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Efficacy of quinoa (*Chenopodium quinoa*) saponins against golden apple snail (*Pomacea canaliculata*) in the Philippines under laboratory conditions

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Abstract

A novel product for managing *Pomacea canaliculata*, golden apple snail (GAS), containing quinoa saponins (*Chenopodium quinoa*), was evaluated under laboratory conditions for the protection of newly sprouted rice seeds. Experimental methods mimicked conditions found in direct-seeded rice cultivation in the Philippines, but with a very high GAS density (90 snails/m^2). Protection of newly sprouted seeds was directly proportional to saponin concentration in rice water. At 9 and 11 ppm saponin, seedling protection after 48 h against GAS of different sizes was 93% and 95%, respectively. Seedling recovery after 5 d with 11 ppm saponin was 93%. This value declined to 0% and 4%, for the control (untreated) and niclosamide, a synthetic chemical molluscicide, respectively. The results indicated that although niclosamide provides high efficacy against GAS (100% mortality, 24 h), it has a serious detrimental effect on rice seedlings. Mean GAS mortality with 11 ppm saponin was low at 24 h (45%), but increased to 94% at 48 h. Thus, seedling protection was probably due to an almost immediate closure of the snail's opercula when exposed to saponin solutions, followed by significant death rates within 24 and 48 h. The product also exhibits ovicidal effects, particularly with 1–5 d old egg masses; older egg masses were less susceptible to the product. The use of 11 ppm saponin slightly affected shoot growth, but this effect disappeared with time and the plants attained normal development. Saponin application rates at 10 ppm saponin in the rice water correspond to ca. 6 kg product/ha under cultivation conditions used in the Philippines. These results suggest that quinoa saponins may represent a commercially feasible environmentally benign alternative to synthetic chemical molluscicides against GAS, particularly in direct-seeded rice culture.

Keywords: Golden apple snail; *Pomacea canaliculata*; *Chenopodium quinoa*; Saponin

1. Introduction

Golden apple snail, *Pomacea canaliculata*, is an invasive alien pest that seriously affects rice cultivation in many Asian countries (Ranamukhaarachchi and Wickramasinghe, 2006). These freshwater mollusks devour young rice seedlings, causing extensive damage to both transplanted and direct-seeded rice (Wada et al., 1999; Sin, 2003). Most Asian farmers resort to short-term solutions by using molluscicides that have negative impacts on non-target organisms, aquatic biodiversity, and the environment (Cheng and Kao, 2006). For example, tea seed cake powder, a residue derived from the extraction of oil from seeds of the Chinese plants *Camellia sinensis* and *Camellia oleifera*, is widely used as an organic molluscicide to control GAS. Although the product contains saponins that are very effective to control GAS, it is also highly toxic to non-target species such as fish and beneficial snails (Yang et al., 2006). At present, metaldehyde and niclosamide are the most used synthetic molluscicides in many Asian countries (Schnorbach et al., 2006). However, countries such as Japan do not allow their use, due to their negative side effects against non-target species (Wada, 2006).

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Recently, a novel molluscicide derived from the husks of the Andean pseudocereal quinoa, *Chenopodium quinoa*, was discovered and developed (San Martín et al., 2007). Quinoa husks are a by-product with a high content of bidesmosidic saponins, particularly derivates of hederagenin, oleanolic acid and phytolaccagenic acid (Madl et al., 2006; Woldemichael and Wink, 2001). Previous research has shown that quinoa saponins exhibit bacterial and antifungal activities (Dutcheshen, 2004; Environmental Protection Agency, 2005). When tested against GAS, these bidesmosidic saponins showed minimum molluscicidal activity. However, treating the husks under alkaline conditions resulted in more active hydrophobic saponin derivatives that killed 100% of GAS in 24 h under laboratory conditions at 10 ppm saponin (San Martín et al., 2007). The most interesting finding was that this product killed fish (e.g. goldfish and tilapia), at saponin concentrations higher than those that killed GAS. This is a major advantage over other molluscicidal saponins and synthetic chemical molluscicides that are very lethal to non-target aquatic organisms such as fish which are present in rice production systems and surrounding rivers (Cheng and Kao, 2006; Wada, 2006).

The purpose of this research was to determine the degree of protection offered by this saponin product to newly sprouted rice seeds in the presence of GAS, mimicking conditions found in direct-seeded rice cultivation. The effect of this product was also tested on the germination and development of rice seeds. The test protocol was developed by the Philippine Rice Research Institute (PhilRice) and it is extremely relevant to the Philippines, since almost all of its rice fields (ca. 4 million ha) are infested with GAS (Ranamukhaarachchi and Wickramasinghe, 2006).

2. Materials and methods

2.1. Test compounds

The product was prepared following the procedure described by San Martín et al. (2007). It consists of a fine powder of quinoa Real from Bolivia, subject to treatment with aqueous NaOH and then air-dried. The product contained approximately 33% w/w saponins as determined by reverse-phase HPLC, following the method described by San Martín et al. (2007).

2.2. Test organisms

The pest snail species (golden apple snails, *P. canaliculata*) were collected from paddy fields, fishponds and along irrigation and drainage canals at PhilRice-CES. Snail sizes of 11–13 cm were used for the majority of test trials. Also, different sizes (10, 15, 20, 25, 30 mm) of *Pomacea* were sorted using a digital Vernier caliper. Collected snails were placed over dry newspapers to absorb water within 48 h. Such snails became immobile, with operculum sealed by their mucus. After 24 h, the snails were soaked on plastic trays with water, and within 3 d the snails returned to their active phase. Snails were fed with gabi (taro) leaves and duckweed while they were in the plastic tray with water. Only the active snails were chosen for the screening test.

2.3. Molluscicide bioassay and protection of rice seedlings

To determine the molluscicidal effect on GAS and protection of sprouted rice seeds, a preliminary screening test was performed with 4, 6, 9 and 13 ppm saponin, respectively. For comparison, niclosamide (Bayluscide® 250 EC, Bayer) was used at the recommended rate of 10 mL per 16 L of water. Each treatment had 10 replicates. Each test was repeated three times. For each replicate, $15 \times 15 \times 7.5\,cm^3$ trays with 487 g of sterilized garden soil and 650 mL per tray of test solution were used. The solution level was approximately 2 cm above soil level. Mean air temperature in the screen house was 31 °C. Two healthy GAS with a shell height of 11–13 mm were placed in each tray. The GAS were fed for 3 d prior to testing. Each tray was supplied with 10 newly sprouted rice seedlings (ca. 0.4–0.5 cm high). The number of remaining seedlings and dead GAS per tray was checked at 24 and 48 h after treatment application.

A second experiment was conducted to determine the most effective saponin concentration and molluscicidal effect on GAS of different sizes, and the protection of newly sprouted rice seeds. For this purpose, aqueous solutions containing 9, 11 and 13 ppm saponin were tested, as described above. The average air temperature in the screen house was 30–33 °C. Two healthy GAS with a shell height of 10, 15, 20, 25 and 30 mm were placed in each tray containing 10 newly sprouted rice seedlings (height of sprout 0.4–0.5 cm). Each run was performed with 10 replicates. The number of dead snails and remaining seedlings was checked after 24 and 48 h after treatment application.

2.4. Phytotoxicity bioassay of quinoa saponins on growth of rice seedlings

The effect of the product on seedling germination was evaluated using aqueous solutions containing 9, 11 and 13 ppm saponin. Also, the effect of the product on the growth of rice seedlings was tested by measuring shoot and root lengths of rice seedlings after 7 and 12 d. For both tests, each tray contained 10 seeds, with 5 replications per treatment. Water was used as an untreated control. Niclosamide (Bayluscide® 250 EC, Bayer) was used as a standard, at the recommended rate of 10 mL per 16 L of water for comparison.

2.5. Ovicide bioassay

Ovicidal activity on GAS egg masses was performed as described by Joshi et al. (2005). The trial consisted of