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Acute and sub-acute toxicity of insecticide quinalphos on snakehead fish (*Channa striata*) in the Vietnamese Mekong Delta

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ABSTRACT

Insecticide quinalphos is popularly used in Vietnam and detected in different water bodies, specially rice paddy fields where snakehead fish (*Channa striata*) often distribute. Therefore, the species can be at a high risk of exposure to insecticides. In this study, the effects of quinalphos on juvenile snakehead fish were assessed under both laboratory and field conditions. The 96h-LC50 was estimated by conduct in a static non-renewed system. Afterward, two quinalphos treatments (1% and 5% 96h-LC50) and a control were conducted for 96h to assess the sensitivity of brain cholinesterase of snakehead fish to quinalphos. Two rice-field treatments in the VMD were selected and then three cages were randomly placed into each rice field. One field was not applied with insecticide, and the other was applied Kinalux 25EC at once as an indication dose. The results showed that quinalphos is very toxic for snakehead fish (4-5g) with an 96h-LC50 of 49 µg/L. Quinalphos significantly inhibited the brain ChE activity of snakehead fish at a concentration of 0.49 µg/L (#1% 96hLC50). Using Kinalux 25EC for rice at the instructed dose causes serious effects for snakehead fish, including high mortality and high and prolonged ChE inhibition. Although quinalphos was below the detection limit (0.1 µg/L) after 1 d post-spray, activity of brain ChE of the fish species was significantly inhibited up to a week post-spray. Residues of quinalphos in ricefield soil and this species should be concentrated to determine in further study for better understanding the fate of this insecticide after spraying and to assess the safety of snakehead fish meat for food.

INTRODUCTION

Insecticide quinalphos is widely used for agriculture crops in numerous countries over the world **(Srivastava *et al.*, 2016; Khatun *et al.*, 2023)**. In Vietnam, quinalphos is one of the most commonly used pesticides which is applied to rice fields in the Mekong delta (MD) **(Toan & Cong, 2018)**. Presently, twenty-four commercial pesticide products which contain quinalphos are permitted for use in Vietnam **(MARD, 2022)**. To investigate residues of quinalphos in water in the MD, **Toan *et al.* (2014)** found that

maximum water concentration of quinalphos was 0.7µg/ L in rice fields, 0.58µg/ L in internal rice field canals, and 0.12µg/ L in rivers. Collecting water from rice paddy fields 5 minutes after applying Kinalux 25EC (containing 25% quinalphos) revealed a quinalphos concentration of 11.3 ± 0.15µg/ L. This concentration decreased to 5.8 ± 0.6µg/ L after 1 day and further to 1.8 ± 0.1µg/ L after 3 days **(Thinh *et al.*, 2016)**. It means that quinalphos was not only found in the rice-field where it was most applied; it was transported far away from their application sites.

Quinalphos is reported to be highly toxic to aquatic organisms. The 96h-LC50 of this ingredient was 7.5µg/ L for common carp *Cyprinus carpio* (2± 0.2g) **(Chebbi & David, 2010)**, 0.69µg/ L for post-larval (P35) freshwater shrimp *Macrobrachium rosenbergii* **(Cong *et al.*, 2020)**, and 5.35µg/ L for the tilapia *Oreochromis mossambicus* (17.4± 0.68g) **(Medda *et al.*, 2022)**. These species are found in natural water-bodies and in aquaculture systems in the MD. These 96h-LC50 values were already between quinalphos residues, which were reported in water in the MD. Therefore, residues of quinalphos potentially cause lethality for aquatic organisms in natural water-bodies in the MD.

At the sublethal concentration, quinalphos caused significant effects on physical and biochemical parameters of aquatic species. Significant reduction in the activity of ChE in shrimp (*M. rosenbergii*) was found when the species was exposed into quinalphos at a concentration of 1% the 96h-LC50 (0.07µg/ L) **(Cong *et al.*, 2020)**. Exposing common carp (*Cyprinus carpio*) to quinalphos at a concentration of 0.275 µg/L for 20 days resulted in alterations in protein content and changes in the behavior of the species **(Varghese & Thomas, 2023)**. At sublethal concentrations (0.5 and 1.0µg/ L), quinalphos elevated the levels of liver CAT, SOD, GST, MDA, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and growth inhibition in tilapia *O. mossambicus* **(Medda *et al.*, 2022)**. Snakehead fish (*C. striata*) is a local inhabitant in the MD **(Khoa & Huong, 1993)** and was often found in the rice paddy fields, particularly in the integrated rice-fish system **(Vromant *et al.*, 2001)**. This species often reproduces in the paddy field in the rainy season **(Amilhat & Lorenzen, 2005)**. Therefore, snakehead fish is potentially at risk for exposure to insecticides, such as quinalphos. However, the toxicity of quinalphos for this species is still unknown. In the present study, the effects of Kinalux 25 EC containing 25% quinalphos on snakehead fish in both laboratory and rice field conditions were investigated.

MATERIALS AND METHODS

Test animals

The snakehead fish (3 – 4g/individual) were purchased from the hatchery at the College of Aquaculture and Fisheries, Can Tho University, Vietnam. Fish were acclaimed in 600-L composite tanks containing 300L of tap water for two weeks in the

wet-lab at the Department of Environmental Science, Can Tho University. The tank water was continuously aerated, and 10% of the total water was changed daily. Fish were fed two times a day with commercial Cargill food (1.5mm/tablet). Moreover, feeding was stopped a day prior to experimentation.

Chemicals

A commercially available product, Kinalux 25EC contains 25% active ingredient quinalphos was purchased from Loc Troi Joint Stock Company, Vietnam. It was used both for laboratory and paddy field exposures.

Sodium hydrogen phosphate dihydrate ($NaH_2PO_4.2H_2O$) and di-sodium hydrogen phosphate dihydrate ($Na_2HPO_4.2H_2O$) were purchased from Merck. They were used to prepare buffer solutions with pH 7.4 and pH 8.

Acetylthiocholine iodide (Sigma Aldrich, Germany) and 5,5'-dithiobis (2 nitrobenzoic acid) ($C_{14}H_8N_2O_8S_2$) (Sigma Aldrich, Germany) were used for ChE assay.

Experimental design

Acute toxicity testing

Five quinalphos (25, 40, 50, 70 and 100ppb) treatments and a control (dechlorinated tap water) were performed in 60-L-glass tanks in a static non-renewed system for 96h with three replications. Ten healthy snakehead fish were exposed in each tank. The exposed snakehead fish was not fed for over the duration of the experiment. Activity of the exposed fish was observed, and the number of dead fish was recorded at 3, 6, 9, 12, 24, 48, 72 and 9 h after exposure to quinalphos. Dead fish were removed from the tanks to prevent degradation of water quality. Dissolved oxygen, temperature and pH were measured daily using pH and DO meters.

Response of brain cholinesterase in snakehead fish to sub-lethal concentrations of quinalphos

Three quinalphos concentrations (0.49 and 2.45µg/ L equivalent to 1%, 5% 96h-LC50) and the control (de-chlorinated tap water) were designed for determining sensitivity of ChE to sub-lethal concentrations of quinalphos. At each quinalphos concentration, three replications were performed. The experiment was conducted using 60-L glass tanks. After preparing pesticide solutions, the water in the tanks was mixed for 10 minutes and then twenty healthy fish (3.5± 0.42g) were exposed to each replication. The experiment lasted for 96h. Fish were collected at 1, 6, 12, 24, 48, 72, and 96h after exposure. At each sampling time, six fish were collected in each treatment (two fishes/replication) and placed on ice until death for ChE assay. Dissolved oxygen, temperature and pH were measured daily.

Effect of using Kinalux 25EC for rice on activity of brain ChE in snakehead fish

Two rice-fields in Binh Thuy District, Can Tho city were chosen for field exposure study. These rice-fields were isolated and surrounded by earthen dikes (30cm wide, 40cm high) to keep and prevent water exchange during the experimentation period. One tank was left untreated, while Kinalux 25EC was applied to the other. In these rice-fields, rice

variety IR504 was sown at a density of 250kg/ ha. Within each rice field, three plots (1.5 x 1.5m^2) were selected and rice plants were removed and then a cage (1.2x 1.2 x 1.2m^3) made of nylon net (mesh size 0.5cm) was installed into each plot. After being left for sedimentation for 4 days, twenty healthy snakehead fish were gently caught from the acclimated tanks and carefully released into each cage. Fish were fed daily at 08:30 by commercial pellets. After a 7-day adaptation period to the rice-field environment (with rice being 40 days after sowing), farmers were provided with Kinalux 25EC and instructed to apply it at the highest recommended dose according to the label (1.5 L/ha). The Kinalux 25EC was sprayed on the rice plants once.

Water samples were collected around the cage before pesticide application, as well as at 1 hour, and 1, 3, 5, 7, and 14 days after application to measure the actual water concentration of quinalphos using GC-MS **(Parfitt, 2000)**.

Water temperature, pH, and DO were monitored onsite every 2 days at 6:30-7:30 and 14:00-15:00 at three points within each cage using the pH meter and DO meter. After recording temperature, pH, and DO, water level on rice fields were checked using a ruler.

Fish were sampled at 1 day prior applying Kinalux 25EC and 1, 3, 5, 7, 14 days after spraying for checking activity of brain ChE. In each cage two fish were sampled. After sampling, fish from each cage were placed in a plastic bag which already contained ice. Fish samples were preserved in the icebox and then transferred to the laboratory for processing and ChE assay.

Sample preparation

Each brain was separately prepared following the procedures that were described by **Cong *et al.* (2021)**. Brain (78.1± 26.5mg, mean ± S.D) was homogenized in 0.1M phosphate buffer of pH 7.4 at concentration of 20mg/ mL of fresh weight using a glass homogenizer (Uniform, Jencons PLC, Leighton Buzzard, UK). After that, the solution was mixed, one milliner of the solution was removed for centrifuging at 2,000rpm for 20min at a temperature of 4^{o}C and then the upper part was used for the ChE analysis.

Cholinesterase assay

ChE activity was determined by using a spectrophotometer (Hitachi U-2900) at 412nm for 200s based on the method of **Ellman *et al.* (1961)** and modified by **Cong *et al*. (2021)**. All measurements were done in the air-conditioned room with temperature controlled at 25^{o}C and ChE activity was calculated using the method of **Cong *et al.*, (2021)**.

Statistical data analysis

The LC50-96h value of quinalphos was estimated by probit analysis **(Finney, 1971)**. ChE activity data were tested for normal distribution and homogeneity of variance before statistical analysis. Analysis of variance and Duncan test were applied to compare treatments with controls and among treatments with the statistically significant difference at 95% ($P< 0.05$).

RESULTS AND DISCUSSION

1. The 96h-LC50 of quinalphos for fingerling snakehead fish

The measurements showed that among treatments, the water temperature fluctuated between 26.0± 0.4 and 27.3± 0.41, while these fluctuations for the pH were between 6.9± 0.19 and 7.1± 0.08. For dissolved oxygen (DO), it varied between 4.1± 0.12 and 4.9± 0.34mg/ L (Table 1). The environmental parameters did not show significant differences among treatments.

According to **Vivekanandan (1977)**, snakehead fish is an obligate air-breathing fish, the species can live in low DO by increasing air-breath. Snakehead fish can adapt to a wide range of pH (4.25- 9.4) and temperature (11– 40^{o}C) **(Lee *et al*., 1994)**. Therefore, the DO and pH and temperature of the water in these treatments were ideal for living of the experimental fish.

Table 1. Temperature, pH and DO of water in experimental tanks

Quinalphos	Temperature (°C)		pH		DO (mg/L)	
(ppb)	**Morning**	**Afternoon**	**Morning**	**Afternoon**	**Morning**	**Afternoon**
Control	26.0±0.05	26.7±0.27	6.9±0.19	6.8±0.18	4.7±0.43	4.1±0.12
25	26.0±0.04	27.1±0.30	7.0±0.10	7.0±0.13	4.9±0.27	4.2±0.23
40	26.0±0.07	27.1±0.42	7.1±0.06	7.0±0.06	4.6±0.47	4.2±0.08
50	26.2±0.08	27.2±0.40	7.1±0.04	7.0±0.10	4.7±0.40	4.1±0.13
70	26.2±0.06	27.2±0.43	7.1±0.08	7.0±0.15	4.7±0.36	4.2±0.20
100	26.2±0.04	27.3±0.41	7.1±0.02	7.0±0.17	4.9±0.34	4.2±0.11

Data are presented as mean ± SD, n = 12.

No mortality occurred in the control treatment. The mortality of the snakehead fish increased following increases of quinalphos concentrations and duration of exposure (Fig. 1). In the lowest concentration, mortality occurred at 24h, whereas at the higher concentrations the mortality was seen earlier (Fig. 1).

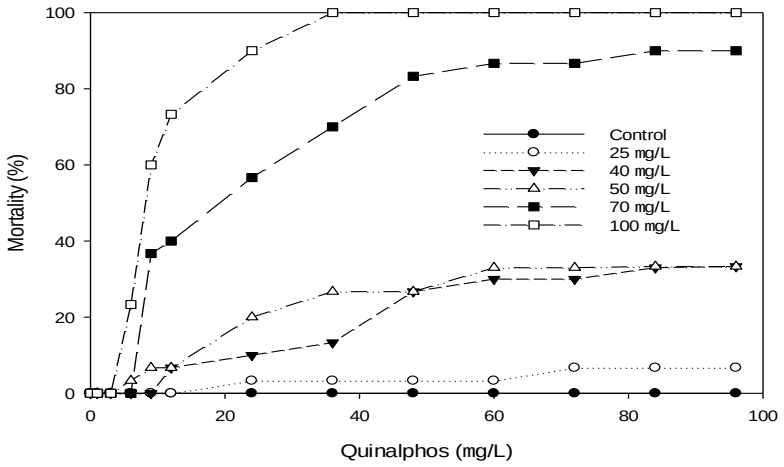

Fig. 1. Trends of snakehead mortality during 96 h of exposure to quinalphos

From the result of mortality, the probit method was applied to estimate the relationship curve between quinalphos concentration and mortality probability (Fig. 2). From this curve, 96h - LC50 of quinalphos for the fingerling snakehead fish was estimated at 49µg/ L, varying between 44 and 54µg/ L (95% interval). It shows that quinalphos is very toxic to *C. striata* with the 96h - LC50 which is less than 1mg/ L. Previous studies reported that the 96h-LC50 of quinalphos was 856µg/ L for *C. carpio* (10- 15g) **(Anh *et al.*, 2012)**, 1,400µg/ L for *Barbonymus gonionotus* (5.23± 1.05g) **(Sadiqul *et al.*, 2017)**, 0.69µg/ L for *M. rosenbergii* (0.17± 0.02g) **(Cong *et al.*, 2020)**, and 24µg/ L for *O. mossambicus* (25± 1.8g) **(Nimila & Joseph, 2022)**. These aquatic species are local inhabitants in the Mekong delta. It is clear that quinalphos was also found very toxic for these species except *B. gonionotus*. For other pesticides, the 96h-LC50 of chlorpyrifos ethyl for the snakehead fish (2.5- 3g/fish) was 27,1ppb **(Tuan *et al.*, 2015)**. It means that an organophosphate chlorpyrifos is more toxic to the snakehead fish than an organophosphate quinalphos.

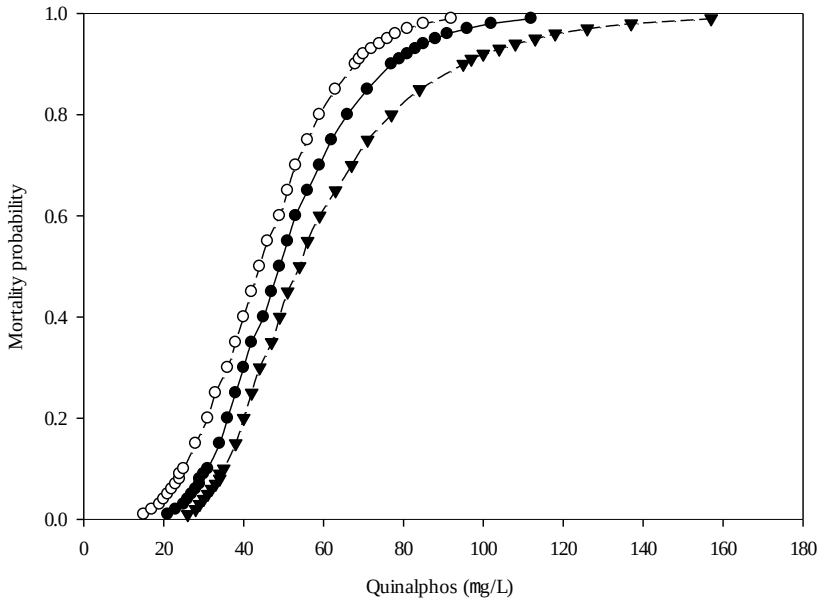

Fig. 2. Relationship between probability of snakehead's mortality and quinalphos concentration (●), 95% lower confidence interval (o), 95% upper confidence interval (▼) at 96h of exposure

2. Effects of sub-acute concentrations of quinalphos on ChE activity

The morning measurements showed that among treatments, the values varied between 25.4± 0.3 and 25.6± 0.3 for temperature, 4.6± 0.19 and 4.8± 0.22mg/ L for DO, and 6.8± 0.09 and 7.0± 0.04 for pH (Table 2). In the afternoon, the temperature values were higher than that in the morning and ranged between 28.1± 0.3 and 28.2± 0.2; Dissolved oxygen among treatments were 4.2± 0.11 and 4.5± 0.16mg/ L, while the pH of the water fluctuated between 6.9± 0.07 and 7.1± 0.03. These environmental parameters

did not show significant difference among treatments and were within the ideal living environment for this species **(Lee *et al.*, 1994)**.

Table 2. Temperature, pH and DO of water in sub-acute experimental tanks

Quinalphos (ppb)	Temperature (°C)		pH		DO (mg/L)	
	Morning	Afternoon	Morning	Afternoon	Morning	Afternoon
Control	25.4±0.3	28.1±0.3	6.8±0.09	6.9±0.07	4.8±0.22	4.5±0.16
1% 96h-LC50	25.5±0.4	28.2±0.2	7.0±0.04	7.0±0.03	4.8±0.06	4.3±0.13
5% 96h-LC50	25.6±0.3	28.2±0.2	7.0±0.04	7.1±0.03	4.6±0.19	4.2±0.11

Data are presented as mean ± SD, $n = 12$.

Brain ChE activity in the fish in the controlled treatment varied between 8.1 and 8.3µMg/ min. In the quinalphos treatments, trends of brain ChE inhibition were significantly increased and peaks were reached at 12h of exposure (Fig. 3). Afterward, they decreased and were insignificant to the controlled treatment from 48h of exposure. The highest ChE inhibition rate in the treatment of 1% 96h-LC50 was 37.7%± 4.1, while this value was 48.1%± 4.7 in the 5% 96h-LC50. It is clearly seen that ChE inhibition is dose dependent; the higher quinalphos concentration, the more brain ChE inhibition. ChE has a function of regulating the nervous system of animals **(Peakall, 1992)**. In many cases, when ChE inhibition exceeds 30%, it leads to abnormal activities in the animals **(Fulton & Key, 2001)**. To expose trout (*Oncorhynchus mykiss*) to organophosphate diazinon or organophosphate malathion for 24 or 96h, **Brewer *et al.* (2001)** found that swimming speed of trout was positively correlated with ChE activity. This suggests that the higher ChE inhibition, the lower swimming speed. Snakehead was found to have muscle convulsion after exposure into diazinon and was easily caught **(Cong *et al.*, 2006)**. Inhibition of swimming speed or muscle convulsion may lead to risk for escaping their predators or hunting their food. In the present study, at the very low quinalphos concentration (1% 96h-LC50 # 0.49 µg/L), brain ChE was significantly inhibited for over 30%. It means that the quinalphos concentration of 1% 96h-LC50 is not safe for the snakehead fish. In the Mekong delta, quinalphos was detected at 0.7µg/ L in rice fields, and 0.58µg/ L in internal rice field canals **(Toan *et al.*, 2014)**. It implies that snakehead fish would not be safe from residues of quinalphos in water in the Mekong delta and will be discussed more in the field experiment.

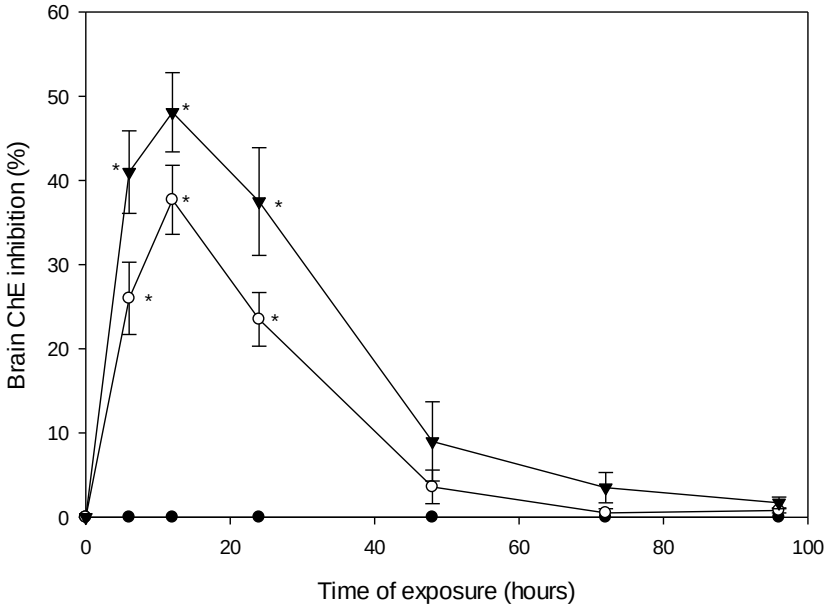

Fig. 3. Sensitivity of brain ChE to quinalphos during 96h of exposure. Data presents mean ± SE, n=5. Control (●), 1% 96h-LC50 (o), 5% 96h-LC50 (▼). At the same time, an asterisk (*) shows a significant difference to control data ($P< 0.05$)

3. Effects of using quinalphos for rice on snakehead fish in paddy field condition

The measurements from ricefields showed that water temperature showed small differences between the control fields and the quinalphos application fields. It ranged from 28.6± 0.3 to 30.6± 0.2^0C in the control fields and from 27.9 ± 0.6 to 30.5 ± 0.7^0C in the pesticide application fields (Table 3). In the morning, the temperature is lower than in the afternoon measurements. Dissolved oxygen was very low in both the control and the pesticide application fields (≤ 2mg/ L) (Table 3). Water pH values showed small differences among fields and varied from 6.6± 0.02 to 6.7± 0.03 in the control field and from 6.7± 0.01 to 6.8± 0.01 in the quinalphos field. Water level on the rice-field fluctuated from 13.7± 0.03 - 14.5± 0.05cm in the control field and from 13.7± 0.02 - 14.2± 0.06cm in the pesticide application field.

Environmental conditions in the experiment rice-fields are different in the laboratory, particularly for DO and temperature. In low DO conditions, fish would increase breathing frequency and uptake more toxicants **(Yang *et al*., 2000)**. In higher temperatures, fish may increase metabolic rate **(Jimenez *et al*., 1987)**. In the present study, DO in rice-fields was less than 2mg/ L and temperature in the afternoon was over 30^oC. These factors would lead fish to increase their breathing frequency and result in uptake more quinalphos into their body.

Table 3. Variation of environmental parameters during experimentation

Parameter	Controlled field		Quinalphos applying field	
	Morning	Afternoon	Morning	Afternoon
Temperature (^{0}C)	28.6±0.3	30.6±0.2	27.9±0.6	30.5±0.7
DO (mg/L)	1.3±0.04	1.8±0.07	1.4±0.02	1.9±0.06
pH	6.6±0.02	6.7±0.03	6.7±0.01	6.8±0.01
Water depth (cm)	14.5±0.05	13.7±0.03	14.2±0.06	13.7±0.02

Before pesticide application, quinalphos in water was less than the detection limit (0.1µg/ L) in both the control and the Kinalux 25EC sprayed fields (Table 4). After 1h post-spraying, quinalphos was 4.72± 1.59µg/ L in the pesticide application field. After 1 day of application, the water concentration of quinalphos quickly declined to 0.91± 0.07µg/ L and then afterward it was below the detection limit.

Table 4. Quinalphos *c*oncentrations in water in the experiment fields

Time	Actual water concentration of quinalphos (µg/L)	
	The control field	The Kinalux 25EC applying field
Before spraying	< *Detection limit*	< *Detection limit*
1 hour post-spraying	< *Detection limit*	4.72±1.59
1 day post-spraying	< *Detection limit*	0.91±0.07
3 days post-spraying	< *Detection limit*	< *Detection limit*
5 days post-spraying	< *Detection limit*	< *Detection limit*
7 days post-spraying	< *Detection limit*	< *Detection limit*
14 days post-spraying	< *Detection limit*	< *Detection limit*

Note: Detection limit = 0.1µg/ L.

Quinalphos has a low water solubility, approximately 17.8mg/ L (20^{o}C) with coefficient log(K_{ow}) of 4.44 **(Panda, 1998)**. Therefore, quinalphos strongly adhere to suspended particles and settle to the soil in the fields, leading to rapid decrease concentration in water. **Thinh *et al.* (2016)** found that concentration of quinalphos in water in the integrated rice – fish after this pesticide applying in the Mekong delta varied within 9 – 11.3µg/ L, and these concentrations reduced and fluctuated between 1.1 and 1.8µg/ L at day 3. Moreover, **Thinh *et al.* (2018)** found that half-life of first and second quinalphos applications for rice fields in the MD were 1.1 and 1.0 days for water, respectively. Actual concentration of quinalphos in the present study is lower than that in the previous study but showed a similar trend.

In the controlled field, ChE fluctuated between 8.01 and 8.23µM/g/ min and showed insignificant difference during sampling times. In the field that Kinalux 25EC was applied at dose 1.5L/ ha, ChE activity was significantly inhibited after 1 day post spraying and then recovered gradually, however at day 7 the activity was still significantly lower than that of control (17.5% inhibition) (Fig. 4). The level of ChE activity was 24.4% of the control or ChE inhibition was 75.6%. Afterward, ChE activity recovered gradually but at day 7 the activity was still significantly inhibited (17.5% inhibition) (Fig. 2). It was seen fully recovered at the last measurement (14 days after application).

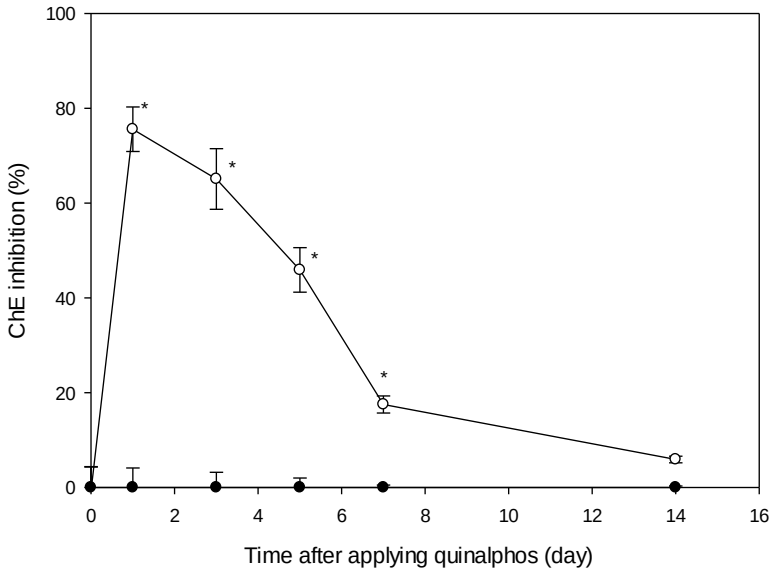

Fig. 4. Trend of brain ChE inhibition during 14 days monitoring after applying quinalphos based-Kinalux 25 EC for rice. The Kinalux 25EC was used at a dose 1.5L/ ha. Data present mean ± SD, n=6. Control rice field (●), quinalphos rice-field (o). At the same time, an asterisk (*) shows a significant difference to the controlled value ($P< 0.05$)

In the present study, brain ChE activity of the snakehead fish declined at the 1st day after applying Kinalux 25EC. Most aquatic organisms could not survive when their ChE was inhibited at over 70% value. In the present study, over 70% ChE inhibition was found at the first sampling times. Mortality did not appear in the control field but it was 37,8% in the quinalphos application field at 1 day after spraying and reached to 43.1% on the day 5 after spraying (Table 5). All dead fish also have high ChE inhibition (>70%). In snakehead fish, high ChE inhibition also resulted in death. Concentration of quinalphos in rice-field water (4.72± 1.59µg/ L after 1h application) in the present study did not reach the range causing lethality (Fig. 2), but low DO and high temperature may have led to the death of fish. In the present study DO was less than 2mg/ L. Although snakeheads can survive in low DO **(Vivekanandan, 1977)**, they would increase breathing **(Yang *et al.*, 2000)** resulting in uptake much quinalphos and cause serious effects such as ChE inhibition and mortality. At higher temperatures (24, 30 and 34^0C), organophosphate diazinon **(Cong *et al.*, 2006)** and quinalphos **(Cong *et al.*, 2024)** caused more brain ChE inhibition for snakehead fish. Therefore, the high temperature in rice field water in the afternoon in the present study would lead snakehead fish to increase uptake more quinalphos and resulted in negative effects, including ChE inhibition and mortality.

Table 5. Mortality (%) of snakehead fish in the rice fields

Rice-field treatment	Cumulative mortality rate (%)					
	Before spraying	Time after spraying (day)				
Control field	0	0	0	0	0	0
Quinalphos application field	0	37.8±9.2	41.1±6.6	43.1±2.5	43.1±2.5	43.1±2.5

In the field conditions of the present study, ChE gradually recovered but remained significantly lower than ChE levels in the control field or pre-exposure levels up to the 7th day after spraying. Given that pesticides were applied 5 to 8 times per crop (100 days) **(Toan & Cong, 2018)**, ChE in fish may not fully recover before subsequent pesticide applications, leading to continued exposure. Quinalphos may also persist in fish. **Thinh *et al.* (2018)** found that the half-life of quinalphos in integrated rice–fish fields in the MD was 2.5 and 1.1 days for silver barb and 1.9 and 1.3 days for common carp, with a bioconcentration factor (log BCF) above 2 for fish. Therefore, detecting quinalphos residues in snakehead fish is crucial for assessing the safety of the fish as food and the potential risk to humans consuming it.

CONCLUSION

The present study found that quinalphos was highly toxic to snakehead fish (4- 5g), with a 96-hour LC50 of 49µg/ L. The brain cholinesterase (ChE) of this species was particularly sensitive to quinalphos, with significant inhibition observed at a concentration of 0.49µg/ L (approximately 1% of the 96-hour LC50). Using Kinalux 25EC (containing 25% quinalphos by weight) on rice at the recommended dose had severe effects on snakehead fish, including high mortality and prolonged ChE inhibition. Even though quinalphos levels fell below the detection limit (0.1µg/ L) one day after spraying, the brain ChE activity of fish in the treated fields remained significantly lower than normal for up to seven days post-spray. This underscores the need to investigate quinalphos residues in soil and fish to better understand its distribution after application and to assess the safety of snakehead fish meat for consumption, as well as the broader impacts of quinalphos use on other aquatic organisms in the paddy ecosystem.

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