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封面图说:相当数量的降雪与低温严寒是冰川发育的主要因素,地球上的冰川除南北两极外,只有在高海拔的寒冷山地才能存在。喜马拉雅山造山运动使中国成为了世界上中低纬度冰川最为发育的国家,喜马拉雅山地区雪峰连绵、冰川广布,共有现代冰川17000多条,是世界冰川发育的中心之一。

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Fang G F. Toxicity of three pesticides and their effects on carboxylesterase activity of *Propsilocerus akamusi*. Acta Ecologica Sinica, 2011, 31 (17): 4914-4918.

三种农药对红裸须摇蚊毒力和羧酸酯酶活性的影响

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摘要: 摆蚊是一种世界性分布的水生昆虫, 常作为监测水环境污染指示生物。以红裸须摇蚊为对象, 测定了氧化乐果、毒死蜱和三氟氯氰菊酯对红裸须摇蚊4龄幼虫毒力和体内羧酸酯酶活性的影响。结果表明, 氧化乐果、毒死蜱和三氟氯氰菊酯12 h致死中浓度 LC_{50} 分别为12.508、2.478和0.046 μg/L。低浓度(0.05 μg/L)氧化乐果处理3 h和12 h以及各浓度(除8 μg/L)氧化乐果处理48 h, 摆蚊体内羧酸酯酶活性均高于对照, 表现为诱导作用, 其余各浓度各处理时间均表现为抑制减少。除0.125 μg/L和0.25 μg/L毒死蜱处理12 h对羧酸酯酶有诱导作用外, 毒死蜱均抑制羧酸酯酶活性; 而三氟氯氰菊酯也均抑制羧酸酯酶活性。因此, 摆蚊羧酸酯酶可作为一种监测农药污染的生物化学标志物加以利用。

关键词: 摆蚊; 农药; 羧酸酯酶(CarE); 毒力; 活性

Toxicity of three pesticides and their effects on carboxylesterase activity of *Propsilocerus akamusi*

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Abstract: Chironomids are a globally-distributed family of insects that can serve as biological indicators of environmental pollution. Pollution due to pesticide usage is of particular importance due to the heavy application of these chemicals. Three routinely-applied insecticides, omethoate, chlorpyrifos, and cyhalothrin, were selected for our study to investigate their toxicity against *Propsilocerus akamusi* and to determine their effects on carboxylesterase activity of 4th-instar larvae. After 12 h of exposure, the LC_{50} values of omethoate, chlorpyrifos and cyhalothrin were 12.508, 2.478, and 0.046 μg/L, respectively, indicating cyhalothrin has the highest level of toxicity to *P. akamusi*. The omethoate induced carboxylesterase activity at 3 and 12 h post-application when *P. akamusi* was challenged with 0.05 μg/L. When challenged with the higher rates, 0.05, 0.125, 0.25, 0.5, 1, 2 and 4 μg/L of omethoate, carboxylesterase activity was found to increase after 48 h. In addition, carboxylesterase activity was inhibited at 24 h and 48 h for the 0.05 μg/L of omethoate while enzyme inhibition was observed for the earlier time points of 3, 12 and 24 h for all doses tested. The inhibition carboxylesterase activity ranged from 5.149%–50.587% and 3.225%–36.403% under treatments with 0.125, 0.25, 0.5, 1, 2, 4, and 8 μg/L at 3 and 12 h, respectively. Overall, at 24 h, the inhibition of carboxylesterase activity by omethoate ranged from 20.441%–48.523%. In comparison, when chlorpyrifos was applied with the concentration of 0.125, 0.25, 1, 2 and 4 μg/L, the level of carboxylesterase was inhibited dramatically after 3 h treatment. This trend was also found at 12 h with the exception of the lower concentrations of 0.125 and 0.25 μg/L. Overall, the inhibition of carboxylesterase was found by the treatment of chlorpyrifos with the concentration of 0.125, 0.25, 1, 2 and 4 μg/L ranged from 14.145%–51.254% at 24 h and 9.772%–39.659% at 48 h. For the cyhalothrin test, carboxylesterase activity was inhibited by 0.00625, 0.0125, 0.05,

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0.25, and 1 μg/L of cyhalothrin treatments at 3, 12, 24, and 48 h. The magnitude of the inhibition caused by cyhalothrin was found to be decreased following the cyhalothrin concentrations and treatment time. The differences in the carboxylesterase activities caused by omethoate, chlorpyrifos, and cyhalothrin indicated different responses of *P. akamusi* to different dose and time of these insecticides. The poisoning symptoms also indicated differences between the three insecticides tested. The symptoms of *P. akamusi* to omethoate and chlorpyrifos poisons in *P. akamusi* were overall excitation followed by a loss of consciousness and ultimately death. The poisoning symptoms of *P. akamusi* to cyhalothrin, however, showed an initial increase in activity followed by a period of inactivity, apparent recovery and finally death. These results suggest that the carboxylesterase activity of chironomids can be used as a biochemical marker to monitor pesticide pollution.

Key Words: chironomid; pesticide; carboxylesterase (CarE); toxicity; activity

化学农药的大量使用,已对生态环境特别是水环境造成很多负面影响。农药进入水体后,除直接毒杀水生生物外,其在食物链上传递和富集,最终影响人类健康^[1]。目前,有关农药对水生生物的影响主要集中在对测试生物的急性毒性和抗氧化防御酶方面^[2-5],对水生生物解毒酶系影响的研究还很少。本文以红裸须摇蚊为模式生物,在研究农业生产上常用的氧化乐果、毒死蜱和三氟氯氰菊酯对摇蚊幼虫急性毒性基础上,进一步研究了3种农药对羧酸酯酶活性影响的剂量时间效应,旨在探讨羧酸酯酶作为监测有机磷和拟除虫菊酯农药污染的生物化学标志物的可能性。

1 材料和方法

1.1 供试昆虫

红裸须摇蚊(*Propsilocerus akamusi* Tokunaga)购自哈尔滨大发花鸟虫鱼市场,于室内用暴氯3 d的自来水(pH 7.58;溶解氧6.02 mg/L;总硬度80 mg/L),水温(25±1)℃,自然光照饲养,驯化3d后挑选健壮、大小一致的4龄幼虫用于测定。

1.2 农药与试剂

40% 氧乐果乳油(天津农药研究所);48% 毒死蜱乳油(商品名:陶丝本,东莞市瑞德丰生物科技有限公司);5% 三氟氯氰菊酯微乳剂(商品名:瑞功,东莞市瑞德丰生物科技有限公司);固蓝B盐(Fast Blue B Salt)购自美国Sigma公司;α-乙酸萘酯(α-NA),考马斯亮蓝G-250,牛血清白蛋白(BSA),购自国药集团化学试剂有限公司。

1.3 摆蚊毒性测定及致毒处理

采用药液培养法进行毒性测定。将氧化乐果、毒死蜱和三氟氯氰菊酯用蒸馏水分别配成8个浓度梯度,以蒸馏水为空白对照,将健壮、大小一致的摇蚊4龄幼虫放入盛有50 mL药液的透明塑料杯中,每处理20头,3个重复,于12 h后统计死亡数,以探针触动摇蚊尾部不动弹视为死亡。另将健壮、大小一致的4龄摇蚊幼虫分别放入0.05、0.125、0.25、0.5、1、2、4、8 μg/L 氧化乐果;0.125、0.25、1、2、4 μg/L 毒死蜱和0.00625、0.0125、0.05、0.25、1 μg/L 三氟氯氰菊酯药液中,致毒处理3、12、24和48 h后取样,随机挑取活泼的幼虫20头,蒸馏水润洗,冷冻于-80℃冰箱中用于蛋白质含量和羧酸酯酶活性测定。

1.4 羧酸酯酶(CarE)活性测定

取如1.3方法处理的摇蚊4龄幼虫,加入1 mL 0.04 mol/L 预冷的磷酸盐缓冲液(pH 7.0),冰浴匀浆,4℃,12000r/min离心15 min后取上清,抽滤后即为粗酶液。取0.05 mL待测酶液,2 mL 3×10⁻⁴ mol/L α-NA混匀,于30℃水浴保温15 min,加入1 mL显色剂(1% 固蓝B盐:5% 十二烷基硫酸钠;2:5)终止反应,于600nm波长下测定光吸收值。酶活性以每mg蛋白质每min分解底物的纳摩尔数(nmol·min⁻¹·mg⁻¹蛋白质)表示。蛋白质含量测定参照Bradford的考马斯亮蓝G-250法^[6]。

1.5 数据分析

采用 POLO 软件计算农药对摇蚊幼虫致死中浓度 LC_{50} 及 95% 置信区间。运用 GraphPad InStat 软件对同一时间处理下不同浓度对 CarE 活性影响差异采用 Tukey 方法进行比较分析, 比较的显著水平为 $P=0.05$ 。

2 结果与分析

2.1 3 种农药对摇蚊幼虫毒力

氧化乐果、毒死蜱和三氟氯氰菊酯对摇蚊 4 龄幼虫毒力如表 1 所示。氧化乐果、毒死蜱和三氟氯氰菊酯对幼虫 12 h 致死中浓度 LC_{50} 分别为 12.508 $\mu\text{g}/\text{L}$ 、2.478 $\mu\text{g}/\text{L}$ 和 0.046 $\mu\text{g}/\text{L}$, 毒力大小依次为三氟氯氰菊酯 > 毒死蜱 > 氧化乐果, 说明 3 种常用农药对水生昆虫摇蚊均具有高的生态毒性。

表 1 3 种农药对摇蚊 4 龄幼虫毒力

Table 1 Toxicity of three pesticides applied to 4th-instar larval chironomids

药剂 Pesticide	LC_{50} (95% 置信区间)/($\mu\text{g}/\text{L}$) LC_{50} (95% Confidence interval)	斜率 Slope \pm SE	卡方值 χ^2
氧化乐果 Omethoate	12.508 (10.564—14.881)	2.425 \pm 0.261	20.018
毒死蜱 Chlorpyrifos	2.478 (1.956—3.100)	2.292 \pm 0.265	23.510
三氟氯氰菊酯 Cyhalothrin	0.046 (0.037—0.055)	4.427 \pm 0.565	22.090

2.2 3 种农药对摇蚊 CarE 活性影响

氧化乐果对摇蚊体内羧酸酯酶活性影响如表 2 所示。0.05—8 $\mu\text{g}/\text{L}$ 氧化乐果作用 48 h, 对摇蚊体内羧酸酯酶影响趋势基本一致, 均表现为高—低—高变化。氧化乐果处理 3 h, 除 0.05 $\mu\text{g}/\text{L}$ 处理羧酸酯酶活性显著高于对照, 表现诱导增加外, 其余浓度处理羧酸酯酶活性均低于对照, 抑制范围为 5.149%—50.587%, 其中抑制率最大的浓度为 0.5 $\mu\text{g}/\text{L}$ 。氧化乐果处理 12 h 羧酸酯酶活性变化与 3 h 类似, 0.05 $\mu\text{g}/\text{L}$ 氧化乐果对羧酸酯酶的诱导率为 14.727%, 其余浓度处理羧酸酯酶活性均低于对照, 抑制率为 3.225%—36.403%, 抑制率最大的浓度为 8 $\mu\text{g}/\text{L}$ 。而 24 h 处理组羧酸酯酶活性均低于对照, 抑制率为 20.441%—48.523%。氧化乐果处理 48 h, 除 8 $\mu\text{g}/\text{L}$ 对羧酸酯酶活性抑制减少外, 其余浓度均诱导羧酸酯酶活性增加。

表 2 氧化乐果对摇蚊 4 龄幼虫 CarE 活性影响

Table 2 Effects of omethoate on carboxylesterase activity of 4th-larval chironomids

时间 Time/h	CarE 比活力 Carboxylesterase activity/(nmol \cdot min $^{-1}$ \cdot mg $^{-1}$ 蛋白质)							
	0 $\mu\text{g}/\text{L}$	0.05 $\mu\text{g}/\text{L}$	0.125 $\mu\text{g}/\text{L}$	0.25 $\mu\text{g}/\text{L}$	0.5 $\mu\text{g}/\text{L}$	1 $\mu\text{g}/\text{L}$	2 $\mu\text{g}/\text{L}$	4 $\mu\text{g}/\text{L}$
3	38.29 \pm 1.03bc	46.13 \pm 1.50a	36.32 \pm 1.30b	26.63 \pm 3.53ef	18.92 \pm 1.53g	23.65 \pm 1.94fg	31.23 \pm 1.50e	31.34 \pm 0.63de
12	68.46 \pm 0.63bc	78.55 \pm 1.42a	57.55 \pm 3.15de	51.70 \pm 5.20e	56.41 \pm 1.58e	68.33 \pm 4.83c	64.57 \pm 1.07cd	66.26 \pm 0.83c
24	62.79 \pm 3.45a	49.95 \pm 0.94c	54.15 \pm 1.90bc	39.22 \pm 0.91d	35.41 \pm 2.33d	37.94 \pm 0.09d	48.37 \pm 5.40c	47.36 \pm 0.42c
48	30.76 \pm 1.56bc	34.02 \pm 5.08abc	39.68 \pm 6.35ab	40.44 \pm 0.50a	32.12 \pm 1.03abc	33.40 \pm 2.93abc	38.95 \pm 1.02abc	36.86 \pm 1.76abc

表中数据为平均值 \pm 标准差; 同一行中不同字母表示差异显著($P<0.05$)

与氧化乐果作用不同, 毒死蜱对摇蚊幼虫羧酸酯酶的影响主要表现为抑制作用如表 3 所示。0.125—4 $\mu\text{g}/\text{L}$ 毒死蜱处理 3 h 对羧酸酯酶活性均表现为抑制, 且随着浓度增加抑制作用增强, 抑制率范围为

表 3 毒死蜱对摇蚊 4 龄幼虫 CarE 活性影响

Table 3 Effects of chlorpyrifos on carboxylesterase activity of 4th-larval chironomids

时间 Time/h	CarE 比活力 Carboxylesterase activity/(nmol \cdot min $^{-1}$ \cdot mg $^{-1}$ 蛋白质)					
	0 $\mu\text{g}/\text{L}$	0.125 $\mu\text{g}/\text{L}$	0.25 $\mu\text{g}/\text{L}$	1 $\mu\text{g}/\text{L}$	2 $\mu\text{g}/\text{L}$	4 $\mu\text{g}/\text{L}$
3	39.32 \pm 0.72a	37.60 \pm 1.03a	33.76 \pm 0.52b	28.86 \pm 2.37c	30.47 \pm 1.47bc	27.85 \pm 0.56c
12	43.15 \pm 1.32b	46.34 \pm 0.05a	44.32 \pm 0.45b	37.00 \pm 0.27c	33.29 \pm 0.56d	26.39 \pm 0.35e
24	36.72 \pm 0.31a	31.53 \pm 0.40b	30.09 \pm 0.87b	24.09 \pm 1.58c	17.90 \pm 1.21d	19.76 \pm 0.97d
48	37.32 \pm 1.12a	33.68 \pm 0.95b	28.56 \pm 0.44c	20.55 \pm 2.50d	23.57 \pm 1.36d	22.52 \pm 0.23d

4.357%—29.156%。0.125 μg/L 和 0.25 μg/L 毒死蜱处理 12 h 对羧酸酯酶表现为诱导作用,但高浓度(1—4 μg/L)毒死蜱对羧酸酯酶抑制作用增强。处理 24 h 和 48 h,各浓度毒死蜱均抑制羧酸酯酶活性,抑制率范围分别为 14.145%—51.254% 和 9.772%—39.659%。

表 4 显示了三氟氯氰菊酯对羧酸酯酶活性的影响。三氟氯氰菊酯处理 3、12、24 h 和 48 h,0.00625—1 μg/L 浓度处理羧酸酯酶活性均比对照低,表现为抑制作用,但随着浓度的增加和处理时间延长抑制率有减小趋势。

表 4 三氟氯氰菊酯对摇蚊 4 龄幼虫 CarE 活性影响

Table 4 Effects of cyhalothrin on carboxylesterase activity of 4th-larval chironomids

Time/h	CarE 比活力 Carboxylesterase activity/(nmol·min ⁻¹ ·mg ⁻¹ 蛋白质)					
	0 μg/L	0.00625 μg/L	0.0125 μg/L	0.05 μg/L	0.25 μg/L	1 μg/L
3	33.37±0.19a	29.44±2.21ab	28.83±1.94b	21.09±1.26c	21.46±1.66c	16.86±0.13d
12	44.53±0.90a	37.21±1.58bc	36.64±0.81c	39.71±1.11b	38.63±1.11b	43.09±1.00a
24	39.31±1.39a	37.21±1.58a	35.42±3.39ab	34.40±2.18ab	29.36±2.36b	33.98±2.36ab
48	48.96±2.23a	48.39±0.37ab	44.24±2.91bc	47.88±0.25abc	38.41±0.49d	43.86±0.12c

3 讨论

本文研究发现菊酯类农药三氟氯氰菊酯对红裸须摇蚊 4 龄幼虫毒力高于有机磷类农药氧化乐果和毒死蜱,而 2 种有机磷类农药间也存在差异,毒死蜱毒性高于氧化乐果。这主要是由于不同农药类型作用靶标不同以及同类型农药不同化学结构导致不同毒力,菊酯类农药作用靶标为干扰 Na⁺通道的门控动力学,引起神经传导受阻,而有机磷农药作用靶标为抑制神经系统突触上乙酰胆碱酯酶活性,引起突触传递受阻而导致昆虫死亡。这种毒力差异也表现在受试虫体中毒症状上,氧化乐果和毒死蜱处理后摇蚊中毒症状表现为开始处于兴奋状态,8 字运动的幅度和频率明显高于对照,随后兴奋程度下降,慢慢进入昏迷、麻痹期,虫体瘫软,最终虫体皱缩死亡。而三氟氯氰菊酯处理后的中毒症状可明显的分为活跃期,麻痹期,复苏期和死亡期。为了从蛋白水平分析农药对摇蚊影响,本研究测定了羧酸酯酶解毒酶对 3 种农药的响应。羧酸酯酶属于 α/β 水解酶系中 β 酯酶,通过水的介导催化羧酸酯的水解,研究报道能够被有机磷类和拟除虫菊酯类农药抑制,但羧酸酯酶表达和活性存在物种和组织特异性,其水平和活性变化很大^[7]。研究结果表明氧化乐果对羧酸酯酶影响变化较大,低浓度短时间暴露及长作用时间均能诱导羧酸酯酶活性增加,这也可能是氧化乐果对摇蚊毒性低于毒死蜱和三氟氯氰菊酯的原因。毒死蜱和三氟氯氰菊酯对羧酸酯酶的作用主要表现为抑制作用,通过抑制羧酸酯酶对农药的降解而杀死昆虫。例如,1 μg/L 氧化乐果处理 3 h 和 48 h 对羧酸酯酶抑制率分别为 38.247% 和 -8.592%;1 μg/L 毒死蜱处理 3 h 和 48 h 对羧酸酯酶抑制率分别为 26.598% 和 44.944%;1 μg/L 三氟氯氰菊酯处理 3 和 48 h 对羧酸酯酶抑制率分别为 49.486% 和 10.418%。已有研究报道羧酸酯酶通过与有机磷、氨基甲酸酯和拟除虫菊酯相互作用对农药的有效性和降解起着重要的作用^[8-9]。同时生物体内羧酸酯酶表达水平变化性和相关同工酶的丰富性产生了农药的选择毒性^[10-12]。本文从生物化学标志物羧酸酯酶活性分析有机磷和菊酯农药对其影响效应,而有关基因水平影响以及对其他重要生物化学标志物(如:细胞色素 P450、谷胱甘肽 S-转移酶)影响研究将具有重要的意义。

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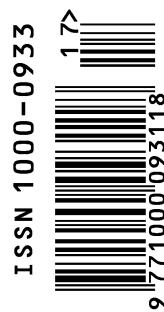
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