Assessment of acute toxicity of thiamethoxam (Actara® 25WG) to *Achatina fulica* and its potential ecological applications

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ABSTRACT

Achatina fulica is considered as one of the world's worst invasive species and is known to cause ecological disruption as well as agricultural and health problems in the Philippines. The study evaluated the use of the insecticide thiamethoxam (Actara® 25WG) in the control of *A. fulica* populations. This was done by performing an acute toxicity test of thiamethoxam to *A. fulica*. Six individuals each were exposed to thiamethoxam concentrations of 0, 50, 100, 200, and 400 µg/L for 72 hours. The percent mortality was then determined after the exposure period. Probit analysis was used to determine the LD_{50} of thiamethoxam to *A. fulica*. The LD_{50} was found to be 662.95 ± 172.98 µg/L. Using the mean body weight of the snails, the LD_{50} per body weight of *A. fulica* was determined to be 90.09 ± 23.60 µg/kg. This is 70 times lower than the recommended application of thiamethoxam on field. Thus, normal application would eliminate *A. fulica*. However, because the LD_{50} to *A. fulica* is higher than that for other beneficial non-target species such as honey bees (0.03 µg/bee), the use of thiamethoxam in the control of *A. fulica* populations is only recommended when in conjunction with the control of target pest insects.

Keywords: Ecotoxicology, acute toxicity

INTRODUCTION

The prevalence of invasive alien species is a global trend and is considered to be one of the major causes of biodiversity loss, economic damage (Lowe and others 2000), and potentially detrimental environmental changes (Leung and others 2002). One such species is *Achatina fulica* (Achatinidae), the giant African snail, a terrestrial gastropod with a widespread distribution in the humid tropics (Fontanilla 2010). The International Union for Conservation of Nature lists *A. fulica* as one of the 100 world's worst invasive species (Lowe and others 2000).

A. fulica originated in East Africa but now occupies the Indian subcontinent, Southeast Asia, the Pacific, and the Caribbean (Fontanilla 2010). Its introduction into non-native habitats has been documented to be both intentional (i.e. as a food source) and accidental (Venette and Larson 2004). The reproductive biology of *A. fulica* has largely aided its success as an invasive species, with the snail reaching maturity at five to eight months and producing 10 to 400 eggs per clutch and up to 1800 eggs per year (Fontanilla 2010). It is also a voracious herbivore and its wide host range makes it a threat to native flora as well as agricultural crops (Venette and Larson 2004). *A. fulica* may also spread diseases, as it is known to be an intermediate host of the rat lungworm *Angiostrongylus cantonensis*, which can infect humans in its third juvenile stage. *A. cantonensis* can cause eosinophilic meningo-encephalitis (EME) or angiostrongyliasis (Fontanilla 2010). Black pod disease, a plant disease caused by *Phytophthora palmivora*, may also be spread through the snail's feces (Venette and Larson 2004).

In the Philippines, *A. fulica* populations are known to attack and feed on grown crops, gardens and agricultural farms of *ampalaya* (*Momordica charantia*), arrowroot (*Canna edulis*), banana (*Musa paradisiaca*), cabbage (*Brassica* spp.), *Canna* sp., cassava (*Manihot esculenta*), *Citrus* sp., coffee (*Coffea* spp.), sunflower (*Cosmos* spp.), cucumber (*Cucumis sativus*), eggplant (*Solanum melongena*), *upo* (gourd: *Lagenaria leucantha*), *gumamela* (*Hibiscus* spp.), *malunggay* (*Moringa olifeira*), papaya (*Carica papaya*), ramie (*Boehmeria nivea*), squash (*Cucurbita* sp.), sweet potato (*Ipomoea batatas*), *patola* (*Luffa* sp.), and yam (*Dioscorea alata)* among others (Sherley 2000, Raut and Barker 2002, Fontanilla 2010).

Various strategies for controlling *A. fulica* populations have been proposed and attempted, but so far, no single control measure has been successful in the eradication of the pest. One strategy that has been attempted in the Philippines involved the purposeful introduction of the *Platydemus manokwari* (Turbellaria: Rhynchodemidae), a non-native invertebrate enemy of *A. fulica,* to Bugsuk Island. Although the giant African snails were eradicated in some areas, the introduction of *P. manokwari* was said to have adverse effect on indigenous gastropod fauna (Sherley 2000).

A more common strategy involves chemical control with the use of toxicants, repellents, or pesticides. Despite the development of new molluscicidal agents, very few are targeted against *A. fulica* (Raut and Barker 2002). However, even non-target pesticides such as brodifacoum, which is aimed at rodents and vertebrate pests, have been found to be toxic to *A. fulica* (Booth and others 2001, Hoare and Hare 2006). Thus, using non-target pesticides as chemical control agents for *A. fulica* may have great potential.

The median lethal dose (LD_{50}) is the most frequently used measure of the acute toxicity of a substance. Expressing toxicity as LD_{50} provides a relative measure that can be used to compare substances with different mechanisms based solely on their lethal effect. The measurement of LD_{50} also assumes that it estimates the toxicity of the most hazardous constituent in the mixture (Spencer and Colonna 2003). A smaller LD_{50} value means relatively greater toxicity, indicating that a smaller amount of the substance is required for the death of the test organism (Girard 2010).

This study aimed to determine the acute toxicity, expressed as median lethal dose (LD_{50}), of thiamethoxam (Actara® 25WG) to *Achatina fulica*, which is a non-target species of the insecticide. The study also assessed whether thiamethoxam would be effective in the pest control of the snail.

MATERIALS AND METHODS

Achatina fulica

Ninety *Achatina fulica* individuals were obtained from around the University of the Philippines Diliman campus, weighed, and then placed in 15 identical glass containers (30 cm x 30 cm x 45 cm), with six individuals in each container. Nylon tulle netting was used as lids to contain the samples. Three centimeters of loamy soil were added to the container and kept moist throughout the study by spraying with distilled water. Ten grams of tomato leaves were also placed per container to serve as food for the snails for the entire duration of the study. The snails were given a 24-hour acclimatization period before the experiment proper.

Thiamethoxam

Thiamethoxam, 3-(2chloro-thiazol-5-ylmethyl)-5-methyl-{1,3,5}oxadiazinan-4-yldene-N-nitroamine, was obtained from Syngenta Philippines, Inc. (Makati City, Philippines). Formulated granules of thiamethoxam (Actara® 25WG) were dissolved in distilled water and thiamethoxam solutions were prepared in four concentrations of 50, 100, 200, and 400 µg/L each. The concentrations were calculated based on the formulation of the granules used (250 g thiamethoxam per kg). The pH and temperature of the solutions were then measured.

Toxicity test

Toxicity tests were performed to determine the LD_{50} of thiamethoxam to *A. fulica*. Ten mL of the thiamethoxam solutions was applied by spraying on the soil surface and the sides of the containers. The snails were left to feed on tomato leaves during the 72-hour toxicity test period. Concentrations tested were 50, 100, 200, and 400 µg/L of thiamethoxam in the insecticide solutions applied per container. A control treatment was also made, with distilled water used as control. After 72 hours, percent mortality was determined by examining the containers for dead snails. Three replicates of the toxicity test were performed. Using the mean weight of *A. fulica* used per treatment, the thiamethoxam concentrations were also expressed in µg/kg (Table 1). Using IBM SPSS Statistic v.19, the LD_{50} (in µg/L and µg/kg) was determined by probit analysis. Chi-square goodness-of-fit test was also performed in SPSS to check if the probit model fits the data adequately.

Table 1. Concentration of thiamethoxam applied per body weight of *Achatina fulica*

Treatment	Mean Weight (kg)	Thiamethoxam Concentration (µg/kg)
Control	0.0130 ± 0.0545	0
50 µg/L	0.0109 ± 0.0519	7.678532
100 µg/L	0.0137 ± 0.4650	13.79039
200 µg/L	0.0149 ± 0.0264	22.33958
400 µg/L	0.0121 ± 0.3497	59.18589

RESULTS

The temperature of the insecticide solutions ranged from 23.6-23.7°C, while a pH of 6.87 was measured for all the solutions. The percent mortality of *A. fulica* in the different treatments is shown in Table 2. The probit transformation output from SPSS Statistic v.19 is shown in Figure 1 (thiamethoxam concentration expressed in µg/L) and Figure 2 (thiamethoxam concentration expressed in µg/kg). From the probit analysis graph, the 72-hr median lethal dose (LD_{50}) of thiamethoxam to *A. fulica* was determined to be 662.95 ± 172.98 µg/L (90.09 ± 23.60 µg/kg). A Chi-square goodness-of-fit test was also performed with a null hypothesis that the probit analysis model fits the dose response data (Table 3).

DISCUSSION

Thiamethoxam is a neonicotinoid that is capable of mimicking acetylcholine. It acts selectively on the nicotinic acetylcholine receptors of insects, ultimately damaging the nervous system and leading to the death of the organism (Nauen and others 2003). Neonicotinoids are popular insecticides because the neural pathway affected is more common in invertebrates than in other animal groups (Kindemba 2009).

Table 2. Percent mortality of *Achatina fulica* in the five treatments of thiamethoxam

Treatment	Percent Mortality
Control	0
50 µg/L	5.555556
100 µg/L	16.66667
200 µg/L	22.22222
400 µg/L	38.88889

Table 3. The 72-hr median lethal dose (LD_{50}) of thiamethoxam to *Achatina fulica*

LD_{50}	Chi-Square Significance
662.95 ± 172.98 µg/L	0.980
90.09 ± 23.60 µg/kg	0.983

The toxicity (LD_{50}) of thiamethoxam has been previously determined in other species. The 24-hr LD_{50} was found to be 0.46 µg/g (460 µg/kbw) in houseflies (*Musca domestica*), 10.84 µg/g (10,840 µg/kbw) in German cockroaches (*Blatella germanica*) (Eremina and Lopatina 2005), 0.03 µg/bee in honey bees (*Apis* mellifera) (Iwasa and others 2004), and 0.024 µg/g (24 µg/kbw) in bumblebees (*Bombus terrestris*) (NRA 2001). For mice and rats, the LD_{50} values were 1,563 mg/kbw (1,563,000 µg/kbw for 59-day exposure) in rats, 783 mg/kbw (783,000 µg/kbw for 14-day exposure) in male mice, and 964 mg/kbw (964,000 µg/kbw for 14-day exposure) in female mice (NRA 2001). Thiamethoxam applied by contact or ingestion is particularly highly toxic to honey bees and bumblebees, and has been implicated along with other pesticides as possible cause of colony collapse disorder (Kindemba 2009, NRA 2001).

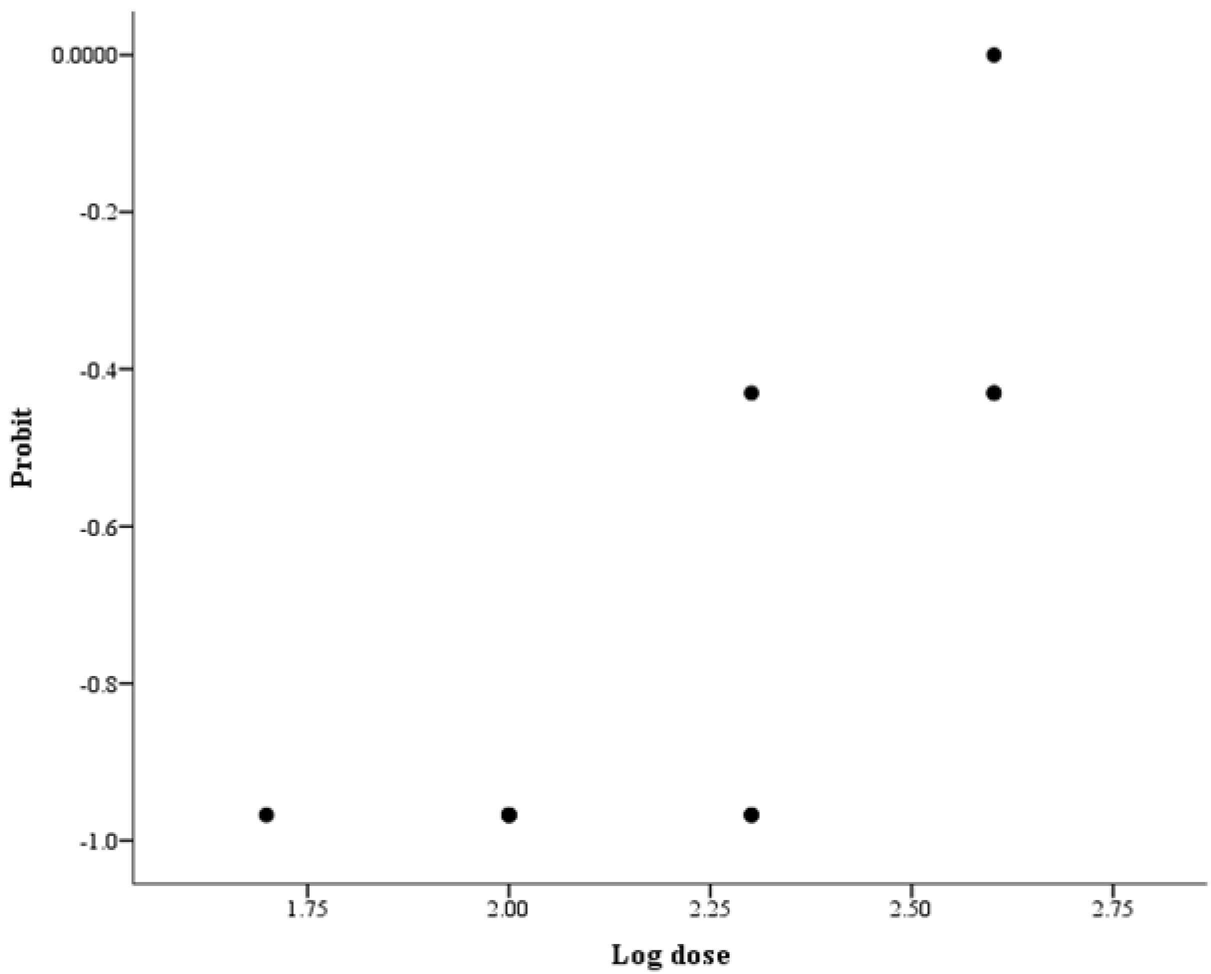

Figure 1. Probit transformation output for the determination of the 72-hour median lethal dose (LD_{50}) (µg/L) of thiamethoxam to *A. fulica*

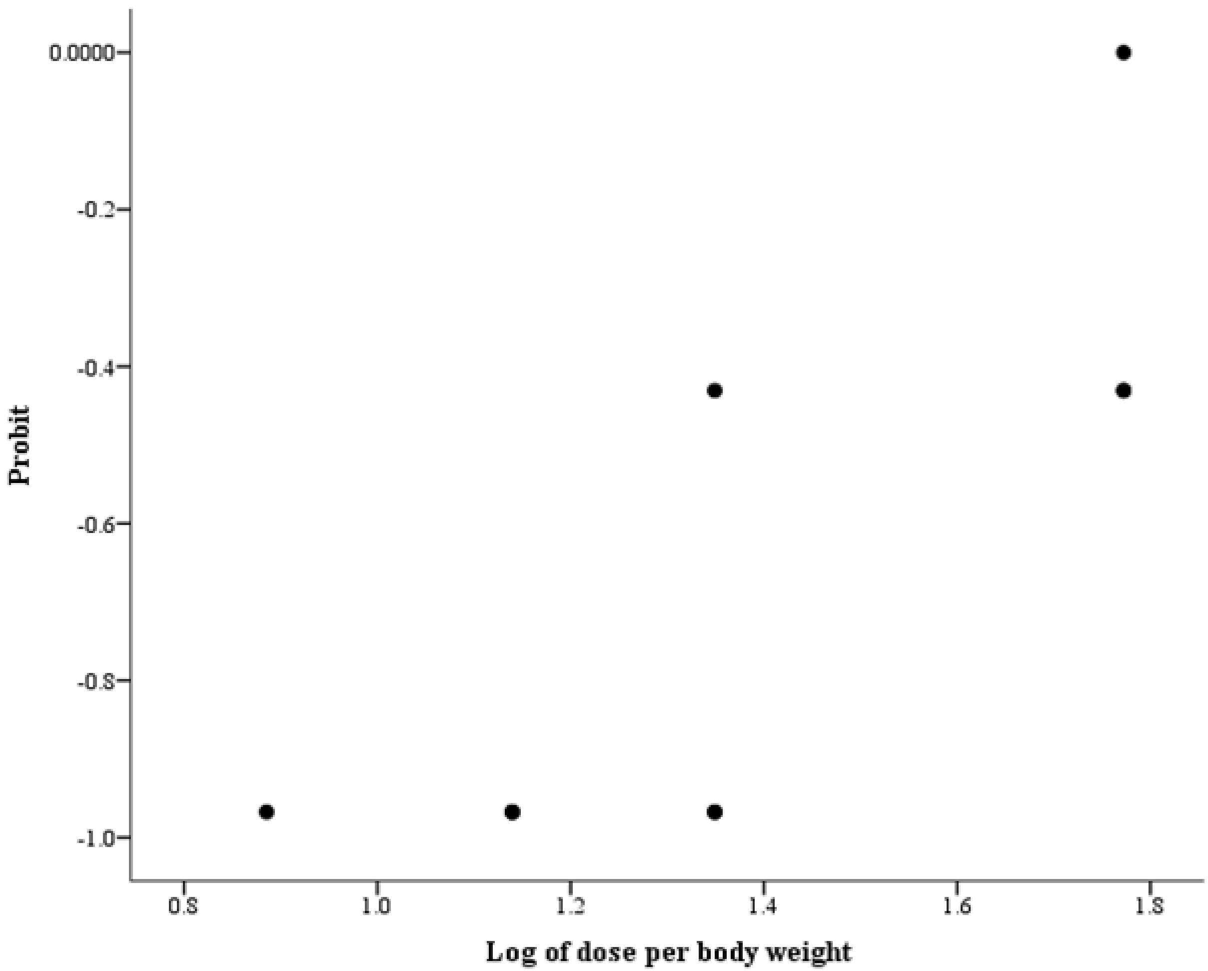

Figure 2. Probit transformation output for the determination of the 72-hour median lethal dose (LD_{50}) (µg/kg) of thiamethoxam to *A. fulica* per body weight (µg/kg)

The recommended application of Actara® 25WG for tomatoes, potatoes, and sugar beets is 20 g/hl (Syngenta 2011), which is effectively 50,000 µg/L thiamethoxam. Degradation of thiamethoxam is primarily caused by aqueous photolysis, while photolytic degradation of thiamethoxam absorbed into the soil is not significant. In soil/water systems, thiamethoxam accumulates in the sediment phase while degradation is continuous (NRA 2001). The half-life of thiamethoxam in soil ranges from 7 to 109 days, with longer persistence under dry conditions. Thiamethoxam also has the potential to leach down under heavy rainfall conditions (NRA 2001, Gupta and others 2008).

The LD_{50} of thiamethoxam to *A. fulica* obtained was not within the range of concentrations used in the study (50 to 400 µg/L). Typically, a range-finding experiment is performed to find a more specific range of concentrations prior to the toxicity test proper. The range-finding experiment was not performed because of scarcity of test animals due to the dry weather during the entire study period. This was also the reason why only six individuals were used per dose of thiamethoxam. Instead of a range-finding experiment, previous literature on the toxicity of thiamethoxam and other similar insecticides on other land snails and mollusks were used as the basis for the range of concentrations prepared (Booth and others 2001, Salama and others 2005, Minakshi and Mahajan 2012).

The recommended application is about 70 times the LD_{50} determined in this study. This prescribed application would be able to eliminate *A. fulica* pests, if present. However, the use of thiamethoxam solely as a molluscicidal to *A. fulica* is not recommended due to the lower LD_{50} of the insecticide to other non-target organisms and to the persistence of thiamethoxam, especially in dry soil. Furthermore, since thiamethoxam may potentially leach under heavy rainfall, contamination of nearby water bodies and other ecosystems becomes a concern. The potential effect on other non-target organisms, especially native and beneficial species, should also be considered. Thus, the use of thiamethoxam in the control of *A. fulica* populations is only recommended in conjunction with the control of target pest insects.

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