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TECHNICAL NOTE

Toxic Effect of Herbicide 2,4-D on the Earthworm *Eutyphoeus waltoni* Michaelsen

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Abstract The toxicity of herbicide 2,4-D (2′-4′ dicholoro phynoxy acetic acid) was studied on earthworms in different combinations of biological wastes and different types of soils as feed materials under laboratory conditions. *Eutyphoeus waltoni* were exposed to different concentrations of 2,4-D (200, 300, 400, 450 mg/kg) in feed material (i.e., buffalo dung, wheat straw and gram bran) and different concentrations of 2,4-D (150, 300, 350, 400 mg/kg) in different types of soil (i.e., loamy soil, clay soil and sandy soil). Observations were recorded from 24 h up to 240 h. It was observed that the toxic effect of herbicide 2,4-D on the earthworm *Eutyphoeus waltoni* was both time and dose dependent. Maximum toxicity was observed in the sandy soil, whereas minimum in the feed material of buffalo dung with gram bran. There was no mortality observed in control.

Keywords Herbicide · 2,4-D · *Eutyphoeus waltoni* · Soils · Buffalo dung · Agro-wastes · Toxicity

1 Introduction

Soil environments are contaminated by the indiscriminate use of pesticides and herbicides, which affect the soil flora and fauna population (Gobi and Gunasekaran 2010). Earthworms were used as model experimental organisms for toxicity as well as bioaccumaltion assessment (Nusetti et al. 1999). 2,4 dichlorophenoxy acetic acid (2,4-D) is a low cost, easily available, and early and extensively effective used herbicide worldwide. Ville et al. (1997) reported that the 2,4-D has toxic effects on mammals, including neurological dysfunction, pulmonary oedema, hepatic and renal dysfunctions or symptoms of tetanus. Generally 2,4-D is used in agricultural fields for the purpose of controlling broad-leaf weeds (Munro et al. 1992). The present work studies the toxicity of 2,4-D on earthworm *Eutyphoeus waltoni* in soil, because this herbicide has poor biodegradability, several metabolic alterations and tissue necrosis in non-target organisms, including important members of the food chain organisms, such as fish (Gallagher and Di Giulio 1991; Chingombe et al. 2006).

The abundant use of herbicides does not affect only selected weeds; herbicides can also destroy non-target species in the agricultural fields and also affect the texture and physico-

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chemical properties of soils (Robidoux et al. 1999). The use of specific herbicides, fungicides, insecticides in the agricultural field can be highly toxic to earthworm population (Williamson 2000; Zhou et al. 2007). The exclusive major role of earthworms in paedogenesis is through mixing of the particles during digesting, depositing their casts throughout the soil column, and improving aeration and drainage of the agricultural soils (Kavitha et al. 2011). Earthworms are also important contributors to the recycling of carbon and nitrogen in the ecosystem, so, they are used as bioindicators (Callahan 1988; Goats and Edwards 1988).

Correia and Moreira (2010) reported that the effects on growth, survival and reproduction rates of earthworms were verified for different exposure times. Earthworms kept in glyphosate treated soil remained alive in all evaluations, but showed gradual and significant reduction in mean weight (50 %) at all test concentrations. The combinations of animal dung with different agro-wastes are the best suitable feed material for better growth and development of earthworm *Eisenia fetida*. The combination of buffalo dung with wheat straw and gram bran resulted in maximum biomass, weight and length (Nath et al. 2009; Chauhan and Singh 2012; Kumar and Singh 2013). Singh and Kumar (2014) reported that the earthworm *Eutyphoeus waltoni* is found abundantly in agricultural fields of different localities of eastern Uttar Pradesh. Correia and Moreira (2010) reported that 100 % mortality of epigeic earthworm was observed in soil treated with 500 and 1000 mg/kg of 2,4-D; at 14 days, 30–40 % mortality levels were observed for all treated concentrations. No cocoons or juveniles were found in soil treated with the herbicide. Glyphosate and 2,4-D demonstrated severe effects on the development and reproduction of *Eisenia foetida* in laboratory tests in the range of test concentrations (Correia and Moreira 2010).

The aim of the present study was to investigate the toxic effect of herbicide 2,4-D on the earthworm *Eutyphoeus waltoni* in different soils, and in combination of feed material of buffalo dung with agro-wastes and soil alone as feed materials in laboratory conditions.

2 Materials and Methods

Collection of the Earthworm: The cultured earthworm *Eutyphoeus waltoni* was used in the experiment.

Collection of animal dung and agro-wastes: The buffalo dung, wheat straw and gram bran were collected from different part of Gorakhpur district of U.P. India.

Herbicide: The commercially available herbicide 2,4-D (2′-4′ dicholoro phynoxy acetic acid), purchased from EarthCare Pvt. Ltd India, was used in the experiment at different concentrations. The composition of 2,4-D is 2,4-D Acid Technical 59 % w/w (based on 97 % w/w ai.a), dimethyl amine 30 % w/w, Seqvesting agent (lignin sulphonate) 1 % w/w, Divent acqua sufficient 10 % w/w.

Table 1 Concentrations used for toxicity determination against earthworm (*Eutyphoeus waltoni*)

Name	Combinations	Concentration (mg/kg)
2,4-D	BD+Ws	200, 300, 400, 450
	BD+Gb	200, 300, 400, 450
	BD+Ws+Gb	200, 300, 400, 450
	Loamy soil	150, 250, 350, 400
	Clay soil	150, 250, 350, 400
	Sandy soil	150, 250, 350, 400

Table 2 Toxicity of herbicide 2,4-D against earthworm *Eutyphoeus waltoni* in different feed materials

Period	Combinations	LC_{50}	Lower limit	Upper limit	Slope value	t-ratio	g-value	Heterogeneity
24 h	BD+Ws	540.20	477.06	696.39	4.06	4.19	0.218	0.28
	BD+Gb	540.44	475.89	673.04	3.44	6.30	0.097	0.14
	BD+Ws+Gb	510.81	458.33	609.59	3.77	6.71	0.085	0.09
	Loamy soil	470.38	416.14	572.38	3.43	6.95	0.079	0.08
	Clay soil	490.92	427.67	617.82	3.18	6.61	0.088	0.10
	Sandy soil	445.56	392.93	543.98	2.99	6.79	0.083	0.09
48 h	BD+Ws	456.72	416.55	523.62	3.67	7.19	0.074	0.11
	BD+Gb	460.73	414.85	543.85	3.19	6.51	0.090	0.08
	BD+Ws+Gb	474.57	425.11	567.12	3.17	6.37	0.095	0.07
	Loamy soil	392.55	349.76	466.22	2.74	6.79	0.083	0.12
	Clay soil	399.48	356.15	474.41	2.82	6.87	0.081	0.08
	Sandy soil	380.35	338.24	452.59	2.59	6.58	0.088	0.18
72 h	BD+Ws	399.54	368.19	445.09	3.48	7.33	0.071	0.12
	BD+Gb	404.11	364.83	468.91	2.74	6.02	0.106	0.12
	BD+Ws+Gb	409.61	370.43	474.44	2.83	6.15	0.102	0.12
	Loamy soil	310.52	278.56	354.04	2.45	6.58	0.088	0.15
	Clay soil	300.84	271.86	337.55	2.64	7.05	0.077	0.12
	Sandy soil	313.15	280.08	359.27	2.38	6.41	0.093	0.37
96 h	BD+Ws	342.65	312.78	378.72	2.94	6.60	0.088	0.07
	BD+Gb	353.42	314.08	408.84	2.24	5.13	0.145	0.05
	BD+Ws+Gb	343.99	311.58	384.30	2.70	6.12	0.102	0.10
	Loamy soil	250.07	219.84	280.22	2.34	6.46	0.092	0.24
	Clay soil	244.28	216.61	271.06	2.56	7.03	0.078	0.26
	Sandy soil	251.15	222.52	279.81	2.47	6.77	0.084	0.42
120 h	BD+Ws	289.06	261.97	313.90	3.26	7.34	0.071	0.04
	BD+Gb	304.16	265.79	341.67	2.30	5.31	0.136	0.06
	BD+Ws+Gb	299.74	267.23	330.74	2.70	6.17	0.101	0.11
	Loamy soil	212.82	182.97	238.13	2.48	6.82	0.083	0.28
	Clay soil	201.05	171.64	225.25	2.56	7.01	0.078	0.38
	Sandy soil	210.34	179.75	235.89	2.45	6.73	0.085	0.21
240 h	BD+Ws	252.63	225.33	275.08	3.51	7.79	0.063	0.04
	BD+Gb	271.54	230.52	304.43	2.34	5.40	0.131	0.10
	BD+Ws+Gb	268.97	233.40	297.99	2.66	6.09	0.103	0.07
	Loamy soil	190.72	160.07	215.17	2.53	6.89	0.081	0.28
	Clay soil	182.97	156.61	204.38	2.92	7.76	0.064	0.78
	Sandy soil	176.60	160.01	191.04	4.58	10.57	0.034	0.47

Mortality was determined every 24 h. Each set of experiment was replicated six times. Product momentum correlation showed that there was significant negative coefficient ($p<0.05$) observed between exposure time and different value of LC_{50} of 2,4-D

Determination of LC_{50}: The toxicity experiment was performed following the method of Agarwal and Singh (1988). Ten adult earthworms were kept in vermibeds of one kg feed material. The vermibeds were exposed to different concentration of herbicides (Table 1). Six

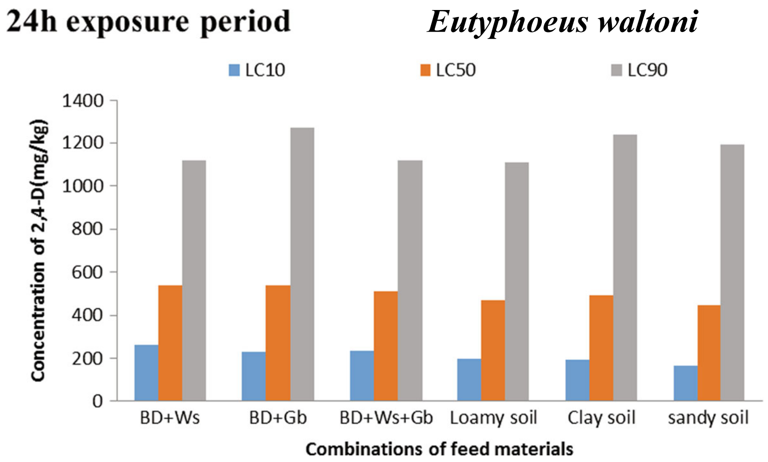

Fig. 1 The toxicity of 24 h exposure of herbicide 2,4-D against earthworm *Eutyphoeus waltoni*

vermibeds were set up for each dose of herbicides. Vermibeds without any treatment were used as control. Mortality was recorded at different exposure periods. 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. (1977).

Statistical Analysis: All the studies were replicated at least six times. Product momentum correlation coefficient was determined between exposure time and different values of LC. Analysis of variance was used to analyze the significant difference between different combinations and exposure time. (Sokal and Rohlf 1973).

3 Results

Laboratory toxicity was evaluated for different concentrations of 2,4-D against earthworm *Eutyphoeus waltoni* in different combination of buffalo dung with agro-wastes

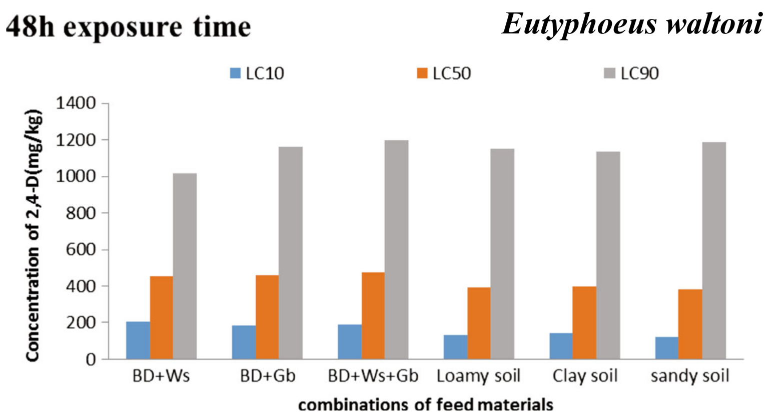

Fig. 2 The toxicity of 48 h exposure of herbicide 2,4-D against earthworm *Eutyphoeus waltoni*

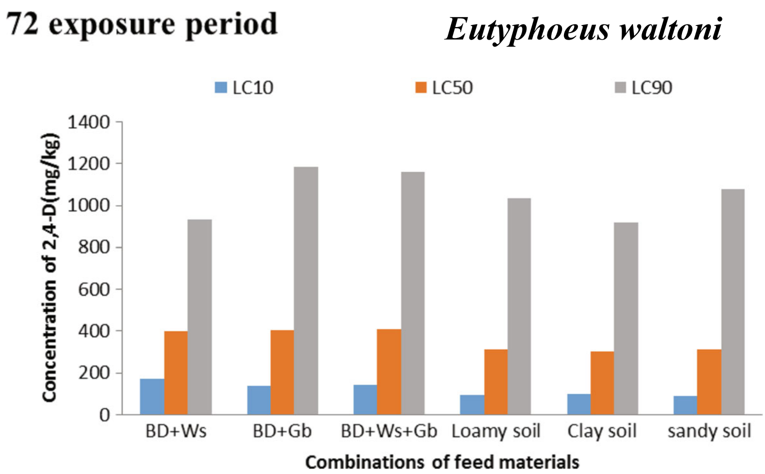

Fig. 3 The toxicity of 72 h exposure of herbicide 2,4-D against earthworm *Eutyphoeus waltoni*

and soil as feed materials. The toxicity of 2,4-D was both time and dose dependent against earthworm *Eutyphoeus waltoni* in all the exposures. There was a negative significant product momentum coefficient ($P<0.05$) observed between exposure time and different value of LC_{50} of 2,4-D (Tables 2 and 4; Figs. 1, 2, 3, 4, 5 and 6). The toxicity of 2,4-D against *Eutyphoeus waltoni* was higher in sandy soil in exposure periods of 24 h to 240 h, whereas, minimum in the feed material of combination of buffalo dung with gram bran (Tables 2 and 4; Fig. 1).

Toxicity of 24 h exposure of 2,4-D against the earthworm *Eutyphoeus waltoni* was higher in sandy soil than other combinations of 2,4-D at different exposure periods against *Eutyphoeus waltoni* as 445.56 mg/kg LC_{50}. The order of 24 h

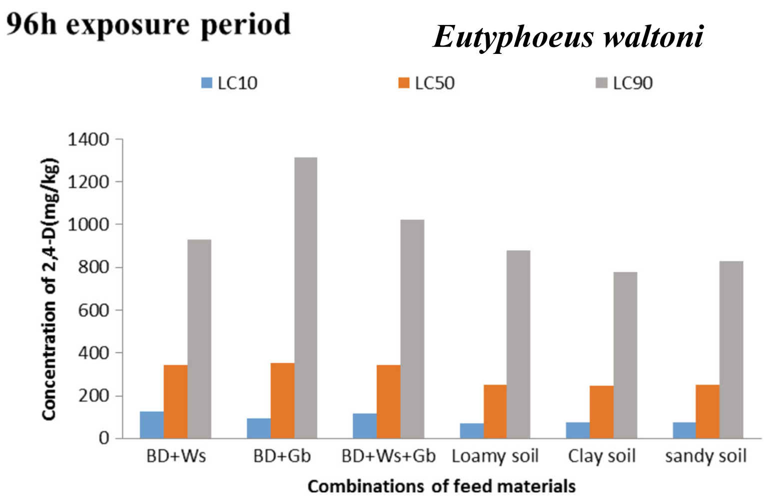

Fig. 4 The toxicity of 96 h exposure of herbicide 2,4-D against earthworm *Eutyphoeus waltoni*

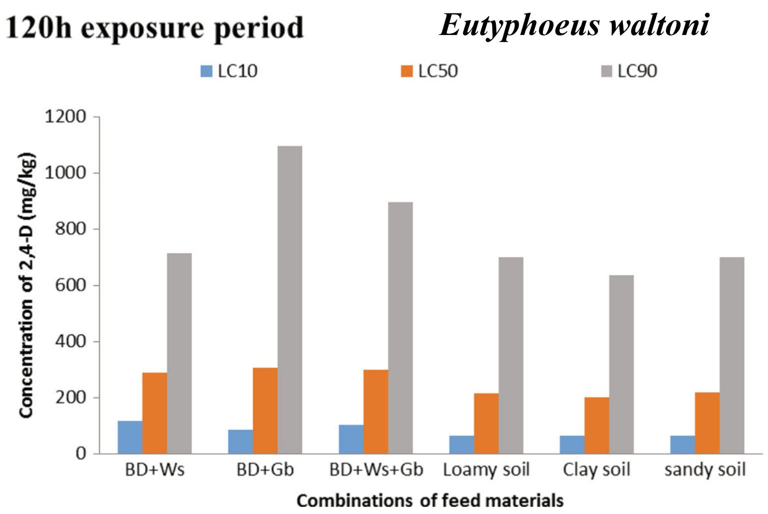

Fig. 5 The toxicity of 120 h exposure of herbicide 2,4-D against earthworm *Eutyphoeus waltoni*

exposure toxicity of 2,4-D was sandy soil>loamy soil>clay soil>BD+Ws+Gb> BD+Ws>BD+Gb. The slope value given in Tables 2 and 3 was steep and separate estimation of LC of different combinations was found to be within the 95 % confidence limits. The t-ratio was greater than 1.96 and heterogeneity less than 1. The g-value was less than 0.5 at all probability levels. A significant correlation coefficient (r) between exposure time and LC_{50} value of 2,4-D was obtained (Tables 2 and 4). There was no mortality recorded after 240 h exposure period in all the treatments (Tables 2 and 4; Fig. 7). Analysis oif variance (ANOVA) was used to analysed the significant difference between different combinations of feed materials and exposure period (Table 3).

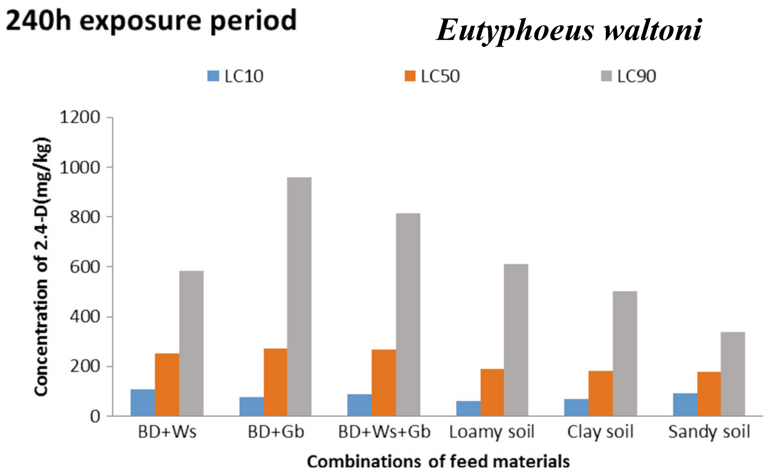

Fig. 6 The toxicity of 240 h exposure of herbicide 2,4-D against earthworm *Eutyphoeus waltoni*

Table 3 Summary of computation of analysis of variance (ANOVA) of the data of Tables 2 and 4

Source of variation	Toxicity of different combination of wastes				
	DF	SS	σ2	F	P
Between treatment	5	62909.1	12581.8	33.6	$P<0.001$
Between Time	5	337386.3	67477.3	180.0	$P<0.001$
Error	10	3748.7	374.9		
Total	20	404044.1			

4 Discussion

It is evident from the results that the studied toxicity of 2,4-D was time and dose dependent against earthworm *Eutyphoeus waltoni*. Thus, the earthworms have been used as model animals for studying the effects of agrochemicals on soil fauna (Cock et al. 1980; Gobi et al. 2004). Herbicides have been reported to have adverse effect on the survival of earthworms (van Gestrel and van Dis 1988; Robidoux et al. 1999). The herbicide acetochlor caused adverse effect on the sperm number and DNA of *Eisenia fetida* (Xiao et al. 2006). Several other studies have demonstrated the lethal activity of herbicides and pesticides on earthworms and histopathological effects (Gupta and Sundaraman 1988; Sorour and Larink 2001; Lydy and Linck 2003; Gobi et al. 2004; Rombke et al. 2007; Mosieh 2009). Correia and Moreira (2010) reported that 100 % mortality was observed a few hours after exposure of those organisms when exposed to 1000 mg/kg of 2,4-D. 2,4-D is toxic against earthworms according to Roberts and Dorough (1984). Brown (1978) also reported that some herbicides are directly toxic to earthworms.

Herbicides affect the feeding behaviour of earthworms, which was reflected in the weight loss and reproduction capacity (Venter et al. 1988; Bustos–Obregon and Goicochea 2002). Indiscriminate use of pesticides may affect non-target organisms in the soil and can cause serious damage to ecosystem (Reinecke and Reinecke 2007). Smith et al. (1992) reported that soil animals, especially earthworms, are one of the best bioindicators of pesticide contamination. The agrochemical concentration is higher in surface layers; earthworm activity is very much reduced in the soil surface layer (Keogh and Whitehead 1975; Cock et al. 1980).

The toxicity of 2,4-D against earthworm *Eutyphoeus waltoni* was higher in the sandy soil at 24 h up to 240 h exposure periods, because sandy soil have less organic content and other nutritional components than other combinations of agro-wastes and soil. Nath et al. (2009) reported that the feed material of buffalo dung with gram bran have rich organic nutrients. The combination of buffalo dung with gram have minimum toxicity of 2,4-D against *Eutyphoeus waltoni* because it is possible that this combination have rich amount of organic nutrients which tolerate the toxicity of 2,4-D. There was no mortality recorded after 240 h exposure period in all treatments, which may be due to the development of tolerance power against 2,4-D in earthworms.

It is evident from the results, that the herbicide 2,4-D has toxic effects against earthworm population, but the use of vermicompost of different combinations of buffalo dung with agro-wastes in soil protect the earthworms against toxic effects of herbicides. The direct use of different combinations of buffalo dung with agro-wastes

Table 4 Lethal concentrations of herbicide 2,4-D against earthworm *Eutyphoeus waltoni* in different feed materials

Period	Combinations/Soils	LC_{10}	LC_{50}	LC_{90}
24 h	BD+Ws	261.30	540.28	1116.75
	BD+Gb	229.70	540.44	1271.51
	BD+Ws+Gb	233.75	510.81	1116.36
	Loamy soil	199.35	470.38	1109.90
	Clay soil	194.40	490.92	1239.70
	Sandy soil	166.33	445.56	1193.49
48 h	BD+Ws	204.71	456.72	1018.96
	BD+Gb	183.00	460.73	1159.96
	BD+Ws+Gb	187.58	474.57	1200.64
	Loamy soil	133.90	392.55	1150.81
	Clay soil	140.52	399.48	1135.67
	Sandy soil	121.77	380.35	1188.06
72 h	BD+Ws	171.51	399.54	930.74
	BD+Gb	137.88	404.11	1184.40
	BD+Ws+Gb	144.59	409.61	1160.38
	Loamy soil	93.39	310.52	1032.44
	Clay soil	98.61	300.84	917.82
	Sandy soil	91.03	313.15	1077.23
96 h	BD+Ws	125.91	342.65	932.45
	BD+Gb	94.85	353.42	1316.38
	BD+Ws+Gb	115.61	343.99	1023.49
	Loamy soil	71.03	250.06	880.38
	Clay soil	77.44	244.28	778.58
	Sandy soil	76.27	251.15	827.01
120 h	BD+Ws	117.12	289.06	713.44
	BD+Gb	84.32	304.16	1097.17
	BD+Ws+Gb	100.49	299.74	893.99
	Loamy soil	64.83	212.82	698.66
	Clay soil	63.74	201.05	634.18
	Sandy soil	63.18	218.34	700.22
240 h	BD+Ws	109.02	252.63	585.42
	BD+Gb	76.98	271.54	957.81
	BD+Ws+Gb	88.77	268.97	814.96
	Loamy soil	59.43	190.72	612.04
	Clay soil	66.74	182.97	501.65
	Sandy soil	92.77	176.60	336.18

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

in the agricultural fields are suitable feed materials for earthworms which provide better nourishment to tolerate the toxic effect of the herbicides. Production and use of vermicompost from biological wastes promotes plant growth and productivity, as well as protects the earthworms, and helps the growth and development of its population.

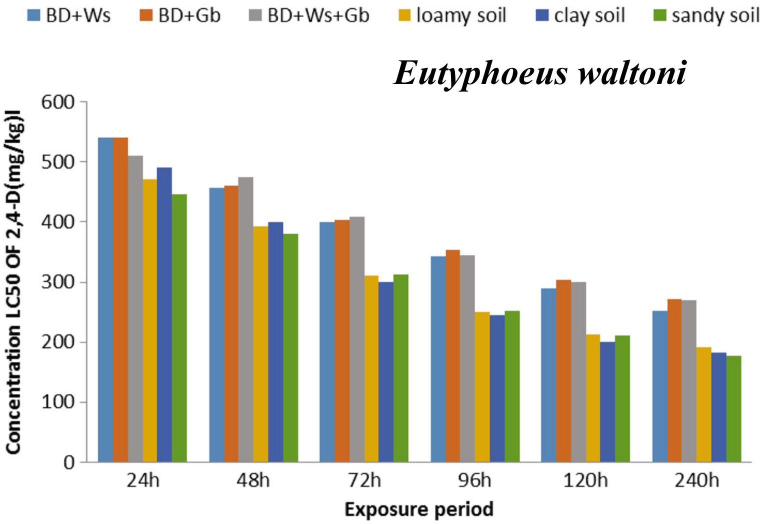

Fig. 7 Effect of different combinations of feed material of exposure of herbicide 2,4-D on earthworm *Eutyphoeus waltoni* in different exposure period

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