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Dose-dependent effects of Abamectin in acute embryotoxicity and morphological defects of sea urchin (*Strongylocentrotus* sp.) gametes

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Abstract. Using sea urchin models, various studies have been conducted to determine possible adverse effects of insecticides towards gametogenesis and fertilization. A widely-used insecticide used in the agricultural setting is avermectin, a group of compounds shown to have exceptionally high parasitic and anthelminthic activity. In this study, we present a descriptive comparison of concentration-dependent effects of avermectin in lytic activity and gamete morphology for male and female *Strongylocentrotus* sp. and its influence on sperm-egg recognition ability.
Key Words: sea urchin, insecticide, embryotoxicity, cell lysis.

Introduction. For many years, humans have used both natural and synthetic chemicals as various forms of insecticides for the control and extermination of pests threatening the production quality of many agricultural crops. Avermectins, a novel class of macrocyclic lactones, is a popular insecticide derived from the soil bacterium *Streptomyces avermitilis.* It has shown exceptional parasitic and anthelminthic activity against nematodes and arthropods respectively (Stapley & Woodruff 1982).

Since its discovery in 1976, avermectins (i.e. abamectin, ivermectin, doramectin) have greatly influenced the arsenal of chemicals available for control of both household and agricultural arthropod pests, as well as in mammalian parasites (Lasota & Dybas 1991). These compounds interfere with neurotransmission in many invertebrates, causing paralysis and death by neuromuscular attacks (Dent et al 1997). Ivermectin was the first to be commercialized, followed subsequently by abamectin (ABM). The latter was marketed as an acaricide and insecticide for fruits, vegetables, ornamental plants and also as anthelmintic for animals (Campbell 1989; Ali et al 2000).

It is widely acknowledged how pesticide use in agriculture can cause undesirable effects on both humans and the natural environment (Van der Werf 1996). In fact, one of the major problems of applying pesticides to crops is the likelihood of contaminating aquatic ecosystems by drift or runoff (Novelli et al 2012). In this respect, laboratory tests devised for testing the toxicity of pesticides towards certain marine animals have shown that pesticide use still cause damage even at the lowest levels as long as the exposure is sufficiently long (Butler 1966). Although not directly used in aquatic ecosystems, ABM may have adverse effects on aquatic biota due to direct application as well as through runoff from experimentally contaminated plots (Vieira 2010).

The sea urchin (Phylum Echinodermata, Class Echinoidea) has long been used as a model organism for developmental biological studies (Semenova et al 2006). Although they can be found on almost any sea coast, the Philippines have been recently dubbed as the center of sea urchin diversity (Munguia & Mooi 2013).

Essential factors contributing to the sea urchin's suitability in many biological tests include the following: straightforward artificial spawning, fertilization and rearing, rapid synchronous development, embryo optical transparency, and well understood embryogenesis. As a result, sea urchin embryos have been successfully used in studying effects of various antiproliferative agents (Korkina et al 2000; Nishioka et al 2003).

Many marine organisms have external modes of fertilization to ensure the survival of progeny for the next generation. When spawning, they simultaneously release both male and female gametes into the water regardless of whether it is a fresh or salt water environment. The threat lies, however, with the chemical run-offs mixing with water - creating hostile environments that alter sperm motility, and even the restriction of ovum fertilization. Even during actual fertilization, various physiological, chemical and morphological changes may still occur. These changes usually damage or arrest the fertilization process and results to offspring with either dead or defective cells (Weis & Weis 1987; Buznikov et al 2001; Kabir et al 2011; Celik-Ozenci et al 2011).

Though various studies have investigated the effects of insecticides toward morphology and physiology of sea urchins, we aim to present a description of the possible damage in developed eggs and sperm before and after gamete fusion when exposed to varying concentrations of commercially available abamectin, a mixture of avermectins containing > 80% avermectin B1a and < 20% avermectin B1b (Meister 1992).

Material and Method. The common sea urchin, *Strongylocentrotus* sp., was collected at early dawn from the shores of Kauswagan, Lanao del Norte, Philippines in March 2015. Samples were immediately stored in a cooler filled with sufficient amounts of sea water obtained from the sampling site. They were then transported carefully to the biology laboratory of the Department of Biological Sciences, Mindanao State University – Iligan Institute of Technology. After arrival, samples were heavily aerated as needed, to provide sufficient oxygen supply throughout the entire experimentation.

Spawning was induced in adult sea urchins by injecting 0.2 mL of 0.5M KCL solution in the soft membrane around the mouth (intracoelomic) using a 1 mL-tuberculin syringe. Within a few minutes, secretions indicating the release of gametes were observed: the sperm are off-white, while eggs are tan to orange. The gametes were inspected microscopically to confirm gamete type and transferred to separate Petri dishes.

Abamec™ (abamectin) was prepared as three different concentrations used as the fertilization medium for sea urchin gametes. Using serial dilution, the following concentrations are obtained: $1x10^{-2}$, $1x10^{-4}$ and $1x10^{-6}$. Sperm and unfertilized eggs were collected from freshly spawned sea urchins and placed in individual Petri dishes as triplicates containing approximately 5 mL of the said dilutions. A fourth concentration containing filtered sea water was used as the control set-up. Gametes were observed within an eight hour period at four hours interval, any morphological changes among transferred gametes were viewed under a compound light microscope and recorded accordingly. Furthermore, a ten hour long observation time was also made to determine the effect of ABM dosage towards sperm to egg recognition ability and ultimately, the likelihood of successful gamete fusion and fertilization. Sperm were added to eggs already placed within the modified fertilization media.

Upon visual examination, morphological changes were observed and described for both eggs and sperm. The presence and absence of cell lysis were tallied and graphed versus abamectin concentrations at increasing observation time.

Results and Discussion. In order to find detectable and quantifiable developmental changes caused by the chemical compound abamectin, we studied the effect of varying concentrations of the compound on several sea urchin and gametes.

Abamectin is highly toxic to fish and extremely toxic to aquatic invertebrates. Its 48-hour LC50 in acute fresh water invertebrate, 0.22 ppb. Meanwhile, the LC50 in shrimp is 0.002 ppb (E.P.A. Pesticide Fact Sheet 1989). A study in rats given 0.40 mg kg^{-1} day^{-1} of abamectin showed decreased lactation, increased stillbirths and an increased likelihood of producing unhealthy offspring, demonstrating a strong chance of similar effects in humans at high enough doses (Meister 2000). These studies support the results showing the insecticide's lethality to the gametes of sea urchins. Sperm activity was greatly affected even at the lowest concentrations causing immotility and death.

In the experimental set-up, sperm-to-egg recognition is unsuccessful because of the lacking formation of the vitelline membrane, indicative of gamete fusion (Figure 1). This may be due to the inability of the sperm to survive in the concentrations of abamectin or in such a weakened state, unable to penetrate the walls of the egg. The pattern continues in increasing doses where most of the cells have been arrested and lysis becomes more evident. Lysis and leaking of cytoplasmic material is highly observable in ova of the sea urchins found in the set-up with the highest concentration (Figure 2).

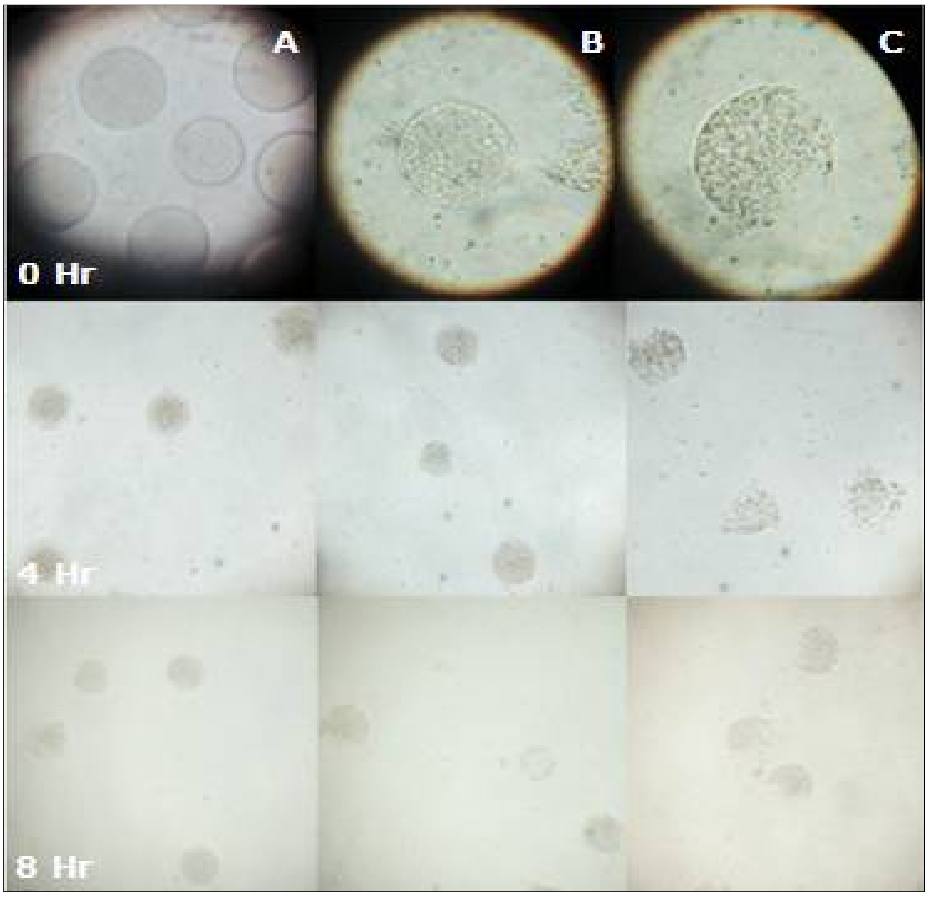

Figure 1. Outcome for sperm-to-egg recognition in increasing abamectin concentrations as observed in 4 hour intervals: A - 0.00001 ppm; B - 0.001 ppm; and C - 0.1 ppm.

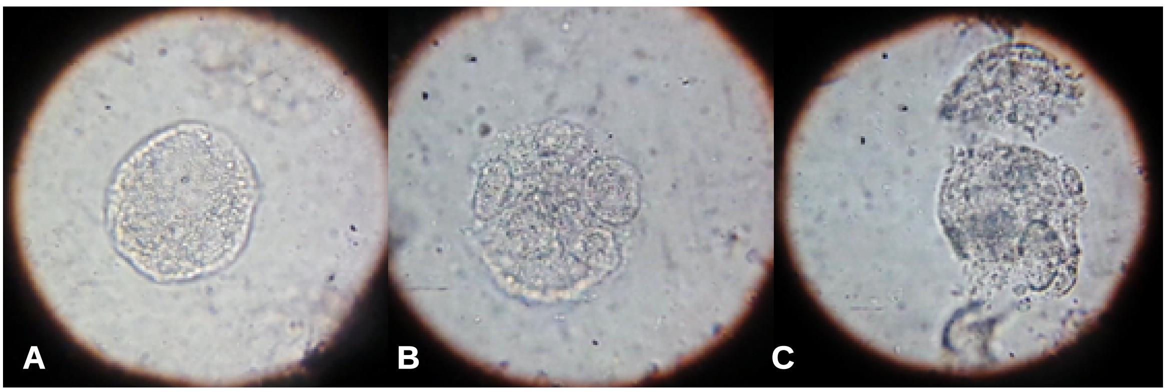

Figure 2. Observations on the unfertilized egg at 10 hours in different concentrations. A - 0.00001 ppm; B – 0.001 ppm; C – 0.1 ppm.

At 10 hours after the unfertilized eggs were subjected to the concentrations of the compound, it was observed that as ABM concentration increased, so did the incidence of lysis in the cells. Deformations in the cell and the organelles were observable at 0.001 ppm and complete lysis in the concentration of 0.1 ppm.

The percentage of lysed cells was computed and the results show a positive relationship between the number of lysed cells and the strength of ABM concentration (Table 1). The tendency for the cell to lyse is higher in the 0.1 ppm while the lowest rate of lysis is found at 0.00001ppm. Erosion of the egg membranes is evident, for both the fertilized and unfertilized groups.

Table 1

Cell count and percentage of lysis in increasing abamectin concentrations

Abamectin concentration	*Presence of cell lysis*	*Absence of cell lysis*	*% of lysed eggs*
0.1 ppm	443	69	87%
0.001 ppm	246	279	47%
0.00001 ppm	49	405	12%

Increasing abamectin concentrations after entire 10-hour observation has highest lytic activity on sea urchin eggs at 0.1 ppm.

Although concentrations of 0.1 ppm to 0.00001 ppm may be relatively high for marine invertebrates, however, this study represents the impact of the compound to the embryos when exposed to larger concentrations in a larger scale. In the environment, its half-life is about 1 week on an unshaded soil surface and about two weeks to two months underneath the soil while in water, half life is four days in pond water and two to four weeks in pond sediment (ETN 1996). Much is yet to be known regarding its persistence as chemical run-off towards other water bodies like rivers and seas.

It is likely that if fertilization were successfull, cleavage and development would be hampered as observed in deformations occuring even at the 0.00001 ppm concentration. If the embryos were able to survive, minor complications are likely to develop as offspring.

Conclusions. The results of the study provide helpful insight on the effects of insecticide use towards gametogenic development of important marine dwelling organisms like sea urchin species. Abamectin is shown here to be highly toxic to these aquatic invertebrates as observed in lytic activity on sea urchin eggs and sperm. It hampers fertilization by preventing gamete fusion as sperm motility weakens dramatically, preventing sperm to egg contact. These findings imply similiar adverse effects for higher animals if administered at higher levels. Such an assumption merits further investigation that can be carried out to determine the chronic effect of abamectin and other insecticides towards other developmental stages in sea urchins and other animal models.

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