of control] was observed in earthworms exposed to 80% of 240h LC_{50} of butachlor [Table 2].

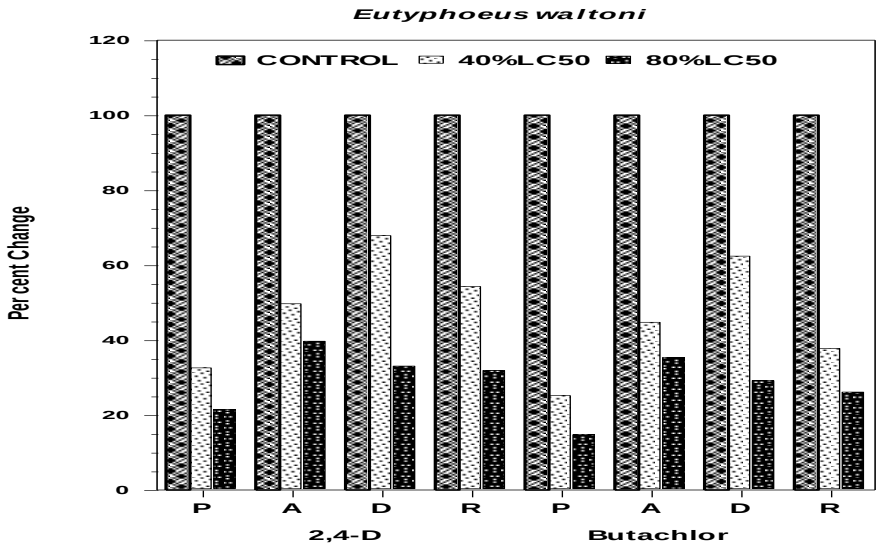

Fig. 2 : Effect of sublethal exposure (40% and 80% of 240h LC_{50}) of 2,4-D and butachlor on the per cent change in protein, amino acids, DNA, RNA level in the gonad of earthworm *Eutyphoeus waltoni*. P= Protein, A= Amino acid, D= DNA, R= RNA

There was a significant dose dependent reduction in the DNA level of gonads in *Eutyphoeus waltoni* exposed to 40% and 80% of 24h and 240h LC_{50} of butachlor and 2,4-D [Fig.1,2]. Treatment of 80% of 24h LC_{50} of 2,4-D caused a significant reduction in DNA level to [59.08%] of control [Table 1]. Maximum reduction to [29.16% of control] was observed in the gonads of earthworms exposed to 80% of 240h LC_{50} of butachlor [Table 2].

[Fig. 1] shows that the exposure of 40% and 80% of 24 h LC_{50} of butachlor, 2,4-D caused reduction in the RNA level of *Eutyphoeus waltoni*. Maximum reduction in the RNA level to [36.34 of control] was observed in the gonads of earthworm exposed to 80% of 24 h LC_{50} butachlor. Treatment of 40% and 80% of LC_{50} of 2,4-D and butachlor caused a significant reduction in RNA level of gonads of *Eutyphoeus waltoni*.[Table 1, 2].

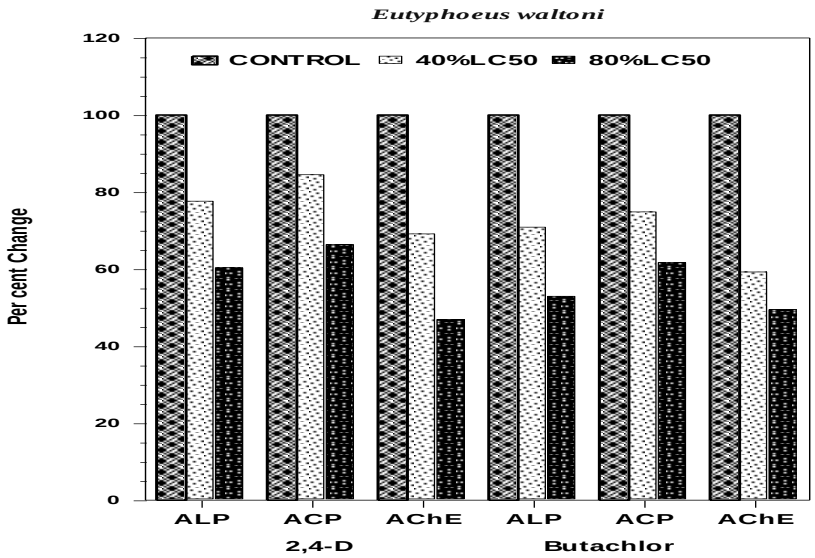

Fig. 3: Effect of sublethal exposure (40% and 80% of 24h LC_{50}) of 2,4-D and butachlor on the per cent change in ALP= Alkaline phosphatase, ACP= Acid phosphatase, and AChE= Acetylcholineesterase activity in the gonad of earthworm *Eutyphoeus waltoni*

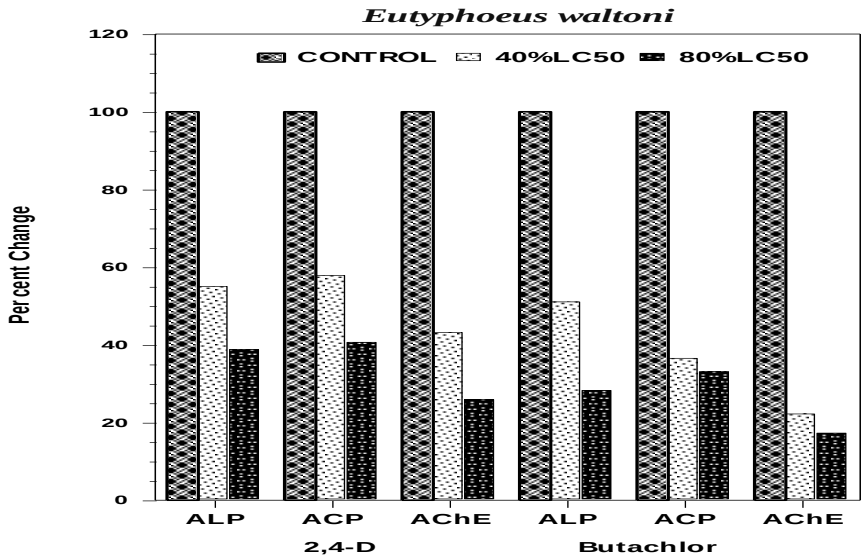

Fig. 4: Effect of sublethal exposure (40% and 80% of 240h LC_{50}) of 2,4-D and butachlor on the per cent change in ALP= Alkaline phosphatase, ACP= Acid phosphatase, and AChE= Acetylcholineesterase activity in the gonad of earthworm *Eutyphoeus waltoni.*

[Fig. 3 and Fig. 4] shows that there was a significant dose dependent of acid/ alkaline phosphatase and AChE activity in the gonads of earthworms exposed to 2,4-D and butachlor. Treatment of 80% of 24h LC_{50} of butachlor and 2,4-D caused inhibition in alkaline phosphatase to 52.95%of control, respectively [Table-3]. Acid phosphatase activity was inhibit to 66.37%, 61.69% in the gonads of *Eutyphoeus waltoni* exposed to 80% of 24h LC_{50} of 2,4-D and butachlor, respectively. [Fig . 3] shows that the treatment of 40% and 80% of 240h LC_{50} of 2,4-D and butachlor caused significant inhibition in acid and alkaline phosphatase activity in the gonads of *Eutyphoeus waltoni*. Maximum inhibition in alkaline phosphatase activity [28.27% of control] and acid phosphatase [33.04% of control] was observed in the gonads of *Eutyphoeus waltoni* exposed to 80% of 240h LC_{50} of butachlor [Table 4].

The earthworms treated with sub lethal exposure [40% and 80% LC_{50}] of 2,4-D and butachlor showed time and dose dependent inhibition of acetylcholinesterase activity in gonads of *Eutyphoeus waltoni.* Treatment of 80% of 24h LC_{50} of butachlor and 2,4-D caused the inhibition in AChE activity was observed [Table 3, 4]. [Fig 4.] Maximum inhibition was observed in 80% of LC_{50} of 240h in butachlor [17.28%] than the sublethal exposure of 2,4-D. Whereas minimum inhibition in AChE activity was observed 40% LC_{50} of 2,4-D [43.20%]. [Table 3, Fig. 3, 4].

In this withdrawal experiment attempts have been made to observed the level of different biochemical parameters [Protein, amino acid, DNA, RNA, acid and alkaline phosphatase and AChE activities] in the gonads of *Eutyphoeus waltoni* after sub lethal exposure of 80% of 24h LC_{50} treatment of butachlor and 2,4-D for 7 days. There was no recovery in the level of protein, amino acid, DNA, RNA and acid/ alkaline phosphatase activity and AChE activity in the gonads of *Eutyphoeus waltoni.*

DISCUSSION

It is evident from results that the 2,4-D and butachlor significantly change the different biochemical parameters i.e protein, amino acid, DNA, RNA, acid, alkaline phosphatase and AChE activity in the gonads of *Eutyphoeus waltoni* within 24h and 240h.The effect of 2,4-D and butachlor in 240h of exposure on different biochemical parameters in gonads of earthworm *Eutyphoeus waltoni* was more pronounced. It seems that increase in exposure periods from 24h to 240h may cause penetration of earthworm body which cause more reduction in protein, amino acid, DNA, RNA, and acid/alkaline phosphatase and AChE activity with respect to *Eutyphoeus waltoni.*

Data obtained from the withdrawal experiment clearly indicates that effect of 2,4-D and butachlor is non reversible as the level of protein, amino acid, DNA, RNA, and acid/alkaline phosphatase and AChE activity within 7 days. Mommensen and Walsh [29] reported that Proteins which are the main source of the nitrogenous metabolism, are mainly involved in the architecture of the cell and during chronic periods of stress they are also a source of energy. Increment in the free amino acids level was the result of breakdown of protein for energy requirement and impaired incorporation of amino acids in protein synthesis. The herbicide acetochlor caused adverse effect on the sperm number and DNA in *Eisenia fetida* [30]. Gobi et al.[31]. Reported that they were glandular cell enlargement and vaccualization in the

intestine of the earthworm *Perionyx sansibaricus* exposed to sub lethal concentration of herbicide butachlor.

AChE inhibition of different animals by many organophosphate insecticides is well established. It reduced the survival rate of earthworms in agricultural soils. The present study assessed the sublethal exposure 40% and 80% LC_{50} of 2,4-D and butachlor to under laboratory conditions with special reference to the biochemical changes of earthworms. Navarro and Obregon [32]. reported that treated *Eisenia fetida* with organophosphate insecticide, Malathion and found that decreased the spermatic viability in spermathecae, altering the cell proliferation and modifying the DNA structure of spermatogonia.

This pesticideis known to retard growth and reproduction in earthworms such as *Eisenia fetida* [33]. and *Perionyx sansibaricus* as well as damage epithelial tissue in *Eisenia fetida* [34]. Butachlor is neurotoxic to land snails and genotoxic to toad and frog tadpoles, flounder, and cat fish where it causes DNA strand break induction in erythrocytes [35-38]. It is also genotoxic to cultured mammalian cells where it causes DNA strand breaks and both micronucleus and chromosomal aberration inductions [39,40].

The earthworm *Eutyphoeus waltoni* treated with sub lethal exposure [40% and 80% LC_{50}] of 2,4-D and butachlor showed time and dose dependent inhibition of acetylcholinesterase activity in gonads of *Eutyphoeus waltoni*. AChE is responsible for the termination of cholinergic impulse by the hydrolysis of acetylcholine esterase [AChE] in the choline and acetic acid [25].

The enzyme AChE units have two active sites, an anionic site, which attracts the cationic part of the substrate or inhibitor by coulombic or hydrophobic force, and anesteric site. AChE has been secreted into the synapse it binds to receptor sites on the next nerve cell, causing the latter to propagate the nerve impulse. Before the transmission of second impulse through the synapse, ACh secreted after the first impulse must be hydrolised by the AChE in the junction. Inhibition of AChE resulted in abnormal accumulation of acetylcholine which cause eventual paralysis of the muscle. Death occurs as a result of asphyxia caused by the paralysis of respiratory muscle [41]. Result obtained from the this section indicate that 2,4-D and butachlor inhibit the acetylcholinesterase of *Eutyphoeus waltoni* in dose dependent manner.

The dose dependence effect on AChE activity could be due to serveral factors like role of penetration in the animal, rate of inactivation, variability or increased competition with the natural substrate at the active sites [42,43]. Singh and Agarwal [44]. also reported that carbamate and organophosphate compounds [e.g. mexacarbate, phorate] also show a dose related response presumably due to conversion to more toxic metabolites in the body of animals. Denoyelle et al. [45]. reported that AChE activity significant decreased in earthworm from treated orchards. Jarden et al. [46]. reported that inhibition of cholinesterase activity after treatment of pesticide azinphos- methyl on juvenile earthworm *[Eisenia andrei].*

CONCLUSION

Among all treatment of sub-lethal exposure of LC_{50} of herbicides 2,4-D and butachlor against *Eutyphoeus waltoni* in the different feed materials, the tertiary combination of buffalo dung with wheat straw and gram bran have more potency to increase the tolerance power of earthworm *Eutyphoeus waltoni* in agricultural fields. The use of vermicompost of buffalo dung with wheat straw and gram bran is beneficial for better productivity of crops as well as provided better nourishment to enhance the population and tolerance power against herbicides and other chemicals.

CONFLICT OF INTEREST
There is no conflict of interest.

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FINANCIAL DISCLOSURE
None

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