JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

pubs.acs.org/JAFC Article

Control of the Invasive Agricultural Pest *Pomacea canaliculata* with a Novel Molluscicide: Efficacy and Safety to Nontarget Species

Weisi Wang, Shuijin Huang, Fengquan Liu, Yang Sun, Xiangyun Wang, Junmin Yao, Shizhu Li,* Yuhua Liu, Bingrong Luo, Xia Zhang, Hehua Hu, Zhuohui Deng, and Liping Duan*

Cite This: *J. Agric. Food Chem.* 2022, 70, 1079−1089 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: The golden apple snail *Pomacea canaliculata* is an invasive pest that causes extensive damage to agricultural production. *P. canaliculata* is also an intermediate host of *Angiostrongylus cantonensis*, which causes human eosinophilic meningitis. In this study, the molluscicidal activity and safety profile of a novel molluscicide PBQ [1-(4-chlorophenyl)-3-(pyridin-3-yl)urea] were evaluated. PBQ exhibited strong molluscicidal potency against adult and juvenile snails (LC_{50} values of 0.39 and 0.07 mg/L, respectively). In field trials, PBQ killed 99.42% of the snails at 0.25 g a.i./m^2. An acute toxicity test in rats demonstrated that PBQ is a generally nonhazardous chemical. PBQ is also generally safe for nontarget organisms including *Brachydanio rerio*, *Daphnia magna*, and *Apis mellifera* L. Transcriptomics analysis revealed that PBQ had a significant impact on the carbohydrate and lipid metabolism pathways, which provided insights into its molluscicidal mechanism. These results suggest that PBQ could be developed as an effective and safe molluscicide for *P. canaliculata* control.

KEYWORDS: *Pomacea canaliculata, invasive pest, molluscicide, paddy field, toxicity, transcriptomics*

1. INTRODUCTION

The freshwater snail, *Pomacea canaliculata*, also known as the golden apple snail, is an invasive agricultural pest that is listed among "100 of the world's worst invasive species".[1] Originally from South America, *P. canaliculata* is now widespread in Asia,[2] North America,[3] and the Pacific islands.[4] The invasiveness is closely related to its broad range of food options, high fecundity, and strong adaptability. *P. canaliculata* has invaded the rice-growing areas of most East and Southeast Asian countries.[2,5,6] Snails feed on young rice seedlings and can devastate transplanted or directly seeded rice. They cause great yield losses, replanting costs, and control expenditures.[7] *P. canaliculata* also threatens wetland ecosystems through decimating macrophytes, outcompeting native snails, and altering the phytoplankton community composition.[8−10]

P. canaliculata is also an important intermediate host of the rat lungworm, *Angiostrongylus cantonensis*. *A. cantonensis* is a zoonotic parasitic nematode that is the most common cause of human eosinophilic meningitis (neural angiostrongyliasis).[11,12] Humans are accidental hosts, who acquire infection mainly through the ingestion of raw or undercooked mollusks contaminated by infective larvae. *A. cantonensis* infection in humans may prove fatal if not diagnosed and treated early.[13] In China, Southeast Asia, and Pacific islands, *P. canaliculata* has been implicated in several outbreaks of *A. cantonensis* infection and is responsible for thousands of cases.[14−16]

A number of measures have been used to control *P. canaliculata*. In many countries, the most widely used measure for snail control is the application of chemical molluscicides.[17−19] Niclosamide is the only commercial molluscicide recommended by the World Health Organization (WHO). However, niclosamide is highly toxic to nontarget aquatic organisms, e.g., fish and crustaceans, and this limits its application in aquatic environments.[20,21] Alternative eco-friendly molluscicides are needed. Nicotinanilide (Figure 1) is a molluscicide first reported in 1969 with very good safety to fish, nontarget organisms, and mammals.[22,23] We have examined the molluscicidal potential of nicotinanilide against multiple snail species (including *P. canaliculata*, *Biomphalaria straminea*, and *Oncomelania hupensis*), while it revealed only low to moderate molluscicidal ability, as well as a slow molluscicidal action. To improve the potency, a urea group, which alone could increase snail mortality through toxicity,[24] was introduced into the chemical structure of nicotinanilide to replace the amide linker. In addition, the substituted groups and their positions on the phenyl ring were investigated in our previous studies, which yielded a novel molluscicide PBQ [1-(4-chlorophenyl)-3-(pyridin-3-yl)urea, Figure 1].[25] PBQ exhibited strong molluscicidal potency against *B. straminea*[25] and *O. hupensis*.[26] In addition, PBQ has low toxicity to local fishes,[25,26] suggesting its relative safety to nontarget organisms. The aim of this work was to expand our knowledge of the molluscicidal spectrum of PBQ to *P. canaliculata*. The molluscicidal activity of PBQ against *P. canaliculata* adult and juvenile snails was determined under laboratory conditions, and its snail control efficacies in plot experiments and field trials were evaluated in paddy fields. Studies were

Received: December 8, 2021
Revised: January 6, 2022
Accepted: January 11, 2022
Published: January 21, 2022

https://doi.org/10.1021/acs.jafc.1c07847
J. Agric. Food Chem. 2022, 70, 1079−1089

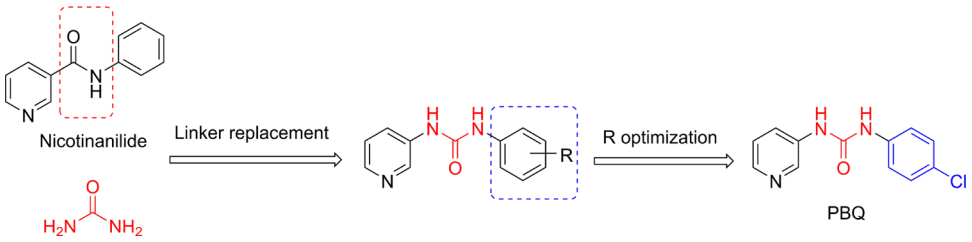

Figure 1. Design strategy for the molluscicide PBQ.

conducted to determine the toxicological effects of PBQ on mammals and other nontarget species. In addition, to understand the possible molluscicidal mechanism, the global gene expression in *P. canaliculata* snails following treatment with PBQ was analyzed using RNA-seq technology.

2. MATERIALS AND METHODS

2.1. Chemicals. PBQ [1-(4-chlorophenyl)-3-(pyridin-3-yl)urea, purity >99%] was synthesized in-house as previously described.[25] The 20% PBQ sulfate floating granules [PBQ (20% FG)] were prepared in-house, containing 20% PBQ sulfate (a.i.), 7% YUS-FS1, 7% YUS-CH8100, 3% white carbon black, 35% floating glass beads, and 28% sodium gluconate. The 50% niclosamide ethanolamine salt wettable powder [niclosamide (50% WP)] was purchased from Senliang Pharmaceuticals Co., Ltd. (Suzhou, China).

2.2. Snail Maintenance. For laboratory assays, adult *P. canaliculata* snails (30−40-mm shell length) were collected from Ruili City, Yunnan Province, southwestern China. Snails were kept in an aquarium (800 mm × 600 mm × 600 mm) containing dechlorinated water and maintained under controlled conditions [24 ± 2 °C, 12:12 h (L:D) cycle]. Snails were fed with lettuce *ad libitum* once a day. The aquarium was cleaned at least twice a week, and water was drained and refilled as needed to maintain water quality. Snails were acclimated to the laboratory conditions for 14 days. During the maintenance, a few female snails laid egg masses on the walls of the aquarium. The naturally hatched juvenile snails were collected and reared in another aquarium using the same procedure used for the adults.

2.3. Molluscicidal Assay. Molluscicides were first dissolved in dimethyl sulfoxide (DMSO) and then added to the test glass tanks (500 mm × 280 mm × 300 mm) containing 40 L dechlorinated water to reach the corresponding final concentrations (PBQ: 0.25, 0.5, 1, 2, and 4 mg/L; niclosamide: 0.1, 0.125, 0.25, 0.5, 1, and 2 mg/L; DMSO ≤ 0.01% v/v). Adults were collected from the aquarium and randomly assigned to test tanks (n = 10, five male and five female individuals per tank) and immersed in the PBQ solutions at the test concentrations. After 48 h of exposure, the snails in each tank were collected, rinsed with water three times, and then reared in dechlorinated water for another 24 h as a recovery period prior to scoring mortality. A snail was scored as dead when it showed no movement and its operculum did not contract after a slight touch of a needle. The control group received DMSO (0.01% v/v) only. The experiments were independently performed three times.

Three- to seven-day-old juvenile snails were randomly assigned to test glass beakers (n = 10 per beaker) and immersed in the PBQ solutions (250 mL) at final concentrations of 0.063, 0.075, 0.1, 0.15, 0.25, and 0.5 mg/L (DMSO < 0.01% v/v) for 48 h. Molluscicide treatment and mortality assessment were performed the same way as that with the adults.

2.4. Plot Experiments. Plot experiments were conducted in Yujiang County, Jiangxi Province, China, in May 2020. Forty-four rectangle-shaped plots (4 m^2/plot, 4 m length × 1 m width) were marked out in a paddy field. Each plot was enclosed with low soil dikes together with 0.4 m high plastic fences to avoid water exchange and the contamination of other treatments. Each plot was surrounded by a fine mesh to prevent snail migration. Water was introduced and maintained at a depth of ∼5 cm. Prior to the experiments, all snails naturally presented in each plot were manually removed. Rice seedlings (variety: Ganwanxian 37, 20 days old) were transplanted into the plots at a density of 25 clumps/m^2. At 7 days after transplantation, a total of 40 adult snails (25−40 mm shell length) collected from the experimental pond of Jiangxi Academy of Agricultural Sciences, China, were placed in each plot. After 1 h, PBQ (20% FG) was applied to the study plots using a broadcast application or a spot treatment method at doses of 0.125, 0.25, 0.5, 1.0, and 1.5 g a.i./m^2. For broadcast application, PBQ (20% FG) was evenly mixed with fine soil (20 g) and manually scattered over the entire plot area. For spot treatment, PBQ (20% FG) was placed at the center of the plot and allowed to self-diffuse. At 3 days after molluscicide application, snails in each plot were collected and mortality was evaluated at the research site. After the examination, all of the snails (dead or alive) were returned to the corresponding plots. At 7 days post application, the collected snails of each plot were placed in a plastic box with water and transported from the paddy field to the laboratory where mortality was determined as previously described. The study had a randomized complete block design, and each treatment was replicated four times. The arrangement included negative (distilled water) and positive [niclosamide (50% WP), 0.1 g a.i./m^2, spraying] controls.

2.5. Field Trials. Field trials were carried out in paddy fields (variety: Ganwanxian 37) heavily infested with *P. canaliculata* in Nanchang County, Jiangxi Province, China, in July 2020. Three paddy fields were selected for PBQ (20% FG) application. Each field covered an area of about 1334 m^2 and was surrounded by low soil dikes and plastic fences. Another paddy field (about 667 m^2) treated with niclosamide (50% WP) served as the positive control. A negative control field (about 667 m^2) was molluscicide-free, which was selected in a separate area with similar ecological conditions to avoid the interference of molluscicides. To avoid dilution of the molluscicide, water circulation from the irrigation channels in each field was stopped before the start of the trial, and this condition was maintained during the entire efficacy trial. Before molluscicide application, the snail density of each field was investigated using a square frame (1 m^2) along the two diagonals of the rectangle-shaped fields, one frame by one frame. All snails within the frames were counted. PBQ (20% FG) was applied in three fields by the broadcast application method at doses of 0.25, 0.5, and 1 g a.i./m^2. Niclosamide (50% WP, 0.1 g a.i./m^2) was sprayed with a mist sprayer (3WBD-20 L, Xuzhou Lanyi Plant Protection Equipment Co. Ltd., Xuzhou, China). At 3 days post application, snails were sampled using the same protocol used for snail density investigation. The collected snails from each field were taken to the laboratory for mortality assessment. Snail control efficiency was calculated as follows

$$\text{control efficiency}(\%) = \frac{\text{snail density}_{\text{pre-application}} - \text{snail density}_{\text{post-application}}}{\text{snail density}_{\text{pre-application}}} \times 100\%$$

2.6. Cytotoxicity Assay. The HepG2 cell line was obtained from the Cell Bank of the Type Culture Collection of the Chinese Academy of Sciences. Cells were cultured in DMEM (Gibco, Cat# 12430054, Grand Island, NY) with 10% fetal bovine serum (Biological Industries, Cat# 04-001-1ACS, Beit Haemek, Israel) and penicillin/streptomycin (Gibco, Cat# 15140122). HepG2 cells were

https://doi.org/10.1021/acs.jafc.1c07847
J. Agric. Food Chem. 2022, 70, 1079−1089

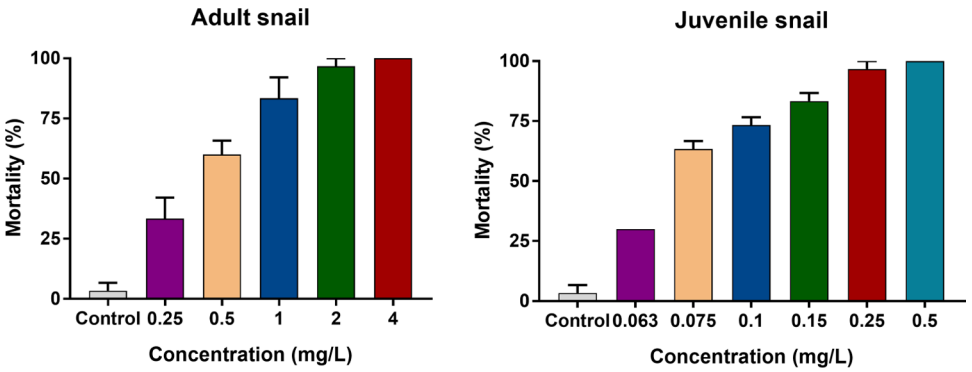

Figure 2. Mortality rates of *P. canaliculata* adult and juvenile snails treated with PBQ for 48 h. The data are presented as means of three biological replications ± SEM.

seeded into 96-well plates at a density of 5000 cells/well. PBQ was made up to a 10× stock solution with DMSO. The cells were treated with PBQ at final concentrations of 25, 50, 60, 70, 80, 100, 125, 150, and 175 μM for 48 h at 37 °C in 5% CO_2. The cell viability was measured by the cell counting kit-8 (CCK8, Beyotime Biotechnology, Cat# C0038, Shanghai, China) according to the manufacturer's instructions. DMSO (0.1% v/v) was used as the negative control.

2.7. Acute Oral Toxicity in Rats. An acute oral toxicity test was performed by the Zhejiang Yangtze River Delta Chemical Safety Evaluation Co., Ltd. (Hangzhou, China), a fully certified laboratory with a Good Laboratory Practice (GLP) certificate and a Pesticide registration test unit certificate (certificate No.: SD2020026) issued by the Ministry of Agriculture and Rural Affairs of China. Healthy male and female SD rats (aged 8−10 weeks, 217−232 g for males, 190−204 g for females) were purchased from Zhejiang Vital River Laboratory Animal Technology Co., Ltd. (Jiaxing, China). Animals were housed in sterilized stainless steel cages (350 mm × 300 mm × 170 mm) with sawdust bedding and maintained in the animal care facility under controlled conditions [23−25 °C, 45−70% humidity, 12:12 h (L:D) cycle]. The rats were fed standard rat and mouse food and provided with water *ad libitum*. Animal care and experimental procedures complied with the Chinese Laboratory Animal Administration Act (2017). All animal experiments and procedures were approved by the Institutional Animal Care and Use Committee of Zhejiang Yangtze River Delta Chemical Safety Evaluation Co., Ltd. (Approval No. S20180050-01-2-0194). Animal studies were reported in compliance with ARRIVE guidelines.[27]

The acute oral toxicity test of PBQ in rats was carried out according to the China National Standards GB/T 15670.2-2017: Toxicological test methods for pesticides registration—Part 2: Acute oral toxicity test—Horn's method. After 1 week of acclimatization, the rats were randomly assigned to four groups (n = 10, five male and five female individuals per group). Animals were challenged with a single dose of PBQ suspension in corn oil (1000, 2150, 4640, and 10 000 mg/kg) by gavage (1.5 mL/100 g.bw). After the administration of PBQ, animals were observed individually with special attention paid to the first 6 h (observation time points: 0.5, 1, 2, 4, and 6 h post administration) and once daily thereafter, for a total of 14 days. At the end of the observation period, all animals were euthanatized, and necropsy examination was performed. The 50% lethal doses (LD_{50}) in male and female rats were calculated using Horn's method.

2.8. Acute Toxicity Test in Zebrafish. The acute fish toxicity test was carried out according to the guidelines of the Organization for Economic Cooperation and Development (OECD) Test No. 203.[28] Zebrafish (*Brachydanio rerio*, 25 ± 5 mm) were obtained from Zhejiang Academy of Agricultural Sciences, China. Zebrafish were reared in an aquarium (800 mm × 600 mm × 600 mm) containing dechlorinated water (pH 7.5−7.7, water hardness 68−78 mg/L $CaCO_3$) and maintained at 23 ± 2 °C with oxygenation under a 14:10 h (L:D) photoperiod. The fish were fed with commercial fish food once daily. The acclimation continued for at least 10 days, and feeding was stopped 24 h prior to experiments. The tests were performed in glass beakers (n = 10 fish per beaker). Zebrafish were exposed to PBQ (20% FG) solutions (2 L) at series concentrations of 8.33, 10, 12, 14.5, 17.3, 20.8, and 25 mg a.i./L or to niclosamide (50% WP) solutions (2 L) at series concentrations of 0.120, 0.192, 0.307, 0.491, and 0.786 mg a.i./L. Each test solution was renewed every 24 h. The control group received an equal amount of dechlorinated water. Throughout the 96 h exposure period, the mortality rate of each group was recorded every 24 h. Dead fish were removed when death was confirmed.

2.9. Acute Immobilization Test in *Daphnia magna*. The acute immobilization test was carried out according to the guidelines of OECD Test No. 202.[29] *D. magna* (less than 24 h old) were obtained from Zhejiang Academy of Agricultural Sciences, China. *D. magna* were maintained in dechlorinated water (pH 7.8−8.0, water hardness 140 mg/L $CaCO_3$) under controlled conditions [20 ± 1 °C, 16:8 h (L:D) cycle]. The tests were performed in crystallizing dishes (n = 5 per dish). *D. magna* were exposed to PBQ (20% FG) solutions (50 mL) at series concentrations of 3.48, 4.17, 4.99, 5.96, 7.21, 8.64, and 10.4 mg a.i./L or to niclosamide (50% WP) solutions (50 mL) at series concentrations of 0.004, 0.0064, 0.0102, 0.0163, and 0.0261 mg a.i./L. Each test was repeated four times. The test solutions were renewed every 24 h. The control group received only dechlorinated water. Throughout the 48 h exposure period, *D. magna* were inspected every 24 h and considered immobile if they were unable to swim within 15 s after a gentle agitation of the test dish.

2.10. Acute Contact and Oral Toxicity Tests in Honeybees. Acute contact and oral toxicity tests were performed according to the guidelines of OECD Test Nos. 214 and 213.[30,31] Adult worker honeybees (*Apis mellifera* L.) were provided by the Institute of Apicultural Research, Chinese Academy of Agricultural Sciences. The bees were randomly assigned to cages (n = 20 per cage) and held in the dark at a temperature of 30 °C and a humidity of 58−64%.

2.10.1. Contact Test. Series test solutions of PBQ or niclosamide (2×10^3, 1×10^4, 2×10^4, 1×10^5, and 2×10^5 mg a.i./L, equivalent to 1, 5, 10, 50, and 100 μg a.i./bee, respectively) were prepared in acetone. The bees were anesthetized with CO_2 for 3 min and placed ventral side up in a glass Petri dish. Then, a 0.5 μL drop of the PBQ test solution was applied on the pronotum of each bee using a micropipettor. After treatment, the bees were returned to the cages, fed with a 50% sucrose solution, and allowed to recover. Control bees were treated with acetone only.

2.10.2. Oral Test. PBQ or niclosamide was dissolved with acetone to prepare a stock solution, and six test solutions (2000, 3000, 4000, 6000, 8000, and 10 000 mg a.i./L, equivalent to 20, 30, 40, 60, 80, and 100 μg a.i./bee, respectively) were obtained by diluting the stock solution with a 50% (w/v) sucrose solution. All bees were fasted for 2 h before the tests started. Each cage was fed with a feeding tube containing the corresponding PBQ test solution (200 μL). After 4 h, all of the test solutions had been consumed, and the feeding tubes were replaced by similar tubes containing 50% sucrose solution only. The control group received an equal amount of the 50% sucrose solution containing acetone (1% v/v).

https://doi.org/10.1021/acs.jafc.1c07847
J. Agric. Food Chem. 2022, 70, 1079−1089

Table 1. Molluscicidal Activity of PBQ against *P. canaliculata* under Laboratory Conditions

molluscicide	snail stage	LC_{50} (mg/L, 95% CI[a])	regression equation	χ^{2b}	p^b
PBQ	adult	0.39 (0.33−0.44)	$y = 1.039 + 1.093(\ln(x))$	0.97	0.81
	juvenile	0.07 (0.06−0.08)	$y = 4.069 + 1.547(\ln(x))$	13.51	0.04
niclosamide	adult	0.24 (0.22−0.26)	$y = -2.119 + 8.967x$	3.24	0.36

[a]95% confidence interval. [b]Pearson chi-square goodness-of-fit test.

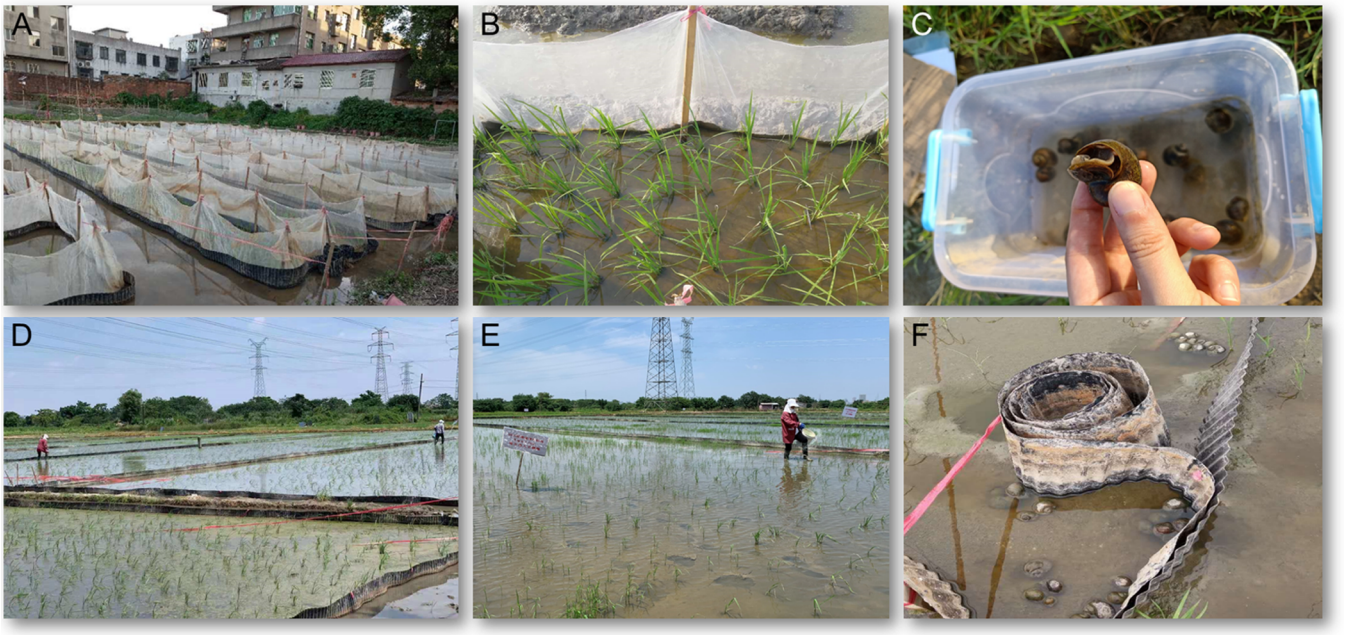

Figure 3. Snail control with PBQ (20% FG) in plot experiments (A−C) and field trials (D−F). (A, B) Plot construction; (C) snail collection and mortality assessment; (D) field trials scheme; (E) molluscicide application; and (F) dead snails floating on the water surface.

For both assays, each treatment was repeated three times. Mortality was recorded at 24 and 48 h post treatment. Bees that did not move when touched with a blunt probe were considered dead.

2.11. Data Analysis. Unless otherwise specified, the 50% lethal concentration/dose (LC_{50}/LD_{50}) and 50% effective concentration (EC_{50}) were calculated by probit analysis using SPSS 19.0 software. If the significance (p) of the Pearson chi-square goodness-of-fit test was less than 0.15, a heterogeneity factor was used in the calculation of confidence limits.

3. RESULTS

3.1. PBQ Potently Kills Adult and Juvenile Snails. In the laboratory immersion assay, PBQ exhibited potent molluscicidal activity against *P. canaliculata* adult and juvenile snails in a dose-dependent manner (Figure 2). For adults, PBQ showed an LC_{50} value of 0.39 mg/L, which was comparable to that of niclosamide (LC_{50} = 0.24 mg/L, Table 1). A five-fold stronger molluscicidal ability of PBQ was seen in juveniles (LC_{50} = 0.07 mg/L, Table 1) compared with that in adults.

3.2. PBQ Exhibits Significant Molluscicidal Activity in Plot Experiments Using Broadcast Application and Spot Treatment Methods. A 20% FG formulation of PBQ was prepared for application to the snail habitat. The molluscicidal activity of PBQ (20% FG) was first evaluated in plot experiments (Figure 3). The plots were mutually independent study areas that were similar to the paddy field. Two application methods, broadcast application and spot treatment, were used. PBQ produced significant molluscicidal potency in the study plots compared with the untreated plots (Table 2). The snail mortality in different treatments showed

Table 2. Molluscicidal Effect of PBQ (20% FG) in Plot Experiments

			snail mortality rate (%)	
molluscicide	method	dose (g a.i./m^2)	day 3 post application	day 7 post application
PBQ (20% FG)[a]	broadcast application	0.125	38.63	63.84
		0.25	79.64	95.76
		0.5	90.39	99.62
		1.0	100	100
	spot treatment	0.125	17.08	39.05
		0.25	71.95	96.37
		0.5	88.01	100
		1.0	100	100
		1.5	98.15	100
niclosamide (50% WP)[b]	spraying	0.1	100	100
control			0	0

[a]20% PBQ sulfate floating granules. [b]50% niclosamide ethanolamine salt wettable powder.

clear dose-dependent effects. For the broadcast application plots, snail mortality rates of 38.63, 79.64, 90.39, and 100% were achieved at 3 days following PBQ (20% FG) treatment at doses of 0.125, 0.25, 0.5, and 1.0 g a.i./m^2, respectively. Similar efficacy was observed in the corresponding spot treatment plots. The molluscicidal activity of PBQ (20% FG) increased with exposure time. Snail mortality rates at 0.125 and 0.25 g a.i./m^2 determined at 7 days post treatment were all higher than those at 3 days, irrespective of the application method

https://doi.org/10.1021/acs.jafc.1c07847
J. Agric. Food Chem. 2022, 70, 1079−1089

Table 3. Snail Control Efficiency of PBQ (20% FG) in Paddy Fields

molluscicide	method	dose (g a.i./m^2)	survey area (m^2)	pre-application no. of snails	pre-application snail density	day 3 post application no. of snails	day 3 post application snail density	control efficiency (%)
PBQ (20% FG)[a]	broadcast application	0.25	102	174	1.71	1	0.01	99.42
		0.5	102	270	2.65	0	0	100
		1	102	186	1.82	0	0	100
niclosamide (50% WP)[b]	spraying	0.1	60	104	1.73	7	0.12	93.06
control			55	340	6.18	463	8.42	

[a]20% PBQ sulfate floating granules. [b]50% niclosamide ethanolamine salt wettable powder.

(Table 2). At doses equal to or greater than 0.25 g a.i./m^2, PBQ (20% FG) treatment produced *P. canaliculata* snail mortality over 95% at 7 days. This result was comparable to the niclosamide treatment.

3.3. PBQ Demonstrates Excellent Snail Control Efficiency in Field Trials. The snail control effect of PBQ (20% FG) was also studied in paddy fields heavily infested with *P. canaliculata* (Figure 3). PBQ demonstrated a fast onset of action. At 3 days after application, PBQ (20% FG) significantly reduced the snail population (Table 3). Almost 100% control efficiency was achieved in all three test fields at doses of 0.25, 0.5, and 1 g a.i./m^2. The PBQ formulation was judged to be as potent as the positive control niclosamide.

3.4. PBQ Displays a Good Safety Profile in Mammals. The cytotoxicity of PBQ against the human cell line HepG2 was studied. PBQ had a negligible cytotoxic level (CC_{50} = 103.3 μM, Figure 4). To further evaluate the safety of PBQ *in vivo*, acute toxicity was determined in rats. Groups of rats were orally administered with a single dose of PBQ and then monitored for 14 days. All rats survived a dose of 1000 mg/kg for up to 14 days. Reduced activity and sluggish behavior were observed shortly after treatment, but these symptoms disappeared at 6 h post treatment. At dose levels of 2150, 4640, and 10 000 mg/kg, mortality values were 30, 100, and 100%, respectively. The acute oral LD_{50} for PBQ in rats was determined to be 2710 mg/kg (95% CI: 2000−3690 mg/kg) in males and 2330 mg/kg (95% CI: 1600−3990 mg/kg) in females.

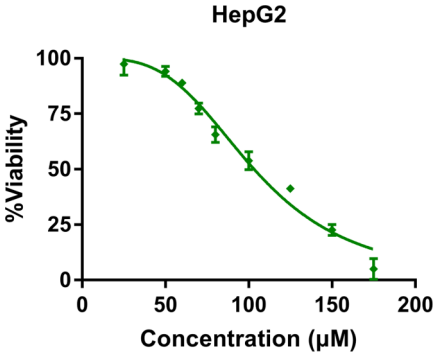

Figure 4. Cytotoxicity of PBQ against HepG2 cells. HepG2 cells were treated with PBQ at indicated concentrations for 48 h. Cell viability was evaluated using the CCK8 assay and normalized to the vehicle controls. The data are presented as means of three determinations ± SEM.

3.5. Acute Toxicity against Nontarget Aquatic Organisms. *B. rerio* and *D. magna* were selected as representative nontarget aquatic organisms to evaluate the acute aquatic toxicity of PBQ (Table 4). For *B. rerio*, the mortality in the treatment groups increased with the concentration and the exposure time. No zebrafish died at a concentration of 8.33 mg/L, but signs of hypoactivity were observed. Zebrafish that were exposed to test concentrations >8.33 mg/L showed symptoms of poisoning. Initially, their swimming speed declined, then the equilibrium was lost, and finally some of them died. The LC_{50} values of PBQ for zebrafish at three sampling times are listed in Table 4. PBQ revealed slight toxicity against *B. rerio* with a 96-h LC_{50} value of 11.96 mg/L, which was 66-fold lower than the value of niclosamide (96-h LC_{50} = 0.18 mg/L). In addition, PBQ exhibited moderate toxicity against *D. magna* in an acute immobilization test. The 48-h EC_{50} value of PBQ was 5.97 mg/L, indicating substantially less toxicity (497-fold) than niclosamide (48-h EC_{50} = 0.012 mg/L).

3.6. PBQ is Practically Nontoxic to Honeybees. The toxicity of PBQ against adult worker honeybees *A. mellifera* L. occurred in a dose- and time-dependent manner (Table 5). PBQ exhibited little acute contact toxicity with a contact 48-h LD_{50} > 100 μg/bee (equivalent to 2 × 10^5 mg a.i./L). PBQ also revealed very low acute oral toxicity against bees (oral 48-h LD_{50} = 77.65 μg/bee, equivalent to 7765 mg a.i./L).

3.7. Comparative Transcriptomics Suggests the Molluscicidal Mechanism of PBQ. *3.7.1. Transcriptome Assembly and Annotation.* Using Illumina paired-end

Table 4. Acute Toxicity of PBQ against *B. rerio* and *D. magna*

organism	molluscicide	time (h)	LC_{50}/EC_{50} (mg/L, 95% CI[a])	regression equation	χ^{2b}	p^b	SI[c]
B. rerio	PBQ	48	12.43 (11.91−12.98)	$y = -6.423 + 0.517x$	8.27	0.14	
		72	12.43 (11.91−12.98)	$y = -6.423 + 0.517x$	8.27	0.14	
		96	11.96 (11.66−12.26)	$y = -6.410 + 0.536x$	5.37	0.37	31
	niclosamide	96	0.18 (0.13−0.23)	$y = 2.195 + 1.294(\ln(x))$	6.49	0.09	0.75
D. magna	PBQ	24	8.71 (7.84−10.15)	$y = -4.346 + 2.008(\ln(x))$	9.16	0.10	
		48	5.97 (5.40−6.57)	$y = -4.654 + 2.605(\ln(x))$	14.87	0.01	15
	niclosamide	48	0.012 (0.010−0.014)	$y = 3.189 + 0.714(\ln(x))$	1.12	0.77	0.05

[a]95% confidence interval. [b]Pearson chi-square goodness-of-fit test. [c]SI, selectivity index = $LC_{50\ B.\ rerio}$ or $EC_{50\ D.\ magna}/LC_{50\ adult\ snail}$.

https://doi.org/10.1021/acs.jafc.1c07847
J. Agric. Food Chem. 2022, 70, 1079−1089

Table 5. Toxicity of PBQ to *A. mellifera* L

test	molluscicide	time (h)	LD_{50} (μg/bee, 95% CI[a])	regression equation	χ^{2c}	p^c
contact toxicity	PBQ	24	>100	F[b]		
		48	>100	F[b]		
	niclosamide	48	>100	F[b]		
oral toxicity	PBQ	24	96.02 (85.55−113.63)	$y = -2.596 + 0.027x$	6.84	0.14
		48	77.65 (74.08−81.27)	$y = -3.502 + 0.045x$	5.41	0.25
	niclosamide	48	>100	F[b]		

[a]95% confidence interval. [b]F, failed to determine, as the mortality rates of the groups treated with the highest test concentration (100 μg/bee) were still <50%. [c]Pearson chi-square goodness-of-fit test.

sequencing technology, six independent cDNA libraries (PBQ_1-3 and Control_1-3) constructed from PBQ-treated and control snails were sequenced and produced a mean of 47 672 975 clean reads for each library (Table S1). Data quality assessment showed that the Q30 percentages of all samples were over 95%, and the GC counts ranged from 44.94 to 46.77%, indicating good data quality. All clean reads in six libraries were merged and *de novo* assembly was performed using Trinity software. The assembly produced a total of 53 805 unigenes with an average length of 1428 bp and an N50 length of 2659 bp (Table S2).

To obtain the functional information of the unigenes, seven known databases (Nr, KOG, GO, Swiss-prot, eggNOG, KEGG, and Pfam; Supporting Information) were employed for unigene annotation and classification. Of the 53 805 unigenes, 24 877 unigenes (46.24%) had at least one significant match with a gene sequence in the searched databases (Table S2).

3.7.2. Functional Enrichment Analysis of Differentially Expressed Genes. To understand the effect of PBQ treatment on the gene expression profiles of *P. canaliculata*, the expression levels of unigenes were compared among the control and PBQ treatment libraries. As shown in the volcano plot (Figure 5), a total of 1280 differentially expressed genes (DEGs) were identified, including 524 upregulated and 756 downregulated genes (Table S3).

GO term and KEGG pathway enrichment analyses were performed to analyze the functions of DEGs in *P. canaliculata* (Figure 5). GO enrichments (Table S4) revealed that the DEGs involved in the biological process category were mostly enriched in cytolysis, glycolipid biosynthetic process, and glucose metabolic process. In the cellular component category, respiratory chain, cytoplasmic microtubule, and extracellular region were the predominantly enriched GO terms for DEGs, and guanyl-nucleotide exchange factor activity, glycoprotein-N-acetylgalactosamine 3-β-galactosyltransferase activity, and hydrolase activity were the most enriched GO terms in the molecular function category.

For KEGG pathway enrichment, the DEGs were grouped into 124 known pathways (Table S5). The most significantly enriched 10 KEGG pathways are shown in Figure 5, which involved apoptosis, fatty acid and carbohydrate metabolism, phagosome, bile secretion, and bile acid biosynthesis. Among them, five pathways were related to metabolism, two were connected with cellular processes, two were associated with organismal systems, and one was related to environmental information processing. Particularly, the pathways related to carbohydrate and fatty acid biosynthesis/metabolism, including "glycolysis/gluconeogenesis", "mucin type O-glycan biosynthesis", "linoleic acid metabolism", and "primary bile acid biosynthesis", were remarkably downregulated.

4. DISCUSSION

As a well-known agricultural pest, *P. canaliculata* devastates a wide range of crops, the most notable is rice. One adult snail can consume 5−24 rice seedlings per day.[32] *P. canaliculata* can result in an annual rice yield loss ranging from 5 to 100%.[2,5] In addition, the damage of *P. canaliculata* to wetland ecosystems may be underestimated and has not been adequately evaluated. An additional concern is a substantial increase in the global distribution of human angiostrongyliasis, with reported cases in more than 30 countries.[13] The global distribution of human angiostrongyliasis almost coincides with the geographical distribution of the intermediate snail hosts. The aggressive invasion of *P. canaliculata* to new locations poses a significant threat to human health. Niclosamide has been the most effective and widely used molluscicide for snail control. Despite its powerful molluscicidal activity, the high toxicity of niclosamide to nontarget aquatic organisms often makes it inappropriate for the control of freshwater snails such as *P. canaliculata*. Less toxic alternatives are therefore needed.

PBQ exhibited substantial molluscicidal activity against adult *P. canaliculata* snails under laboratory conditions. Our previous study demonstrated that PBQ is also very toxic to *B. straminea* (LC_{50} = 0.50 mg/L), another invasive freshwater snail.[25] Relatively weaker potency was observed in *O. hupensis*, probably due to its amphibious habitat and thicker shell.[26] We also found that the susceptibility of *P. canaliculata* to PBQ was dependent on the developmental stage. A substantially lower LC_{50} was recorded for juveniles, indicating their higher susceptibility to PBQ. This would be beneficial in actual field situations. Egg masses may hatch several days after PBQ application. After days of dissipation, the residual concentration of PBQ may be insufficient to kill adults but probably sufficient to kill juveniles and control population growth.

A floating granule formulation of PBQ (20% FG) was developed to facilitate the application in paddy fields. This formulation showed a time- and dose-dependent lethal effect on *P. canaliculata* in the plot experiments using broadcast applications and spot treatments. At a dose of 0.25 g a.i./m^2, PBQ resulted in over 70% snail mortality after 3-day exposure, while niclosamide (0.1 g a.i./m^2) killed all snails. Extended exposure time increased the total snail mortality. For example, at a dose of 0.25 g a.i./m^2, over 95% mortality occurred in PBQ plots at 7 days with both application methods. In addition, over 99% snail control efficacy was achieved in field trials. PBQ reduced the snail population by almost 100% at a dose of only 0.25 g a.i./m^2. Thus, the recommended field application dose of PBQ for *P. canaliculata* control would be 2500 g a.i./ha or less, as the WHO cutoff for the efficacy of a molluscicide formulation against adult snails is ≥80% mortality.[33] According to Chinese Agricultural Industry Standards, the recommended doses of a few common molluscicides in paddy fields are as

https://doi.org/10.1021/acs.jafc.1c07847
J. Agric. Food Chem. 2022, 70, 1079−1089

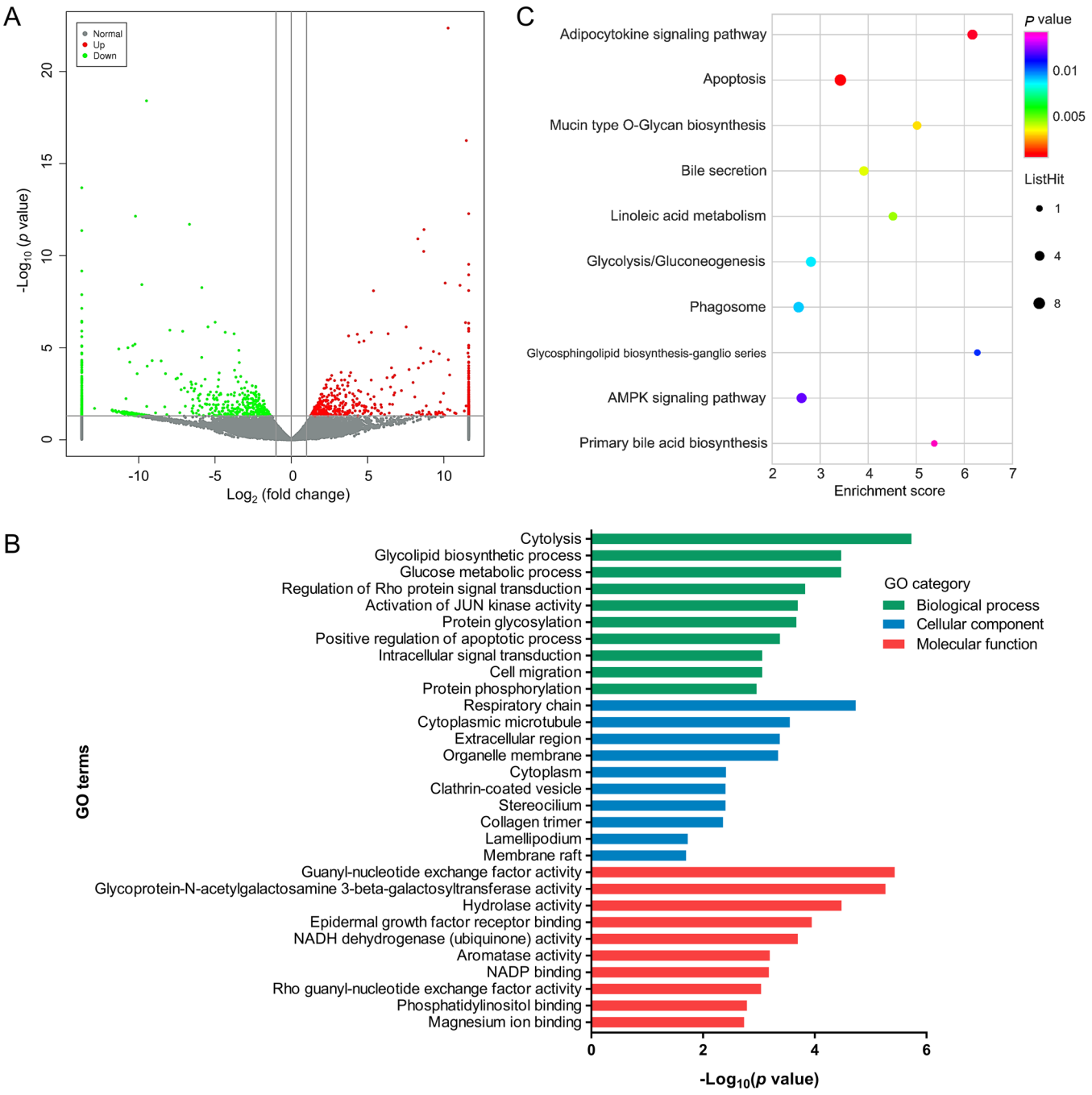

Figure 5. Functional enrichment analysis of DEGs. (A) Volcano plot of DEGs between PBQ-treated and control *P. canaliculata* snails. Upregulated genes are shown in red and downregulated genes are shown in green. (B) Histogram indicating the top 10 GO terms in the enrichment analysis of DEGs. Green bars represent the biological process category, blue bars represent the cell component category, and red bars represent the molecular function category. (C) The top 10 significantly enriched KEGG pathways are shown together with the *p* value (color), enrichment factor (*X*-axis), and the number of involved DEGs (size of circles).

follows: niclosamide: 630 g a.i./ha; metaldehyde: 7500 g a.i./ha; and tea saponin: 30 000 g a.i./ha. In terms of field application rate, PBQ has a reasonable value compared to these molluscicides. We also observed that the snails typically closed their opercula about 30 min after PBQ application. Although these snails may still be alive, they are no longer capable of damaging rice seedlings. This behavior was also noted in other molluscicides against *Pomacea* snails in the Philippines, Spain, and Brazil.[34,35]

The field trial results were promising, but temperature remains a factor to consider. Temperature plays a crucial role in several aspects of the life cycle of *P. canaliculata*, including growth, feeding, and reproduction.[36−38] In this study, the range of temperature in the locations where field trials were conducted was 25−35 °C, which allows the snails to remain active[39] and suggests that a molluscicide in this temperature range would exhibit good performance. However, it is unclear if the molluscicidal efficacy of PBQ would be affected by reduced temperatures. For example, the molluscicide metaldehyde is ineffective when the water temperature falls below 10 °C.[40] Field trials in different seasons of rice seeding and

https://doi.org/10.1021/acs.jafc.1c07847
J. Agric. Food Chem. 2022, 70, 1079−1089

Table 6. DEGs That Coding for Rate-Limiting or Essential Enzymes Present in Carbohydrate and Lipid Metabolism/Biosynthesis Processes

gene ID	description	enriched pathway	fold change	up/down
TRINITY_DN31156_c0_g1_i4_2	6-phosphofructokinase (PFK)	glycolysis/gluconeogenesis	0.19	down
TRINITY_DN25002_c0_g2_i4_2	cholesterol 7 α-hydroxylase (also known as CYP7A1)	primary bile acid biosynthesis	0.0005	down
TRINITY_DN31519_c0_g1_i8_2	core 1 synthase glycoprotein-N-acetylgalactosamine 3-β-galactosyltransferase 1 (C1GALT1)	mucin type O-glycan biosynthesis	0.015	down

cultivation will be necessary to determine the relationship between temperature and the snail control efficacy of PBQ.

The safety profile of PBQ appears satisfactory. PBQ revealed a slight risk of cytotoxicity in human cells and had very low acute oral toxicity in rats. According to the Globally Harmonized System (GHS),[41] PBQ is classified as a Category 5 (2000 mg/kg < LC_{50} ≤ 5000 mg/kg) chemical to rats, ensuring a wide safety range for its application. In addition, data for contact and oral toxicity tests on *A. mellifera* L. suggest that PBQ is generally nontoxic to this species, and it is within the U.S. EPA toxicity category of "practically nontoxic" (LD_{50} > 11 μg/bee).[42] Fish and crustaceans are considered surrogate species covering a range of trophic levels and taxa. Ecotoxicological studies on *D. magna* and *B. rerio* suggested that the use of PBQ molluscicide would be reasonably safe for aquatic ecosystems. PBQ is classified as a GHS Category 3 (10 mg/L < LC_{50} ≤ 100 mg/L, slightly toxic) chemical to *B. rerio* and a GHS Category 2 (1 mg/L < LC_{50} ≤ 10 mg/L, moderately toxic) chemical to *D. magna*, demonstrating that it is substantially safer than niclosamide [GHS Category 1 (LC_{50} ≤ 1 mg/L, highly toxic) for both organisms].[43] Laboratory studies revealed that a *P. canaliculata* mortality of 50% was attained at 0.39 g/L of PBQ, which was 31-fold lower than the LC_{50} for zebrafish and 15-fold lower than the EC_{50} for *D. magna* (Table 4). This is particularly important since many rice fields are also used as aquaculture systems, e.g., fish or crab farming, and the paddy water containing molluscicides could be discharged into nearby aquaculture ponds. In this regard, PBQ use could be advantageous compared to the currently used molluscicides in paddy fields. Further studies are needed to evaluate the toxicity of PBQ to other species (such as shrimp and crabs) present in semicommercial and commercial aquaculture ponds close to rice fields.

Transcriptome sequencing is widely used for genome analysis and functional gene identification. In this work, comparative transcriptomics analyses were conducted to study the genetic response of *P. canaliculata* snails to the exposure of PBQ. A total of 1280 DEGs were identified, which provided a basis for understanding the molluscicidal mechanism of PBQ. Functional enrichment analyses of DEGs revealed that the significantly affected biological processes and pathways were strongly associated with carbohydrate and lipid metabolism, especially in "glycolysis/gluconeogenesis", "mucin type O-glycan biosynthesis", "linoleic acid metabolism", and "primary bile acid biosynthesis" pathways. The two pathways of "glycolysis/gluconeogenesis" and "linoleic acid metabolism" were also significantly enriched in the transcriptome profile of *O. hupensis* snails treated with niclosamide.[44,45] Furthermore, we found that PBQ treatment significantly downregulated several DEGs that coding for rate-limiting or essential enzymes present in carbohydrate and lipid metabolism/biosynthesis processes, such as 6-phosphofructokinase (PFK), cholesterol 7 α-hydroxylase (also known as CYP7A1), and core 1 synthase glycoprotein-N-acetylgalactosamine 3-β-galactosyltransferase 1 (C1GALT1, Table 6). In the glycolytic pathway, PFK is the key regulatory enzyme, which catalyzes the rate-limiting committed and irreversible step, fructose-6-phosphate to fructose-1,6-bisphosphate. As such, PBQ-induced downregulation of PFK could slow down the conversion from glucose to pyruvic acid and consequently produce less energy, resulting in the disordered regulation of glycolytic flux within snail cells. Our finding is consistent with the results of previous studies showing a significant reduction in PFK in other snail species, e.g., *Biomphalaria alexandrina* and *Lymnaea natalensis* following the treatment of plant extracts.[46,47] A similar trend of suppression in the expression of proteins involved in glycolysis has also been reported in a proteomics study of *P. canaliculata* during prolonged estivation (a strong stressor to this species).[48] Cholesterol 7 α-hydroxylase (also known as CYP7A1) is the rate-limiting enzyme in the synthesis of bile acid from cholesterol, catalyzing the formation of 7-α-hydroxycholesterol. Although little is known about CYP7A1 in *P. canaliculata*, it is expected to serve similar functions in the snails. CYP7A1 gene was dramatically downregulated (2000-fold) following the exposure of PBQ, indicating that the primary bile acid biosynthesis process of the snails underwent significant alterations, and this is consistent with the remarkable downregulation of the "bile secretion" pathway. It suggests that the molluscicidal effect may be resulted from the reduced bile acid production or consequent cholesterol metabolism disorder in *P. canaliculata*.

Integrated pest management is appropriate for *P. canaliculata* snail control. Paddy-upland rotation cropping systems are recommended in China to prevent snail spread and reproduction. For heavily infested rice fields, reduced toxicity molluscicides, such as PBQ, should be applied without delay. Biological control is a long-term strategy that is beneficial to reduce the snail population through the introduction of predators, including ducks, fish, and turtles.[49−51] These control actions should be followed by containment measures based on physical barriers to prevent the spread of snails through watercourses and channels. The technological components of integrated pest management still remain to be verified in the field.

In summary, PBQ is a molluscicide with potent activity against the invasive agricultural pest *P. canaliculata*. A 20% PBQ sulfate FG formulation demonstrated excellent snail control efficacy in field trials, and the results were comparable to the WHO-recommended molluscicide niclosamide. The recommended application dose of PBQ (20% FG) in paddy fields is 2500 g a.i./ha. PBQ has a good safety profile, exhibiting low toxicity against mammals, nontarget aquatic organisms, and insects. Comparative transcriptomics analysis indicated that PBQ treatment had substantial effects on the carbohydrate and lipid metabolism of *P. canaliculata*, which were likely the main cause of snail mortality. The findings of

https://doi.org/10.1021/acs.jafc.1c07847
J. Agric. Food Chem. 2022, 70, 1079−1089

this study indicate that PBQ could be a molluscicide candidate for the control of *P. canaliculata* with a slight effect on nontarget organisms and the environment.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.1c07847.

Sample collection, RNA extraction, and cDNA library construction; transcriptome assembly and functional annotation; identification of differentially expressed genes and enrichment analyses; availability of data; summary of RNA-seq data generated from *P. canaliculata* snails with or without PBQ treatment (Table S1); and summary of the statistics for sequencing of the *P. canaliculata* transcriptome (Table S2) (PDF)

Differentially expressed genes (Table S3) (XLSX)

GO terms for differentially expressed genes (Table S4) (XLSX)

KEGG pathways for differentially expressed genes (Table S5) (XLSX)

■ AUTHOR INFORMATION

Corresponding Authors

Shizhu Li − *NHC Key Laboratory of Parasite and Vector Biology, WHO Collaborating Centre for Tropical Diseases, National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, Shanghai 200025, China*; Email: lisz@chinacdc.cn

Liping Duan − *NHC Key Laboratory of Parasite and Vector Biology, WHO Collaborating Centre for Tropical Diseases, National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, Shanghai 200025, China*; orcid.org/0000-0002-0686-1931; Email: duanlp@nipd.chinacdc.cn

Authors

Weisi Wang − *NHC Key Laboratory of Parasite and Vector Biology, WHO Collaborating Centre for Tropical Diseases, National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, Shanghai 200025, China*

Shuijin Huang − *Institute of Plant Protection, Jiangxi Academy of Agricultural Sciences, Nanchang 330200, China*

Fengquan Liu − *Jiangsu Key Laboratory for Food Quality and Safety-State Key Laboratory Cultivation Base of Ministry of Science and Technology, Institute of Plant Protection, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, China*; orcid.org/0000-0002-2029-0012

Yang Sun − *Institute of Plant Protection, Jiangxi Academy of Agricultural Sciences, Nanchang 330200, China*

Xiangyun Wang − *Ministry of Agriculture and Rural Affairs Key Laboratory for Pesticide Residue Detection, Institute of Agro-Products Safety and Nutrition, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China*

Junmin Yao − *NHC Key Laboratory of Parasite and Vector Biology, WHO Collaborating Centre for Tropical Diseases, National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, Shanghai 200025, China*

Yuhua Liu − *Dali Institute of Schistosomiasis Prevention and Control, Dali 671099, China*

Bingrong Luo − *Dali Institute of Schistosomiasis Prevention and Control, Dali 671099, China*

Xia Zhang − *Jiangling Institute of Schistosomiasis Prevention and Control, Jingzhou 434100, China*

Hehua Hu − *Jiangling Institute of Schistosomiasis Prevention and Control, Jingzhou 434100, China*

Zhuohui Deng − *Guangdong Provincial Center for Disease Control and Prevention, WHO Collaborating Centre for Surveillance, Research and Training of Emerging Infectious Diseases, Guangzhou 511430, China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jafc.1c07847

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was funded by the National Natural Science Foundation of China (Grant Number 82072309) and the National Key Research and Development Program of China (Grant Numbers 2021YFC2300800, 2021YFC2300803, 2021YFC2300804, and 2017YFC1200604). The authors thank Shanghai OE Biotech Co., Ltd. (Shanghai, China) for providing transcriptomics services.

■ REFERENCES

(1) Global Invasive Species Database. 100 of the World' Worts Invasive Alien Species, 2013. http://www.iucngisd.org/gisd/100_worst.php.

(2) Naylor, R. Invasions in agriculture: assessing the cost of the golden apple snail in Asia. *AMBIO* **1996**, *25*, 443−448.

(3) Rawlings, T. A.; Hayes, K. A.; Cowie, R. H.; Collins, T. M. The identity, distribution, and impacts of non-native apple snails in the continental United States. *BMC Evol. Biol.* **2007**, *7*, No. 97.

(4) Cowie, R. H.; Hayes, K. A.; Tran, C. T.; Levin, P. Distribution of the invasive apple snail *Pomacea canaliculata* (Lamarck) in the Hawaiian islands (Gastropoda: Ampullariidae). *Bishop Museum Occasional Papers*, 2007, Vol. *96*, pp 48−57.

(5) Halwart, M. The golden apple snail *Pomacea canaliculata* in Asian rice farming systems: Present impact and future threat. *Int. J. Pest Manage.* **1994**, *40*, 199−206.

(6) Joshi, R. C.; Sebastian, L. S. *Global Advances in Ecology and Management of Golden Apple Snails*; Philippine Rice Research Institute, 2006.

(7) Invasive Speices Compendium for Golden Apple Snail. https://www.cabi.org/isc/datasheet/68490 June 14th, 2021.

(8) Carlsson, N. O. L.; Brönmark, C.; Hansson, L. Invading herbivory: The golden apple snail alters ecosystem functioning in Asian wetlands. *Ecology* **2004**, *85*, 1575−1580.

(9) Horgan, F. G.; Stuart, A. M.; Kudavidanage, E. P. Impact of invasive apple snails on the functioning and services of natural and managed wetlands. *Acta Oecol.* **2014**, *54*, 90−100.

(10) Fang, L.; Wong, P. K.; Lin, L.; Lan, C.; Qiu, J. Impact of invasive apple snails in Hong Kong on wetland macrophytes, nutrients, phytoplankton and filamentous algae. *Freshwater Biol.* **2010**, *55*, 1191−1204.

(11) Wang, Q. P.; Wu, Z. D.; Wei, J.; Owen, R. L.; Lun, Z. R. Human Angiostrongylus cantonensis: an update. *Eur. J. Clin. Microbiol. Infect. Dis.* **2012**, *31*, 389−395.

(12) Barratt, J.; Chan, D.; Sandaradura, I.; Malik, R.; Spielman, D.; Lee, R.; Marriott, D.; Harkness, J.; Ellis, J.; Stark, D. *Angiostrongylus cantonensis*: a review of its distribution, molecular biology and clinical significance as a human pathogen. *Parasitology* **2016**, *143*, 1087−1118.

(13) McAuliffe, L.; Fortin Ensign, S.; Larson, D.; Bavaro, M.; Yetto, J.; Cathey, M.; Mukaigawara, M.; Narita, M.; Ohkusu, K.; Quast, T.; Volk, C. Severe CNS angiostrongyliasis in a young marine: a case report and literature review. *Lancet Infect. Dis.* **2019**, *19*, e132−e142.

(14) Lv, S.; Guo, Y. H.; Wei, F. R.; Zhang, Y.; Xiao, N.; Zhou, X. N. Control of eosinopilic meningitis caused by *Angiostrongylus cantonensis* in China. *Adv. Parasitol.* **2020**, *110*, 269−288.
(15) Johnston, D. I.; Dixon, M. C.; Elm, J. L.; Calimlim, P. S.; Sciulli, R. H.; Park, S. Y. Review of cases of angiostrongyliasis in Hawaii, 2007-2017. *Am. J. Trop. Med. Hyg.* **2019**, *101*, 608−616.
(16) Lv, S.; Guo, Y.; Nguyen, H. M.; Sinuon, M.; Sayasone, S.; Lo, N. C.; Zhou, X.; Andrews, J. R. Invasive Pomacea snails as important intermediate hosts of Angiostrongylus cantonensis in Laos, Cambodia and Vietnam: Implications for outbreaks of eosinophilic meningitis. *Acta Trop.* **2018**, *183*, 32−35.
(17) Olivier, H. M.; Jenkins, J. A.; Berhow, M.; Carter, J. A pilot study testing a natural and a synthetic molluscicide for controlling invasive apple snails (*Pomacea maculata*). *Bull. Environ. Contam. Toxicol.* **2016**, *96*, 289−294.
(18) Yang, C.; Chang, X.; Zhang, M.; Ni, X.; Lv, T.; Gong, G.; Yue, G.; Sun, X.; Chen, H. Active compounds of stem bark extract from *Schima superba* and their molluscicidal effects on *Pomacea canaliculata*. *J. Pest Sci.* **2018**, *91*, 437−445.
(19) San Martín, R.; Ndjoko, K.; Hostettmann, K. Novel molluscicide against *Pomacea canaliculata* based on quinoa (*Chenopodium quinoa*) saponins. *Crop Prot.* **2008**, *27*, 310−319.
(20) Andrews, P.; Thyssen, J.; Lorke, D. The biology and toxicology of molluscicides, Bayluscide. *Pharmacol. Ther.* **1982**, *19*, 245−295.
(21) Oliveira-Filho, E. C.; Paumgartten, F. J. R. Toxicity of *Euphorbia milii* Latex and niclosamide to snails and nontarget aquatic species. *Ecotoxicol. Environ. Saf.* **2000**, *46*, 342−350.
(22) Dunlop, R. W.; Duncan, J.; Ayrey, G. Quantitative structure-activity relationships for nicotinanilide molluscicides. *Pestic. Sci.* **1980**, *11*, 53−60.
(23) Parashar, B. D.; Kaushik, M. P.; Prakash, S.; Rao, K. M. Toxicity of nicotinanilide and its analogues to the freshwater snail Indoplanorbis exustus, mediator of animal schistosomiasis, and to non-target organisms. *J. Med. Appl. Malacol.* **1990**, *2*, 135−140.
(24) Stuart, A. M.; Nogues Palenzuela, A.; Bernal, C. C.; Ramal, A. F.; Horgan, F. G. Effects of fertiliser applications on survival and recruitment of the apple snail, *Pomacea canaliculata* (Lamarck). *Crop Prot.* **2014**, *64*, 78−87.
(25) Wang, W.; Mao, Q.; Yao, J.; Yang, W.; Zhang, Q.; Lu, W.; Deng, Z.; Duan, L. Discovery of the pyridylphenylureas as novel molluscicides against the invasive snail *Biomphalaria straminea*, intermediate host of *Schistosoma mansoni*. *Parasites Vectors* **2018**, *11*, No. 291.
(26) Chen, Z.; Wang, W.; Yao, J.; Li, S.; Zhang, X.; Hu, H.; Liu, X.; Luo, B.; Liu, Y.; Xu, H.; Duan, L. Toxicity of a molluscicide candidate PPU07 against *Oncomelania hupensis* (Gredler, 1881) and local fish in field evaluation. *Chemosphere* **2019**, *222*, 56−61.
(27) du Sert, N. P.; Hurst, V.; Ahluwalia, A.; Alam, S.; Avey, M. T.; Baker, M.; Browne, W. J.; Clark, A.; Cuthill, I. C.; Dirnagl, U.; Emerson, M.; Garner, P.; Holgate, S. T.; Howells, D. W.; Karp, N. A.; Lazic, S. E.; Lidster, K.; MacCallum, C. J.; Macleod, M.; Pearl, E. J.; Petersen, O. H.; Rawle, F.; Reynolds, P.; Rooney, K.; Sena, E. S.; Silberberg, S. D.; Steckler, T.; Würbel, H. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *PLoS Biol.* **2020**, *18*, 3793−3801.
(28) OECD. Test No. 203: Fish, Acute Toxicity Test, 2019. https://www.oecd-ilibrary.org/environment/test-no-203-fish-acute-toxicity-test_9789264069961-en.
(29) OECD. Test No. 202: Daphnia sp. Acute Immobilisation Test, 2004. https://www.oecd-ilibrary.org/environment/test-no-202-daphnia-sp-acute-immobilisation-test_9789264069947-en.
(30) OECD. Test No. 214: Honeybees, Acute Contact Toxicity Test, 1998. https://www.oecd-ilibrary.org/environment/test-no-214-honeybees-acute-contact-toxicity-test_9789264070189-en.
(31) OECD. Test No. 213: Honeybees, Acute Oral Toxicity Test, 1998. https://www.oecd-ilibrary.org/environment/test-no-213-honeybees-acute-oral-toxicity-test_9789264070165-en.
(32) Oya, S.; Hirai, Y.; Miyahara, Y. Injuring habits of the apple snail, Ampullarius insularus D'ORBIGNY, to the young rice seedlings (in Japanese). *Kyushu Plant Prot. Res.* **1986**, *32*, 92−95.
(33) *Guidelines for Laboratory and Field Testing of Molluscicides for Control of Schistosomiasis*; World Health Organization: Geneva, 2019.
(34) Castillo-Ruiz, M.; Cañon-Jones, H.; Schlotterbeck, T.; Lopez, M. A.; Tomas, Á.; San Martín, R. Safety and efficacy of quinoa (*Chenopodium quinoa*) saponins derived molluscicide to control of *Pomacea maculata* in rice fields in the Ebro Delta, Spain. *Crop Prot.* **2018**, *111*, 42−49.
(35) San Martíns, R.; Gelmi, C.; de Oliveira, J. V.; Galo, J. L.; Pranto, H. Use of a saponin based molluscicide to control *Pomacea canaliculata* snails in Southern Brazil. *Nat. Prod. Commun.* **2009**, *4*, 1327.
(36) Bae, M.; Chon, T.; Park, Y. Modeling behavior control of golden apple snails at different temperatures. *Ecol. Model.* **2015**, *306*, 86−94.
(37) Seuffert, M. E.; Martin, P. R. Juvenile growth and survival of the apple snail *Pomacea canaliculata* (Caenogastropoda: Ampullariidae) reared at different constant temperatures. *SpringerPlus* **2013**, *2*, No. 312.
(38) Koch, E.; Winik, B. C.; Castro-Vazquez, A. Development beyond the gastrula stage and digestive organogenesis in the apple-snail *Pomacea canaliculata* (Architaenioglossa, Ampullariidae). *Biocell* **2009**, *33*, 49−65.
(39) Seuffert, M. E.; Burela, S.; Martín, P. R. Influence of water temperature on the activity of the freshwater snail *Pomacea canaliculata* (Caenogastropoda: Ampullariidae) at its southernmost limit (Southern Pampas, Argentina). *J. Therm. Biol.* **2010**, *35*, 77−84.
(40) Cheng, E. Y. Control Strategy for the Introduced Snail, *Pomacea lineata*, in Rice Paddy. *Monograph − British Crop Protection Council*, 1989, Vol. *41*, pp 69−75.
(41) Acute Toxicity. In *Globally Harmonized System of Classification and Labelling of Chemicals (GHS)*; United Nations, 2019.
(42) *Technical Overview of Ecological Risk Assessment − Analysis Phase: Ecological Effects Characterization*; U.S. Environmental Protection Agency, 2021.
(43) Hazardous to the Aquatic Environment. In *Globally Harmonized System of Classification and Labelling of Chemicals (GHS)*; United Nations, 2019.
(44) Zhao, Q. P.; Xiong, T.; Xu, X. J.; Jiang, M. S.; Dong, H. F. De Novo transcriptome analysis of *Oncomelania hupensis* after molluscicide treatment by next-generation sequencing: implications for biology and future snail interventions. *PLoS One* **2015**, *10*, No. e118673.
(45) Xiong, T.; Jiang, N.; Xu, S.; Li, S. Z.; Zhang, Y.; Xu, X. J.; Dong, H. F.; Zhao, Q. P. Metabolic profiles of *Oncomelania hupensis* after molluscicidal treatment: Carbohydrate metabolism targeted and energy deficiency. *Acta Trop.* **2020**, *210*, No. 105580.
(46) Bakry, F. A.; El-Hommossany, K.; Mossalem, H. S. Immunological and physiological parameters of *Biomphalaria alexandrina* snails exposed to *Azadirachta indica* plant. *Eur. Rev. Med. Pharmacol.* **2012**, *16*, 133−143.
(47) Bakry, F. A.; Eleiwa, M. E.; Taha, S. A.; Ismil, S. M. Comparative toxicity of Paraquat herbicide and some plant extracts in *Lymnaea natalensis* snails. *Toxicol. Ind. Health* **2016**, *32*, 143−153.
(48) Sun, J.; Mu, H.; Zhang, H.; Chandramouli, K. H.; Qian, P.; Wong, C. K. C.; Qiu, J. Understanding the regulation of estivation in a freshwater snail through iTRAQ-based comparative proteomics. *J. Proteome Res.* **2013**, *12*, 5271−5280.
(49) Liang, K.; Zhang, J.; Fang, L.; Zhao, B.; Luo, M.; Parajuli, P.; Ouyang, Y. The biological control of *Pomacea canaliculata* population by rice-duck mutualism in paddy fields. *Biocontrol Sci. Technol.* **2013**, *23*, 674−690.
(50) Dong, S.; Zheng, G.; Yu, X.; Fu, C. Biological control of golden apple snail, *Pomacea canaliculata* by Chinese soft-shelled turtle, Pelodiscus sinensis in the wild rice, *Zizania latifolia* field. *Sci. Agric.* **2012**, *69*, 142−146.

https://doi.org/10.1021/acs.jafc.1c07847
J. Agric. Food Chem. 2022, 70, 1079−1089

(51) Ip, K. K. L.; Liang, Y.; Lin, L.; Wu, H.; Xue, J.; Qiu, J. Biological control of invasive apple snails by two species of carp: Effects on non-target species matter. *Biol. Control* **2014**, *71*, 16−22.

https://doi.org/10.1021/acs.jafc.1c07847
J. Agric. Food Chem. 2022, 70, 1079−1089