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Physiological Effects of Carbofuran on Earthworm *Pheretima javanica* Gates

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Abstract Carbofuran is a carbamate pesticide that has known to possess adverse effects in soil organisms including earthworms that potentially lead to changes in nutrient cycling. However, little is known about the effects of carbofuran, either dirrectly or indirrectly, on the earthworm *Pheretima javanica.* This research is intended to know the effect of carbofuran at sublethal doses on the food consumption rate, assimilation efficiency and growth rate of *Pheretima javanica worms.* Six concentration levels of treatment were prepared by concecutively adding carbofuran granules as much as 0 mg (for control), 0.1mg, 0.2mg, 0.3mg, 0.4mg and 0.5mg into the worm foods (100 mg of cow manure). The feeding parameters were based on the dry weight of the worms, food given and remnant food, and worm feces (casts) during three days of treatment. Growth rate of the worms was based on the weekly weight gain of the young worm treated for eight weeks. The results showed the consumption rate decreased, assimilation efficiency increased and growth rate decreased significantly by the increased concentration of the pesticide ($\alpha = 0.05$). Thus, it can be conclusively suggested that carbofuran (Furadan 3G) highly harmful to earthworms, even at low concentrations.

Keywords Carbofuran, Furadan G3, Carbamate pesticide, Earthworm, *Pheretima javanica*

1. Introduction

In Indonesia, carbofuran (2,3-dihydro-2, 2-dimethyl benzofuran-7-yl methylcarbamate), is marketed under the trade mark of Furadan 3G, Curater 3G, Indofuran 3G in granular form. In this country the carbamate pesticide is commonly used to eradicate pests of citrus, cotton, clove, pepper, potato, rice, sugarcane, and tobacco. Because of its widespread use, the residues of this compound are potential to contaminate the terrestrial and aquatic environments. The evidence of high carbofuran residues in soil and water due to intensive use of carbamate pesticides and its impact on biological system have been indicated by both field and laboratory studies from many parts of he world.

In aquatic environment, carbofuran has caused sporadic, and sometimes extensive, field kills of fish, wildlife, and invertebrates. Through laboratory tests carbofuran at concentrations of about 200 ppb cause significant death rates for sensitive species of aquatic biota including Ostracods, Cladocerans and aquatic Oligochaetes [1, 2]. A report from Kenya indicated that intensive use of Furadan in farming causing high residues of carbofuran (3-hydroxycarbofuran and 3-ketocarbofuran) in soil, water, and plants that lead to massive dead of white-backed vultures (*Gyps africanus*) [3].

From India it was reported that carbofuran use in rice field at the dose of 9 kg/acre (normal dose) and 12kg/ acre (high dose) causing an adverse effect on the growth of soil microflora such as actinomycetes, bacteria and fungi [4] and poses hazard to the consumers [5]. In Bangladesh carbofuran residues found to present in water sample collected from paddy and vegetable farming areas [6]. Such existence of carbofuran in the environment also evident in Malaysia, carbofuran even detected in river, sea and ground water in pollution-free areas [7]. Another description of the high threat of carbamate pesticide residues, particularly carbofuran, is reported from the Czech Republic that although distribution of carbofuran was prohibited in 2007, no decline in the number of intoxicated animals in the following two years [8].

Based on the above facts, it is reasonable to worry that the use of pesticide especially carbofuran has potential adverse ecological effects at all levels of biological organization that lead to loss in production, changes in growth, development and/or behavior, changes in system processes (such as nutrient cycling), and losses of valuable species [9]. One of soil organism groups possessing great role and services for both ecological and farming systems is earthworms. Earthworm, due to capable of producing cellulase enzyme in its gut make this Oligochaeta worm contributes to the nutrient cycling in terrestrial ecosystem [10] and affects physicochemical properties of soil [11]. In a review article

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on the benefits of earthworms for cropping system, Bertrand et al. [12] summarizing that earthwoms improve soil structural stability and soil porosity and reducerunoff, modify soil organic matter (SOM) and nutrient cycling, allegedly contribute to crop production, enhance organic amendments. However, due to feeding at soil surface earthworm most exposed to pesticides and other agrochemiclas.

Given the importance of earthworms in the soil community on the one hand, but the high agrochemical threats on the other, studies on the impact of pesticides on each type of earthworm are important. Different types of pesticides such as cyfluthrin, carbaryl, chlorpyrifos, fipronil, imidacloprid, pendimethalin, pretilachlor, and cypermethrin have been tested for their effect on earthworms. Various species of earthworms have been used as test animals including *Lumbricus terrestris*, *Aporrectodea giardi*, *Pheretima posthuma*, *Pheretima* (Metaphire) *hilgendorfi*, *Perionyx excavatus, Eisenia fetida*, *Enchytraeus luxuriosus*, and *Enchytraeus albidus.* However, the pesticide effect test on the *Pheretima javanica* worm has not been performed. This research is intended to know the effect of carbofuran at sublethal dose on the physiological aspects of *Pheretima javanica* worms such as feeding rate, assimilation efficiency and growth rate.

2. Materials and Methods

The Pesticide

The pesticide carbofuran (2,3-dihydro-2, 2-dimethylbenzofuran-7-yl methylcarbamate) used in the experiment was Furadan 3 G produced by PT. Bina Guna Kimia, Indonesia, Registration No.: RI. 010101197416. This pesticide containing active ingredient of 3% carbofuran in silica sand as the carrier material.

Earthworms collection and rearing

Earthworms (*Pheretima javanica* Gates) used in the study were collected from suburb area of Palembang and Inderalaya, district of Ogan Ilir, the province of South Sumatra, Indonesia. The worms obtained by digging the ground to a depth of 10-15 cm and then separated from the soil by hand sorting. The sorted soil fragments were collected to serve as a medium for rearing the worms. In the laboratory the worms were kept in a plastic tray sized of 20 x 30 x 10 cm, with the soil water content range 30-40% at room temperature, and fed on cow manure *ad libitum*.

Experimental design

By using a completely randomized design, six levels of treatments were prepared based on carbofuran concentration namely: 0% (as the control), 0.1%, 0.2%, 0.3%, 0.4% and 0.5%. The concentration levels of treatment were prepared as follows. Furadan granules were ground into fine powder. Active ingredient powder of carbofuran as much as 0 mg (for control), 0.1mg, 0.2mg, 0.3mg, 0.4mg and 0.5mg respectively were added into 100 mg cow manure and re-ground in mortar until evenly mixed (homogenous).

Treatments and parameters

1. Food consumption and assimilation

Earthworms tested were taken from the stock reared in the plasctic trays, having length and weight range of 14 -18cm and 3.75 - 4.85g respectively. Each test worm was placed on a wet filter paper in a Petri dish. After being fasted for 24 hours the worms were fed with cow manure containing carbofuran according to the concentration that was set for three days. After three days of treatment all worms, remnant food, and casts secreted by the worms were collected, dried, and the dry weight was assessed. The food consumption rate and assimilation efficiency consecutively determined according to Seenappa [13] using formula 1 and 2 below.

$$\text{Consumption rate (CR)} = \frac{C}{T\ x\ A} \qquad (1)$$

where
C= dry weigth of food consumed (mg)
T= time (days)
A= worm body weight (g)

$$\text{Assimilation Efficiency (AE)} = \frac{(C-F)}{C} x\ 100\% \qquad (2)$$

where
C= dry weigth of food consumed (mg)
F= worm casts dry weight (mg)

2. Growth rate

To investigate effects of carbofuran on the growth of *Pheretima javanica*, the test worm was transferred into a black plastic pot (diameter: height = 20cm: 15cm) containing 1000g of soil with water content range of 30 – 40 %. Criteria of worms eligible to use was having body length of 3.75 - 3.85 cm and clitelum length of 0.6 – 0.8 cm. Into each pot was added 250g of cow manure containing carbofuran of different concentration according to levels that was set. Once a week, for 8 weeks, the body weight of all worms were measured and expressed as weekly growth rate (mg/week) [13].

Statistical analysis

The data of food consumption and growth rate were analyzed using one-way ANOVA followed by Duncan Multiple Range Test (DMRT) as the post hoc test at the 95% confidence level.

3. Results

The effect of carbofuran on the consumption rate and the assimilation efficiency of test worms are presented in Table 1. The ANOVA and DMRT test results for both dependent variables, consumpstion rate and assimilation efficiency, showed highly siginificant differences of mean values between treatment levels ($\alpha = 0.05$). Table 2 showed effects of carbofuran treatment on the growth rate of the test worms. Statistical analysis (ANOVA) and post hoc test (DMRT) made for the mean values between treatments showed significant difference at α = 0.05.

Table 1. Food consumption rate and assimilation efficiency of *Pheretima javanica* by carbofuran treatments

Carbofuran concentration (%)	Consumption rate (mg/g.day)	Assimilation efficiency (%)
0	2.53±0.05[a]	40.78±2.56[a]
0.1	1.30±0.03[b]	50.01±4.59[b]
0.2	1.05±0.07[c]	62.36±3.75[c]
0.3	0.65±0.04[d]	76.46±6.24[d]
0.4	0.45±0.06[e]	86.21±5.15[e]
0.5	0.23±0.02[f]	90.76±3.67[f]

Data were presented as Mean±SD. Values in the same column followed by the same superscript are not different at α =0.05

Table 2. Growth rate of *Pheretima javanica* by carbofuran treatments

Carbofuran concentration (%)	Growth rate (mg/week)
0	50.57±1.25[a]
0.1	30.25±2.50[b]
0.2	20.36±1.75[c]
0.3	15.20±2.24[d]
0.4	10.21±2.15[e]
0.5	7.12±1.67[f]

Data were presented as Mean±SD. Values in the same column followed by the same superscript are not different at α =0.05

4. Discussion

This study findings clearly indicated that pesticide carbofuran (Furadan 3G) impairing physiological process of *Pheretima javanica* that lead to growth retardation of the worms. However, the data in Table 1 is somewhat difficult to explain because the food consumption rate parameter appears to be inconsistent with the assimilation efficiency parameters. As has been suggested, earthworm feeding strategy is generally one of high consumption and low assimilation of poor-quality food material [14]. Logically, if food consumption rate decrease, assimilation efficiency should also decrease. It is hard, if not impossible, to find literature that directly supports this study results, especially in the context of pesticide carbofuran and *Pheretima javanica*. Fortunately, the carbamate pesticide tests on other vertebrate and invertebrate animals including Oligochaete worms have been available in relatively large quantities and some of which may appropriate for explaining the data of this study.

The decrease food consumption rate in worms exposed to carbofuran with increased doses may be due to the following. Food consumption rate of earthworm is affected by the quantity as well as quality of food given. Food quality involves taste, smell, chemical composition that influenced palatability [15]. In addition, factors such as favourableness of environmental conditions for earthworm activity, also a determining factor [16]. In this experiment, the cow manure (food source) was mixed with carbamate carbofuran. The presence of the pesticides seemed to change food palatability of cow manure to the worms. Furthermore, given the test animals are kept in Petri dish the worms have no choice for food to consume other than the carbofuran-polluted manure. In natural soil habitat, in fact, the worm does not feed on soil indiscriminately but is able to select an organic matter-enriched diet from the medium [17].

In an experiment using earthworm *Eisenia fetida* to test effects of lindane and deltamethrin on mortality, growth, and cellulase activity in earthworms, Shi et al. [18] suggested that the decrease in worm growth by pesticides possibly correlated with an earthworm’s strategy for natural survival, reducing food intake to avoid the toxins. As the food consumption rate decreases, it is usually followed by decreases in other feeding parameters including assimilation rates and assimilation efficiency. For *Millsonia anomala*, a tropical geophagous earthworm, food assimilation rates were depended on the physical properties of organic matter consumed. Assimilation rates were highest with fresh organic matter (undecomposed plant debris) and with leaf material rather than root material due to the high content of watersoluble compounds and the high N availability in fresh organic matter [19].

At this point, there is still no satisfying answer regarding why assimilation efficiency increases, whereas food consumption rate decrease with increasing doses of carbofuran given. The most plausible explanation is, carbamate carbofuran in addition to lowering the palatability of food that lead to decreased food intake, also causing food retention in the gut of worms increased. The increased of such food retention is not due to normal conditions, but because of systemic impairments in the worm's body, either histologically as well as biochemically. As has been indicated, carbamate pesticide along with organophosphates, strongly inhibits acetylcholinesterase (AChE) activity in a number of invertebrate species [20]. In earthworms, inhibition of AChE activity causing decrease in growth rate [21].

The mechanism of AChE inhibition is involved hydrolysis and carbamylation of the nucleophilic serine, followed by release of an alcohol leaving group [22]. In rats, influence of carbofuran metabolism on acetylcholinesterase can be depicted as follows. Carbofuran absorption (peak plasma levels < 7 min), distribution, and elimination ($t_{½}$ = 29 min) occurred rapidly. A significant oxidative metabolite of carbofuran with anticholinesterase activity, 3-hydroxycarbofuran, was rapidly formed and subject to entero hepatic circulation (plasma $t_{½}$ = 64 min). Rapid RBC AchE recovery closely paralleled carbofuran metabolism and the primary in vivo disposition of 3-hydroxycarbofuran was metabolic conjugation [23].

Lastly, based on data in Table 2, there is no doubt that carbofuran is highly toxic to earthworms. Even at a concentration of 0.2% can inhibit the growth rate to more than 50% (α = 0.05). The carbofuran-induced fatalities are, as already known for decades, due to these compounds developed two type of lesions in worms namely a multi segment swelling which often ulcerated and a discrete nodular mass protruding from the surface of the worm [24]. Side-effects of carbofuran applications causes dramatic effects on many nontarget organisms mainly insectivorous organisms including frogs and birds; some birds that consumed spilled granules died immediately [25].

Although there are many aspects that still need to be clarified from the data inconsistency of feeding variables, it can be conclusively suggested that carbofuran (Furadan 3G) highly harmful to earthworms, even at low concentrations below 0.5%. Given the data Further studies are certainly still needed to assue food retention time in the worm gut using a variety of carbofuran concentrations below 0.1%.

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