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STUDY ON TOXICITY EFFECT OF BISPYRIBAC-SODIUM HERBICIDE ON EARTHWORMS BY FILTER PAPER AND SOIL MIXING METHOD

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ABSTRACT

Two techniques were used to assess the toxicity of the herbicide bispyribac-sodium on earthworms (*Aporrectodea caliginosa)* by filter paper contact test at 24, 48 and 72 h and soil mixing test at 5 and 10 day. The results obtained in filter paper contact test showed that the concentrations of ≤ 1 µg a.i mL^{-1} for herbicide caused 0% mortality at 24 h and 0.01 µg a.i mL^{-1} caused 0% mortality at 48 h. BS showed the highest intrinsic toxicity to the worms with an LC_{50} value of 0.062 µg a.i mL^{-1} at 72-h compared to LC_{50} at 48 h was 38.9 µg a.i mL^{-1} and LC_{50} at 24 h were 518.32 µg a.i mL^{-1}. The toxicity of BS on earthworms by soil mixing technique expressed as LC_{50} was increased when the exposure time was increased. The LC_{50} was reduced from 152.92 to 18.59 in clay soil, from 311.60 to 29.63 in clay soil: sandy clay loam soil (1:1) and from 448.23 to 33.67 in sandy soil at 5 and 10 days after treatment, respectively. The toxicity of tested pesticide was greater in clay soil at both time intervals than clay soil: sandy clay loam soil (1:1) and sandy clay loam soil.

Keywords: Toxicity; Bispyribac-sodium; Soil; Earthworms; *Aporrectodea caliginosa*

1. INTRODUCTION

Bispyribac-sodium (CAS No. 125401-75-4), sodium 2,6-bis [(4,6-dimethoxy-2-pyrimidinyl)oxy] benzoate, is a common systematic post-emergence herbicide used for the effective control of annual and perennial grasses, sedges and broad leaf weeds, such as Echinochloa species, in rice fields. Advantageous and unavoidable uses, herbicides often contaminate the water, soil and agricultural products causing public health hazards due to their high toxicity and long persistence (Qamruzzaman and Nasar, 2019). This gives rise to increasing interest in analyzing and monitoring the BS residue in rice and environments, i.e. soil, water and side effects on non-target organisms (Pareja *et al.*, 2012). Reduction in the height of rice plants due to application of BS

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has been observed. Hence, the agricultural application of BS is associated with a significant risk to the soil, aquatic system and water resources. Thus, the treatment of BS, after the fulfillment of its herbicidal applications, is essential to eliminate or at least minimize its negative impact especially on water resources and earthworms in soil (Qamruzzaman and Nasar, 2019). Earthworms are the most important soil invertebrates, accounting for 60-80% of the total soil biomass, and widely distributed in different soils of the world (Benckiser, 1997; Sun *et al.*, 2019). Due to their digging, digesting and excreting activities, earthworms have strong abilities to transform soil structure, improve soil physio-chemical characters, microbial remove the activities and promote reactions, thereby directly and indirectly promote biodegradation of organic contaminants. Earthworms could also retard the binding of organic contaminants to soils, release previously soil-bound contaminants for subsequent degradation, and enhance the bioavailability of organic pollutants (Zhao *et al.*, 2016). Previous researches have suggested that earthworms could assist the microbial degradations of many pesticides in soil, e.g., 2-Methyl-4-chlorophenoxyacetic acid (Liu *et al.*, 2011), pentachlorophenol (Li *et al.*, 2015) and atrazine (Lin *et al.*, 2018). Thus, it is supposed that addition of earthworms will possibly strengthen the microbial degradation of BS in soil. The characterization of contaminated sites and evaluation of remediation techniques usually involves the determination of contaminant concentration in the soil or other contaminated media (sediment, semisolid waste, etc.). However, for several decades it has become desirable, and in some cases truly necessary, to include bioassays for such characterization also. This is due to two factors. First, in the biodegradation or chemical transformation of soil contaminants, the contaminating compounds often are not mineralized completely, but other intermediates are produced. Usually, not all of the metabolites are known, and their toxicity is unknown. Second, many weathering processes, as well as bioremediation/chemical treatments, may not greatly reduce the concentration of the parent compound, but may reduce the bioavailability to sufficiently low levels to be only of minimal risk to public health and the environment. Thus, to characterize a site or treated material adequately, bioassays are needed in addition to chemical determinations (Domínguez-Rodríguez *et al.*, 2020). The objectives of this study were to: (1) comparison of the toxicity of BS on earthworms (Aporrectodea caliginosa) by different techniques by filter paper contact test and soil mixing technique; and (2) effect of soil type on the toxicity of BS for *Aporrectodea caliginosa.*

2. MATERIALS AND METHODS

2.1 Bispyribac-sodium

IUPAC name: Sodium 2,6-bis[(4,6-dimethoxypyrimidin-2-yl)oxy] benzoate. The chemical class: Pyrimidinyl oxy benzoic acid. Product: Technical 98% a.i. Solubility (20 ºC): Water 7330 mg/L. Pesticide type: Herbicide. Production Company: Sundat, AGROCHEM. Formulations:

Bispyribac-sodium 1% SC. Uses: Control of grasses, sedges and broad-leaved weeds, especially *Echinochloa spp.*, in direct-seeded rice and weeds in non-crop situations.

2.2 Tested soils

There are two common types of the Egyptian soil, alluvial and calcareous. The soil samples were collected from the surface layer (0-20 cm) from different locations that had no history with the pesticides. The alluvial soil (clay soil) was collected from the Agricultural Research Station, Abis farm of the Faculty of Agriculture, University of Alexandria and the calcareous soil (sandy clay loam soil) collected from the Elnahda region, Elamria, Alexandria Governorate. The physical and chemical properties were determined at the Department of Soil and Water Sciences, Faculty of Agriculture, University of Alexandria and the data are presented in Tables (1 and 2). Soil samples were air-dried, ground and passed through a 2-mm sieve use. The soil texture was determined by the hydrometer method (Gee *et al.*, 1986). Soil pH was measured using 0.01 M calcium chloride ($CaCl_2$) in a 1:2 w/w soil: solution slurry. The OM content was determined by dichromate oxidation according to the Walkley-Black method (Nelson *et al.*, 1996).

Table 1: Physical properties of the tested soils

Soil code	**Soil type**	**Particle size distribution (%)**			**Texture class**	**Water holding capacity (mL/100g)**
		Clay	Silt	Sand		
1	Alluvial	42	18	40	Clay	46
2	Calcareous	20	13	67	Sandy clay loam	38

Table 2: Chemical properties of the tested soils

Chemical properties	**Claysoil**	**Sandy clay loamsoil**
EC (m mohs/cm) at 25˚C	1.32	5.03
Soil pH	8.25	8.15
Organic matter content (%)	3.31	1.54
Total carbonate (%)	7.87	44.64
Soluble cations conc. (meq/L):		
Ca^{++}	3.8	18.7

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Mg^{++}	5.0	8.8
Na^{+}	9.4	22.5
K^{+}	0.5	0.3
Soluble anions conc. (meq/L):		
CO_3^{--}	1.6	0.8
HCO_3^{-}	2.6	4.6
Cl^{-}	8.5	21.0
SO_4^{--}	0.6	23.9

2.3 Earthworm

Earthworms was used in this study belonged to species commonly found in Egypt (*Aporrectodea caliginosa*). Individual worms were collected from fields around Alexandria Governorate and reared in artificial soil in large plastic containers (38 x60 x 10 cm) covered with muslin cloth to reduce water evaporation, as described by Heimbach (1984). The worms were maintained in the artificial soil at 23 ± 2ºC for one month before the experiments. Earthworms used in this study were adults. As earthworms are hermaphrodite, no sexual differences are taken into account. The adults were removed from the artificial soil 24 h before use and stored in Petri dishes on damp filter paper (in the dark at 23 ± 2˚C) to void gut contents (Li *et al.*, 2020).

2.4 Experiments

2.4.1 Contact toxicity of tested pesticide on earthworm by filter paper contact test

The standard OECD Guidelines for contact toxicity protocol (OECD, 1984) was followed, glass Petri dishes and filter paper, 11.0 cm diameter were used (Addison and Holmes, 1995). The filter papers were treated with tested BS (Technical 98 %) solutions in acetone (0.01, 0.1, 1, 10, 50, 100, 200, 500, 1000, 2000 and 4000 μg/ml) to obtained final concentrations of BS in filter paper; 600, 300, 150, 75, 30, 15, 1.5, 0.15, 0.015 and 0.0015μg/cm^2. After solvent evaporation, 1.5 mL water was added, and one earthworm (*Aporrectodea caliginosa*) was placed in each Petri dish to direct contact with pesticide, four earthworms were used as a replicate. With a plastic film to permit inner aeration, plates were covered and holed (Figure 1). Three replicates for each tested concentration as well as control were kept in a big box at the darkness to ambient temperature for 72 h. Every 24 h, mortality and general aspect of all individuals was recorded and the LC_{50} values were calculated by LdP line software (Singh and Singh, 2015; Cheng *et al.*, 2020).

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The earthworms was placed in glass Petri plates with a known diameter (to know the exact quantity of pesticide - μg/cm^{-2})

The Petri dish was treated with tested BS solution (0.01, 0.1, 1, 10, 50, 100, 200, 500, 1000, 2000 and 4000 μg/ml)

↓

After solvent evaporation, 1.5 mL water was added and earthworms were placed (concentrations pesticide in Petri plates; 600, 300, 150, 75, 30, 15, 1.5, 0.15, 0.015 and 0.0015 μg/cm^2)

↓

Three replicates for each concentration was kept at the darkness up to 72 h

↓

Every 24 h, mortality and general aspect of all individuals was recorderd and the LC_{50} values was calculated

Fig. 1: Schematic diagram of LC_{50}assessment of tested bispyribac-sodium on earthworm by residual film technique

2.4.2 Toxicity of tested BS on earthworm by soil mixing test

In the soil mixing bioassay, the earthworms were adapted in the laboratory using artificial soil. The artificial soil was prepared using 70% sand, 20% kaolin clay, and 10% sphagnum peat moss, and the pH was adjusted to 6.0 ± 0.5 by the addition of $CaCO_3$. Then, 200 g of artificial soil was placed in plastic boxes (5 x 10 cm), the moisture content was adjusted to 35% of the final weight. In similar, using the tested claysoil, sandy clay loams soil and clay soil: sandy clay loams soil (1:1), the boxes were treated with aqueous solutions of herbicide BS formulations (1%SC) to obtain 1000, 500, 200, 100, 10 and 1 μg/g soil. Mature individuals weighting between 0.6 and 0.7 g were selected. Four prewashed and ventilated mature earthworms were then introduced into each box then (Three replicates for each concentration), covered with Parafilm and holed for aeration and placed in an incubation chamber at a temperature of 23 ± 2ºC with a 12:12 photoperiod. The control was prepared in a similar way except that only water was added to the soil. Lost moisture was replaced during assessment on a lost weight basis and the lost weight was replaced with distilled water (Figure 2). The percentage of mortality was monitored after 5 and 10 days. Earthworms were regarded as dead when they did not react to a mild mechanical stimulus. Also, the LC_{50} value of BS was calculated by LdP line software (Singh and Singh, 2015; Kavitha *et al.*, 2020).

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200 g of soil was placed in plastic boxes (5X10 cm), moistened with distilled water (40 mL / box) and then treated with BS (1% SC)

↓

Four adults previously weighed was placed in each box, covered with Para film and holed for aeration

↓

Three replicates (boxes) by concentration was used, and other for control

↓

Other box was prepared to control humidity

↓

All boxes was kept into a large wooden box in the darkness to ambient temperature

↓

The percentage of mortality by each tested concentration was monitored and the LC_{50} values were calculated

Fig. 2: Schematic diagram of LC_{50} assessment of tested bispyribac-sodium on earthworm by soil mixing technique

2.5 Statistical analysis

Experimental data presented as LC_{50} statistical analysis was performed by the Ldp line software (Wang *et al.* 2012; Mohammed, 2020). A probit analysis developed by Chi (1997) was employed to assess the acute toxicity of BS to earthworm.

3. RESULTS AND DISCUSSION

3.1 Toxicity of tested BS on earthworm by filter paper contact test

Laboratory tests play an essential role in the risk assessment of chemicals such as pesticides toward earthworms and are considered valuable if they predict the impacts on earthworms under field conditions (Zhou *et al.*, 2008). Among these, acute toxicity tests are considered the most relevant for laboratory testing, and earthworm mortality has been the main end point (Kandalkar and Naik, 2004). Although considering it to be of low ecological relevance, Heimbach (1992) found a reasonable correlation between the results of acute toxicity tests and the effects observed in the field. The results of acute toxicities of tested bispyribac-sodium by filter paper contact test demonstrated that widely varied in their contact toxicities to *Aporrectodea caliginosa* (Table 3).

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The results obtained showed that the concentrations of ≤ 0.15 µg cm^{-2}(1 µg a.i mL^{-1}) for herbicide caused 0% mortality at 24 h and concentration of 0.0015 µg cm^{-2}(0.01 µg a.i mL^{-1}) caused 0% mortality at 48 h. The effect on earthworm death rate was negligible with lower mortality at 24 h, indicating a very weak effect on earthworm compared to mortality at 48 and 72 h. In addition, the concentration of 500 µg a.i mL^{-1} for the tested pesticide causes lower than 50% mortality at 24 h. Shown LC_5, LC_{50}, LC_{95} and other parameters in Table 4. BS showed the highest intrinsic toxicity to the worms with an LC_{50} value of 0.062 (0.161-0.030) µg a.i mL^{-1} at 72-hcompared to LC_{50} at 48 h was 38.91 (96.57-15.29)µg a.i mL^{-1} and LC_{50} at 24 h was 518.32 (1046.64-258.67) µg a.i mL^{-1}. The toxicity as mortality percentages were increased and LC_{50} value was reduced when the exposure time was increased for, indicating a positive correlation between the pesticide toxicity and exposure time (Tables 3 and 4).

Table 3: Toxicity of tested bispyribac-sodium (Mortality % ±SE) on earthworm by filter paper contact test

Conc. (µg/cm^2)	24 h	48 h	72 h
0.0015	0.00 ± 0.00	0.00 ± 0.00	25.00 ± 0.00
0.015	0.00 ± 0.00	8.33 ± 0.33	41.67 ± 0.00
0.15	0.00 ± 0.00	16.67 ± 0.33	100.00 ± 0.00
1.5	8.33 ± 0.33	25.00 ± 0.58	100.00 ± 0.00
7.5	16.67 ± 0.67	33.33 ± 0.33	100.00 ± 0.00
15	25.00 ± 0.58	50.00 ± 1.15	100.00 ± 0.00
30	33.33 ± 0.33	75.00 ± 0.00	100.00 ± 0.00
75	41. 67 ± 0.33	83.33 ± 0.67	100.00 ± 0.00
150	58. 33 ± 0.33	100.00 ±0.00	100.00 ± 0.00
300	75.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
600	83. 33 ± 0.33	100.00 ± 0.00	100.00 ± 0.00

Table 4: Toxicity indeces and their parameters for herbicide bispyribac-sodium on earthworm by filter paper contact test

Time (h)	24	48	72
LC_5 [a](µg/mL)	9.10	0.23	0.003
Confedence limits at 95%	48.41-1.56	1.99-1.78	0.0184-0.0003
LC_{50} [b](µg/mL)	518.32	38.91	0.062
Confedence limits at 95%	1046.64-258.67	96.57-15.29	0.161-0.030
LC_{95} (µg/mL)	29495.26	6508.98	1.41

Confedence limits at 95%	221156.10-4382.72	65740.66-938.60	14.36-0.25
Slope[d]	0.937±0.0397	0.740±0.021	1.212±0.117
$\chi^{2\ e}$	0.725	6.962	3.164
P	0.994	0.324	0.419

a: Concentration causing 5% mortality. b: Concentration causing 50% mortality. c: Concentration causing 95% mortality. Results ofLC_5, LC_{50} and LC_{95} are expressed as mean of three replicates ± standard error (SE). d: Slope of the concentration-mortality regression line. e: Chi square value.

3.2 Toxicity of tested herbicide BS on earthworm by soil mixing test

The soil mixing test was used to evaluate the toxicity of tested herbicide BS on earthworm, *Aporrectodea caliginosa*. The contact filter paper test is simpler, cheaper, and faster, and is designed in such a way that the earthworms are exposed to the toxicant both by contact and in the aquatic phase (Tripathi *et al.*, 2010). This test is reported to be an excellent screening technique to assess relative toxicity (Grumiaux *et al.*, 2010). In contrast, the soil mixing test is more representative of the natural earthworm environment, and the chemicals are absorbed mainly by the gut in this method (Udovic and Lestan, 2010). Thus, the soil mixing test is more practical when the pesticide toxicities to earthworms are assessed (Wang *et al.*, 2012). Table (5) shows the mortality percentages of earthworms exposed to bispyribac-sodium (SC 1%) at concentrations of 0, 1, 10, 100, 500 and 1000 µg/g soil at time 5 and 10 days after treatment in clay, clay : sandy clay loam (1:1) and sandy clay loam soil. The highest mortality percentage for BS at 1000 µg/g soil, was 100 % at 10 days in three soils while she was 91.67, 83.33 and 66.67 % at 5 days in clay, clay: sandy clay loam (1:1) and sandy clay loam soil, respectively. The lowest tested concentration of BS (1 µg/g soil) caused 0.00 and 25.00 % mortality in clay soil, 0.00 and 16.67 % mortality in clay : sandy clay loam (1:1) and sandy soil at 5 and 10 days after treatment, respectively. Toxicity data of BS from the soil mixing test showed a clear concentration-dependent relationship, and the mortality of earthworms was increased when the exposure time was increased. BS herbicide has been exhibited different levels of toxicity to earthworms, this result is in agreement with those obtained by Cang *et al.* (2017). Also, Singh and Singh (2015), pointed out that the toxicity of 2,4-D on earthworm (*Eutyphoeus waltoni*) was both time and dose-dependent. The toxicity of BS on earthworm by soil mixing technique expressed as LC_{50} was increased when the exposure time was increased. The LC_{50} was reduced from 152.92 (479.5-48.37) to 18.59 (68.54-4.37) in clay soil, from 311.60 (977.11-102.64) to 29.63 (93.78-8.72) in clay soil: sandy clay loam soil (1:1) and from 448.23 (4431.55-56.99) to 33.67 (115.51-9.16) in sandy soil at 5 and 10 days after treatment, respectively. The lower the LC_{50} value the more toxic the chemical (Karasu and Koksal, 2005). The toxicity of tested pesticide was greater (lower LC_{50}) in clay soil at both time intervals than that in clay soil: sandy clay loam soil (1:1) and sandy clay loam soil (Table 6).

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Table 5: Toxicity of bispyribac-sodiumon earthworm (Mortality % ±SE) by soil mixing test

Soil	Conc. (µg/g soil)	1	10	100	500	1000
Clay	5th day	0.00 ± 0.00	25.00 ± 0.58	33.33 ± 0.88	50.00 ± 0.00	91.67 ± 0.33
	10th day	25.00 ± 0.00	41.67 ± 0.33	58.33 ± 0.33	75.00 ± 0.58	100.00 ± 0.00
Clay : Sandy clay loam (1:1)	5th day	0.00 ± 0.00	16.67 ± 0.33	25.00 ± 0.58	41.67 ± 1.20	83.33 ± 0.33
	10th day	16.67 ± 0.33	33.33 ± 0.67	66.67 ± 0.67	66.67 ± 0.33	100.00 ± 0.00
Sandy clay loam	5th day	0.00 ± 0.00	25.00 ± 0.58	25.00 ± 0.58	41.67 ± 0.33	66.67 ± 0.33
	10th day	16.67 ± 0.33	41.67 ± 0.33	50.00 0.58	66.67 ± 0.67	100.00 ± 0.00

Table 6: Toxicity indeces and their parameters for herbicide bispyribac-sodium on earthworm by soil mixing test

Soil Types	Clay Soil	Clay soil : Sandy Clay Loam Soil (1:1)	Sandy Clay Loam Soil
Time (day)	5th day		
LC5	1.16	3.17	0.16
Upper/Lpower	35.70-0.03	59.217-0.123	288.84-0.00002
LC50	152.92	311.60	448.23
Upper/Lpower	479.5-48.37	977.11-102.64	4431.55-56.99
LC95	20165.11	30667.8	1288728
Upper/Lpower	854408-733	1418364-973.998	0.00-99.80
Slope	0.776±0.07	0.825±0.079	0.48±0.07
Chi Square	4.281	3.547	1.09
Probability (P)	0.118	0.17	0.58
Time (day)	10th day		
LC5	0.06	0.18	0.13
Upper/Lpower	1.599-0.0007	2.625-0.006	2.60-0.00
LC50	18.59	29.63	33.67
Upper/Lpower	68.54-4.37	93.78-8.72	115.51-9.16

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LC95	5963.65	5012.7	9227.94
Upper/Lpower	174740-453	85539.48-519.75	264815-660.33
Slope	0.654±0.029	0.738±0.031	0.675±0.03
Chi Square	2.986	3.756	4.278
Probability (P)	0.687	0.562	0.585

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