



Response of *Suidasia pontifica* (Acaridida: Suidasiidae) to phosphine fumigation

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Abstract

The present study evaluated the level of phosphine toxicity of various strains of *Suidasia pontifica*, infesting stored agricultural products in the Philippines. The bioassays for the efficacy of phosphine fumigation followed the FAO standard method. A full assay comprised eight concentrations ranging from 0.005 to 1.00 mg/L with 20, 72 and 144-h exposure periods. A total of 15 strains were collected, representing 15 provinces in the country. Results revealed that the most susceptible strain (MR12gsn) was recorded from a private owned warehouse in General Santos City—LC₅₀ and LC₉₉ were 0.009 and 0.025 mg/L, respectively. On the other hand, the most resistant strain (LR3tar) was collected from a private owned warehouse in Tarlac City—LC₅₀ and LC₉₉ were 1.501 and 2.407 mg/L, based on discrimination dose tests. Eggs were more tolerant than the adults; complete mortality of eggs was achieved at 0.50 mg/L for 72 h and 0.35 mg/L for 144 h, whereas complete mortality of adults was obtained with 0.10 and 0.20 mg/L for 144 and 72 h exposure, respectively. The estimated minimum effective concentration (MEC) of phosphine to give complete kill of *S. pontifica* at various exposure periods must exceed 0.50 mg/L for 72 and 144 h. These results simply illustrate the increased efficiency (i.e., increased mortality) of phosphine fumigation of longer exposure of *S. pontifica* to lower concentrations.

Keywords *Suidasia pontifica* · Phosphine · Fumigation · Philippines · Storage mites

Introduction

Suidasia pontifica (Acaridida: Suidasiidae) is commonly found in most tropical countries where temperature and humidity are high throughout the year; mostly associated with stored agricultural products such as cereals, animal feed and even inside our houses (Colloff 2009). Clinical relevance of *S. pontifica* in an asthmatic population was observed in 73.2% of asthmatic patients had positive IgE reactivity to *Suidasia medanensis* (= *pontifica*)

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(Mercado et al. 2001; Puerta et al. 2005). Moreover, it produces potent allergens causing serious diseases like dermatitis and rhinitis (Samung et al. 2006; Yap et al. 2014). According to Ho (2002), the presence of *S. pontifica* was dwell and propagated inside the tympanum of human ear. Moreover, most storage mites have the ability to survive outside warehouses with small amounts of available food and re-infest the commodity under favorable conditions (Nayak 2006). Thus, the economic and medical impact of this species supports the need for its management and control.

Hydrogen phosphide (PH₃), locally known as phosphine, is the most commonly used fumigant in the world. Fumigation using phosphine, is ideal in stored product protection because of its high efficacy and potency for the eradication of pest infestation in stored agricultural commodities (Daglish 2004; Collins 2005; Wang et al. 2006). It has multiple advantages, including low cost of application, accepted as residue-free in international trade, ease of use, and it has been proven effective against storage pests (Emery et al. 2003; Opit et al. 2012; Kaur et al. 2015; Zhao et al. 2015). Further, when low levels of phosphine were absorbed by the commodity, most, if not all, phosphine retained was converted to phosphate and so it leaves no harmful residue into the stored commodities.

There are two registered fumigants in the Philippines: methyl bromide and phosphine. However, methyl bromide is an ozone-depletor, hence, one of the subjects of the Montreal Protocol signed by most countries, which bans its use by the year 2005 in developing nations (UNEP 2000; Fields et al. 2002). The continuous use and the high dependence on phosphine fumigation have led to the development of resistance in major storage insect pests (Nayak et al. 2012, 2019; Nguyen et al. 2015; Rajan et al. 2017). There are numerous reports available in the literature on the efficacy of phosphine, but detailed data on the effect of phosphine fumigation against *S. pontifica* remained lacking up to date. Therefore, the present study aimed to understand the biological response of *S. pontifica* to phosphine fumigation and to carry out a preliminary assessment on the appropriate dosage with respect to developmental stages (eggs and adults) and the time factor for achieving the miticidal potency of phosphine fumigation. Further, to recommend minimum effective concentration (MEC) that could kill all the stages of *S. pontifica* infesting stored agricultural products.

Materials and methods

Collection and rearing

Suidasia pontifica were collected from 15 provinces in the Philippines (Table 1). Locations were selected based on the frequency of phosphine used for the collection of tolerant and susceptible populations.

Adults were mass reared in formulated media with a mixture of oatmeal, corn powder and dried yeast extracts (10:20:1). In 50-mL containers, 100 adult mites were carefully transferred and allowed to oviposit on fresh medium for 48 h. After oviposition, the eggs from parent colonies were carefully picked up using a soft and fine camel's hairbrush under dissecting microscope (×8 magnification) to ensure the uniformity of test mites. A thin layer of Fluon® was smeared on the inner surface of culture containers and placed on aluminum trays with a thin layer of mineral oil to prevent movement of mites and avoid cross contamination. Culture containers were set-up weekly and

Table 1 Collected strains of *Suidasia pontifica*

Strains	Location	Date of collection	Date of the last fumigation ^d
LR5alb ^b	Albay	April–June 2018	June 2016
LR4btg ^b	Batangas	April–June 2018	March 2016
VR7bjl ^a	Bohol	September–December 2017	October 2015
LR3bul ^b	Bulacan	January–March 2018	May 2016
MR10cdo ^a	Cagayan de Oro	September–December 2017	December 2017
VR7ceb ^b	Cebu City	April–June 2018	May 2018
MR11dav ^a	Davao City	September–December 2017	April 2015
MR12gsn ^b	General Santos City	September–December 2017	March 2014
LR1ilN ^b	Ilocos Norte	April–June 2018	December 2015
LR2isa ^a	Isabela	April–June 2018	April 2015
LNCR ^a	Quezon City	September–December 2017	Dec 2017
LR3ne ^b	Nueva Ecija	January–March 2018	July 2015
VR7neg ^a	Negros Oriental	April–June 2018	February 2016
LR1png ^a	Pangasinan	January–March 2018	April 2015
LR3tar ^c	Tarlac	January–March 2018	March 2018

^aGovernment owned commercial store^bPrivate owned commercial store^cMultinational corporation^dbased on the last fumigation in stores/warehouses for certain locations

kept at controlled temperature of 28 ± 2 °C at $70 \pm 5\%$ RH. All cultures were maintained and reared in the laboratory. Eggs and adults of *S. pontifica* were used in the tests.

Preparation of phosphine gas source

Phosphine (ca. 86% pure) gas was generated through commercial aluminum phosphide tablets (Quickphos®, Bayer) and dipped into acidified (5% sulfuric acid) water solution under a laboratory fume hood, specifically designed for the production of phosphine gas. Commercial Quickphos tablets contain ammonium carbamate, which also reacts with water liberating ammonia and carbon dioxide. As the gas generated from a phosphine tablet is not pure, it has to be quantified before use (Valmas and Ebert 2006). This was done by injecting (using a gas-tight syringe) a known volume of gas into a gas-tight desiccator (5 mL:6L) with known volumes, calculated gravimetrically serving as fumigation chambers; this could be used to determine the concentration of the original gas stock (Graver 2004; Valmas and Ebert 2006). Concentration was measured using a calibrated phosphine meter (Uniphos-250 Phosphine Monitor, United Phosphorus, Mumbai, India). Recording of phosphine concentration was done after 5 min at which time the mixture was homogenous (Valmas and Ebert 2006). Test mites were carefully transferred in polypropylene Petri plates (15 × 60 mm) with perforated cup head. Each treatment was placed in separate desiccators. Concentration of phosphine gas was monitored and recorded during and after the end of experiment.

Discriminating dose test

Fifty adult (unsexed) test mites (1–3 days old) were used, replicated 3× with three trials. The polypropylene Petri plates were kept inside the gas-tight desiccators. Test mites were exposed for 20 h testing protocol to separate susceptible from tolerant adult strains, following a general procedure by Food and Agriculture Organization (FAO 1975; Collins et al. 2002; Agrafioti et al. 2019). This method has been used recently with slight modification to diagnose resistance in field populations in the USA (Opit et al. 2012; Cato et al. 2017; Afful et al. 2018).

A full assay comprised eight concentrations ranging from 0.005 to 1.0 mg/L. All assays were conducted at 25 °C and 70% RH. After exposure, test mites were transferred to fresh media for a post-treatment period of 7 days before mortality was assessed. Basis for response is knock down, which is the inability of test species to stand or walk normally. Mortality in control was corrected using the Abbot's formula.

Extended exposure tests

The response of the most resistant strain was investigated using extended exposure tests for the periods of 3 days (72 h) and 6 days (144 h). Three g of rearing media were added to each cup for the whole duration of exposure (72 and 144 h). The response of the most tolerant developmental stages such as eggs were also investigated to establish effective fumigation concentrations and exposure periods. Assessment of mortality after the post-treatment period was the same as in the 20-h test.

Egg fumigation

Suidasia pontifica adults were reared and maintained at 28 °C and 70% RH. Adults were allowed to oviposit for 48 h in rearing containers with a mixture of oatmeal, corn powder and dried yeast extracts (10:20:1). Fifty mite eggs (1–2 days old) were used, replicated 3× with three trials. Eggs were counted under a microscope and carefully placed in perforated polypropylene Petri plates (60×15 mm), then fumigated for 72 and 144 h at 25 °C and 70% RH. At the end of each fumigation period, eggs were monitored to record nymphal hatching and adult emergence. Mortality was assessed after the 7 days post treatment period.

Statistical analyses

Probit analysis was applied to evaluate the LC_{50} and LC_{99} values (i.e., the lethal concentration, at which 50 and 99%, respectively, of the organisms die) of phosphine (Finney 1971) using SPSS Statistics v.21.0 for Windows. Number of responding *S. pontifica* was corrected for natural mortality using Abbott's formula. Lethal concentration test and 95% confidence intervals (95% CI) were used to determine significant differences between the response of test and reference strains at LC_{50} and LC_{99} for discriminating dose test. When confidence intervals (lower and upper levels) of the ratio overlap, then the LC values were considered not to be significantly different (Robertson et al. 2017). However, for extended exposure

period test, all data were subjected to multivariate analysis of variance (MANOVA), followed by means comparisons using Tukey's honestly significant differences (HSD) test ($\alpha=0.05$).

Results

The response toxicities of the 15 collected strains, subjected to full assay tests, were analyzed by probit analysis, using LC_{50} and LC_{99} (Table 2). Discriminating dose test revealed that the most susceptible strain (MR12gsn) was recorded from a private owned warehouse in General Santos City; LC_{50} and LC_{99} were 0.009 and 0.025 mg/L, respectively. On the other hand, the most tolerant strain (LR3tar) was collected from a private owned warehouse in Tarlac City; LC_{50} and LC_{99} were 1.501 and 2.407 mg/L, respectively.

Strains with the highest level of phosphine toxicity on discriminating dose test (20 h) were subjected for extended exposure period of 72 and 144 h (Fig. 1). Complete mortality of adults was recorded at 0.20 mg/L phosphine concentration for 72 h exposure, but a lower concentration (0.10 mg/L) was needed when exposure was longer (144 h). Eggs were more tolerant than the adults. Complete mortality was achieved at 0.50 mg/L for 72 h and 0.35 mg/L for 144 h exposure. These results simply illustrate the increased efficiency (i.e., increased mortality) of phosphine fumigation of longer exposure of *S. pontifica* to lower

Table 2 Response of adult *Suidasia pontifica* strains (total number of mites per strain: 1050) exposed to phosphine with an exposure period of 20 h

Strain	df	Mortality of control (%)	Slope ($\pm$ SE)	LC_{50} (mg/L) (95% CI)	LC_{99} (mg/L) (95% CI)	χ^2	P
MR12gsn ^a	22	0	6.30 ($\pm$ 0.44)	0.006 (0.005–0.009)a	0.023 (0.019–0.028)a	6.46	0.32
LR1iIN	22	0	7.13 ($\pm$ 0.26)	0.010 (0.008–0.011)a	0.029 (0.024–0.030)a	1.32	0.51
VR7bjl*	22	0	5.50 ($\pm$ 0.42)	0.017 (0.015–0.019)b	0.045 (0.039–0.054)b	5.01	0.31
LR3bul	22	0	1.59 ($\pm$ 0.10)	0.050 (0.046–0.054)c	0.123 (0.114–0.134)c	3.42	0.23
LR1png*	22	0	1.42 ($\pm$ 0.10)	0.052 (0.049–0.056)c	0.131 (0.123–0.140)c	2.38	0.49
MR11dav*	22	0	2.42 ($\pm$ 0.14)	0.054 (0.051–0.058)c	0.135 (0.127–0.144)c	2.76	0.26
LR5alb	22	0	1.59 ($\pm$ 0.10)	0.056 (0.049–0.063)c	0.159 (0.143–0.180)c	6.26	0.34
LR4btg	22	0	3.93 ($\pm$ 0.29)	0.057 (0.040–0.080)c	0.178 (0.134–0.301)c	2.33	0.21
VR7neg*	22	0	2.71 ($\pm$ 0.12)	0.061 (0.054–0.067)c	0.155 (0.141–0.173)c	5.57	0.49
LR2isa*	20	0	3.97 ($\pm$ 0.28)	0.064 (0.049–0.084)c	0.246 (0.154–0.298)c	3.52	0.29
LR3ne	22	0	1.89 ($\pm$ 0.10)	0.066 (0.051–0.087)c	0.248 (0.164–0.310)c	3.77	0.24
LNCR*	20	0	2.24 ($\pm$ 0.21)	0.071 (0.064–0.077)c	0.252 (0.198–0.321)c	4.53	0.21
VR7ceb	22	0	3.29 ($\pm$ 0.33)	0.075 (0.060–0.094)c	0.255 (0.206–0.349)c	5.77	0.33
MR10cdo*	22	0	2.73 ($\pm$ 0.23)	0.380 (0.305–0.387)d	0.988 (0.893–1.111)d	5.38	0.49
LR3tar ^b	20	0	2.39 ($\pm$ 0.16)	1.501 (1.461–1.540)e	2.407 (2.319–2.513)e	2.82	0.39

Means within a column followed by the same letter are not significantly different, based on ration tests [95% CI for LC_{50} and LC_{99}] (Robertson et al. 2017)

*Government owned warehouse

^aSusceptible population

^bMost resistant population

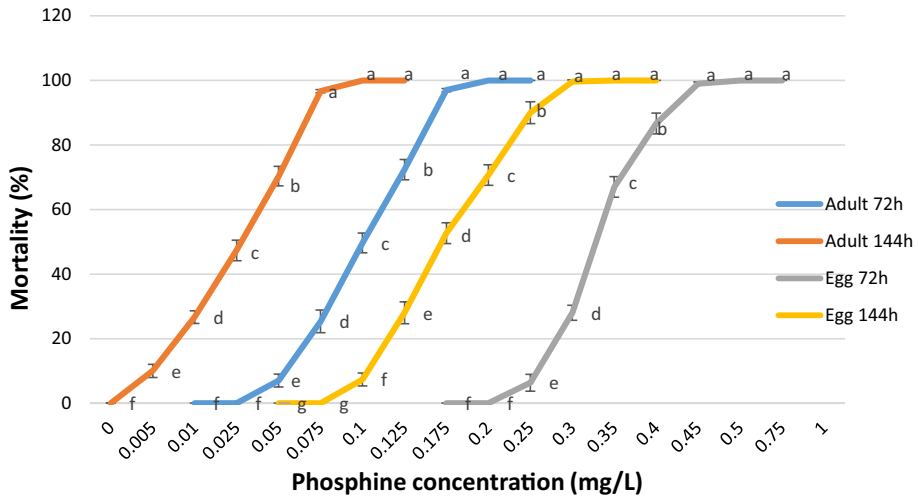


Fig. 1 Response of eggs and adults of the most resistant strain of *Suidasia pontifica* (LR3tar) exposed for 72 or 144 h to phosphine fumigation. Means within a colored line marked by the same letter are not statistically different (Tukey's HSD test: $P > 0.05$). Vertical bars are standard error of means ($n = 1200$)

concentrations. Meanwhile, the phosphine concentration required to achieve 50% mortality of adults at extended exposure were 0.032 and 0.103 mg/L for 144 and 72 h, respectively. Eggs, however, required much higher concentrations of 0.151 and 0.331 mg/L for 144 and 72 h exposure, respectively, to attain LC_{50} . MANOVA showed that adult and egg stages of *S. pontifica* were significantly affected by phosphine fumigation at either concentration and for both 72 and 144 h exposure periods (Table 3).

Discussion

The current investigation indicates that phosphine fumigation was able to control *S. pontifica* populations. The efficacy of phosphine fumigation against major stored product insects and mites has been investigated since the 1960s (Sinha et al. 1967; Jalil et al. 1970; Winks 1985; Daglish et al. 2004; Collins et al. 2005; Opit et al. 2012; Zhao et al. 2015; Nayak et al. 2019). Phosphine is a simple molecule (PH_3); the chemistry of phosphorus is central to its toxicity. The associated hydride causes related symptoms to cellular and organismal physiology through suppressing the energy metabolism and toxic changes to the redox state of the cell (Nath et al. 2011). It also inhibits oxygen respiration by penetrating the body thru spiracles (Chaudry 1997; Jian et al. 2000). According to Nath et al. (2011), there is strong proof that phosphine fumigation disrupts the energy metabolism, particularly the mitochondrial function, in both egg and adult *S. pontifica*. The degree of inhibition is most significant when mitochondrial respiration is in the active state (Chefurka et al. 1976) by the addition of either chemical uncoupler or precursors to ATP synthesis (Nath et al. 2011).

Suidasia pontifica adults showed greater susceptibility to phosphine fumigation at extended exposure times (144 and 72 h) rather than the 20 h exposure and as compared to the egg stage. According to Nayak et al. (2012), phosphine toxicity can be enhanced by altering either the concentration or exposure period of time. Moreover, the efficacy of a fumigant varies depending on the developmental stage, temperature and humidity; hence,

Table 3 Multivariate analysis of variance (MANOVA) showing the effect of phosphine fumigation on the adult and egg stages of *Suidasia pontifica*

Source	Stage	SS	df	MS	F
Exposure (E)	Adult	14966.69	1	14966.69	4592.05*
	Egg	21760.30	1	21760.30	6152.13*
Concentration (C)	Adult	295781.87	17	17398.93	5338.31*
	Egg	376681.26	17	22157.72	6264.49*
Trial (T)	Adult	2.24	1	2.24	0.69
	Egg	3.63	1	3.63	1.03
E*C	Adult	31410.32	17	1847.67	566.89*
	Egg	44267.70	17	2603.98	736.20*
E*T	Adult	1.50	1	1.50	0.46
	Egg	1.19	1	1.19	0.33
C*T	Adult	29.43	17	1.73	0.53
	Egg	15.04	17	0.89	0.25
E*C*T	Adult	12.83	17	0.76	0.23
	Egg	22.82	17	1.34	0.38
Error	Adult	469.33	144	3.26	
	Egg	509.33	144	3.54	
Total	Adult	342674.20	215		
	Egg	443261.26	215		

SS sum of square, df degrees of freedom, MS mean squares

*Significantly different (Tukey's test: $P < 0.05$)

it is vital to assess susceptibility under these conditions (Schortemeyer et al. 2011; Nayak et al. 2012; Lee et al. 2018).

Eggs, however, were consistently more difficult to kill than the adult stage. High tolerance of stored product insect and mite eggs to fumigant has been reported before: *Liposcelis* sp. (Ho et al. 1995; Nayak et al. 2003; Athanassiou et al. 2012), *Tribolium castaneum* (Rajendran 2000; Manivannan 2015), *Rhyzopertha dominica* (Kaur et al. 2012), *Cryptolestes ferrugineus* (Konemann et al. 2017), *Tetranychus urticae* (Lee et al. 2018). This might be due to either lack or limited number of aeropyles present in most stored product pest eggs including Acaridida (Kucerova et al. 2009; Witalinski 1993). Aeropyles are functional openings facilitating respiration in mite eggs. Further, egg stage treated with phosphine would possibly affect or disrupt its cellular structure that leads into death or delayed hatching; the extent of damage may vary according to concentration (Rejendran et al. 2001). Mortality also correlated positively with exposure time and concentration. As exposure time increased (72 and 144 h), the required concentrations to achieve complete mortality decreased.

On the other hand, *S. pontifica* response to the FAO 20-h test gave an indication of the characteristics of the resistant strains in the probit-mortality lines and is useful for comparative purposes. The extended exposure tests, meanwhile, explored the response of *S. pontifica* under conditions more closely related to field conditions. Phosphine gas from commercially prepared tablet evolves fully after 24 h.

Based on the current results, the estimated minimum effective concentration (MEC) of phosphine to give complete kill of *S. pontifica* at different exposure periods must be higher than 0.50 mg/L for 72 and 144 h. The Association of South East Asian Nations (ASEAN) estimated MEC approximates 1.0 mg/L recommended concentration by some fumigators

at the end of 7 days fumigation (Van et al. 1994). *Suidasia pontifica* developed from egg to adult within a period of 6.62–7.92 days (Balmes-Parcia et al. 1998). For this reason, 72 h fumigation is shorter than the longest possible time for eggs to develop into the more susceptible adult. Survival after fumigation with phosphine occurs if the length of exposure is insufficient to bridge the longest tolerant phase in the life cycle of an insect. The 144 h exposure period is more relevant for field application. Given this scenario, the MEC of the current results is lower than the recommended rate of the ASEAN regions. Therefore, the possible resurgence of *S. pontifica* after fumigation can be averted.

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