AES BIOFLUX

Advances in Environmental Sciences - International Journal of the Bioflux Society

Effect of *Cymbopogon citratus* (lemon grass) crude leaf extracts on the developmental stages of *Pomacea canaliculata* (golden apple snail)

[1,4]Meljan T. Demetillo, [2,4]Mirasol L. Baguio, [4]Dysa E. Limitares, [3,4]Genelyn G. Madjos, [4]Liza R. Abrenica-Adamat

[1] Caraga State University, Butuan City, Philippines; [2] Adventist Medical Center College, Iligan City, Philippines; [3] Department of Biology and Natural Sciences, College of Science and Mathematics, Western Mindanao State University, Zamboanga City, Philippines; [4] Department of Biological Sciences, College of Science and Mathematics, MSU-Iligan Institute of Technology, Iligan City, Philippines. Corresponding author: M. T. Demetillo, meljan_demetillo@yahoo.com

Abstract. Some exogenous compounds found in the environment alter the development of an organism from its usual path and worst, can cause embryonic abnormalities. Specifically, this study sought to determine the effect of crude leaf extract of *Cymbopogon citratus* (lemon grass) on the developmental stages of *Pomacea canaliculata* (golden apple snail). Three experimental treatments (*i.e.* 20%, 50% and 100% *C. citratus*), one negative control (water) and one positive control (molluscicide) were employed in the study. Results showed that crude extracts of *C. citratus* alters the normal growth of *P. canaliculata* by preventing the development of the embryo, altering the incubation or hatching period of the egg and increasing mortality rates on both adult and juvenile stages. Moreover, leaf extracts of *C. citratus* is a valuable molluscicide since *P. canaliculata* is considered an invasive rice field pest. *C. citratus* is a potential organic substitute to synthetic molluscicide with no harmful effects to humans and to the environment.
Key Words: *Cymbopogon citratus*, *Pomacea canaliculata*, anti-hatching, molluscicidal effect.

Introduction. Environmental cues play a role in the development of an organism. Exogenous compounds found in the environment can divert and alter development of an organism from its usual path and cause abnormal effects (Gilbert 2000). Many of the embryos express their abnormality so early that they fail to develop further into its next stage in its life cycle. Others develop but fail to hatch. In a similar manner, developmental stages of *Pomacea canaliculata* (golden apple snails) are sensitive to these environmental exogenous compounds.

Several studies have been reported that hatching of the snail eggs are inhibited by reducing the air supply to embryos in the egg and decreasing of temperature of incubation water (Schnorbach 1995; Horn et al 2008), or by blocking the penetration of water into the egg (Pizani et al 2005). These related studies which alter the embryonic development of the snails are so important since *P. canaliculata* is considered a major exotic rice pest. It is highly prolific and reproduces ten times faster than the native species (Marwoto & Isnaningsih 2011; Suharto 2002). Since golden apple snails reproduce rapidly, egg destruction is a very effective control strategy, hence altering its normal embryonic development.

P. canaliculata is an invasive species native to South America (Halwart 1994) and recently posed problems to both public and private sectors of rice industry in Asia, including the Philippines (Taguiling 2015). If no control measure is taken, they can completely destroy 1 sq. m. of rice field overnight. This damage could lead to more than 50% yield loss. Aside from the devastating effect of *P. canaliculata* on rice, the community that surrounds rice farms may be also invaded and be affected, such as other local fauna species in the area (Chaichana & Sumpan 2014).

Medicinal plants represent the oldest and most widespread form of medication known to man which include the use of plant extracts as molluscicides (Salawu & Odaibo 2011) since they are less expensive and less hazardous to the environment than their synthetic counterparts (WHO 1985). In fact, the first pesticides in the group of organotin compounds used to eradicate *P. canaliculata* were banned because of its unregistered used as molluscicide and its harmful health effects to farmers. Plants cause behavioral and physiological effects on pest and vectors of diseases because they possess defensive chemicals of various categories, *e.g.* terpenoids, alkaloids, glycosides, phenols and tannins (Singh et al 2010).

Cymbopogon citratus, commonly known as lemon grass, is a perennial plant with long thin leaves and is one of the largely cultivated medicinal plants for its essential oils in parts of tropical and subtropical areas of Asia, Africa and America (Chanthal et al 2012). The leaves of *C. citratus* exhibit lemony characteristic flavor due to its main content known as citral which posed great importance to the industry (Ranitha et al 2014). It has also been reported to have therapeutic and insecticidal properties (Masamba et al 2003; Eifert 2004; Soonwera & Sinthusiri 2014). Several studies and different strategies have been done to explore plant as chemical control to snail populations (El-Sherbini et al 2009; Chantal et al 2012), some are not effective while other are moderately effective (Hendarsih et al 1994). This is supported by the study of Musman et al (2013) which emphasized the molluscicidal activity of *Baringtonia racemosa* seed extracts on *P. canaliculata* due to the presence of saponins and flavonoids. However, only few publications are known for its molluscicidal effects on certain species of snails which shows that relatively little work has been carried out on their application on the field.

Hence, the objective of the present study was to examine the effect of crude leaf extracts of *C. citratus* on different developmental stages of *P. canaliculata* which can support organic farming and may be a substitute to the synthetic pest control measures used by farmers which are expensive and harmful. By altering its normal development, this offers potential management technique.

Material and Method. This study employed field sampling and followed by laboratory set-up.

Collection of P. canaliculata and maintenance. Mature male and female of *P. canaliculata* were randomly collected in the rice field of Barangay Sta Elena of Iligan City (coordinates 08º11.954 N and 124º13.606 E), province of Lanao del Norte, Mindanao, Philippines. *P. canaliculata* were allowed to lay eggs in a pail half-full of rice field water *in vitro* from April 22 to May 6, 2015 with temperature ranging from 27-30°C. Masses of eggs were collected and were considered as experimental eggs in several treatments. These cluster of eggs (200-277 eggs per cluster) were then isolated with a scalpel and were put on top of wire gauze set on top of the basin above water level. Adults were fed with rice plant and *Arum* leaves during the egg gathering, after which they were also exposed to the different treatments (Figures 1 A-C).

Figure 1. Experimental set-up: (A) newly hatched eggs of *P. canaliculata* inside the pail; (B) separated eggs as experimental treatments, (C) adult apple snail inside the pail.

Collection and preparation of plant extracts. *C. citratus* leaf was collected from the nearby garden along Barangay Hinaplanon, Iligan City, Philippines. It was rinsed with distilled water to remove dust and unwanted materials. The fresh *C. citratus* was then cut into small pieces using knife and were grinded with mechanical grinder. It was then squeezed using white cheese cloth and was filtered through Whatman No. 1 filter paper to obtain its pure extract. Treatments were then prepared in three replication viz. 100%, 50% and 20%. Distilled water was utilized to make the solutions. The plant extract was used immediately after the extraction and was refrigerated to ensure their freshness. Control set-up includes molluscicide (positive control) and rice field water (negative control). Niclosamide (SURE KILL®) served as the reference molluscicide.

Exposure of eggs to treatments. Egg clusters in each set-up were exposed to 5 different treatments, with three replicates each, as follows: treatment 1 (100% *C. citratus*, treatment 2 (50% *C. citratus*), treatment 3 (20 % *C. citratus*), treatment 4 (molluscicide) and treatment 5 (water). Using syringe, 5 mL were then sprayed to each replicates every 4:00 PM for 7 consecutive days until the eggs were hatched. Hatched eggs were counted for each treatment, after which each remaining unhatched egg was checked for undeveloped embryo and possible developed embryo but unsuccessful in hatching even after the maximum incubation time (14 days). Observations were recorded and photographs were taken (Figures 2 A-B).

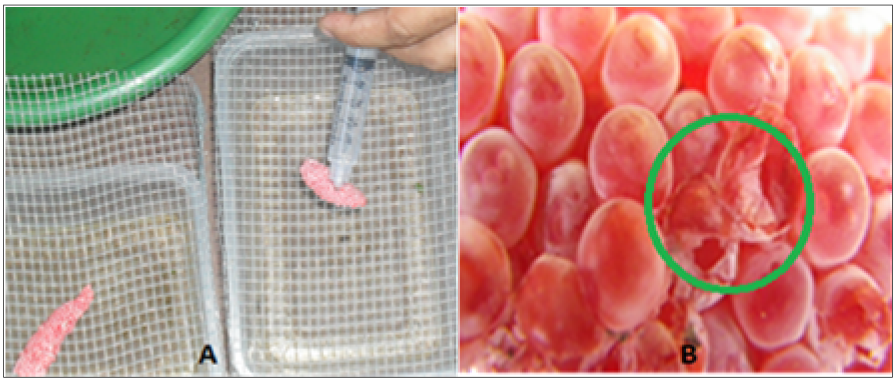

Figure 2. Exposure of eggs to treatment: (A) spraying of treatments using syringe and (B) hatched eggs (encircled part).

Exposure of juvenile P. canaliculata to treatments. Ten juvenile snails (1 day old) were exposed to the same treatments for 24 hours (Figures 3 A-B). These juvenile snails were taken from the untreated eggs. Thereafter, mortality counts were done and recorded.

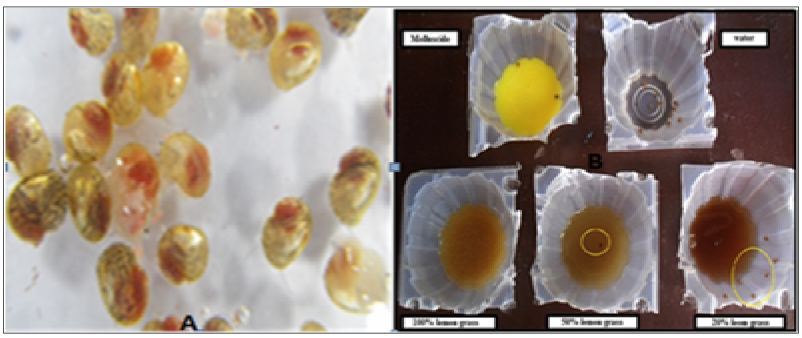

Figure 3. (A) Newly-hatched juveniles; (B) Juvenile stage exposed to different treatments (encircled oval shows floating, dead juveniles and circular shape shows behavioral escape of the juveniles from the treatment).

Exposure of adult P. canaliculata to treatments. Similarly, ten adults of equal sizes were exposed to the same treatments for 24 hours. After 24 hours of exposure, mortality of each treatment was counted. Mortality counts were based on negative retraction exhibited by the operculum when picked with a pointed object (Figures 4 A-B).

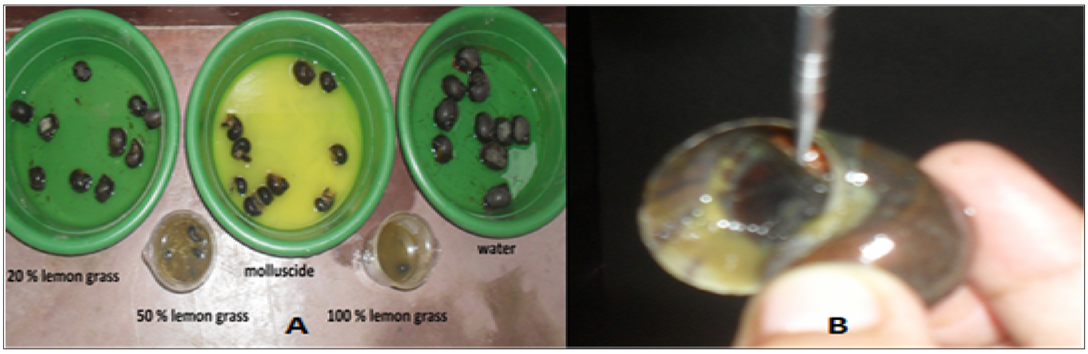

Figure 4. (A) Exposure of adult snails to treatments; (B) Retraction response as basis of mortality counting.

Results and Discussion. This part focuses on the effect of *C. citratus* crude leaf extracts on the different life cycle stages of *P. canaliculata*, in the following order: egg, juvenile and adult stage.

Effect of the treatments to the egg development. After the application of the different treatments during the incubation period of the eggs, there were many eggs that did not develop to embryos in some treatments. It was also observed that some eggs developed into embryos but did not hatched and some were successful in their development until they finally hatched into juvenile snails. Table 1 shows these effects (in percent) on the development of *P. canaliculata* eggs.

Table 1

Effect of the different treatments on the development of *P. canaliculata* eggs

Treatments of eggs	*Percent of eggs with empty embryo*	*Percent of eggs with developed embryo but did not hatch*	*Percent hatched eggs*
Treatment 1	88.32 %	11.68%	0%
Treatment 2	76.92 %	23.08%	0%
Treatment 3	67.36 %	20.10%	12.54%
Treatment 4	98.38%	1.62%	0%
Treatment 5	31.54 %	0%	68.46%

Among the 5 treatments, only treatments 3 and 5 exhibit successful hatching. However, both still contained 67.36% and 31.54% undeveloped eggs respectively. Treatment 5 also exhibits the highest percentage in terms of the hatched eggs since this is the negative control (68.46%). This result is lesser than the 80% hatchability rate of the *P. canaliculata* in the field as stated by De la Cruz et al (2001). This could be due to the developmental symbionts of these snail eggs which is the photosynthetic algae in the field. As stated by Gilbert (2000), symbiosis between egg masses and photosynthetic algae is critical for its development. When eggs are packed together in tight masses, the supply of oxygen limits the rate of development, and the development of those embryos on the inside of the cluster is retarded compared with those near the surface. While there is a steep gradient of oxygen from the outside of the cluster to the deep within it, the embryos seem to get around this problem by coating themselves with a thin film of photosynthetic algae. Thus, the algae "rescue" the eggs by their photosynthesis.

Treatments 1 and 4 obtain the highest percentage of eggs with empty embryos (88.32% and 98.38% respectively). This means that these treatments significantly altered the normal development of the egg. Treatment 2 obtained the highest percentage of developed embryos but did not hatched. Accordingly, in the developmental process of the organism, many of the embryos express their abnormality so early that they fail to hatch or be implanted (Gilbert 2000). Treatments 1, 2 and 4 did not exhibit hatching as reflected in the 0% of hatched eggs. It also shows that the higher the concentration of the plant extract, the greater it will alter the development of the embryo. *C. citratus* has phytochemical components such as saponin and flavonoids which prevent the development of the apple snail embryo and permits anti-hatching (Musman et al 2013).

Effect of treatments on juvenile stage. Table 2 shows zero mortality in treatment 5. All juvenile snails were active and alive in the control treatment after 24 hours. One juvenile snail died in treatment 3, obtaining 10% percent mortality. One of the bench-side observations in terms of its behavioral development, juvenile snails tried to crawl away from the treatment. But in the treatments 1, 2 and 4, the juveniles were not able to escape and all of them died, thus 100% mortality rate.

Table 2

Percent mortality of juvenile *P. canaliculata* exposed to different treatments

Treatments	*Percent mortality*
Treatment 1	100
Treatment 2	100
Treatment 3	10
Treatment 4	100
Treatment 5	0

Effect of treatments on adult stage. Table 3 shows the percent mortality of adult *P. canaliculata* exposed to different treatments. Higher concentrations of extracts (treatments 1 and 2) caused significant percent mortality (100% and 80%, respectively).

Table 3

Percent mortality of adult *P. canaliculata* exposed to different treatments

Treatments	*Percent mortality*
Treatment 1	100
Treatment 2	80
Treatment 3	0
Treatment 4	100
Treatment 5	0

In treatment 3, no mortality was observed however it exhibits inactivity. They were observed to be withdrawing into their shell but became active after 30-45 minutes and moved around the container with their foot extended. Immediately less than 30 minutes, snails treated with treatments 1 and 4 died. There was a visible swelling of the cephalopodal mass. High doses of the active plant extracts caused the cephalopodal mass of each snail to become severely swollen and turgid and mucous secretion was observed over most of the foot. After 24 hours, the toxic effects of the plant extract became evident. There was either a partial retraction (withdrawal response) in the partially dead snail or no retraction in the dead snails (Figures 5 A-B).

Figure 5. Effect of treatments to adult *P. canaliculata* (A) recovery from inactivity of adult snails exposed to 20% lemon grass extract; (B) dead adult *P. canaliculata* after treated with 100% *C. citratus* extract, showing swollen tissues and mucus secretion.

On the overall account of the life cycle stages of *P. canaliculata*, higher concentrations of *C. citratus* alters the development of the test organisms. This study supports the results conducted by Musman et al (2013) that the seed extracts of *B. racemosa* has a molluscicidal activity to *P. canaliculata* due to the presence of saponins and flavonoids which had a significant effect on mortality of the freshwater golden apple snails. These components are found also in the leaf extracts of *C. citratus*. According to Wu et al (2005), these components could reduce the air supply to the embryos in the egg, thus, altering the normal development of the golden apple snail embryo.

Other noticeable observations*.* Other important factors that affect hatching success of eggs include temperature, water immersion and cannibalism. Egg predation by red harvester ants (*Solenopsis invicta*) and cannibalism was also observed in this study. According to Hochberg et al (1992), red harvester ant collect seeds, dead insects and eggs of invertebrate animals and store them in the nests as food for the colony. They can easily get foods because their mouthparts are designed for chewing.

In a study by Horn et al (2008), hatching success of *P. canaliculata* eggs was reduced 75% by immersion in water and was negatively correlated with time submerged. Both underwater egg predation and low immersion tolerance may be exploited to limit its spread. Figures 6 A-B show some of these observed factors during the conduct of the experiment. In this incident, egg clusters were replaced and important precautionary measures were taken to prevent it from happening again.

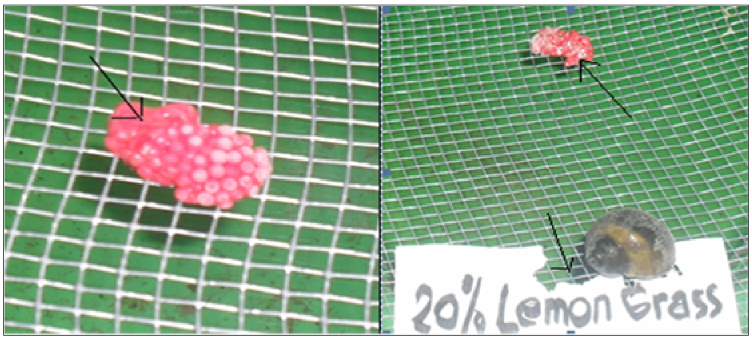

Figure 6. (A) Eaten portion of the egg by red ants; (B) cannibalism observed by an adult snail which escaped from captivity, with labeled paper also eaten (arrows indicate the eaten part)

These observations support the common traits possessed by invasive species as stated by Joshi (2010) such as being generalist (can eat wide variety of food such as its own eggs and paper), fast growth (7 days incubation time), rapid reproduction (can mate anytime and can lay between 25-350 eggs per week). Another significant observation was the distinct characteristics of the snail eggs. When sprayed with water (control), the water was not able to penetrate into the cluster of eggs, it just flows to the basin container. This is contrary to the observations on the other treatments (1, 2 and 4) which allowed a slight penetration or diffusion to the egg, and caused its shrinkage. According to Singh et al (2010), egg shells of the *P. canaliculata* are composed mainly of calcium carbonate and small amount of organic matter holding on it and responsible for any color of the eggs. Water cannot penetrate to the calcium carbonate shells, thus *P. canaliculata* eggs are waterproof. Unlike those eggs treated with the rest of the plant extracts, organic compounds from the *C. citratus* can stick to the calcium carbonate component of the shell, thereby causing the shrinkage of the egg masses. Clustering of the snail's eggs could be considered a significant adaptation because only the outermost part is being affected easily by exogenous materials and by its predators.

The male snail is more aggressive and somehow displays courtship by climbing over the shell of the female which also opens its operculum. Mating occurs when the adult male *P. caniculata* attaches to the female operculum with a maximum of 2 hours in a day and detaches thereafter. Prior to egg-laying, gravid female started crawling to the upper portion of the pail. In this *in vitro* experiment, egg clusters ranges from 200 to 277. Egg attaches itself to twigs, stems and stones (or in this case, plastic pail). Eggs are bright pink when hatched and turn to light pink then to light brown when about to hatch.

Incubation is 7-8th days. The juveniles ressemble like miniature adults in some ways, however, the shell is softer and it is a very squat, transparent shell rather than the elongated spire.

Conclusions. The leaf extracts of *C. citratus* caused alterations on the development of *P. canaliculata* by increased percentage of the undeveloped embryo and greater anti-hatching ability of its eggs. It also caused significant mortality on the juvenile and adult stages of *P. canaliculata*. The results obtained demonstrate that *C. citratus* is a potential plant molluscicide, a less costly and environment-friendly pesticide management technique that may improve the maximum yield of rice production and other crops.

Acknowledgements. The authors would like to acknowledge the scholarship grants from the Department of Science and Technology (DOST) and Commission on Higher Education (CHED).

References

Chaichana R., Sumpan T., 2014 The potential ecological impact of the exotic snail *Pomacea canaliculata* on the Thai native snail *Pilas cutata*. Science Asia 40:11–15.

Chanthal S., Prachakoli S., Ruangviriyachai C., 2012 Influence of extraction methodologies on the analysis of five major volatile aromatic compounds of citronella grass and lemongrass grown in Thailand. Journal of AOAC International, 95:763-769.

De la Cruz M., Joshi R. C., Martin A. R., 2001 Basal application of fertilizer reduces golden apple snail population. International Rice Research Notes 26(1):20-21.

Eifert J., 2004 The effectiveness of lemon grass as a natural mosquito repellent. California State Science Fair: Project Summary, 1 pp.

El-Sherbini G. T., Zayed R. A., El-Sherbini E. T., 2009 Molluscicidal activity of some *Solanum* species extracts against the snail *Biomphalaria alexandrina*. Journal of Parasitology Research vol. 2009, article ID: 474360, 5 pp.

Gilbert S. F., 2000 Environmental regulation of normal development. In: Developmental biology. 6th edition, Sunderland (MA), Sinauer Associates, pp. 213-215.

Halwart M., 1994 The golden apple snail *Pomacea canaliculata* in Asian rice farming systems: present impact and future threat. International Journal of Pest Management 40:199-206.

Hendarsih S., Suriapermana A., Fagi A., Manwan I., 1994 Potential of fish in rice-fish culture as a biological control agent of rice pests. In: Role of fish in enhancing ricefield ecology and in integrated pest management. dela Cruz C. R. (ed), ICLARM Conference Proceedings, pp. 32-33.

Hochberg M. E., Thomas J. A., Elmes G. W., 1992 A modelling study of the population dynamics of a large blue butterfly, *Maculinea rebeli*, a parasite of red ant nests. Journal of Animal Ecology 61(2):397-409.

Horn K. C., Johnson S. D., Boles K. M., Moore A., Siemann E., Gabler C. A., 2008 Factors affecting hatching success of golden apple snail eggs: effects of water immersion and cannibalism. Wetlands 28:544-549.

Joshi R. C., 2010 Invasive alien species (IAS): concerns and status in the Philippines. Philippine Rice Research Institute (PhilRice) Maligaya, Science City of Muñoz, Nueva Ecija 3119, Philippines, 23 pp.

Masamba W. R. L., Kamanula J. F. M., Henry E. M. T., Nyirenda G. K. C., 2003 Extraction and analysis of lemongrass (*Cymbopogon citratus*) oil: an essential oil with potential to control the larger grain borer (*Prostephanus truncatus*) in stored products in Malawi. Malawi Journal of Agricultural Science 2(1):56-64.

Marwoto R. M., Isnaningsih N. R., 2011 Notes on the distribution of invasive freshwater snail *Pomacea canaliculata* (Lamarck, 1822) and *P. insularum* (D'Orbigny, 1835) in Indonesia. Biotropia 18(2):123-128.

Musman M., Kamaruzzaman S., Karina S., Rizqi R., Arisca F., 2013 A preliminary study on the anti hatching of freshwater golden apple snail *Pomacea canaliculata* (Gastropoda: Ampullariidae) eggs from *Barringtonia racemosa* (Magnoliopsida: Lecythidaceae) seed extract. AACL Bioflux 6(4):394-398.
Pizani N. V., Estebenet A. L., Martin P. R., 2005 Effects of submersion and aerial exposure on clutches and hatchlings of *Pomacea canaliculata* (Gastropoda: Ampullariidae). American Malacological Bulletin 20:55-63.
Ranitha M., Abdurahman H. N., Sulaiman A., Nour A., Thana R., 2014 A comparative study of lemongrass (*Cymbopogon citratus*) essential oil extracted by microwave-assisted hydrodistillation (MAHD) and conventional hydrodistillation (HD) method. International Journal of Chemical Engineering and Applications 5(2):104-108.
Salawu O. T., Odaibo A. B., 2011 The molluscicidal effects of *Hyptis suaveolens* on different stages of *Bulinus globosus* in the laboratory. African Journal of Biotechnology 10(50):10241-10247.
Schnorbach H. J., 1995 The golden apple snail (*Pomacea canaliculata* Lamarck) an increasingly important pest in rice, and methods of control with Bayluscid®. Pflanzenschutz-Nachrichten Bayer 48:313-346.
Singh S. K., Yadav R. P., Singh A., 2010 Molluscicides from some common medicinal plants of eastern Uttar Pradesh, India. Journal of Applied Toxicology 30:1-7.
Soonwera M., Sinthusiri J., 2014 Thai essential oils as botanical insecticide against house fly (*Musca domestica* L.). International Conference on Agricultural, Ecological and Medical Sciences, Bali, Indonesia Feb. 6-7.
Suharto H., 2002 Golden apple snail, *Pomacea canaliculata* (Lamarck) in Indonesia. In: Proceedings of the Meeting of the Special Working Group on the Golden Apple Snail (*Pomacea* spp.) at the Seventh International Congress on Medical and Applied Malacology, Los Baños, Laguna. Wada T., Yusa Y., Joshi R. C. (eds), SEAMEO Regional Center for Graduate Study and Research in Agriculture. Los Baños, Laguna: SEAMEO Regional Center for Graduate Study and Research in Agriculture, pp. 69-73.
Taguiling N. K., 2015 Effect of combined plant extracts on golden apple snail (*Pomacea canaliculata* (Lam.)) and giant earthworm (*Pheretim*a sp). International Journal of Agriculture and Crop Sciences 8(1):55-60.
WHO, 1985 The control of Schistosomiasis. Technical Report: Geneva, Switzerland, 114 pp.
Wu D. C., Yu J. Z., Chen B. H., Lin C. Y., Ko W. H., 2005 Inhibition of egg hatching with apple wax solvent as a novel method for controlling golden apple snail (*Pomacea canaliculata*). Crop Protection 24:483-486.

Received: 11 May 2015. Accepted: 02 June 2015. Published online: 11 June 2015.
Authors:
Meljan T. Demetillo, Biology Department, College of Arts and Sciences, Caraga State University, Ampayon, National Highway, NH1, Butuan City, 8600 e-mail: meljan_demetillo@yahoo.com
Mirasol L. Baguio, General Education Department, School of Health and Allied Sciences, 9200 San Miguel street, Iligan City, Philippines, e-mail: solbagz@yahoo.com
Dysa E. Limitares, Department of Biological Sciences, College of Science and Mathematics, Mindanao State University - Iligan Institute of Technology, 9200 Andres Bonifacio, Iligan City, Philippines, e-mail: dysa.limitares@gmail.com
Genelyn G. Madjos, Department of Biology and Natural Sciences, College of Science and Mathematics, Western Mindanao State University, Baliwasan, Normal Road, 7000 Zamboanga City, Philippines, e-mail: genelyn_madjos@yahoo.com
Liza A. Adamat, Department of Biological Sciences, College of Science and Mathematics, Mindanao State University - Iligan Institute of Technology, 9200 Andres Bonifacio, Iligan City, Philippines, e-mail: lizadamat@gmail.com

How to cite this article:
Demetillo M. T., Baguio M. L., Limitares D. E., Madjos G. G., Abrenica-Adamat L. R., 2015 Effect of *Cymbopogon citratus* (lemon grass) crude leaf extracts on the developmental stages of *Pomacea canaliculata* (golden apple snail). AES Bioflux 7(3):460-467.