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Susceptibility of Non-target Aquatic Macro-invertebrates and Vertebrates to *Piper guineense* (Piperaceae) and *Spilanthes mauritiana* (Asteraceae) Powder in Kilifi District, Kenya

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Abstract: Powders of *Piper guineense* and *Spilanthes mauritiana,* potential mosquito larvicides, were assessed to determine their impact on non-target aquatic macro-invertebrates and vertebrates. Field tests were conducted in Kilifi District, Kenya on field populations of damselfly nymph (Gomphilidae), dragonfly nymph (Coenagrionidae), macro-dytiscids, micro-dytiscids (Dystiscidae), notonectids (Notonectidae), freshwater shrimps (Palaemonidae), tadpoles (Ranidae) and tilapia fish (Cichlidae). Artifical habitats were sampled and populations determined before and after application of the plant powder. Four doses (8.571, 5.714, 2.875 and 1.429 g L^{-1}), were used in the trials and mortalities monitored after 24, 48 and 72 h. After 24 h, at 1.429 g L^{-1} of the powder from *P. guineense* and *S. mauritiana*, less than 20% mortality was obtained against gomphilids, coenagrionids, dytiscids, notonectids and palaemonids. At 48 h, powder of *P. guineense* gave 8.8 and 45% mortality against micro-dytiscids and tadpoles at 8.571 g L^{-1}, respectively. After 72 h, at 2.847 g L^{-1}, powder of *S. mauritiana* caused 6.9 and 35% mortality against micro-dytiscids and fish, respectively. The LD_{50}s (24 h) for the organisms varied from 12.2 to 39.2 g L^{-1} and 13.6 to 41.82 g L^{-1} for *P. guineense* and *S. mauritiana*, respectively. Both plant powders showed slight negative effects on non-target aquatic macro-invertebrates and vertebrates.

Key words: Macro-invertebrates, vertebrates, non-target, aquatic, powder

INTRODUCTION

Plant-derived products are increasingly being used to combat pests and vectors because they are natural and are often assumed to be safe for the environment. These plant-derived products, however, can have a detrimental effect on the environment. Powders of *P. guineense* and *S. mauritiana* can be used as mosquito larvicides (Okinyo, 2002; Ohaga, 2003). *Piper guineense* Schum and Thonn is a dicotyledonous plant that belongs to the family Piperaceae. Extracts of this plant have been studied and proved to be potential control agents against many destructive arthropods, (Ivbijaro, 1990; Ivbijaro and Bolaji, 1990; Mbata *et al*., 1995). Substantial evidence from chemical studies has shown that

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P. guineense contains naturally-occurring piperine-type alkaloids (Addae-Mensah *et al*., 1977). The alkaloids isolated from *P. guineense* were found to be very active on *Aedes aegypti* larvae (Addae-Mensah and Achieng, 1986). Consequently, under laboratory and semi-field conditions, the same extracts have been shown to have high larvicidal activity against *Anopheles gambiae s.l.* (Okinyo, 2002; Ohaga, 2003). *Spilanthes mauritiana* Rich belongs to the family Asteraceae. The plant has been reported to have many medicinal properties (Fabry *et al*., 1996, 1998). The plant owes its activity to the antiseptic alkaloid spilanthol and immune-stimulating alkylamides (Fabry *et al*., 1998). Jondiko (1989) and Ohaga (2003) reported the larvicidal properties of *S. mauritiana* under laboratory and semi-field conditions. The activity was found to be due to long chain fatty amides such as N-isobuyl-2E, 4E, 8Z, 10Z-dodeca-2, 4, 8,10-tetraenamide (Jondiko, 1989). Powders of *P. guineense* and *S. mauritiana* have shown high larvicidal activity against mosquitoes (Okinyo, 2002; Ohaga, 2003). If powders of these plants were to be used for mosquito larvae control, possibility exists that some components may sip into the ground water and migrate into nearby rivers and streams. Therefore, the effects of these powders on the environment and non-target aquatic fauna must be studied in greater detail before they are widely integrated into mosquito control programs. No information has been reported on the effects of these plant powders on non-target aquatic macro-invertebrates and vertebrates. This research was conducted to determine the effects of *P. guineense* and *S. mauritiana* powder on non-target aquatic macro-invertebrates and vertebrates. We tested the hypothesis that if these powders are applied directly to the aquatic ecosystems, they will adversely affect and ultimately cause death to non-target aquatic macro-invertebrates and vertebrates.

MATERIALS AND METHODS

Study Site

The study was conducted along Jaribuni stream (03° 36.81°S and 03° 949.28°E) of Jaribuni village in Kilifi District, Kenya. The selection of this site was dependent on 3 factors; known aquatic habitats for macro-invertebrates and vertebrates, presence of relatively high populations of non-target aquatic macro-invertebrates and vertebrates and the relative permanence of aquatic habitats in the area.

Plant Collection and Preparation

Green leaves of *P. guineense* and *S. mauritiana* were collected from Kakamega forest in Western Kenya and dried under shade for 30 days. The dry dark leaves were separated from the leaf petioles and ground into a fine powder by motor driven hammer mills. The powdered material was further filtered through a series of sieves with small (1 μm) mesh sizes to give the final material for bioassay.

Selection of Test Macro-invertebrates and Vertebrates and Collection Sites

Six taxons of aquatic invertebrates and two vertebrates were used for bioassays. These included: damselfly nymph (Gomphilidae), dragonfly nymph (Coenagrionidae), macro-dytiscids, micro-dytiscids (Dystiscidae), notonectids (backswimmers) (Notonectidae), freshwater shrimps (Palaemonidae), tadpoles (Ranidae) and tilapia fish (Cichlidae). The taxonomic determinations were made with the help of John Carlson of the Department of Tropical Medicine, Tulane University, USA. These taxons were chosen because of the following reasons: (1) abundance at Jaribuni site; (2) their representation of different Orders and Families (3) previous identification of some as predators of mosquito larvae that play a great role in larval population regulation; (4) ease of identification under field conditions. All aquatic non-target organisms tested were collected from aquatic habitats along the river. The test organisms were collected on each day by the use of standard dipping technique (Service, 1993) or aquatic net. They were placed in white plastic trays for the identification of the organisms with individuals of the taxons to be tested being removed manually using pipettes and sieves and then placed in the basins.

Bioassays Procedures

A total of 104 circular pools of 35 cm in diameter and depth of 15 cm were dug 1 m from the edge of the stream. One hundred and four plastic wash basins (35×13 cm) with a capacity of 3500 mL smeared with mud to mimic the natural aquatic test organisms soil habitats found in the area were inserted into each pool. Water (3500 mL) from the river was introduced into each pool and 96 of the pools treated with a known amount of the plant-derived powder and 8 used as untreated controls. Into each of the artificial habitats, a known number of test organism, 60 damselfly nymphs, 25 dragonfly nymphs, 60 macro-dystiscids, 160 micro-dystiscids, 40 notonectids, 60 shrimps, 60 fish and 20 tadpoles per basin were introduced. To 3500 mL water in each basin containing the test organism, a known amount of the plant powder was added giving a known dose. The doses used were 8.571, 5.714, 2.857 and 1.429 g L^{-1}, respectively. These doses were selected based on the results obtained from the preliminary laboratory experiments (Okinyo 2002; Ohaga, 2003). Mortalities were monitored after 24, 48 and 72 h. Test species were considered dead if they showed no movement after being agitated for 10 sec. There were four replicates for each dose and control.

Data Analysis

To determine the lethal doses (LD) for each powder, acute toxicity data were analyzed by Probit analysis (Finney, 1981). In all tests, percentage reduction of the test organisms were determined and the percentage mortality calculated indirectly using Abbott's formula, taking into account mortality in the controls (Abbott, 1925).

$$P_T = \frac{P_O - P_C}{100 - P_C} \times 100$$

Where,
P_T = Corrected % mortality
P_O = Observed % mortality
P_C = Control % mortality

Percentage data were transformed using square root (x+1) prior to analysis of variance (ANOVA). Treatment means were compared and separated by Least Significant Difference (LSD) test at $p = 0.05$. Statistical analyses were performed using the statistical package SAS (SAS, 2003) and Microsoft© Excel 2000.

RESULTS

At a dose of 1.429 g L^{-1}, after 24 h of treatment with *P. guineense* powder, less than 20% mortality was observed among the aquatic macro-invertebrates and vertebrates (Table 1). Powder of *S. mauritiana* gave less than 25 and 35% mortality at 2.847 and 8.571 g L^{-1}, respectively. At a dose of 2.847 g L^{-1}, less than 25% mortality was achieved with both powders. At 5.714 g L^{-1}, *S. mauritiana* powders caused 1.25, 10, 20 and 26% mortalities against micro-dytiscids, notonectids, dragonfly nymphs and fish, respectively. At a dose of 8.571 g L^{-1}, *P. guineense* powder caused 1.88, 12.5, 25 and 30% mortalities against micro-dytiscids, notonectids, damselfly nymphs and tadpoles, respectively. After 48 h of exposure, aquatic macro-invertebrates and vertebrates treated with *P. guineense* and *S. mauritiana* powders showed a slight increase in mortality. At 1.429 and 2.847 g L^{-1}, less than 30 and 35% mortality was achieved with *P. guineense* and *S. mauritiana* powders, respectively (Table 2). At 5.714 and 8.571 g L^{-1}, both powders caused mortalities of between 8 and 45%. Powder of *P. guineense* gave 8 and 30% mortality in dragonfly nymph and

Table 1: Percent mean (±SE) mortality of non-target aquatic macro-invertebrates and vertebrates introduced into artificial pools treated with *Piper guineense* and *Spilanthes mauritiana* powder for the duration of 24 h

	Non-target organism		Dose (g L^{-1})			
Order	Family	Common name	1.429	2.847	5.714	8.571
P. guineense						
Odonata	Gomphilidae	Damsel fly nymph	13.33±1.12a	13.33±1.88a	20.00±1.60a	25.00±0.55b
Odonata	Coenagrionidae	Dragon fly nymph	4.00±1.20a	8.00±2.68a	12.00±1.60b	20.00±0.48c
Coleoptera	Dytiscidae	Macro-dytiscids	11.67±1.78a	15.00±1.76a	16.67±1.09a	18.33±0.64a
Coleoptera	Dytiscidae	Micro-dytiscids	0.00±0.00a	1.25±2.11a	1.88±1.20a	1.88±1.20a
Hemiptera	Notonectidae	Backswimmers	5.00±1.37a	7.50±0.89a	12.5±1.46b	12.50±0.49b
Decapoda	Palaemonidae	Freshwater shrimps	10.00±1.18a	11.67±0.89a	15.00±1.17b	18.33±0.50b
Perciformes	Cichlidae	Tilapia fish	20.00±1.56a	21.67±1.01a	26.67±3.60a	35.00±0.58c
Anura	Ranidae	Tadpoles	5.00±1.50a	25.00±3.49b	30.00±3.23b	30.00±0.06b
S. mauritiana						
Odonata	Gomphilidae	Damsel fly nymphs	6.67±0.68a	13.33±0.54a	23.33±1.30b	25.00±0.54b
Odonata	Coenagrionidae	Dragon fly nymph	8.00±0.42a	8.00±0.43a	20.00±0.71b	26.67±0.48b
Coleoptera	Dytiscidae	Macro-dytiscids	5.00±0.66a	11.67±0.48a	18.33±1.69b	20.00±0.64b
Coleoptera	Dytiscidae	Micro-dytiscids	0.00±0.00a	1.25±0.55a	1.25±1.10a	1.88±0.64a
Hemiptera	Notonectidae	Backswimmers	5.00±0.43a	7.50±0.95a	10.00±1.29a	12.50±0.53a
Decapoda	Palaemonidae	Freshwater shrimps	8.33±0.43a	10.00±1.23a	18.33±0.87b	18.33±0.42b
Perciformes	Cichlidae	Tilapia fish	21.67±0.36a	20.00±0.94a	26.00±2.57a	35.00±0.50b
Anura	Ranidae	Tadpoles	25.00±0.39a	25.00±1.30a	25.00±0.64a	30.00±0.61a

Values in row for each organism followed by different letter(s) are significantly different ($p \leq 0.05$, LSD test)

Table 2: Percent mean (±SE) mortality of non-target aquatic macro-invertebrates and vertebrates introduced into artificial pools treated with *Piper guineense* and *Spilanthes mauritiana* powder for the duration of 48 h

	Non-target organism		Dose (g L^{-1})			
Order	Family	Common name	1.429	2.847	5.714	8.571
P. guineense						
Odonata	Gomphilidae	Damsel fly nymph	23.33±0.55a	25.00±0.73a	35.00±4.50b	36.67±3.23b
Odonata	Coenagrionidae	Dragon fly nymph	8.00±0.56a	12.00±0.86a	28.00±0.75b	28.00±0.55b
Coleoptera	Dytiscidae	Macro-dytiscids	20.00±0.59a	21.67±0.11a	28.33±0.70a	38.33±2.57b
Coleoptera	Dytiscidae	Micro-dytiscids	4.38±0.64a	4.38±0.96a	5.00±0.59a	8.75±0.59a
Hemiptera	Notonectidae	Backswimmers	15.00±0.91a	15.00±0.86a	25.00±0.75b	27.00±1.42b
Decapoda	Palaemonidae	Freshwater shrimps	18.33±4.50a	20.00±1.42a	28.33±0.79b	28.33±0.63b
Perciformes	Cichlidae	Tilapia fish	26.67±0.83a	35.00±0.74b	45.00±0.69c	45.00±2.11c
Anura	Ranidae	Tadpoles	30.00±0.75a	30.00±0.88a	40.00±0.76b	45.00±0.64b
S. mauritiana						
Odonata	Gomphilidae	Damsel fly nymphs	15.00±0.71a	25.00±0.12b	35.00±0.12c	36.00±0.11c
Odonata	Coenagrionidae	Dragon fly nymph	20.00±0.31a	24.00±0.13a	28.00±0.11a	24.00±0.08b
Coleoptera	Dytiscidae	Macro-dytiscids	15.00±0.61a	20.00±0.15a	38.00±0.14b	35.00±0.12b
Coleoptera	Dytiscidae	Micro-dytiscids	3.75±0.11a	4.38±0.09a	8.75±0.10b	10.63±0.12b
Hemiptera	Notonectidae	Backswimmers	15.00±0.88a	17.50±0.13a	27.50±0.09b	27.50±0.90b
Decapoda	Palaemonidae	Freshwater shrimps	18.33±0.10a	21.67±0.10a	25.00±0.08ab	28.33±0.10b
Perciformes	Cichlidae	Tilapia fish	35.00±0.15a	35.00±0.15a	45.00±0.10b	45.00±0.08b
Anura	Ranidae	Tadpoles	30.00±0.15a	30.00±0.13a	35.00±0.12a	40.00±0.10a

Values in row for each organism followed by different letter(s) are significantly different ($p \leq 0.05$, LSD test)

tadpoles, respectively, at 1.429 g L^{-1} but 28 and 45% mortality in dragonfly nymph and tadpoles, respectively at 8.571 g L^{-1}. Powders of *S. mauritiana* caused 4.38 and 45% mortality of micro-dystiscids and fish, respectively at 2.847 g L^{-1}, but mortality of 8.75 and 45%, respectively of micro-dystiscids and fish at a higher dose (5.714 g L^{-1}) was recorded. At the lowest dose (1.429 g L^{-1}) after 72 h of treatment with the powders, aquatic macro-invertebrates and vertebrates had mortalities of between 4-35% and 3.7-36% for *P. guineense* and *S. mauritiana*, respectively (Table 3). The powder of *S. mauritiana* caused 13.3 and 55% mortalities of micro-dystiscids and tadpoles at 8.571 g L^{-1}, respectively. The powder of *P. guineense* showed 4.3 and 35% mortality in micro-dystiscids and fish at 1.429 g L^{-1}, whereas 11.9 and 60% mortalities were shown against micro-dystiscids and fish at 8.571 g L^{-1}, respectively.

Table 3: Percent mean (±SE) mortality of non-target aquatic macro-invertebrates and vertebrates introduced into artificial pools treated with *Piper guineense* and *Spilanthes mauritiana* powder for the duration of 72 h

	Non-target organism		Dose (g L^{-1})			
Order	Family	Common name	1.429	2.847	5.714	8.571
P. guineense						
Odonata	Gomphilidae	Damsel fly nymph	26.67±0.09a	31.00±0.08a	41.67±0.80b	43.33±0.07b
Odonata	Coenagrionidae	Dragon fly nymph	24.00±0.10a	24.00±0.10a	32.00±0.10ab	36.67±0.11b
Coleoptera	Dytiscidae	Macro-dytiscids	23.33±0.11a	26.67±0.10a	35.00±0.09b	46.67±0.11c
Coleoptera	Dytiscidae	Micro-dytiscids	4.38±0.10a	5.63±0.10a	5.63±0.08a	11.88±0.09a
Hemiptera	Notonectidae	Backswimmers	17.50±0.07a	20.00±0.10a	32.50±0.78b	32.50±0.09b
Decapoda	Palaemonidae	Freshwater shrimps	21.67±0.09a	26.67±0.09ab	35.00±0.10b	35.00±0.11b
Perciformes	Cichlidae	Tilapia fish	35.00±0.08a	45.00±0.10b	51.67±0.09b	60.00±0.11c
Anura	Ranidae	Tadpoles	30.00±0.08a	45.00±0.10b	45.00±0.09b	55.00±0.11c
S. mauritiana						
Odonata	Gomphilidae	Damsel fly nymphs	16.67±0.15a	26.67±0.17b	35.00±0.11b	45.00±0.61c
Odonata	Coenagrionidae	Dragon fly nymph	20.00±0.10a	24.00±0.11a	40.00±0.10b	44.00±0.13b
Coleoptera	Dytiscidae	Macro-dytiscids	18.33±0.13a	28.33±0.15b	43.33±0.10c	45.00±0.14c
Coleoptera	Dytiscidae	Micro-dytiscids	3.75±0.12a	6.88±0.12a	10.00±0.13a	13.13±0.11a
Hemiptera	Notonectidae	Backswimmers	22.50±0.10a	30.00±0.10a	32.50±0.12ab	42.50±0.13b
Decapoda	Palaemonidae	Freshwater shrimps	23.33±0.09a	26.67±0.11a	28.33±0.14ab	36.67±0.10b
Perciformes	Cichlidae	Tilapia fish	36.67±0.10a	45.00±0.09ab	48.33±0.11b	48.33±0.88b
Anura	Ranidae	Tadpoles	35.00±0.08a	35.00±0.80a	40.00±0.09a	55.00±0.13b

Values in row for each organism followed by different letter(s) are significantly different ($p \leq 0.05$, LSD test)

Table 4: Probit analyses of mortality, LD_{50} using *Piper guineense* powder for 24 h against aquatic macro-invertebrates and vertebrates

Order	Family	Common name	Intercept±SE	Slope±SE (Pearson χ^2 of the slope)	Pearson χ^2 for goodness-of-fit-test	LD_{50} g L^{-1} (95% CI)
Odonata	Gomphilidae	Damsel fly nymph	-1.26±0.78	0.06±0.01 (21.38, p<0.0001)	3.40NS	20.16 (15.54-31.44)
Odonata	Coenagrionidae	Dragon fly nymph	-1.79±0.10	0.07±0.02 (22.53, p<0.0001)	8.08NS	22.51 (17.39-34.69)
Coleoptera	Dytiscidae	Macro-dytiscids	-0.96±0.07	0.06±0.04 (25.75, p<0.0001)	2.73NS	15.31 (12.56-2.01)
Coleoptera	Dytiscidae	Micro-dytiscids	-1.21±0.08	0.03±0.01 (6.04, p<0.01)	1.82NS	35.18 (21.63–154.18)
Hemiptera	Notonectidae	Backswimmers	-2.57±0.20	0.07±0.03 (4.24, p<0.05)	6.21NS	39.15 (22.69-692.48)
Decapoda	Palaemonidae	Freshwater shrimps	-1.67±0.09	0.07±0.02 (16.45, p<0.0001)	5.55NS	25.50 (18.44–44.61)
Perciformes	Cichlidae	Tilapia fish	-1.29±0.08	0.04±0.01 (7.91, p<0.001)	2.53NS	32.24 (20.91-95.24)
Anura	Ranidae	Tadpoles	-1.29±0.14	0.11±0.02 (17.83, p<0.0001)	50.65S	12.23 (9.58-19.83)

NS, non significant ($p > 0.05$). Chi-square test for goodness-of-fit. S, significant. Chi-square test for goodness-of-fit

The Intercept, Slope, Pearson's goodness-of-fit test and the lower and upper confidence limits of the LD_{50}s of the powders at 24 h post exposure are indicated. In this test, the *P. guineense* powder showed toxicity with LD_{50}s of 20.2 and 22.5 g L^{-1} against damselfly and dragonfly nymph, respectively (Table 4). Among the macro-dytiscids and micro-dytiscids, LD_{50}s of 15.3 and 35.2 g L^{-1}, respectively was observed. Similarly, the powder showed toxicity with LD_{50}s of 32.2 and 12.2 g L^{-1} against fish and tadpoles, respectively. The highest toxicity with LD_{50}s of 39.2 g L^{-1} was achieved against notonectids. The powder of *S. mautitiana* showed toxicity with LD_{50}s of 37 g L^{-1} and 26.5 g L^{-1} against notonectids and palaemonids, respectively (Table 5). Toxicity with LD_{50}s of 14.9 g L^{-1} and 17.3 was recorded against macro-dytiscids and micro-dytiscids, respectively. Between the damsel and dragon fly nymphs, toxicity with LD_{50}s of 13.4 and 13.9 g L^{-1}, respectively was observed. The highest toxicity with LD_{50}s of 41.8 g L^{-1} against tadpoles was recorded.

Table 5: Probit analyses of mortality, LD_{50} using *Spilanthes mauritiana* powder for 24 h against aquatic macro-invertebrates and vertebrates

Order	Family	Common name	Intercept±SE	Slope±SE (Pearson χ^2 of the slope)	Pearson χ^2 for goodness-of-fit-test	LD_{50} g L^{-1} (95% CI)
Odonata	Gomphilidae	Damsel fly nymph	-1.56±0.08	0.01±0.01 (66.59, p<0.0001)	19.42NS	13.55 (11.85-16.26)
Odonata	Coenagrionidae	Dragon fly nymph	-1.59±0.08	0.11±0.01 (65.03, p<0.0001)	12.17NS	13.89 (12.11-16.74)
Coleoptera	Dytiscidae	Macro-dytiscids	-0.98±0.07	0.07±0.01 (28.33, p<0.0001)	1.87NS	14.89 (12.05-20.86)
Coleoptera	Dytiscidae	Micro-dytiscids	-1.51±0.08	0.09±0.01 (36.69,p<0.0001)	17.18NS	17.26 (14.21-23.13)
Hemiptera	Notonectidae	Backswimmers	-2.61±0.20	0.07±0.03 (4.74, p<0.05)	5.53NS	36.96 (22.04-319.14)
Decapoda	Palaemonidae	Freshwater shrimps	-1.67±0.09	0.06±0.02 (15.10, p<0.0001)	0.85NS	26.46 19.23-48.23
Perciformes	Cichlidae	Tilapia fish	-1.36±0.08	0.05±0.01 (10.92, p=0.001)	7.36NS	28.37 19.56-62.57
Anura	Ranidae	Tadpoles	-0.73±0.06	0.02±0.01 (2.03, p<0.154)	1.82NS	41.82[a]

NS, non significant (p>0.05). Chi-square test for goodness-of-fit. Fiducial limits not available with SAS (SAS Institute, 2003) for this data set

DISCUSSION

One ecological interest in mosquito control programs is for control agents to be sufficiently target specific that they do not cause damage to non-target species or food webs (Secord and Kareiva, 1996). Since mosquito larvicides are applied to water for the control of larval stages of mosquitoes, aquatic ecosystems are of the immediate concern.

A phototoxin extracted from marigolds, alpha-terthie-nyl, was shown to be extremely insecticidal against mosquitoes but did not affect non-target organisms like the ostracod, caddisfly and *Physa* sp. (Philogene *et al*., 1985). However, in a field evaluation of alpha-terthienyl, Dosdall *et al*. (1991) found that their application in stream ecosystems caused catastrophic drift of several species of non-target aquatic insects and behaviour changes in crayfish. Neem (*Azadirachta indica*) is most selective against Lepidopteran pests and has less effect on parasitoids or predators (Stark *et al*., 1990). However, adverse effects of azadirachtin against beneficial organisms have been reported (Schmutterer and Holst, 1987; Price and Schuster, 1991). In the current study, the powders of the two plants were relatively safe to some of the tested invertebrates and vertebrates. Fish was the least tolerant even in the controls with high mortality within 24 h of exposure to the powder. The mortality of the fish in the controls could be attributed to unfavourable environmental conditions in the artificial pools and the problems of handling during sampling. Notonectids and micro-dystiscids were the least susceptible to the powders. The observed reduction in the number could be due to the fact that they can fly away once exposed to the powder, indeed, the beetles were observed to fly away on the application of the powder. The plant powders may be repellent to the beetles.

The range of responses for the non-target organisms tested can be attributed to many factors. The individual test organisms used in this study were native populations and probably had a high degree of genetic, physiological and behavioural variability within each taxa. In general, the results indicate that these powders had less toxic effect on predatory aquatic macro-invertebrates and vertebrates studied. The less-interference of the tested powders with the predators accommodates supplementary contribution of the predators towards population regulation of mosquitoes. However, more information need to be collected of firm conclusions to be drawn on consequences or benefits from the use of these powders as replacements/supplements to more commonly used larvicides in areas adjacent

to streams and river systems. At the doses tested, the powder may not be harmful to non-target macro-invertebrates and vertebrates in aquatic environments. However, the vertebrate toxicology and its effects on a wide range of non-target aquatic organisms need further study before they can be seriously considered as an alternative or complementary to current mosquito larvicides. It is only after this that the economics of their use in mosquito control could be evaluated. It could also be necessary to assess their suitability for mosquito control in habitats used as sources of drinking water.

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