TOXICITY OF TWO INSECTICIDES TO *Bactrocera dorsalis* AND THEIR EFFECTS ON KEY ENZYME ACTIVITIES

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SUMMARY

The oriental fruit fly, Bactrocera dorsalis *(Diptera: Tephritidae), is an important quarantine pest worldwide, known for its high invasiveness and destructive impact. To assess the sensitivity and effectiveness of pesticides against* Bactrocera dorsalis*, the lethal concentration (*LC_{50}*) of two insecticides was determined. The* LC_{50} *values for Acetamiprid and beta-cyhalothrin were 19.432mg/L and 5.245mg/L, respectively. Beta-cyhalothrin exhibited the highest insecticidal efficacy, demonstrating a superior toxic effect compared to Acetamiprid. The toxicity of both chemical pesticides showed a clear dose-response relationship in* Bactrocera dorsalis *mortality. Specifically, the corrected mortality rate of adult B.* dorsalis *progressively increased with rising concentrations of the pesticide treatments. Additionally, the impact of these insecticides on enzyme activity in B.* dorsalis *was investigated. Acetamiprid primarily influenced the activity of Carboxylesterase (CarE) and glutathione S-transferase (GST) enzymes, while beta-cypermethrin significantly altered the activity of GST and P450 enzymes, exhibiting a no trend of increasing impact with prolonged treatment duration. These results provide valuable insights for the selection of effective pest control pesticides and the understanding of the mechanisms underlying insect response to pesticide exposure. They lay the groundwork for ongoing monitoring and the development of integrated pest management (IPM) programs targeting this invasive pest in China and other affected regions.*

Introduction

Bactrocera dorsalis (oriental fruit fly), is a member of the Diptera: Tephritidae family and is recognized as a destructive pest that threatens over a hundred plant species worldwide (Clarke *et al*., 2005). As an omnivorous insect, *B. dorsalis* primarily infests various fruits and vegetables, including citrus, apples, and pears (Clarke *et al*., 2019). *B. dorsalis* has a strong dispersal capacity, allowing it to spread widely. With the continued expansion of global trade, *B. dorsalis* has spread to many countries and regions, leading to its classification as a quarantine pest in several areas (Li *et al*., 2024).

The female adults of *B. dorsalis* typically lay their eggs inside fruit, and the larvae feed directly on the fruit after hatching, causing significant

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internal damage. Pre-harvest infestations often result in premature fruit drop, while post-harvest infestations can lead to fruit decay and lesion development, significantly affecting the quality of adjacent fruits. These impacts can result in substantial economic losses to agriculture and trade sectors (Fletcher, 1987; Follett *et al*., 2005; Aluja *et al*., 2007).

As a result, *B. dorsalis* is considered one of the most significant agricultural pests that requires intensive management and control efforts. It was first discovered and recorded in Eastern India in 1794 (Vargas *et al*., 2015; Clarke *et al*., 2019). In China, *B. dorsalis* is now present in nearly all provinces (Jin *et al*., 2011).

In recent years, a wide range of control methods and various pesticides have been employed to manage *B. dorsalis*. However, with prolonged pesticide use, *B. dorsalis* has developed varying degrees of resistance to certain insecticides (Hsu *et al*., 2004; Wang *et al*., 2013; Khan *et al*., 2018). This emerging resistance highlights the critical need for selecting appropriate control strategies and developing more effective insecticides for *B. dorsalis* management. Insects possess complex detoxification and protective enzyme systems that allow them to counteract toxic substances and environmental stresses. When exposed to chemical insecticides, these detoxification enzymes and protective enzyme systems facilitate the breakdown of chemical components within the insect body, or enhance the physiological changes triggered by these agents (Vontas *et al*., 2011; Hsu *et al*., 2012; Gichuhi *et al*., 2020). Consequently, monitoring the responses of these key enzyme systems following insecticide exposure provides a valuable approach for evaluating pesticide efficacy against target insect populations. To clarify the insecticidal effects of different pesticides on *B. dorsalis* and analyze the enzyme responses to these agents, this study evaluated the indoor toxicity of two chemical pesticides against *B. dorsalis* under controlled laboratory conditions. The study also examined the enzyme activity response to pesticide stress, aiming to identify more effective chemical agents and lay the groundwork for developing green and efficient control measures for *B. dorsalis*.

Materials and Methods

Insect rearing

Bactrocera dorsalis specimens were collected from the Insect Molecular Ecology Laboratory at Anhui Agricultural University, Hefei, Anhui Province. The insects were reared for multiple generations on an artificial diet under controlled conditions (26 ±1°C, 14:1 h light-dark cycle, 70% relative humidity). Adults used in the experiments were 1-2 days old, exhibiting high activity and uniform body shape.

Toxicity determination of two chemical pesticides

The test pesticides included 97% acetamiprid and 97% beta-cyhalothrin (SINO-AGRI Union Co., China). Stock solutions were serially diluted with 0.05% Tween-80 to prepare test concentrations of 24mg/L, 20mg/L, 16mg/L, 12mg/L, 8 mg/L, and 4 mg/L for acetamiprid, and 11 mg/L, 9mg/L, 7mg/L, 5mg/L, 3mg/L, and 1mg/L for beta-cyhalothrin. Each concentration group was treated as an experimental group, while a sterile water and acetone mixture containing 0.05% Tween-80 served as the control. For each group, 30 adult insects (1 day post-emergence) were placed in a plastic box with filter paper, sprayed with 3mL of the test solution, and maintained at 26 ±1°C, 70 ±5% relative humidity, and a 14:10 (L:D) h light cycle. Observations were conducted every 12 hours for 5 days, and mortality rates were calculated. Each treatment and control group was replicated three times, involving a total of 84 experimental units.

Enzyme activity assay

Samples were collected at 12, 24, 36, 48, 60, and 72 hours after pesticide exposure. The control group consisted of sterile water and acetone containing 0.05% Tween-80. *B. dorsalis* adults were extracted using phosphate-buffered saline (PBS) according to the instructions provided by the assay kit manufacturer (Nanjing Jiancheng Bioengineering Institute, China). The insect tissue was ground on ice using an electric homogenizer to prepare a uniform homogenate. The homogenates were processed according to the manufacturer's protocols for each enzyme assay. Four key enzyme activities were measured, including acetylcholinesterase (AChE), carboxylesterase (CarE), glutathione-S-transferase (GST), and cytochrome P450. All experiments included three biological replicates.

Statistical analysis

Experimental data were systematically recorded using Excel and statistically analyzed using one-way ANOVA in SPSS 23.0 software. Duncan's new multiple range test was employed to assess the differences between treatments (p <0.05).

Results

Toxicity determination of 2 chemical pesticides on B. dorsalis adults

The toxicity assays for the six concentration gradients of acetamiprid and beta-cyhalothrin demonstrated clear dose-dependent effects over 120 hours. The slopes (b) of the toxicity regression equations were 1.642 for acetamiprid and 3.302 for beta-cyhalothrin. The median lethal concentrations (LC_{50}) were 19.432mg/L for acetamiprid and 5.245mg/L for beta-cyhalothrin (Table I). The corrected mortality rates for six concentrations of acetamiprid at 120 hours were 6.67%, 12.22%, 27.78%, 36.67%, 50.00%, and 66.67%, respectively. For beta-cyhalothrin, these rates were 5.56%, 32.22%, 41.11%, 52.22%, 75.56%, and 83.33% (Table II). The data indicate that higher concentrations of both pesticides correspond to higher corrected mortality rates in *B. dorsalis* adults, confirming a dose-response relationship.

TABLE I
DETERMINATION OF THE TOXICITY OF TWO CHEMICAL PESTICIDES TO *B. dorsalis*

Pesticides	Regression equation	LC_{50}	95% Confidence interval	r
Acetamiprid	y= 1.642+2.606x	19.432	17.073-23.199	0.9712
beta-cyhalothrin	y= 3.302+2.359x	5.245	4.678-5.926	0.9794

TABLE II
THE CORRECTED DEATH RATE OF *B. dorsalis* TREATED BY TWO CHEMICAL PESTICIDES AT 120 H

Pesticides	Concentration (mg/L)	n	120 h Corrected mortality (%)
Acetamiprid	4	90	6.67±1.11f
	8	90	12.22±1.11e
	12	90	27.78±1.11d
	16	90	37.67±1.11c
	20	90	50.00±0.00b
	24	90	66.67±1.92a
beta-cyhalothrin	1	90	10.00±3.84f
	3	90	26.67±0.00d
	5	90	48.89±1.11c
	7	90	81.11±2.93b
	9	90	86.67±5.09ab
	11	90	94.44±1.11a

Different lowercase letters in the same column indicate significant differences under different concentrations of *M. anisopliae* at the same time. (Duncan; p <0.05).

Effects of two chemical pesticide on enzyme activities in adults of B. dorsalis

The effects of two chemical pesticide treatments on the enzyme activities in test insects were evaluated at different time intervals. For acetamiprid treatment, the AChE enzyme activity at 12 hours was significantly lower than that observed in other time groups, while at 6 hours, AChE activity was significantly higher than in the other groups (Figure 1a). CarE activity in the 6-hour and 12-hour groups was significantly higher than in other groups, reaching almost twice the level of the control and 18-hour groups (Figure 1b). The GST activity results indicated that all treated groups had

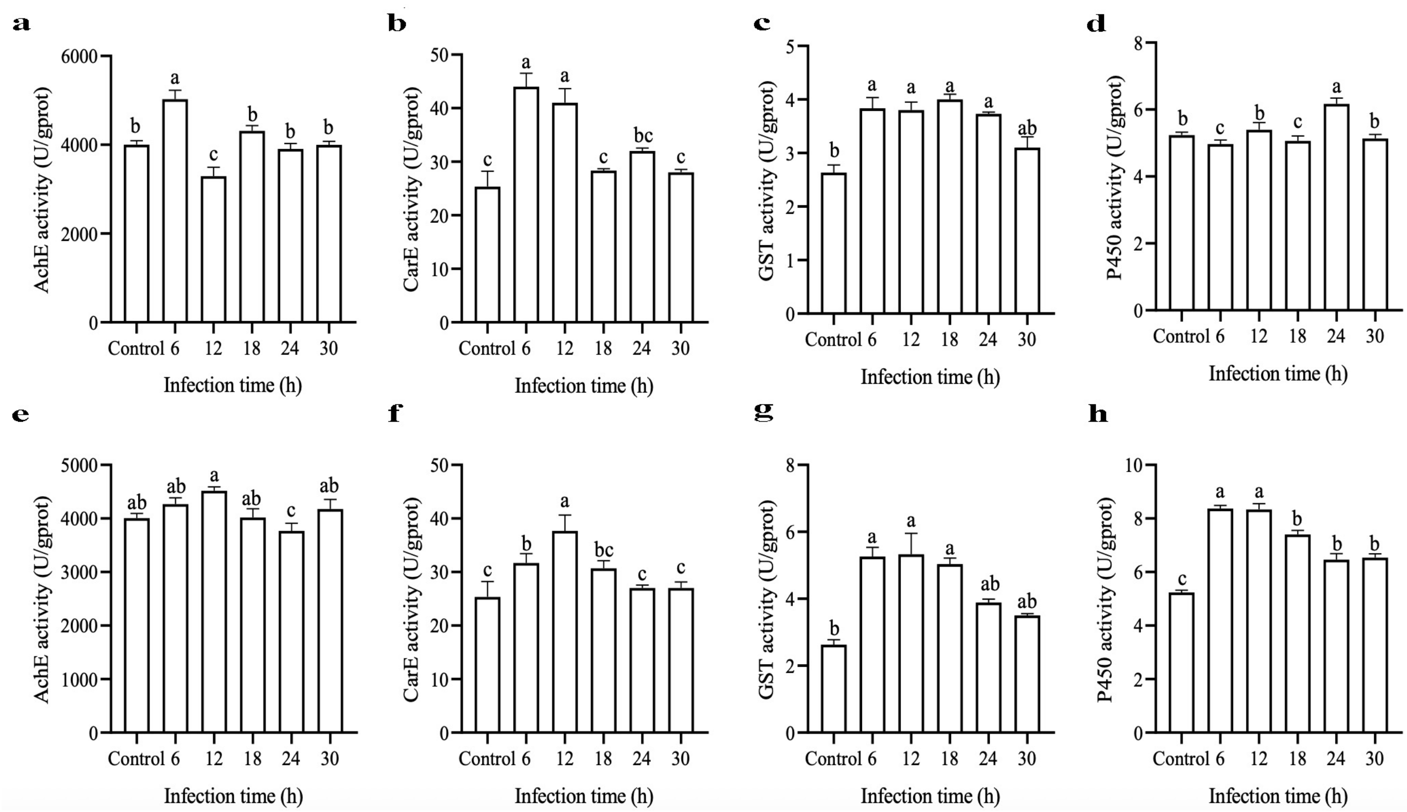

Figure 1. Effect of two chemical pesticide on enzyme activity in *B. dorsalis* adults. A-D: Treated under the acetamiprid; E-H: Treated under the beta-cyhalothrin. A, E: AChE activity, B, F: CarE activity, C, G: GST activity, D, H: P450 activity. Data are presented as mean ± SE. Different lower letters indicate significant differences at p <0.05. CarE: Carboxylesterase; AChE: Acetylcholinesterase; GST: Glutathione S-transferase; P450: Cytochrome P450.

significantly higher activity than the control group (Figure 1c). The P450 enzyme activity peaked at 24 hours in the treated groups, showing a significant increase compared to the other groups (Figure 1d).

For beta-cyhalothrin treatment, AChE activity in the 24-hour group was significantly lower than in all other groups, including the control group (Figure 1e). CarE activity in the 12-hour group was significantly higher than in other groups, reaching approximately 1.5 times the level of the control group (Figure 1f). The results for GST activity indicated that all treated groups exhibited significantly higher levels compared to the control group (Figure 1g). P450 activity followed a similar pattern to GST, with all treatment times showing significant differences from the control group (Figure 1h).

Discussion and Conclusions

Bactrocera dorsalis remains a significant invasive quarantine pest, requiring urgent control measures. The present study evaluated the toxicity of two critical insecticides against *B. dorsalis* and examined their impacts on key enzyme activities. The results indicated that beta-cyhalothrin exhibited the most effective insecticidal action against *B. dorsalis*, despite the emergence of resistance in some populations. Additionally, significant changes in enzyme activities were observed, with GST and P450 responses being particularly pronounced. These findings suggest that these enzymes play a crucial role in the detoxification processes that contribute to insecticide resistance.

This phenomenon may reflect one of the key mechanisms underlying the interactions between insecticides and insect physiological responses. Previous studies have shown that beta-cyhalothrin primarily targets voltage-gated sodium channels in the insect nervous system, potentially inducing the activity of cytochrome P450 monooxygenases and glutathione S-transferases, which are critical for the detoxification of insecticides (Jin *et al*., 2023; Dong *et al*., 2024). Similarly, acetamiprid primarily inhibits acetylcholinesterase (AChE), while also affecting carboxylesterase (CarE) and glutathione S-transferase (GST) activities (Saggioro *et al*., 2019; Wang *et al*., 2020). As a pyrethroid, beta-cypermethrin specifically acts on neuronal sodium channels, disrupting normal nerve impulse transmission (Shen *et al*., 2023). This disruption prevents proper depolarization of nerve cells, leading to continuous muscle contraction, spasm, and eventual death in the affected insects. P450 enzymes play a vital role in the metabolism of exogenous compounds, including insecticides, in insects (Feyereisen, 1999; Feyereisen, 2006).

Notably, acetamiprid also demonstrates complex interactions with various detoxification enzymes. Studies on *Aphis gossypii* revealed a biphasic response in carboxylesterase activity following acetamiprid exposure, including initial inhibition, subsequent activation, and eventual suppression (Afzal *et al*., 2015). This dynamic response indicates that CarE enzymes are involved in acetamiprid metabolism, while GST activation may contribute minimally to resistance development. The primary mode of action for acetamiprid involves competitive inhibition of AChE (Hemingway, 2004). As the enzyme responsible for acetylcholine hydrolysis in synaptic clefts, AChE inhibition leads to neurotransmitter accumulation, resulting in hyperexcitation, disrupted neurotransmission, and eventual insect paralysis and death.

Overall, this study provides valuable insights into the selection of effective pest control agents and highlights the critical role of detoxification enzymes in developing insecticide resistance. These findings establish an important scientific foundation for developing more effective management strategies against this economically significant agricultural pest.

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TOXICIDAD DE DOS INSECTICIDAS PARA *Bactrocera dorsalis* Y SUS EFECTOS EN ACTIVIDADES ENZIMÁTICAS CLAVE

Jinyuan Zhao, Yangjunlu Sheng, Chen Shen, Lijuan Liu, Wenkai Yan, Yujia Guan, Renwen Zheng, Sihan Lu y Qingfeng Tang

RESUMEN

La mosca oriental de la fruta, Bactrocera dorsalis *(Diptera: Tephritidae), es una plaga cuarentenaria importante a nivel mundial, conocida por su alta capacidad invasiva y su impacto destructivo. Para evaluar la sensibilidad y efectividad de los pesticidas contra* Bactrocera dorsalis*, se determinó la concentración letal (LC_{50}) de dos insecticidas. Los valores de LC_{50} para Acetamiprid y beta-cialotrina fueron 19,432mg/L y 5,245mg/L, respectivamente. La beta-cialotrina mostró la mayor eficacia insecticida, demostrando un efecto tóxico superior en comparación con el Acetamiprid. La toxicidad de ambos pesticidas químicos mostró una clara relación dosis-respuesta en la mortalidad de* Bactrocera dorsalis. *En particular, la tasa de mortalidad corregida de adultos de* B. dorsalis *aumentó progresivamente con concentraciones crecientes de los tratamientos con pesticidas. Además, se investigó el impacto de estos insecticidas en la actividad enzimática de* B. dorsalis. *El Acetamiprid influyó principalmente en la actividad de las enzimas Carboxilesterasa (CarE) y glutatión S-transferasa (GST), mientras que la beta-cipermetrina alteró significativamente la actividad de las enzimas GST y P450, mostrando una tendencia de impacto creciente con una mayor duración del tratamiento. Estos resultados proporcionan información valiosa para la selección de pesticidas efectivos para el control de plagas y para comprender los mecanismos subyacentes en la respuesta de los insectos a la exposición a pesticidas. Sientan las bases para el monitoreo continuo y el desarrollo de programas de manejo integrado de plagas (MIP) dirigidos a esta plaga invasora en China y otras regiones afectadas.*

TOXICIDADE DE DOIS INSETICIDAS PARA *Bactrocera dorsalis* E SEUS EFEITOS NAS ATIVIDADES ENZIMÁTICAS CHAVE

Jinyuan Zhao, Yangjunlu Sheng, Chen Shen, Lijuan Liu, Wenkai Yan, Yujia Guan, Renwen Zheng, Sihan Lu e Qingfeng Tang

RESUMO

A mosca oriental da fruta, Bactrocera dorsalis *(Diptera: Tephritidae), é uma praga quarentenária importante em nível global, conhecida por sua alta capacidade invasiva e impacto destrutivo. Para avaliar a sensibilidade e a eficácia de pesticidas contra* Bactrocera dorsalis*, foi determinada a concentração letal (LC_{50}) de dois inseticidas. Os valores de LC_{50} para Acetamiprid e beta-cialotrina foram 19,432 mg/L e 5,245 mg/L, respectivamente. A beta-cialotrina apresentou a maior eficácia inseticida, demonstrando um efeito tóxico superior em comparação ao Acetamiprid. A toxicidade de ambos os pesticidas químicos apresentou uma clara relação dose-resposta na mortalidade de* Bactrocera dorsalis. *Especificamente, a taxa de mortalidade corrigida dos adultos de* B. dorsalis *aumentou progressivamente com concentrações crescentes dos tratamentos com pesticidas. Além disso, foi investigado o impacto desses inseticidas na atividade enzimática de* B. dorsalis. *O Acetamiprid influenciou principalmente a atividade das enzimas Carboxilesterase (CarE) e glutationa S-transferase (GST), enquanto a beta-cipermetrina alterou significativamente a atividade das enzimas GST e P450, apresentando uma tendência de impacto crescente com o aumento da duração do tratamento. Esses resultados fornecem informações valiosas para a seleção de pesticidas eficazes no controle de pragas e para a compreensão dos mecanismos subjacentes na resposta dos insetos à exposição a pesticidas. Eles estabelecem as bases para o monitoramento contínuo e o desenvolvimento de programas de manejo integrado de pragas (MIP) direcionados a essa praga invasora na China e em outras regiões afetadas.*