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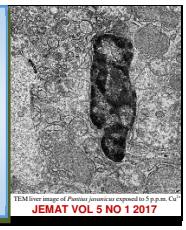

A Confidence Interval Comparison Chart to Assess the Sensitivity of Acetylcholinesterase from *Hemibagrus nemurus* as a Source of Enzyme for Insecticides Detection

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ABSTRACT

In this work, the potential of acetylcholinesterase (AChE) from *Hemibagrus nemurus* (Baung) as a sensitive tool to detect the presence of insecticides was assessed. The affinity partially purified fraction was utilized as a source of AChE for *in vitro* assay of the effect of insecticides on the AChE. The resultant IC_{50} values were then compared with published results. An IC_{50} confidence interval comparison chart coined Shukor's chart was utilized to compare the sensitivity of the AChE from *H. nemurus* to various other published results. In this chart, the IC_{50} confidence interval (95%) values for a particular insecticide is placed at one end of the chart and lined up with published values of IC_{50}. Two lines are then made crossing the entire chart and represent the 95% confidence interval value for *H. nemurus*. Any confidence interval values for targeted insecticides from published results that do not touch these two lines are deemed significantly different ($p<0.05$), either more or less sensitive judging whether they fall below or above the two lines, respectively, whilst any confidence interval values that fall in or touch the two lines are deemed comparable in sensitivity.The results show that the AChE from *H. nemurus* is more sensitive to diazinonoxon and comparable in sensitivity compared to commercial and other local fish sources suggesting that the ACHE from *H. nemurus* can be a cheaper alternative for the current commercially and locally available AChEs.

INTRODUCTION

The use of neurotoxic insecticides such as organophosphate (OP) and carbamate is worrying because of their inhibitive properties towards cholinesterase affecting the nervous systems [1]. Biomarkers such as aquatic organisms are a popular choice in detecting inhibition activity of toxic compounds on cholinesterases [2–4]. Snakehead (*Channa striata*), is an example of fish that has been used to test the effect of pesticides [5]. Native fish species in Malaysia such as *C. striatus, H. nemurus and C. lucius* [6] are similar to *C. striata* and may have the potential to be utilized as a biomarker of insecticides and pesticides. Other aquatic organisms used to detect pollution through the application of cholinesterases includes mussels and snails [7]. Primarily there are classes of cholinesterases, i.e. acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), that can be differentiated by using substrate specificity assay [8–14].

AChE will specifically hydrolyze acetylcholine at a faster rate compared to other cholinesterases and will not react towards butyrylcholinesterase. In contrast, BChE is reactive towards both butyrylcholine as well as acetylcholine at a lower rate [15]. Nonetheless, cholinesterase is a sensitive enzyme towards insecticides which can be utilized as handy and quick *in vitro* tools in the biomonitoring of environmental pollution. Thus, the

use of *H. nemurus* which is easily accessible can be a good alternative of local fish to be used in the detection of insecticide in water. The purpose of this study is to determine the level of sensitivity of *H. nemurus*'s AChE towards organophosphate and carbamate *in vitro*. The results collected will be compared to the sensitivity of AChE from *Electrophorus electricus*, which is a popular commercial choice currently.

MATERIALS AND METHODS

Chemicals

Commercial AChE from eel (*E. electricus*) of 349 units/mg in solid form, Biorad Protein assay and Vivaspin4 were bought from Sigma, Bio-Rad Laboratories Inc. and Vivascience. DTNB and BTC were purchased from Fluka Chemie GmbH. Acephate, bendiocarb, carbaryl, carbofuran, chlorpyrifos, diazinon, dimethoate, malathion, methomyl, parathion, and trichlorfon were bought from Dr Ehrenstorfer (Augsburg, Germany). ATC, PTC, bromine, procainamide hydrochloride, sodium borohydride, 1,4-butanediol diglycidyl ether and β-mercaptoethanol were bought from Sigma-Aldrich. Other chemicals used were of the special or analytical grade.

Specimen

In this study, *H. nemurus* was used as the trial organism. *H. nemurus* of 900-1200 g in weight and about 36 cm in length were acquired from a local aquaculture company in 2015, Selangor, Malaysia. The fish was decapitated, and the whole brain was collected immediately. Brain weighing approximately 1 gram was homogenized with 20% (w/v) of 0.1 M, pH 8 sodium phosphate buffer by using Ultra-Turrax T25 homogenizer fitted with a Teflon pestle. To remove and deactivate unwanted serine proteases phenylmethylsulfonyl fluoride was used. To remove debris, the brain suspension was then homogenized and centrifuged at 15 000xg for 10 min in 4 ºC. To separate cytosol and components of the membrane, the homogenate was spun for 100 000×g using Sorval® Ultra Pro 80-TH-641 within 1 h at 4 ºC. Finally, the supernatant was collected to be further purified.

Preparation of Affinity Chromatography Columns

Epoxy (Bisoxirane) Activation

A modified method was used to prepare affinity procainamide chromatography [16]. A 100 mL of Sephacryl S-1000 (settled gel, Sigma, St. Louis, USA) was briefly washed using 1000 mL of deionized water in sintered glass tunnel, dried and then transferred into 500 mL beaker. The gel was placed in a 75 mL solution of 0.6 M NaOH and 150 mg of sodium borohydride and stirred. An approximately 75 mL of 1,4-butanediol diglycidyl ether was then added slowly and stirred constantly.

The mixture was left to be stirred overnight at room temperature. To remove excess reagent, the activated gel was thoroughly washed using water until there is no presence of an oily film on the surface which indicates the presence of the epoxy compound. To completely remove bisoxirane groups, acetone was used. For ligand coupling, the gel was resuspended in water.

Ligand Coupling of Procainamide–Sephacryl S-1000 gel

Deionized water was used to wash the epoxy-activated Sephacryl S-1000 several bed volumes on a sintered glass filter. The gel slurry was then transferred into12 mM of borate buffer (pH 11.0) containing 0.2 M of procainamide. 1.0 M NaOH was added to adjust the pH to 12. Next, the mixture was incubated for 96 h at 25 °C on a shaking incubator. The gel was then washed in a sequence of 10 volumes each of 0.1 M sodium acetate (pH 4.5), 12 mM sodium borate (pH 10) and deionized water. To block excess active groups, the gel was suspended in 100 ml of 1.0 M ethanolamine, pH 9.0. The mixture was then stirred for a duration of 6 h at room temperature. The gel was finally washed with 1 L of 1.0 M NaCl and 5 L of deionized water.

Screening of carbamates and organophosphates as AChE inhibitor

Before the assay, OPs were first activated using the modified method from Villate et al. [15]. A volume of 25 µl of pesticide was incubated in 5 µl of 0.01 M pure bromine solution for 20 min at room temperature. To stop the activation process, 20 µl of 5% ethanol was added into the mixture. Initial testing showed that at a given concentration, bromine and ethanol did not inhibit the activity of AChE. At least five concentrations of carbamate and OPs were used to determine the half maximal inhibitory concentration (IC_{50}).

A mixture containing 150 µl of potassium phosphate buffer (0.1 M, pH 8.0), DTNB (20 µl, 0.067 mM), carbamate (50 µl) and enzymes (10 µl) were used for the assay. The mixture was then incubated for 10 min in the dark at room temperature. Then, 20 µl of 0.5 mM ATC was added and left to react for 10 min at room temperature before measuring the absorbance at 405 nm [4].

RESULTS AND DISCUSSION

The effect of insecticides on AChE activity

Screening of insecticides showed that all of the carbamates and the OPs inhibited the activity of the AChE. The lowest inhibition was shown by bendiocarb (33.6%) followed by carbofuran (23.9%), parathion oxon (15.3), chlorpyrifos oxon (15.1%), diazinon oxon (12.5%), carbaryl (6.8%), methomyl (4.5%) and malathion oxon (3.9%) (**Fig. 1.**). This concludes that the AChE was mostly affected by malathionoxon followed by methomyl, carbaryl, diazinonoxon, chlorpyrifosoxon, parathionoxon, carbofuran and bendiocarb. The IC_{50} values for these inhibiting insecticides are shown in comparison with other sources of AChE (**Table 1**).

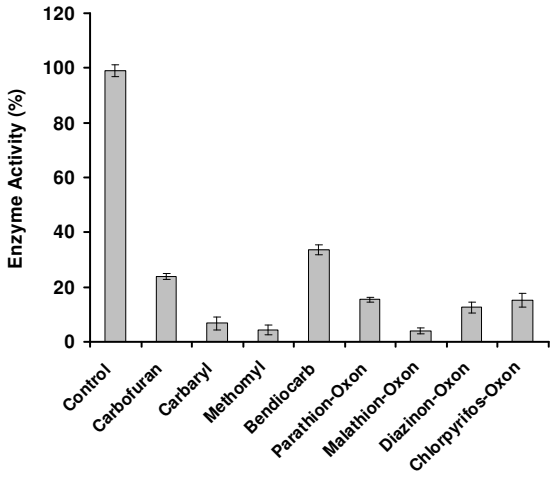

Fig. 1. Effect of various pesticides (1 mg/L) on partially purified AChE from *H. nemurus*. Data is a mean± standard error (n=3).

The significant difference is usually signified by a non-overlap of 95% confidence interval with $p<0.05$ level. The overlapped confidence interval, on the other hand, does not a necessary shows variation or not significant in differences at $p<0.05$ level. More data and study are necessary to assess the non-significance of overlapped confidence interval [17]. The

inhibition study on AChE from *H. nemurus* can be compared in terms of the sensitivity to carbamates and organophosphates from various published results (**Table 2**).

Table 2. Comparisons of the sensitivity of *H. nemurus* AChE to various insecticides in comparison to other fish AChEs.

Fish species	IC_{50} (mg/L) (95% Confidence Interval)								Ref.
	Carbo-furan	Carb-aryl	Metho-myl	Bendio-carb	Para-thion oxon	Mala-thion oxon	Diaz-inon oxon	Chlor-pyrifos oxon	
Electrop-horus electricus	0.0060 (0.0063-0.0065)	0.1330 (0.1220-0.1450)	0.0260 (0.0240-0.0280)	0.0150 (0.0150-0.0160)	0.0680 (0.0660-0.0690)	0.0140 (0.0130-0.0140)	0.1770 (0.1690-0.1860)	0.0600 (0.0550-0.0650)	[18]
Periophtal-modon schlosseri	0.0450 (0.0399-0.0517)	0.1124 (0.1025-0.1245)	0.0567 (0.0504-0.0648)	0.0633 (0.0537-0.0773)	Not done	Not done	Not done	Not done	[19]
Osteochilus hasselti	0.0550 (0.0515-0.0670)	0.0497 (0.0414-0.0620)	0.0845 (0.0747-0.0973)	0.0470 (0.0409-0.0553)	0.0660 (0.0580-0.0766)	0.0681 (0.0592-0.0802)	0.0991 (0.0906-0.1094)	0.0632 (0.0570-0.0709)	[18]
Pangasius sp.	0.006 (0.0058-0.0065)	0.061 (0.043-0.105)	0.016 (0.015-0.017)	0.012 (0.011-0.013)	0.047 (0.041-0.055)	0.011 (0.008-0.015)	0.081 (0.074-0.089)	0.029 (0.023-0.039)	[20] [21]
Channa micropeltes (Toman)	0.0081 (0.0074-0.0089)	0.07922 (0.0697-0.0917)	0.0192 (0.0178-0.0208)	0.0379 (0.0341-0.0427)	0.0316 (0.0279-0.0363)	0.0242 (0.0192-0.0327)	0.0599 (0.0554-0.0652)	0.0522 (0.0418-0.0693)	
Clarias batrachus	0.006(0.006-0.008)	0.130 (0.12-0.14)	Not done	Not done	Not done	Not done	Not done	Not done	[4]
Tor tambroides	0.0643 (0.0482 - 0.0966)	0.0555 (0.0439 - 0.0754)	0.0817 (0.0571-0.1438)	0.0758 (0.0582-0.109)	Not done	Not done	Not done	Not done	[22]
Puntius schwanen-feldii	1.411	7.045	8.335	0.838	Not done	Not done	Not done	Not done	[23]
Puntius javanicus	0.035 (0.030 - 0.045)	0.031 (0.026 - 0.040)	0.090 (0.077 - 0.108)	0.045 (0.039 - 0.054)	0.151 (0.122 - 0.198)	0.063 (0.053 - 0.078)	0.103 (0.084 - 0.132)	0.202 (0.178 - 0.232)	[24]
Hemibagrus nemurus	0.0554 (0.0450-0.0721)	0.080 (0.0619-0.01132)	0.0239 (0.0202-0.0293)	0.0991 (0.0873-0.115)	0.0463 (0.0411-0.0531)	0.0455 (0.0416-0.0501)	0.0392 (0.0354-0.0440)	0.0479 (0.0409-0.0578)	*This study*

Note: All values have a correlation coefficient of at least 0.95.

A visual comparison of whether the current AChE from *H. nemurus* is more sensitive to certain insecticides compared to publish AChEs from other fish is shown in the form of a Shukor's chart (**Figs 2** to **9**). In this chart, the IC_{50} confidence interval (95%) values for carbofuran (Fig. A) from *H. nemurus* is placed at one end of the chart and lined up with published values of IC_{50}. Two lines are then made crossing the entire chart and represent the 95% confidence interval value for *H. nemurus*. Any confidence interval values for targeted insecticides from published results that do not touch these two lines are deemed significantly different ($p<0.05$), either more or less sensitive judging whether they fall below or above the two lines, respectively, whilst any confidence interval values that fall in or touch the two lines are deemed comparable in sensitivity.

Based on this premise, the AChEs from *E. electricus* (EE), *Pangasius* sp. (P), *Channa micropeltes* (CM) and *Clarias batrachus* (CB) showed a greater sensitivity to carbofuran in comparison to *H.nemurus* whilst the rest of the fish species harbours AChEs that exhibit comparable sensitivity to carbofuran to the AChE from *H. nemurus* (**Fig. 2**).

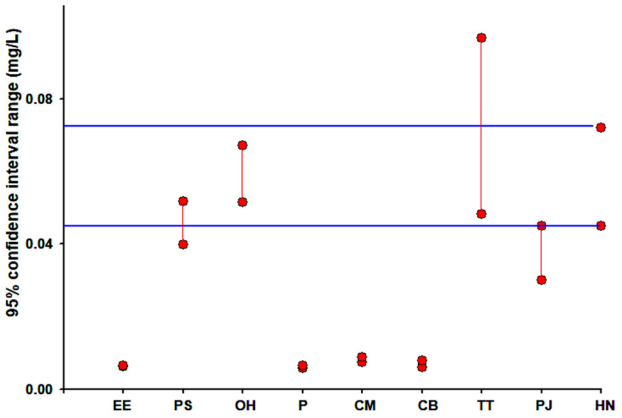

Fig. 2. Comparison of the confidence interval (95%) for the IC_{50} value of AChE's to carbofuran from various fish species (published) including *Electrophorus electricus* (EE), *Periophtalmodon schlosseri* (PS), *Osteochilus hasselti* (OH), *Pangasius* sp. (P), *Channa micropeltes* (CM), *Clarias batrachus* (CB), *Tor tambroides* (TT), and *Puntius javanicus* (PJ) to *H. nemurus* (HN). Nonoverlap confidence interval values are considered statistically significant ($p<0.05$).

Based on the Shukor's chart for carbaryl (**Fig. 3**), the AChEs from *H. nemurus* is more sensitive to carbaryl than the AChEs from *E. electricus* (EE), *Periophtalmodon schlosseri* (PS), *Channa micropeltes* (CM) and *Clarias batrachus* (CB) and comparable in sensitivity to the AChEs from the rest of the fish species.

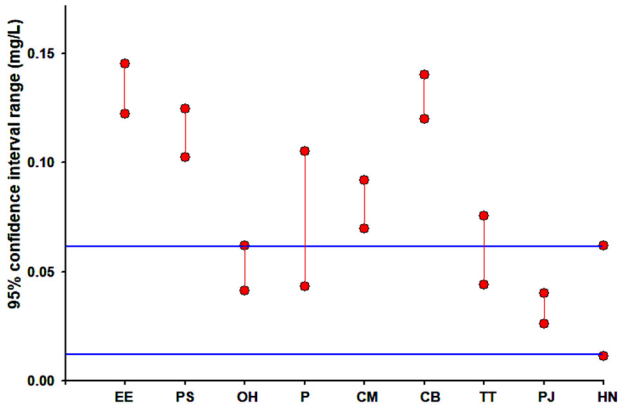

Fig. 3. Comparison of the confidence interval (95%) for the IC_{50} value of AChE's to carbaryl from various fish species (published) including *Electrophorus electricus* (EE), *Periophtalmodon schlosseri* (PS), *Osteochilus hasselti* (OH), *Pangasius* sp. (P), *Channa micropeltes* (CM), *Clarias batrachus* (CB), *Tor tambroides* (TT), and *Puntius javanicus* (PJ) to *H. nemurus* (HN). Nonoverlap confidence interval values are considered statistically significant ($p<0.05$).

Based on the Shukor's chart for methomyl (**Fig. 4**), the AChEs from *H. nemurus* is more sensitive to this insecticide than the AChEs from *Periophtalmodon schlosseri* (PS), *Osteochilus hasselti* (OH), *Tor tambroides* (TT), and *Puntius javanicus* (PJ). The AChEs from *H. nemurus* is less sensitive to methomyl than the AChEs from *Puntius javanicus* (PJ) and comparable in sensitivity to the AChEs from *E. electricus* (EE) and *Channa micropeltes* (CM).

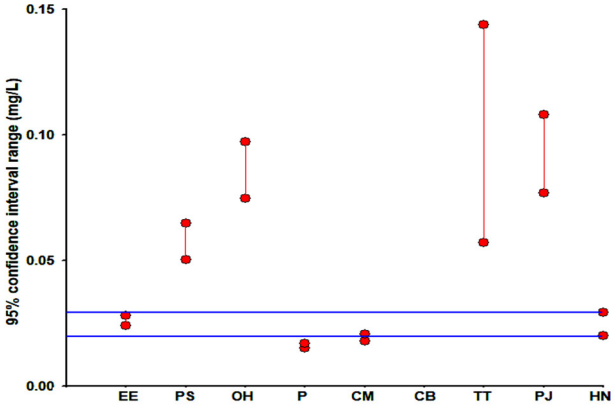

Fig. 4. Comparison of the confidence interval (95%) for the IC_{50} value of AChE's to methomyl from various fish species (published) including *Electrophorus electricus* (EE), *Periophtalmodon schlosseri* (PS), *Osteochilus hasselti* (OH), *Pangasius* sp. (P), *Channa micropeltes* (CM), *Clarias batrachus* (CB), *Tor tambroides* (TT), and *Puntius javanicus* (PJ) to *H. nemurus* (HN). Nonoverlap confidence interval values are considered statistically significant ($p<0.05$). Missing confidence interval values indicates that data for the particular fish species is not available.

Based on the Shukor's chart for bendiocarb (**Fig. 5**), the AChEs from *H. nemurus* is less sensitive to this insecticide than the AChEs from *Electrophorus electricus* (EE), *Periophtalmodon schlosseri* (PS), *Osteochilus hasselti* (OH), *Osteochilus hasselti* (OH), *Pangasius* sp. (P), *Channa micropeltes* (CM) and *Puntius javanicus* (PJ). The AChEs from *H. nemurus* is comparable in sensitivity to the AChE from *Tor tambroides* (TT).

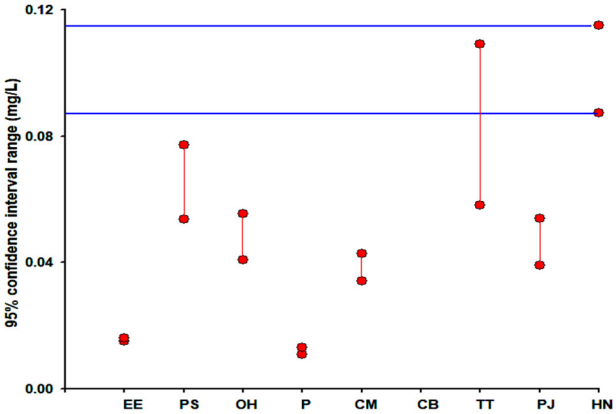

Fig. 5. Comparison of the confidence interval (95%) for the IC_{50} value of AChE's to bendiocarb from various fish species (published) including *Electrophorus electricus* (EE), *Periophtalmodon schlosseri* (PS), *Osteochilus hasselti* (OH), *Pangasius* sp. (P), *Channa micropeltes* (CM), *Clarias batrachus* (CB), *Tor tambroides* (TT), and *Puntius javanicus* (PJ) to *H. nemurus* (HN). Nonoverlap confidence interval values are considered statistically significant ($p<0.05$). Missing confidence interval values indicates that data for the particular fish species is not available.

Based on the Shukor's chart for parathionoxon (**Fig. 6**), the AChEs from *H. nemurus* is more sensitive to this insecticide than the AChEs from *Electrophorus electricus* (EE), and *Osteochilus hasselti* (OH). The AChEs from *H. nemurus* is less sensitive to parathionoxon than the AChE from *Channa micropeltes* (CM) and comparable in sensitivity to the AChEs from *Pangasius* sp. (P).

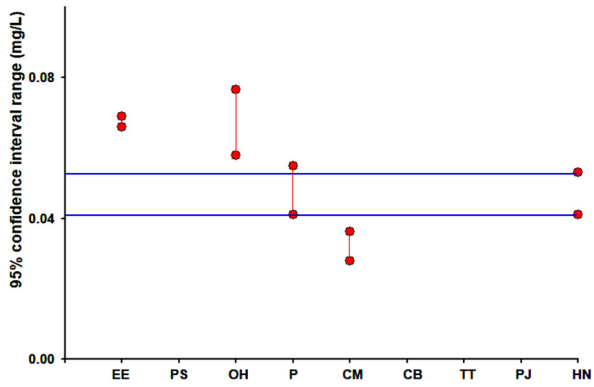

Fig. 6. Comparison of the confidence interval (95%) for the IC_{50} value of AChE's to parathionoxon from various fish species (published) including *Electrophorus electricus* (EE), *Periophtalmodon schlosseri* (PS), *Osteochilus hasselti* (OH), *Pangasius* sp. (P), *Channa micropeltes* (CM), *Clarias batrachus* (CB), *Tor tambroides* (TT), and *Puntius javanicus* (PJ) to *H. nemurus* (HN). Nonoverlap confidence interval values are considered statistically significant ($p<0.05$). Missing confidence interval values indicates that data for the particular fish species is not available.

Based on the Shukor's chart for malathionoxon (**Fig. 7**), the AChEs from *H. nemurus* is more sensitive to this insecticide than the AChEs from *Osteochilus hasselti* (OH) and *Puntius javanicus* (PJ). The AChEs from *H. nemurus* is less sensitive to malathionoxon than the AChEs from *Electrophorus electricus* (EE), *Pangasius* sp. (P) and *Channa micropeltes* (CM).

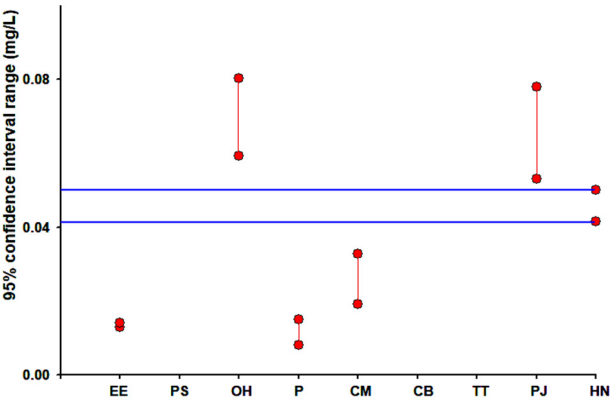

Fig. 7. Comparison of the confidence interval (95%) for the IC_{50} value of AChE's to malathionoxon from various fish species (published) including *Electrophorus electricus* (EE), *Periophtalmodon schlosseri* (PS), *Osteochilus hasselti* (OH), *Pangasius* sp. (P), *Channa micropeltes* (CM), *Clarias batrachus* (CB), *Tor tambroides* (TT), and *Puntius javanicus* (PJ) to *H. nemurus* (HN). Nonoverlap confidence interval values are considered statistically significant ($p<0.05$). Missing confidence interval values indicates that data for the particular fish species is not available.

Based on the Shukor's chart for diazinonoxon (**Fig. 8**), the AChEs from *H. nemurus* is more sensitive to this insecticide than the AChEs from *Osteochilus hasselti* (OH), *Pangasius* sp. (P), *Channa micropeltes* (CM) and *Puntius javanicus* (PJ)

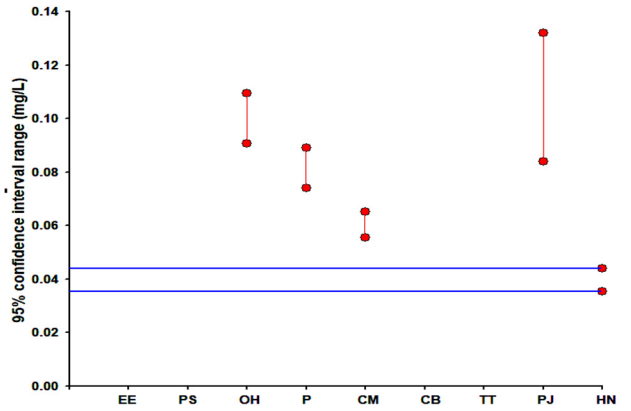

Fig. 8. Comparison of the confidence interval (95%) for the IC_{50} value of AChE's to diazinonoxon from various fish species (published) including *Electrophorus electricus* (EE), *Periophtalmodon schlosseri* (PS), *Osteochilus hasselti* (OH), *Pangasius* sp. (P), *Channa micropeltes* (CM), *Clarias batrachus* (CB), *Tor tambroides* (TT), and *Puntius javanicus* (PJ) to *H. nemurus* (HN). Nonoverlap confidence interval values are considered statistically significant ($p<0.05$). Missing confidence interval values indicates that data for the particular fish species is not available.

Based on the Shukor's chart for chlorpyrifosoxon (**Fig. 9**), the AChEs from *H. nemurus* is less sensitive to this insecticide than the AChEs from *Pangasius* sp. (P). The AChEs from *H. nemurus* is comparable in sensitivity to the AChEs from *Electrophorus electricus* (EE), *Osteochilus hasselti* (OH), *Pangasius* sp. (P) and *Channa micropeltes* (CM).

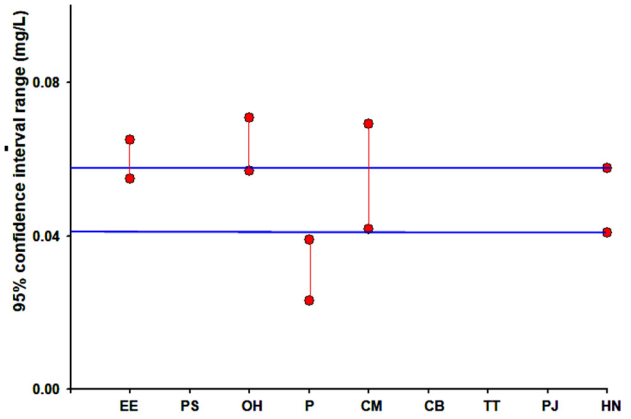

Fig. 9. Comparison of the confidence interval (95%) for the IC_{50} value of AChE's to chlorpyrifosoxon from various fish species (published) including *Electrophorus electricus* (EE), *Periophtalmodon schlosseri* (PS), *Osteochilus hasselti* (OH), *Pangasius* sp. (P), *Channa micropeltes* (CM), *Clarias batrachus* (CB), *Tor tambroides* (TT), and *Puntius javanicus* (PJ) to *H. nemurus* (HN). Nonoverlap confidence interval values are considered statistically significant ($p<0.05$). Missing confidence interval values indicates that data for the particular fish species is not available.

Hemibagrus species is an excellent species to be explored as it is abundant in Southeast Asia including Malaysia. The fish is large in size with high nutritional values is an important source of food and has been aquacultured in Peninsular Malaysia [25]. From this study, the results obtained will be important as baseline data to be compared with different species of local fish and useful for tool development to assay the aquatic pollution from insecticides. The custom-made procainamide based affinity gel used for the purification in this study is an effective technique for partial purification and has been used in other studies [26–28].

The data obtained from the primary screening proved that AChE from *H. nemurus* could be used as a sensitive inhibitive assay for insecticides. The organophosphates were fully oxidized using the bromine oxidation technique. Oxonation using bromine is only limited to OP compounds that require oxidative desulfuration in order to be activated. All of the insecticides tested in this study are also toxic in *vivo* studies, reflecting the relevance of the *in vitro* toxicity assay developed in this study as a rapid assay to detect these insecticides from food or the environment.

Between the CBs, carbofuran is a common and systemic insecticide used globally. Because of its widespread application, carbofuran is a presence in surface, ground and rain water [17]. In Malaysia, carbofuran is used in oil palm plantation, paddy field, fruits and vegetables. Thus the effort to monitor its level in the environment is very important [29]. Carbaryl, another type of CBs is used for the seasonal treatment of oyster beds thus causes concern about the negative impacts that may affect the aquatic environment [30]. A study reported that the carbaryl causes interference to the nervous system in trout through inhibition of acetylcholinesterase. In this study, they reported that the olfactory system of trout is unresponsive to carbaryl, the acetylcholinesterase activity in both brain and muscle were significantly reduced affecting swimming orientation and finally increase death by predation due to the neurobehavioral destruction [31].

The effects of carbamates such as methomyl are also been studied on amphibians; a study reported severe damage on the liver and kidney tadpoles as well as a reduction in growth, metamorphosis period, size and the alteration in biochemical parameters [32]. From the insecticide screening, bendiocarb shows the least inhibition effect compared to other insecticides. A study on the toxicity of agricultural pesticides on selected aquatic species shows that bendiocarb is moderately toxic to blue gill and rainbow trout [33]. Carbamates could be more potent than OPs for in vitro studies. This is because carbamates are reversible while most OPs are non-reversible inhibitors for AChE, this means with OPs, structural conformation through covalent modifications causes the inhibition of enzyme activity to be irreversible. Some OPs are produced in the environment in a less toxic thion form that is more stable.

Organophosphates require activation by oxonation and are not toxic as an individual compound. This is achieved using bromine water and enzymes for in vitro and in vivo testing. Once OPs are absorbed into an organism, they are generally transformed into metabolites which are usually more toxic than the parent compounds or directly induced the enzymes of interest or organs [34]. Malathion which highly inhibited the AChE of *H. nemurus* in this study is usually used for mosquito control and combat agricultural pests. This insecticide has been shown to cause histopathological changes in the brain, liver, ovary, tissues as well as loss of equilibrium due to AChE inhibition in *Ophiocephalus punctatus* [35].

Organophosphate such as parathion has been found to be more toxic as compared to carbosulfan and disrupt the cholinesterases not only in the brain but also in other vital tissues

of *Cirrhinus mrigala* [36]. Other type of OPs, which is Chlorpyrifos is used widely in pest control [37]. Once taken up, it is oxidized by monooxygenase enzymes into chlorpyrifos-oxon which is a toxic form metabolite [38]. Consequently, the effects of most of the pesticides on acetylcholinesterase (AChE) activity are considered to be an irreversible action since the time of re-synthesis of the enzyme is naturally longer than the duration of dissociation of the OP-complex [39].

The compound will phosphorylate the active site of AChE containing the hydroxyl group forming an inactive AChE [40]. Chlorpyrifosoxon and diazinonoxon could cause 10–1000 times stronger effects than those of their parental forms chlorpyrifos and diazinon, respectively [41]. Diazinon is known to be toxic *in vivo* to *Oncorhynchus mykiss* (rainbow trout), *Poecilia reticulata* (guppy), *Brachydanio rerio* (zebra fish) and *Cyprinus carpio* (carp), and guppy exhibited the strongest toxicity due to it having the highest rate of bioactivation of diazinon [42].

CONCLUSION

As a conclusion, the results for the *in vitro* IC_{50} values of most of the AChE from the different species indicated that the AChE from *H. nemurus* is comparable in sensitivity to most of the AChE from local fish species including from the commercial *E. electricus* suggesting that the AChE from *H. nemurus* can be an excellent and cheaper alternative for *E. electricus* as AChE source for the development of biosensor similar to other local fish species [24,43–45]. The importance of screening for more sensitive AChE towards insecticides is because these insecticides display variable sensitivity to the AChEs from fish species including the commercial AChE. For cheap and routine monitoring, the use of two or more AChEs is probably needed to cover the sensitivity required for food and environmental monitoring purposes.

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REFERENCES

1. Gill KD, Flora G, Pachauri V, Flora SJ. Neurotoxicity of organophosphates and carbamates. Hoboken, NJ, USA: John Wiley & Sons, Inc.; 2011. 237–265 p.
2. De la Torre FR., Ferrari L. b, Salibián A. b c. Freshwater pollution biomarker: Response of brain acetylcholinesterase activity in two fish species. Comp Biochem Physiol - C Toxicol Pharmacol. 2002;131(3):271–80.
3. Shaonan L, Xianchuan X, Guonian Z, Yajun T. Kinetic characters and resistance to inhibition of crude and purified brain acetylcholinesterase of three freshwater fishes by organophosphates. Aquat Toxicol. 2004;68(4):293–9.
4. Tham LG, Perumal N, Syed MA, Shamaan NA, Shukor MY. Assessment of *Clarias batrachus* as a source of acetylcholinesterase (AChE) for the detection of insecticides. J Environ Biol. 2009;30(1):135–8.
5. Van Cong N, Phuong NT, Bayley M. Sensitivity of brain cholinesterase activity to diazinon (BASUDIN 50EC) and fenobucarb (BASSA 50EC) insecticides in the air-breathing fish *Channa striata* (Bloch, 1793). Environ Toxicol Chem. 2006;25(5):1418–1425.
6. Esa YB, Japning JRR, Rahim KAA, Siraj SS, Daud SK, Tan SG, et al. Phylogenetic relationships among several freshwater fishes (Family: Cyprinidae) in Malaysia inferred from partial sequencing of the cytochrome b mitochondrial DNA (mtDNA) gene. Pertanika J Trop Agric Sci. 2012;35:307–318.
7. Chitmanat C, Prakobsin N, Chaibu P, Traichaiyaporn S. The use of acetylcholinesterase inhibition in river snails (*Sinotaia ingallsiana*) to determine the pesticide contamination in the upper Ping River. Int J Agric Biol. 2008;10(6):658–60.
8. Radic Z, Reiner E, Taylor P. Role of the peripheral anionic site on acetylcholinesterase: Inhibition by substrates and coumarin derivatives. Mol Pharmacol. 1991;39(1):98–104.
9. Begum G, Vijayaraghavan S. Alterations in protein metabolism of muscle tissue in the fish *Clarias batrachus* (Linn) by commercial grade dimethoate. Bull Environ Contam Toxicol. 1996;57(2):223–8.
10. Venkateswara RJ, Begum G, Pallela R, Usman PK, Nageswara RR. Changes in behavior and brain acetylcholinesterase activity in mosquito fish, *Gambusia affinis* in response to the sub-lethal exposure to chlorpyrifos. Int J Environ Res Public Health. 2005;2(3–4):478–83.
11. Begum G. Enzymes as biomarkers of cypermethrin toxicity: Response of *Clarias batrachus* tissues ATPase and glycogen phosphorylase as a function of exposure and recovery at sublethal level. Toxicol Mech Methods. 2009;19(1):29–39.
12. Narra MR, Begum G, Rajender K, Venkateswara RJ. Sub-lethal effect of chlorpyrifos on protein metabolism of the food fish *Clarias batrachus* and monitoring of recovery. Toxicol Environ Chem. 2011;93(8):1650–8.
13. Narra MR, Rajender K, Rudra RR, Rao JV, Begum G. The role of vitamin C as antioxidant in protection of biochemical and haematological stress induced by chlorpyrifos in freshwater fish *Clarias batrachus*. Chemosphere. 2015;132:172–8.
14. Tham LG, Halmi MIE, Sharif MSA, Perumal N, Begum G, Syed MA, et al. Sensitivity of crude and partially purified acetylcholinesterase from fish to carbamates and organophosphates. J Environ Biol. 2014;35(1):285–7.
15. Villatte F, Marcel V, Estrada-Mondaca S, Fournier D. Engineering sensitive acetylcholinesterase for detection of organophosphate and carbamate insecticides. Biosens Bioelectron. 1998;13(2):157–64.
16. Ralston JS, Main AR, Kilpatrick BF, Chasson AL. Use of procainamide gels in the purification of human and horse serum cholinesterases. Biochem J. 1983;211:243–50.
17. Schenker N, Gentleman JF. On Judging the Significance of Differences by Examining the Overlap Between Confidence Intervals. Am Stat. 2001;55(3):182–6.
18. Sabullah K, Ahmad SA, Ishak I, Sulaiman MR, Shukor MY, Syed MA, et al. An inhibitive assay for insecticides using the acetylcholinesterase from *Osteochillus hasselti*. Bull Environ Sci Manag. 2013;1(1):1–4.
19. Sabullah MK, Ahmad SA, Shukor MY, Syed MA, Shamaan NA. The evaluation of *Periophtalmodon schlosseri* as a source of acetylcholinesterase for the detection of insecticides. Bull Environ Sci Manag. 2013;1(1):20–4.
20. Shukor MS, Sulaiman MR. Assessment of acetylcholinesterase (AChE) from silver catfish (*Pangasius* sp.) as an assay for organophosphates and carbamates. Biosci Biotechnol Res Asia. 2013;10(1):213–8.
21. Sharif MSA, Halmi MIE, Syahir A, Johari WLW, Shukor MY. Assessment of acetylcholinesterase from *Channa micropeltes* as a source of enzyme for insecticides detection. Int J Agric Biol. 2014;16(2):389–94.
22. Ahmad SA, Sabullah MK, Shamaan NA, Abd Shukor MY, Jirangon H, Khalid A, et al. Evaluation of acetylcholinesterase source from fish, *Tor tambroides* for detection of carbamate. J Environ Biol Acad Environ Biol India. 2016 Jul;37(4):479–84.
23. Sabullah MK, Khayat ME. Assessment of inhibitive assay for insecticides using acetylcholinesterase from *Puntius schwanenfeldii*. J Biochem Microbiol Biotechnol. 2015;3(2):26–29.
24. Sabullah MK, Ahmad SA, Shukor MY, Shamaan NA, Khalid A, Gansau AJ, et al. Acetylcholinesterase from *Puntius javanicus* for the detection of carbamates and organophosphates. J Chem Pharm Sci. 2015;8(2):348–53.
25. Adebiyi FA, Siraj SS, Harmin SA, Christianus A. Induced spawning of a river catfish *Hemibagrus nemurus* (Valenciennes, 1840). Pertanika J Trop Agric Sci. 2013;36(1).

26. Talesa V, Contenti S, Principato GB, Pascolini R, Giovannini E, Rosi G. Cholinesterases from *Maia verrucosa* and *Palinurus vulgaris*: A comparative study. Comp Biochem Physiol - C Pharmacol Toxicol Endocrinol. 1992;101(3):499–503.
27. Forget J, Bocquené G. Partial purification and enzymatic characterization of acetylcholinesterase from the intertidal marine copepod *Tigriopus brevicornis*. Comp Biochem Physiol - B Biochem Mol Biol. 1999;123(4):345–50.
28. Gao J-R, Zhu KY. An acetylcholinesterase purified from the greenbug (*Schizaphis graminum*) with some unique enzymological and pharmacological characteristics. Insect Biochem Mol Biol. 2001;31(11):1095–104.
29. Farahani GHN, Zakaria Z, Kuntom A, Omar D, Ismail BS. Persistence of carbofuran in two Malaysian soils. Plant Prot Q. 2008;23(4):179–83.
30. Begum G. Assessment of biochemical markers of carbofuran toxicity and recovery response in tissues of the freshwater teleost, *Clarias batrachus* (Linn). Bull Environ Contam Toxicol. 2008;81(5):480–4.
31. Labenia JS, Baldwin DH, French BL, Davis JW, Scholz NL. Behavioral impairment and increased predation mortality in cutthroat trout exposed to carbaryl. Mar Ecol Prog Ser. 2007;329:1–11.
32. Trachantong W, Saenphet S, Saenphet K, Chaiyapo M. Lethal and sublethal effects of a methomyl-based insecticide in Hoplobatrachus rugulosus. J Toxicol Pathol. 2017;30:15–24.
33. Morgan ER, Brunson MW. Toxicities of Agricultural Pesticides to Selected Aquatic Organisms. South Reg Aquac Cent. 2002;4600:1–28.
34. Belden JB, Lydy MJ. Impact of Atrazine on Organophosphate Insecticide Toxicity. Environ Toxicol Chem. 2000;19(9):2266–74.
35. Pugazhvendan SR, Narendiran NJ, Kumaran RG, Kumaran S, Alagappan KM. Effect of malathion toxicity in the freshwater fish *Ophiocephalus punctatus*-A histological and histochemical study. World J Fish Mar Sci. 2009;1(3):218–224.
36. Ghazala, Mahboob S, Sultana S, Sultana T, Ahmad L, Asi MR. Cholinesterases: Cholinergic biomarkers for the detection of sublethal effects of organophosphorous and carbamates in *Catla catla*. Int J Agric Biol. 2014;16(2):406–10.
37. Sparling DW, Fellers G. Comparative toxicity of chlorpyrifos, diazinon, malathion and their oxon derivatives to larval *Rana boylii*. Environ Pollut. 2007;147(3):535–539.
38. Barata C, Solayan A, Porte C. Role of B-esterases in assessing toxicity of organophosphorus (chlorpyrifos, malathion) and carbamate (carbofuran) pesticides to *Daphnia magna*. Aquat Toxicol. 2004;66(2):125–139.
39. Bocquene G, Galgani F, Truquet P. Characterization and assay conditions for use of AChE activity from several marine species in pollution monitoring. Mar Environ Res. 1990;30(2):75–89.
40. Kousba AA, Sultatos LG, Poet TS, Timchalk C. Comparison of Chlorpyrifos-Oxon and Paraoxon Acetylcholinesterase Inhibition Dynamics: Potential Role of a Peripheral Binding Site. Toxicol Sci. 2004;80:239–248.
41. Flaskos J. The developmental neurotoxicity of organophosphorus insecticides: A direct role for the oxon metabolites: Mini review. Toxicol Lett. 2012;209:86– 93.
42. Keizer J, D'Agostinoa G, Nagel R, Volpe T, Gnemi P, Vittozzi L. Enzymological differences of AChE and diazinon hepatic metabolism: correlation of in vitro data with the selective toxicity of diazinon to fish species. Sci Total Environ. 1995;171:213–20.
43. Hayat NM, Shamaan NA, Sabullah MK, Shukor MY, Syed MA, Khalid A, et al. The use of *Lates calcarifer* as a biomarker for heavy metals detection. Rendiconti Lincei. 2016;1–10.
44. Hayat NM, Shamaan NA, Shukor MY, Sabullah MK, Syed MA, Khalid A, et al. Cholinesterase-based biosensor using *Lates calcarifer* (Asian Seabass) brain for detection of heavy metals. J Chem Pharm Sci. 2015;8(2):376–81.
45. Ahmad SA, Wong YF, Shukor MY, Sabullah MK, Yasid NA, Hayat NM, et al. An alternative bioassay using *Anabas testudineus* (Climbing perch) colinesterase for metal ions detection. Int Food Res J. 2016;23(4):1446–52.