

## Inhibitory Effect of Carbofuran Furadan 3G on the Cocoon Production and Viability in *Pheretima javanica* Gates

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**Abstract** Though carbofuran has known to possess adverse effects in soil organisms including earthworms, little is known about the effects of carbofuran, either directly or indirectly, on the earthworm *Pheretima javanica*. This study aimed to investigate the effect of carbofuran at sublethal doses on the cocoons production and viability of *Pheretima javanica* worms. Six groups of earthworms, each consisted of four worms, were reared in plastic pots and fed on cow manure mixed with carbofuran as much as 0 mg (for control), 0.1mg, 0.2mg, 0.3mg, 0.4mg and 0.5mg respectively. Cocoons produced were hand sorted every two weeks for three months and presented as the number of cocoons per individual per moth. Cocoon viability was determined by observing the hatchling emerged from the cocoons incubated in Petri dish and expressed as percentage of viability. The results showed both cocoons production and cocoon viability of the worms decreased significantly by the increasing concentration of the pesticide given ( $\alpha = 0.05$ ). Thus, it can be conclusively suggested that carbofuran (Furadan 3G) highly harmful to earthworm *Pheretima javanica*, even at low concentrations adversely affect growth and reproduction of the worms.

**Key words:** carbofuran, Furadan G3, carbamate pesticide, earthworm, *Pheretima javanica*

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### I. Introduction

Although many countries have banned the spread and use of carbofuran, here in Indonesia, this carbamate pesticide under the trade mark of Furadan 3G, Curater 3G or Indofuran 3G is still intensively used in agriculture and plantation including rice, citrus, cotton, clove, pepper, potato, sugarcane, and tobacco. Because of its widespread use for many decades the residues of these chemicals 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 the world such as Kenya [1], India [2, 3], and Bangladesh [4]. In Malaysia, carbofuran even detected in river, sea and ground water in pollution-free areas [5]. From Czech Republic it was reported that the number of intoxicated animals did not decline even though carbofuran use has been banned for two years [6].

Research findings mentioned above are the depiction of potential adverse ecological effects of pesticide uses on biological organization that may 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 [7]. One of valuable organism groups possessing great role and services for both ecological and farming systems is earthworms. Earthworm, due to its capability of producing cellulase enzyme in its gut, makes this Oligochaeta worm contribute to the nutrient cycling in the terrestrial ecosystem [8] and affects physicochemical properties of soil [9]. In a review article on the benefits of earthworms for cropping systems, Bertrand et al. [10] summarize that earthworms improve soil structural stability and soil porosity and reduce runoff, modify soil organic matter (SOM) and nutrient cycling, allegedly contribute to crop production, enhance organic amendments. However, due to feeding at soil surface earthworms are most exposed to pesticides and other agrochemicals.

Considering the important role of earthworms in nutrient cycling processes in the terrestrial ecosystem on the one hand, the high agrochemical threats on the other, studies on the impact of agrochemicals on the biological aspects of earthworms are crucial. Different types of agrochemicals such as cyfluthrin, carbaryl, chlorpyrifos, fipronil, imidacloprid, pendimethalin, pretilachlor, cypermethrin, pentachlorophenol, parathion, carbendazim, paraquat, fentin, benomyl, carbaryl, phenmedipham, copper oxychloride, and dieldrin have been tested against earthworms using various biological parameters including reproduction. Though various species of earthworms have been used as test animals including *Lumbricus terrestris*, *Aporrectodea giardi*, *Perionyx excavatus*, *Eisenia fetida*, *Enchytraeus luxuriosus*, *Enchytraeus albidus*, *Pheretima posthuma*, and *Pheretima (Metaphire) hilgendorfi*, but none of the studies involved *Pheretima javanica* as the subject [11]. This study is intended to know the effect of carbofuran (Furadan 3G) at sublethal dose, according to Eisler [12], on the cocoons production and cocoons viability of *Pheretima javanica* worms.

## II. Materials And Methods

### Earthworm collection

Samples of *Pheretima javanica* 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 at the depth of 10-15 cm and then hand-sorted. Soil matter free from litter and worm packed and taken to the laboratory to serve as a medium for rearing the worms in the experiment. Worms for test animal stocks were kept in a plastic tray sized of 20 x 30 x 10 cm at room temperature with the soil water content range 30-40% and fed on cow manure *ad libitum*.

### The Pesticide

The pesticide carbofuran (2,3-dihydro-2, 2-dimethylbenzofuran-7-yl methylcarbamate) used in the experiment was the Furadan 3 G, a granular formulation that contains 3% pure carbofuran, a product from PT. Bina Guna Kimia, Indonesia, Registration No. RI. 010101197416.

### Experimental design

There were six levels of treatments applied in this study based on the concentration of carbofuran namely: 0% (as the control), 0.1%, 0.2%, 0.3%, 0.4% and 0.5%. Each level of concentration was made by mixing 100 mg of cow manure (the worm foods) with the carbofuran granules as much as 0 mg (for control), 0.1mg, 0.2mg, 0.3mg, 0.4mg and 0.5mg respectively. By using a completely randomized design, each treatment involved four replications.

### Cocoon Production

Plastic pots with the diameter of 20 cm and height of 15 cm were used for treating the worms in this study. Soil (1000 g) taken from the original biotope were put into the pot and served as the rearing medium for the worms. The water content in the soil medium on each pot was maintained in the range of 30 – 40%. Into each pot was placed four adult earthworms (two pairs) that fed with cow dung (250 mg) which has been mixed with carbofuran in accordance with the concentration planned. Every two, for total 3 months of rearing, weeks the cocoons deposited were hand sorted. Every two weeks, for a total duration of three months, the deposited cocoons were hand sorted. The cocoon production rate was expressed as the average of cocoon per worm per month.

### Cocoon Viability

All cocoons obtained from each pot of cocoon production experiment were incubated on filter paper spread over water soaked cotton inside a petri-dish. Cocoons were observed in daily basis to record any hatchlings emerged. Hatchability of cocoons (cocoon viability) were presented as percentage assessed using equation belows.

$$\text{Cocoon viability} = \frac{\text{number of juvenile}}{\text{number of cocoons}} \times 100\%$$

### Statistical analysis

The data of cocoons production and cocoon viability were analyzed using one-way ANOVA followed by Duncan Multiple Range Test (DMRT) as the post hoc test at the 95% confidence level.

## III. Results And Discussion

This study findings, as can be seen in Table 1, clearly indicated that pesticide carbofuran (Furadan 3G) significantly inhibit cocoons production and cocoon viability in *Pheretima javanica* worms. Both the cocoons production rate and cocoon viability are significantly decreased by the increasing concentration of carbofuran given ( $\alpha=0.05$ ). These data seem to strengthen what Govindarajan [13] has mentioned that majority of carbamate such as aminocarb, methiocarb, oxamyl, promecarb, aldicarb, bufencarb, carbaryl, carbofuran, methomyl, propoxur and thiofanox are toxic to earthworms and strongly affect population of earthworms.

**Table 1** Cocoons production and viability of *Pheretima javanica* Gates fed on cow dung contaminated with pesticide carbofuran Furadan 3G

Concentration of Carbofuran (%)	Cocoons Production (cocoons/worm/month)	Cocoons viability (%)
0	7.75±1.25 <sup>a</sup>	78.75±1.25 <sup>a</sup>
0.1	4.33±1.02 <sup>b</sup>	50.20±3.02 <sup>b</sup>
0.2	3.20±0.80 <sup>c</sup>	20.20±2.80 <sup>c</sup>
0.3	2.93±0.24 <sup>d</sup>	15.93±3.24 <sup>d</sup>
0.4	1.00±0.15 <sup>e</sup>	10.00±2.15 <sup>e</sup>
0.5	0.25±0.02 <sup>f</sup>	5.75±1.02 <sup>f</sup>
Data are presented as Mean±SD. Values in the same column followed by the same superscript are not different at $\alpha=0.05$		

Based on a study to investigate the effects of butachlor herbicide on earthworm *Eisenia fetida*, Muthukaruppan and Gunasekaran [14] found the earthworm biomass and cocoons production were decreased with increasing herbicide concentration. These data lead the authors hypothesized that butachlor increased the worm demand for energy needed for the regulation and detoxification of herbicide. The decrease in worms biomass might also be related to the low food consumption rate. As mentioned food consumption rate of earthworm is affected by quality of food given such as taste, smell, chemical composition that influenced palatability [15]. In this experiment, the cow manure (food source) was mixed with carbofuran. The presence of the pesticide seemed to change food palatability of cow manure. In addition the test animals are kept in a plastic pot with that make 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 [16]. In *Eisenia fetida* when food quality changed, not only the growth rate of the worms affected, but cocoons production and hatchlings produced significantly decrease as well [17].

Other factors most likely affecting directly cocoon production in earthworms exposed to the pesticides were the histological and cellular effects of the carbofuran. As has been reported, agrochemical Butachlor (a herbicide) found to cause glandular cell enlargement at all the exposed concentrations, which might massively affect food intake and which in turn may indirectly inhibit the earthworm reproductive capacity [18]. Cellular impairment that lead to growth and reproductive inhibition in earthworm due to chemical exposed has been found in *Eisenia andrei* upon treated with cadmium [19]. Another degenerative effect of insecticide (Azodrin) on body tissue of earthworms was reported by Leena [20] which found that spermatid follicles significantly degenerated due to clumping, vacuolization and necrosis.

Lastly, as already known for decades, the carbofuran-induced fatalities are 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 [21]. Besides, as has been indicated, carbamate pesticide along with organophosphates, strongly inhibits acetylcholinesterase (AChE) activity in a number of invertebrate species [22]. In earthworm *Eisenia foetida* this enzyme responsible for stability of lysosomal membrane of coelomocytes [23] and activity of muscle cells in the clitellum [24]. When the muscle cells of clitellum adversely impaired, its major role in cocoons production will ceased and consequently causes low reproductive capacity.

#### IV. Conclusion

Although there are many aspects that still need to be studied in earnest, mainly with regards to the physiological mechanisms of carbofuran in affecting reproductive system of the worms, it can be conclusively suggested that carbofuran (Furadan 3G) highly harmful to earthworms growth and reproduction, even at low concentrations below 0.5%.

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