Investigation note

Control of apple snail with copper chelate and ozonated water in rice cultivation at field level

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Abstract

The pest that has caused the most problems in rice (*Oriza sativa* L.) in recent years is the apple snail, *Pomacea canaliculata* (Lamarck); this pest is considered among the 100 worst invasive species in the world. The present work is an experimental trial at the field level where different doses of copper chelate and ozonated water were applied to control the apple snail in rice cultivation; for which a randomized complete block design (RCBD) was used, with five treatments, including the control and four repetitions. The experimental site was located on a farm in the Salitre canton, in the Guayas province, Ecuador, with tropical climate. The mortality rate of the snails against the proposed treatments was evaluated. Treatment T4, which corresponded to the dose of 2 L ha^{-1} of copper chelate mixed with ozonated water with a concentration of 2 ppm, was the treatment were the best apple snail control was observed, resulting in 94% mortality.

Keywords: apple snail, copper, ozonated water.

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Rice (*Oryza sativa* L.) is considered a staple food for more than half of the world's population (Singh, 2015). Becoming an important agricultural product, generating income throughout the twentieth century, which has evolved from a rainfed crop, to become a highly technical and productive crop (Dugan, 2015). The pest that has caused the most problems in recent years in rice cultivation is the golden apple snail, *Pomacea canaliculata* (Lamarck) and is considered among the 100 worst invasive species in the world (Global Invasive Species Database, 2013; Carvalho, 2019). To produce rice, a large amount of water is required, but this facilitates the spread, feeding and multiplication of the apple snail (Quiroz, 2012).

In rice paddies, *P. canaliculata* has high fecundity and its populations can increase rapidly under favorable conditions (Ding *et al*., 2017). The snail causes more damage after the hatching of the eggs (Gilioli *et al*., 2017). In addition, this pest has been identified as an important vector of *Angiostrongylus cantonensis*, causing eosinophilic meningitis in humans (Huang *et al*., 2019). *P. canaliculata* has significantly reduced rice productivity worldwide, particularly in Asian and Latin American countries (Correoso *et al*., 2017; Castillo *et al*., 2018). According to the Dirección General de Alimentación y Fomento Agroalimentario (2014), losses can reach 60%-90% of plants.

The most important damages occur during the first phenological stages of the seedlings since they devour them, causing extensive damage to both the transplanted rice and the direct seed rice (broadcast seeding) (Ibrahim *et al*., 2017). Its feeding habit is primarily towards the young stems and leaves of paddy rice and it could consume 7 to 24 rice seedlings per day, which would result in extreme damage to rice cultivation (Lee *et al*., 2019). In many countries, the main control measures used by rice farmers are synthetic molluscicides such as metaldehyde and niclosamide, (Hamid *et al*., 2015; Olivier *et al*., 2016).

Niclosamide is currently recognized as one of the most effective pesticides in controlling *P. canaliculata*, as well as other snails, such as *Biomphalaria* and *Oncomelania* (Yang *et al*., 2016). However, these products are toxic to nontarget species such as fish, crustaceans, algae, tadpoles and mosquito larvae (Attademo *et al*., 2016). The use of oxidizing molluscicides based on ozone and peroxides is a good ecological alternative; however, complete control requires near-continuous exposure to oxidizing residues to induce mortality, which may not be possible for all treatment scenarios (Lake and Hofmann, 2019).

Nonoxidizing molluscicides have a higher cost per volume than oxidizing chemicals, but remain profitable due to lower rates of use, short exposure, time requirements and rapid toxicity (The United States Army Corps of Engineers, 2012; Naghma *et al*., 2017). One of them is copper salts and chelates, in addition to being for organic use (Capinera and Dickens, 2016). Copper (Cu^{2+}) is one of the essential micronutrient elements for plants, but excessive Cu^{2+} is toxic to living organisms (Hammond and Ferris, 2019), causing a wide range of harmful effects such as inhibition of photosynthesis and pigment synthesis, damage to plasma membranes and other metabolic alterations (Yruela, 2009).

The mortality of mollusks is because its high concentrations are toxic in nature, causing a final disruption of the structure of DNA and proteins, ultimately resulting in death (de Oliveira-Filho *et al*., 2004). Therefore, it was proposed to evaluate the effect of copper chelate in combination with ozonated water (an oxidizing and a nonoxidizing molluscicide) in the control of the apple snail and thus reduce the load of chemical pesticides used for rice cultivation.

Localization

The trials were carried out on a farm in the Salitre canton, Guayas province. It was carried out on land of the Hacienda 'Delia María' at km 46. These lands were ceded by the owners to be able to carry out the studies. Edaphoclimatic conditions are typical of a tropical climate (Table 1).

Table 1. Edaphoclimatic conditions of the farms located in the Salitre canton.

West longitude	79° 81'00"	Annual rainfall	1 200 mm
South latitude	01° 83'12"	Average annual temperature	25 ºC
Altitude	8 masl	Average annual relative humidity	80%
Soil	Clayey	pH	6.1

Plant material

The work was done with rice variety INIAP-14 (Instituto Nacional de Investigaciones Agropecuarias, 1999). Vegetative cycle of 113-117 days. Plant height of 99-107 cm, long grain, whole milled rice 62%, seed dormancy 4-6 weeks (INIAP, 1999).

Experimental design

The method used in the trial was field experimental, for which a randomized complete block design (RCBD) was used, with Duncan significance test at 5%. The trial was divided into plots, each corresponded to an experimental block with five treatments and four repetitions. The experimental unit on the farm is a plot of 5 x 5 m, which is equivalent to 25 m^2, the total area of the trial was 25 m x 25 m = 625 m^2 and the useful area of the trial was 25 m^2 (useful area of the plot) x 20 plots = 500 m^2.

The treatments under study (Table 2) were grown under a pool system. For each treatment, a total of 50 snails were placed. Five treatments were studied, in which the dose of 1 L and 2 L of copper chelate and the influence of ozone, with a concentration of 2 ppm, on the control of the snail, are evaluated.

Table 2. Copper chelate and ozonated water treatments used in the study.

Treatment	Dose
T1	1 liter copper chelate/200 L water
T2	2 liters copper chelate/200 L water
T3	1 liter copper chelate/200 L ozonated water (C= 2 ppm)
T4	2 liters copper chelate/200 L ozonated water (C= 2 ppm)
T5	Ozonated water (C= 2 ppm)

Water ozonation

To ozonate the water, a corona discharge ozone (O_3) generator of 20 g O_3 h was used, fed with oxygen gas (O_2) of 99.99% purity and, by bubbling, the ozone gas was mixed with the water until reaching 2 ppm. To measure the concentration of ozone in the water, a colorimeter (CHEMetrics®) was used.

Mortality

The mortality of the snails was evaluated the next day after treatment. A total of ten square frames (0.1 m^2) on each plot was studied by systematic random sampling. All the snails within the frames were collected, washed with dechlorinated water and left to recover for 24 h. The snails were considered dead due to the presence of discoloration, the absence of muscle contraction, hemorrhage and deterioration of the body. The mortality percentage (MR= number of dead snails* 100/total number of snails) is based on the observation of dead snails found in a total number of individuals.

Data analysis

Using the statistical program InfoStat, the data collected were subjected to the analysis of variance (Anova) with four repetitions. The significance test used was Duncan’s at 5%.

Copper is a nonoxidizing molluscicide that is effective against the apple snail and is allowed as a molluscicide in organic plantations, this due to its low toxicity (McCartney, 2016; Wang *et al*., 2019). Chelation of metal is important as it makes metal ions more available for absorption by plants or for blocking heavy metals (Clemens *et al*., 1990). The most common chelates used in agriculture are EDTA (Ethylenediaminetetraacetic acid), EDDS (Ethylenediamine-N, N'-disuccinic acid), DTPA (diethylenetriaminepentaacetic acid) and EDDHA (ethylenediamine-N, N’-bis (2-hydroxyphenylacetic acid)) (Murali *et al*., 2018).

Ozone is a molecule with a high electrochemical potential and therefore capable of affecting the cell membranes of living organisms, in addition to providing agronomic benefits to crops (Landa *et al*., 2018; Pandiselvam *et al*., 2019). Table 3 indicates that copper chelate and ozonated water had molluscicidal effects against *P*. *caniculata*.

Table 3. Molluscicidal effect of treatments with copper chelate and ozonated water against *P. canaliculata* in field tests by the immersion method.

Treatment	Mortality (%)
T1	83 ±5.29 b
T2	91.75 ±1.91 cd
T3	88 ±3.65 bc
T4	94 ±3.27 d
T5	10.5 ±1.91 a

Means with a common letter are not significantly different (p>0.05). Mean values represent the mean ± standard deviation.

There is a significant difference between the treatments with chelated copper and the control treatment T5, so we can consider the effect of copper chelate on snails to be effective. It is observed that the dose of 2 L ha^{-1}, which corresponds to treatments T2 and T4 with 91.75% and 94% respectively, is of better effectiveness, against *P. canaliculata*, than treatments T1 and T3 with 83% and 88% respectively, in which a lower dose of 1 L ha^{-1} was used. This is consistent with previous studies that indicate that copper at certain concentrations is toxic to living organisms such as mollusks (Chakraborti *et al*., 2016; Rhys, 2018).

In the study by Osorio *et al*. (2012), they showed that the composition of the formula with chelated copper was effective in reducing the number of snail laying, but not so for the control of adult animals within the plots, giving a mortality of less than 70%. In addition, it is observed that ozone in synergy with copper chelate is better than individual treatments, evidenced in treatments T3 and T4. The treatment that presented the highest mortality rate is T4, where 2 L ha^{-1} of copper chelate with 200 L of ozonated water with 2 ppm of concentration was used, this produced a mortality of 94%, being an alternative to reduce the use of chemical pesticides.

Similar studies conducted by Guzman (2019) in the same area indicate that ozonated water in combination with copper chelate improves the control of *P. canaliculata* in rice cultivations. It is believed that the mechanism of action of copper-based products targets specific physiological processes, such as electron transport in the photosystem, cell division and nitrogen fixation and, when combined with ozonated water, it improves the control of *P. canaliculata*, possibly due to the double oxidizing and nonoxidizing molluscicidal action, as shown in the results in Table 3.

Conclusions

The result of this trial indicates that applying different doses of copper chelate affects the mortality of the apple snail (*Pomacea canaliculata*) in rice cultivation. Treatment 4, which was formulated with 2 L of copper chelate and 200 L of ozonated water with a concentration of 2 ppm, produced the best result since it controlled the apple snail in 94%. It is suggested to investigate at higher concentrations of ozone.

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