

EFFECTS OF METALDEHYDE AND NICLOSAMIDE ON THE BEHAVIOR AND REPRODUCTIVE CAPACITY OF *RADIX QUADRASI* VON MOELLENDORF (BASOMMATOPHORA: LYMNÆIDAE)

PENGARUH METALDEHID DAN NIKLOSAMID TERHADAP PERILAKU DAN KAPASITAS REPRODUKSI *RADIX QUADRASI* VON MOELLENDORF (BASOMMATOPHORA: LYMNÆIDAE)

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INTISARI

Pengaruh molusisida, metaldehid dan niklosamid, terhadap perilaku dan kapasitas reproduksi Radix quadrasi telah diteliti dalam kondisi laboratorium. R. quadrasi diperlakukan dengan dua jenis molusisida, yaitu niklosamid dengan konsentrasi 0; 0,045; 0,058; 0,075 mg/L, dan metaldehid dengan konsentrasi 0, 100, 112, 130 mg/L. Reaksi dan gejala keracunan diamati secara teliti selama berlangsungnya percobaan. Pengamatan dilakukan tiga kali, yaitu awal perlakuan (0 jam), 12 jam, dan 24 jam setelah perlakuan. Setelah perlakuan selesai R. quadrasi yang bertahan hidup dipelihara selama satu bulan untuk diamati kemampuan reproduksinya.

Hasil penelitian menunjukkan adanya reaksi segera setelah R. quadrasi bersentuhan dengan molusisida. Pada konsentrasi rendah dan sedang molusisida menyebabkan meningkatnya kecenderungan keong untuk bergerak meninggalkan air. Kecenderungan meninggalkan air ini juga terjadi pada kontrol meskipun intensitasnya lebih rendah. Sedangkan pada konsentrasi tinggi keong tampak tidak bergerak, dan tinggal di dasar kotak perlakuan. Niklosamid dan metaldehid menyebabkan menurunnya kemampuan reproduksi R. quadrasi. Jumlah telur dan masa telur yang dihasilkan menurun drastis seiring dengan meningkatnya konsentrasi molusisida. Namun demikian masa inkubasi telur, baik yang diperlakukan maupun pada kontrol, tidak berbeda nyata. Niklosamid dan metaldehid berpengaruh nyata terhadap perilaku dan kapasitas reproduksi R. quadrasi. Diantara dua molusisida yang diuji dalam penelitian ini, niklosamid lebih beracun daripada metaldehid.

Kata kunci: Radix quadrasi, molusisida, reproduksi

ABSTRACT

The effects of molluscicides exposure, metaldehyde and niclosamide, on the behavior and reproductive capacity of *Radix quadrasi* was studied under laboratory conditions. *R. quadrasi* were exposed to different concentration-rates of niclosamide: 0, 0.045, 0.058 and 0.075 mg/L, and of metaldehyde: 0, 100, 112 and 130 mg/L. The symptoms of poisoning of treated snails were observed closely throughout the duration of the experiment. Observations were made three times: at the beginning of treatment (0 hour), 12 hours, and 24 hours after exposure. The surviving *R. quadrasi* were maintained for one month to determine their reproductive capacity.

Results indicated that there was immediate reaction of the snails to molluscicide exposure. Lower concentration-rates of molluscicide triggered water-leaving behavior. Such behavior occurred in all of the low and medium concentration-rates of niclosamide and metaldehyde, as well as in some snails from the control group. By contrast, higher concentration-rates caused almost instantaneous immobility. Niclosamide and metaldehyde also significantly reduced the reproductive capacity of *R. quadrasi*. The number of egg masses and eggs laid varied with the treatments. However, the incubation period of the treated eggs was not statistically different from the treated ones. This study showed that both niclosamide and metaldehyde significantly affected the behavior and reproductive capacity of the *R. quadrasi*. Between the two molluscicides used in this experiment, niclosamide was more toxic than metaldehyde.

Keywords: *Radix quadrasi*, molluscicide, reproduction

INTRODUCTION

Chemical pesticides are used worldwide because of their efficacy against a great number of pests. They are readily available, cheap, with quick knock down effect, and easy to apply (Chen & Yeh, 1992). However, their excessive and continuous uses do not only increase the cost of production but also lead to pest resurgence, accumulation of residues in food and the environment, and hazardous effects on humans, animals and the ecosystem. Thus, their effectiveness might be outweighed by these hazards chemical pesticides bring about (Alberto, 1996).

Molluscicides are among the chemical substances released regularly to the environment in big amounts to control mollusk pests. Despite their direct effect on target organisms, the application of molluscicide will also affect non-target organisms in the surrounding environment. According to Waxman (1998), non-target organisms may be harmed by molluscicide in two ways: direct contact with non-target organisms, and formation of a residue that may cause later injuries.

The two most commonly used molluscicides worldwide are metaldehyde and niclosamide. Metaldehyde is relatively safe because of its short life span of one to three days in the environment after application (Calumpang *et al.*, 1995). Field trials using this chemical in a rice paddy ecosystem indicate its efficacy and specificity in controlling *Pomacea canaliculata* (Kayala & Ram, 2000). The mode of action of this molluscicide is by over depressing the central nervous system and causes malfunction of respiratory and vasomotor systems (EXTOXNET, 2001). In addition, as a reaction to metaldehyde mollusk will overproduce mucous which causes dehydration and an inability to move. Meanwhile, niclosamide is a non-cumulative

pesticide that effective against freshwater snails (WHO, 1988). This molluscicide affects the respiratory and digestive organs on snails, and is used to control golden apple snails in rice fields. Mollusks are poisoned either by absorption of niclosamide through the gastrointestinal tract, the skin, or mucous membranes. Once this molluscicide is being absorbed, it causes interference on the ATP production (WHO, 2002).

Mollusks are among the most important animals in the environment because of their dominant biomass and diversity. They are the second largest group in animal kingdom, next to the arthropods. These animals play important roles in the energy flow of the ecosystem. Mollusks also play a role in the balance of nature. Slugs and snails, two groups of mollusks, need particular attention because of the damage they cause in agriculture, horticulture, and forestry (Godan, 1983).

Mollusks do not only attract attention as pests, but may also have commercial value for specific purposes, such as human food. *R. quadrasi* (pond snail) is a non pest mollusk species commonly lives in the rice field and shallow swamp. This species itself is not threatening to the crop, but because it lives in the same habitat as pest mollusk such as *P. canaliculata*, it is considered as a non-target organism. This snail plays an important role in the food chain, but because of its co-occurrence with mollusk pest, it could be threatened by molluscicide application (de Lara (2001), pers. comm). Innumerable species of mollusks are now threatened with extinction as a result of changing environmental conditions, including pollution by chemicals substances (Godan, 1983), which affect mollusks by interfering with fertilization, normal embryonic development, growth, reproductive ability, and normal tissue histology (Thompson & Edwards, 1974; APHA, 1995).

In general, this laboratory study aimed to determine the effects of metaldehyde and niclosamide on the behavior and reproductive capacity of *R. quadrasi*. In specific, this study was focused on the determination of: (1) the effects of metaldehyde and niclosamide on the fecundity and viability of *R. quadrasi* eggs; and (2) the symptoms of poisoning of the two molluscicide on this species.

MATERIALS AND METHODS

Test Organisms. A snail namely *R. quadrasi* (12–14 mm shell length) was obtained from the Azolla ponds of UP Los Baños. The snails were acclimatized for 10 days and maintained at the Malacology Laboratory, Institute of Biological Sciences, College of Arts and Sciences, University of the Philippines Los Baños prior to exposure to the metaldehyde and niclosamide.

Experimental Procedure

1. Range-finding test. A total of 231 *R. quadrasi* snails were used in this particular part of the experiment. The exposure procedure was done by putting seven *R. quadrasi* in each plastic basin (32x25x11 cm) containing three liters of 0, 0.1, 0.5, 1.0, 5.0, and 10.0mg/L of niclosamide; and 25, 50, 100, 200, and 300mg/L of metaldehyde. The duration of treatment was 72 hours. The test solutions were changed every 24 hours in order to keep them fresh, in term of their concentration throughout the experiment. There were three replicates for each concentration. Observations were made three times a day: morning, noon, and afternoon. The number of dead snails was recorded and they were quickly removed from the basin.

2. Short-term toxicity test. The dose range obtained from the range finding test was used as the basis for the short-term toxicity

test in term of low, moderate, and high concentration rates. A semi-static acute toxicity test was conducted following APHA (1995) standard method to determine the LC_{50} 72 hr. A total of 280 *R. quadrasi* was placed in each basin, containing four concentration-rates of niclosamide: 0, 0.045, 0.058, and 0.075mg/L; and four concentration-rates of metaldehyde: 0, 100, 112, and 130 mg/L. There were five replicates per treatment.

The symptoms of poisoning of treated snails were observed closely throughout the duration of the experiment. Observations were made three times a day: at the beginning, 12 hours, and 24 hours after exposure.

3. Fecundity trials. Six *R. quadrasi* survivors from each concentration of niclosamide and metaldehyde were divided into three groups, and were kept in aged tap-water for one month. They were fed with young leaves of lettuce. The number of egg masses and eggs laid, the incubation period and percent hatchability of the eggs were recorded in each group and compared with the control. Egg masses were kept in petri dishes filled-up with 30 ml of aged tap-water.

Data Analysis. The number of eggs laid, incubation period and percent hatchability of the eggs were analyzed using one-way analysis of variance (ANOVA). The Duncan Multiple Range Test (DMRT) was used to make comparison among treatment means.

RESULTS AND DISCUSSION

Symptoms of poisoning. The symptoms of poisoning, particularly the movement of the snails exposed to the molluscicides were visually observed. Tables 1 and 2 show how the snails reacted to each concentration-rate.

Table 1. Symptoms of poisoning of *R. quadrasi* exposed to concentration-rates of metaldehyde

Treatment dose (mg/L)	Toxicity signs	n	%
Control	■ Snails were very active and showed coordinated activity.	21	100
	■ A small number of snails exhibited water-leaving behavior	21	20
100 (Low)	■ Slow movement	21	71
	■ A small number of snails exhibited water-leaving behavior	21	29
	■ Mucus sheet excreted	21	100
	■ Foot did not retract	21	29
112 (Moderate)	■ Slow movement	21	67
	■ Some snails exhibited water-leaving behavior	21	43
	■ Moderate amount of mucus sheet excreted	21	100
	■ Foot did not retract	21	43
130 (High)	■ Writhing movement of the head.	21	19
	■ No sign of movement	21	81
	■ No water-leaving behavior; remained at the bottom of the basin	21	100
	■ Excessive mucus production	21	100

Table 2. Symptoms of poisoning of *R. quadrasi* exposed to concentration-rates of niclosamide

Treatment dose (mg/L)	Toxicity signs	n	%
Control	■ Snails were very active and showed coordinated activity.	21	100
	■ A small number of snails exhibited water-leaving behavior	21	19
0.045 (Low)	■ Slow movement	21	24
	■ Most of snails exhibited water-leaving behavior	21	76
	■ Foot did not retract	21	76
0.058 (Moderate)	■ Some snails were still active	21	62
	■ A small number of snails exhibited water-leaving behavior	21	38
	■ Foot did not retract	21	38
0.075 (High)	■ Snails not active	21	90.5
	■ Slow movement	21	9.5
	■ No water-leaving behavior; remained at the bottom of the basin	21	100

As shown in Table 1 and 2, there was immediate reaction of the snails to molluscicides exposure. Higher concentration-rates caused almost instantaneous immobility, in that the snails remained at the bottom of the plastic basin. By contrast, lower concentration-rates of molluscicide triggered water-leaving behavior. Such behavior occurred in all of the low and medium concentration-rates of both niclosamide and metaldehyde, as well as in some snails from the control groups.

Upon exposure of the snails to low and medium concentration-rates of metaldehyde, some of them remained being active but several exhibited slow movement, while others exhibited water-leaving behavior. Mucus sheets appeared around the foot, which did not retract. The same behavior was also observed in snails exposed to niclosamide, although the mucus sheet did not appear as clearly as in the metaldehyde-treated snails. For those exposed to the moderate concentration-rates, movement was very slow and some snails could be found at the bottom of the basin, and only a few exhibited water-leaving behavior.

In the highest concentration-rates of molluscicide, *R. quadrasi* showed no movement, which obviously meant the absence of water-leaving behavior. The exposure of the snails to high concentration-rates of niclosamide caused them to be immobile. For the metaldehyde treatment, mucus sheets surrounding the foot were very wide. The foot did not retract at all. Some of the snails showed writhing movements of the head from side to side shortly after exposure. If the snails were left in the solution for a long time, their head movements were followed by immobility. Some snails became immobile within 15 minutes but still responded to mechanical stimulation on the foot.

In this study, poisoning symptoms were determined by directly observing the

movement of the snail immediately after exposure to the molluscicide. The presence of molluscicides disturb the activity of the snails, directly affecting their movement. Low and moderate concentration-rates of molluscicides trigger water-leaving or escape behavior in a few snails (Table 1 and 2), with the rest becoming less active than the control. The same reaction was reported on *P. canaliculata*, in which snails were crawling out of treated water to avoid molluscicide exposure (Halwart, 1994). According to Jurberg *et al.* (1995) the repellency of *Biomphalaria glabrata* to niclosamide occurred at the following concentration ranges: 0.01, 0.02 and 0.03 ppm. Giovanelli *et al.* (2002) also reported that niclosamide triggered water-leaving behavior to *B. glabrata* as an escaping mechanism from the molluscicide action.

The highest concentration-rate of molluscicides caused the snails to become immobile and to remain at the base of the container. This behavior might be a defense mechanism as well as an attempt to avoid the effect of molluscicide exposure to the snails, and to reduce the energy expenditure. This result is in line with the finding of Giovanelli *et al.* (2001) on the exposure of snails to *Euphorbia splendens* var. *hislopilii*. After exposure to the molluscicide, many snails retracted into their shells, especially at the highest concentration-rates, with an accompanying reduction in their mobility. However, *R. quadrasi* did not exhibit the same symptom in that the snails did not retract into the shell. After being returned to the control medium, some snails reverted to normal behavior.

In metaldehyde-treated *R. quadrasi*, it was observed that the snails exhibited writhing movements. It is possible that their movements were caused by the action of molluscicide on the nervous system of the snails. This result is in line with the statement of Mills *et al.* (1990) that the symptoms of

metaldehyde poisoning were uncoordinated muscular spasms, which were brought about by the uncoordinated motor output of the central nervous system of the snails. It seems that the motor neurons responsible for the control of body movement were affected by metaldehyde. Lemly and Smith (1986), as cited by Brewer *et al.* (2001), said that the behavior of many organisms is extremely responsive to slight changes in both biotic and abiotic conditions such as lower levels of pollutant. This finding is also in line with the statement of Little and Finger (1990) as cited by Brewer *et al.* (2001) that behavioral responses have been shown to provide integrative measures of neurotoxicity, as well as reflecting biochemical and physiological reactions to the toxicant, since locomotory behavior is commonly affected by contaminants.

Effect of niclosamide and metaldehyde on the reproduction of *R. quadrasi*. Table 3 shows the average number of egg masses and eggs produced by *R. quadrasi* survivors after being reared and maintained in aged tap water for one month. The control snails produced the significantly highest number of egg masses (ranging from 8 to 9), or an average of 8.67 per snail (F value = 76.536, $P < 0.05$). Low and moderate concentration-

rates of metaldehyde and niclosamide had almost the same effect on egg production. On the other hand, the highest concentration-rate of the two molluscicides differed markedly from lower concentration-rates. Even, the survivors of the highest metaldehyde treatment (130 mg/L) produced no eggs at all.

The number of eggs produced by the snails was significantly affected by the treatments, with each concentration-rate differing considerably from the next (F values = 150.7197, $P < 0.05$). Higher concentration-rates of the metaldehyde caused corresponding reductions in egg production. In the case of niclosamide, the highest concentration-rate differed greatly from the two other treatments. However, the total number of eggs produced by survivors of the moderate concentration-rate was not significantly different from those exposed to the lowest dose.

Table 4 shows the mean incubation period and percent hatchability of eggs produced by both control and treated snails. The concentration-rates of the molluscicides did not affect on the incubation period but significantly affect on hatchability (F values = 5.3046, $P < 0.05$). The metaldehyde was so lethal at the rate of 130 mg/L caused no eggs to be produced at all (Table 3).

Table 3. Mean fecundity of *R. quadrasi* surviving after exposure to various concentration-rates of either niclosamide or metaldehyde and cultured to the aged tap-water for one month

Treatment doses (mg/L)	Number of snails	Fecundity *	
		Number of egg masses/snail	Number of eggs/snail
0 (control)	6	8.67a	230.67a
Niclosamide:			
▪ 0.45 (low)	6	2.33 b	35.67 b
▪ 0.58 (moderate)	6	2.67 b	41.33 b
▪ 0.75 (high)	6	0.33 c	6.5 e
Metaldehyde			
▪ 100 (low)	6	1.33 d	17.33 d
▪ 112 (moderate)	6	0.67 cd	7.33 e
▪ 130 (high)	6	0 d	0 f

* Values in columns with the same letters do not differ significantly from each other (DMRT, $P < 0.05$)

Table 4. Mean percentage of incubation period and hatchability of eggs laid by *R. quadrasi* surviving after exposure to various concentration-rates of either niclosamide or metaldehyde and cultured to the aged tap water for one month

Treatment dose (mg/L)	Incubation period (days) *	Percent hatchability (%) *
0 (control)	7.75a	89.77a
Niclosamide:		
■ 0.45 (low)	8.66a	74.83 b
■ 0.58 (moderate)	7.72a	38.66 c
■ 0.75 (high)	8.00a	34.20 c
Metaldehyde:		
■ 100 (low)	8.74a	66.40 d
■ 112 (moderate)	7.51a	63.57 d
■ 130 (high)	-	-

* Values in columns with the same letters do not differ significantly from each other (DMRT, $P < 0.05$)

From the observation, it was obvious that metaldehyde-treated snails attempted to get rid of the chemical by producing copious amount of mucus. This excessive excretion of mucus depleted the energy reserves of the snails. The depletion of energy affected snail reproduction, a process that requires large amounts of energy.

It is also possible that the overall metabolism of the snails was impaired by the molluscicide, so that feeding rhythm was affected. It was observed that control snails ate more actively than the treated ones. Van der Steen *et al.* (1973), as cited by Runham (1993), reported that when more energy in the form of food was provided, more was consumed, and more eggs were produced by *Lymnaea stagnalis*. Similar results were also reported by Jooisse (1979), as cited from Runham (1993), i.e., that low amount of food available for the same species leads to the cessation of egg laying, and finally a resorption of the contents of the gonad. *Thais lapillus*, which lays an average of three clutches of eggs per season, utilizes 81% of the pre-spawning energy content of the body in producing these eggs (Hughes, 1972).

The exposure of the snails to metaldehyde caused severe damage to the

individual gonads. In the present study, oviposition (eggs laid) was delayed in all of the treated snails. Because the snails were treated at the stage preceding sexual maturity, it is possible that the delay in egg production may have been due to its slower rate of gonadal maturation (Ocampo (2001), pers. comm.).

The rate of egg hatchability was also significantly influenced by the molluscicide treatment. This phenomenon suggests that the chemicals exert long-term effects on the reproductive potential of individual snails. It seems that following the period of exposure the molluscicide continues to affect the snails. This result might be attributable to the effect of chemicals on the energy reserve of the snails. Pipe (1985), as cited by Runham (1993), reported that starvation not only delays spawning in *Mytilus edulis* but also decreases the glycogen and protein in the eggs when they are laid.

In general, the resulted effects on the reproductive capacity of *R. quadrasi* to the two molluscicides tested in this study were similar to those caused by carbofuran, in which oviposition and incubation period was delayed, and hat chability was decreased (Pagulayan *et al.*, 1992).

CONCLUSION

The results of this study clearly showed that niclosamide and metaldehyde obviously affected behavior and reproductive capacity of *R. quadrasi*. Treatment with molluscicide caused a variety of effects on the reproductive capacity of this snail. Egg production decreased drastically (72 - 97% for metaldehyde, and 92 - 100% for niclosamide) as compared to control, with increasing molluscicide concentration-rates. The percentage of eggs hatched was significantly affected by the molluscicides. However, the incubation period was not significantly affected by the molluscicides.

The symptoms of poisoning, as indicated by water-leaving behavior, observed on the snail treated with the two molluscicides. Water-leaving behavior was observed not only among those exposed to low and medium concentration-rates of niclosamide and metaldehyde, but also in some snails from the control group. Higher concentration-rates suddenly caused the snails to be immobile, in which they remained at the bottom of the plastic basin. The behavioral reactions indicated that between the two molluscicides used, niclosamide is more toxic than metaldehyde.

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PENGENDALIAN PENYAKIT LAYU FUSARIUM PADA PISANG DENGAN INOKULASI JAMUR MIKORIZA VESIKULAR ARBUSKULAR PADA BIBIT

CONTROL OF FUSARIUM WILT ON BANANA USED SEEDLING WAS INOCULATED VESICULAR ARBUSCULAR MYCORRHIZA

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ABSTRACT

Fusarium wilt disease caused by Fusarium oxysporum f.sp. cubense is the most important and destructive disease on banana. The pathogen is soil borne and can survive in soil without the occurrence of the host.

The Vesicular Arbuscular Mycorrhiza (VAM) is a symbiotic associated fungi and plant roots. The VAM which infected plant can induce the resistance to pathogen.

Banana seedlings obtained from tissue culture one free from pathogen. The seedling was inoculated with VAM to induce the resistance to Fusarium wilt disease. The result showed that the seedling inoculated with VAM can reduce diseases intensity of Fusarium wilt on banana.

Key words: Vesicular Arbuscular Mycorrhiza, Fusarium wilt of banana, Fusarium oxysporum f.sp. cubense

INTISARI

Penyakit layu Fusarium yang disebabkan oleh *Fusarium oxysporum f.sp. cubense* merupakan salah satu penyakit penting pada pisang, dan telah mengakibatkan kerugian yang cukup tinggi karena patogen ini akan menyebabkan kematian tanaman. Pengendalian sangat sulit dilakukan karena patogen mudah tersebar dan mampu bertahan di tanah dalam waktu yang cukup lama meskipun tanpa adanya tanaman inang.

Jamur Mikoriza Vesikular Arbuskular (MVA) merupakan suatu jamur yang bersimbiotik dengan tanaman inangnya. Pada beberapa tanaman infeksi jamur MVA telah terbukti mampu meningkatkan ketahanan tanaman terhadap penyakit.

Penelitian dilakukan dengan menggunakan bibit pisang hasil kultur jaringan yang masih bebas dari patogen. Bibit sebelum ditanam diinokulasi dengan mikoriza dan diharapkan bibit yang telah bermikoriza ini akan mempunyai ketahanan terhadap penyakit layu Fusarium. Hasil penelitian menunjukkan bahwa inokulasi mikoriza mampu menekan intensitas penyakit layu Fusarium.

Kata kunci: Mikoriza Vesikular Arbuskular, penyakit layu Fusarium, *Fusarium oxysporum f.sp. cubense*