



## **Inhibition of Phenoloxidase Activity Delays Development in *Bactrocera dorsalis* (Diptera: Tephritidae)**

Authors: Bai, Ping-Ping, Chen, Er-Hu, Shen, Guang-Mao, Wei, Dong, Wei, Dan-Dan, et al.

Source: Florida Entomologist, 97(2) : 477-485

Published By: Florida Entomological Society

URL: <https://doi.org/10.1653/024.097.0218>

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INHIBITION OF PHENOLOXIDASE ACTIVITY DELAYS DEVELOPMENT IN *BACTROCERA DORSALIS* (DIPTERA: TEPHRITIDAE)

PING-PING BAI, ER-HU CHEN, GUANG-MAO SHEN, DONG WEI, DAN-DAN WEI AND JIN-JUN WANG\*

Key Laboratory of Entomology and Pest Control Engineering, College of Plant Protection, Southwest University, Chongqing 400716, P. R. China

\*Corresponding author; E-mail: jjwang7008@yahoo.com

## ABSTRACT

Phenoloxidase (PO) is a multicopper enzyme, which plays an important role in melanin synthesis. PO is necessary for defense against intruding microorganisms and parasites, and is important in wound healing and cuticle pigmentation. As a specific inhibitor of PO, kojic acid (KA) is commonly used to characterize the functional properties of PO. In this study, we investigated the effect of KA on PO activity during the development of the oriental fruit fly, *Bactrocera dorsalis*, which is a destructive pest of many horticultural crops. After feeding on a KA-containing artificial diet, the larval and pupal developmental periods were significantly prolonged. In addition, the larvae did not grow to normal size and rates of pupation and emergence were decreased when *B. dorsalis* larvae had been fed with KA-containing diet for 6 days. Compared to the control, PO activities from whole bodies of *B. dorsalis* were inhibited, so did in larval cuticles. Consistent with these, kinetic analysis showed that the catalytic capability of PO was significantly reduced. The  $I_{50}$  values of KA for PO activity also indicated that KA was an effective inhibitor of PO in *B. dorsalis*. Biochemical characterization showed that PO from *B. dorsalis* had maximum activity at pH 7.5 and 37 °C. These results provide additional understanding of the role of PO in growth of *B. dorsalis*. As an inhibitor of PO, KA is capable of disrupting the development of this pest insect.

Key Words: *Bactrocera dorsalis*, phenoloxidase, kojic acid, tephritidae, inhibitory effect

## RESUMEN

Fenoloxidasas (FO) es una enzima multicobre que juega un papel importante en la síntesis de melanina. FO es necesaria para la defensa contra los microorganismos intrusos, parásitos y es importante en la cicatrización de heridas, y la pigmentación de la cutícula. Como un inhibidor específico de FO, ácido kójico (AK) es comúnmente utilizado para caracterizar las propiedades funcionales de FO. En este estudio, se investigó el efecto de AK sobre la actividad de FO durante el desarrollo de la mosca oriental de la fruta, *Bactrocera dorsalis*, que es una plaga importante de varios cultivos hortícolas. Después de alimentar con una dieta artificial que tenía AK, los periodos de desarrollo de las larvas y las pupas se prolongaron significativamente en los grupos tratados con AK. Además, las larvas no crecieron a su tamaño normal y se redujeron las tasas de pupación y emergencia cuando las larvas de *B. dorsalis* fueron alimentadas por 6 días con la dieta que tenía AK. En comparación con el control, las actividades de FO fueron inhibidas en los cuerpos enteros y las cutículas de las larvas de *B. dorsalis*. Consistente con estos bloqueos, el análisis cinético mostró que la capacidad catalítica de FO se redujo significativamente. Los valores de  $I_{50}$  de AK para la actividad de FO también indicó que AK fue un inhibidor eficaz de FO en *B. dorsalis*. La caracterización bioquímica mostró que FO de *B. dorsalis* tenían una actividad máxima a pH 7.5 y 37 °C. Estos resultados incrementan nuestra comprensión de la función de FO en el crecimiento de *B. dorsalis*. Como un inhibidor de la FO, AK es capaz de perturbar el desarrollo de esta plaga de insectos.

Palabras Clave: *Bactrocera dorsalis*, fenoloxidasas, ácido kójico, tefritidos, efecto inhibitorio

Phenoloxidase (PO, EC.1.14.18.1) is a copper-containing enzyme that is widely distributed throughout the animal kingdom from bacteria to mammals (Mayer 1986). In arthropods, especially in insects, PO is uniquely associated with many important physiological and biochemical processes,

such as cuticular sclerotization (Sugumaran 1998), melanization (Hiruma et al. 1985), and wound repair (Brey et al. 1993). The function of insect PO is also helpful in combating environmental stress from parasites, fungi, and bacteria as one of their innate immune mechanisms (Ratcliffe et al. 2004).

Among innate immune system factors, PO is critical in insects' defense (Ajamhassani et al. 2012). As a key enzyme in the melanization cascade, PO is usually synthesized and released into the hemolymph as the inactive zymogen prophe-noloxidase (proPO). It is activated by proteolytic cleavage into active PO. Next PO catalyzes the initial step of the melanin biosynthesis pathway by hydroxylation of monophenols into *o*-diphenols, and then the further oxidation *p*-diphenols into *o*-quinones. *O*-quinones can nonspecifically cross-link neighboring molecules to form insoluble melanin (Nappi & Christensen 2005; Charoensapsri et al. 2011). In addition, during quinone sclerotization, quinones generated by PO go through a cascade of enzymatic and non-enzymatic reactions leading to polymerization; and quinones cross link with cuticular structural proteins and chitin, which results in the hardening of the cuticle (Hall et al. 1995). The hardened cuticle is the first line of defense in protecting insects.

The function of PO in cuticle tanning and invertebrate immunity has been extensively studied for decades (Andersen et al. 1996; Soderhall & Cerenius 1998; Taylor, 1969). PO has been successfully extracted and purified from several insect species, including *Sarcophaga bullata* (Parker) (Diptera: Sarcophagidae) (Chase et al. 2000), *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) (Arora et al. 2009), *Eurygaster integriceps* Puton (Hemiptera: Scutellaridae) (Zibae et al. 2011) and *Hyphantria cunea* Drury (Lepidoptera: Arctiidae) (Ajamhassani et al. 2012). In *Bactrocera tau* (Walker) (Diptera: Tephritidae), PO activity in larvae was reported to be higher than in pupae or adults, which suggested that PO was associated with the change in body color that occurs during the various developmental stages (Li & Liu 2011). Since PO is a metalloprotein, its activity could be modified by small changes at the metal center by choosing different metal ions. PO activity could be enhanced by  $Mg^{2+}$  and inhibited by  $Cu^{2+}$  and  $Zn^{2+}$  (Zhao et al. 2010). In addition, PO activity in *Apis mellifera* L. (Hymenoptera: Apidae) could be induced by trypsin and inhibited by protease inhibitors and phenylthiourea *in vitro* (Zufelato et al. 2004). Additional inhibitors of PO activity have been identified in *in vitro* studies and include thiourea, dithiothreitol, and kojic acid (KA) (Goudru et al. 2013). Among these, kojic acid [5-hydroxy-2-(hydroxymethyl)-*y*-pyrone] is a fungal metabolite produced by many species of *Aspergillus* and *Penicillium* (Chen et al. 1991) and it has been used to characterize PO activity (Luna-Acosta et al. 2010). More recently, structural studies have revealed the details of PO-inhibitor binding interactions: kojic acid binds at the entrance to the active site and blocks the entrance to the active site (Sendovski et al. 2011).

The inhibition of PO by synthetic inhibitors could lead to the development of a novel insecti-

cide. These inhibitors could result in a disorder of the insect immune system against pathogens, and cause abnormal body softening (Gholami et al. 2013). Available information on the inhibitors of insect PO is still in the rudimentary stage. So far there is no research reported on the effects of KA on insect PO *in vivo*, and particularly in *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). *Bactrocera dorsalis* is one of the most economically important fruit fly pests in tropical and subtropical areas of the world (Clarke et al. 2005). It is a highly polyphagous pest with the ability to infest more than 250 host plants including various fruits and vegetables (Chen & Ye 2007). Similar to other tephritids, cuticle color and hardness in *B. dorsalis* drastically changes from larval to adult stage and strongly correlates with sclerotization and melanization. The goal of this study was to inhibit pupation and/or adult emergence by altering PO activity using the PO inhibitor, KA.

## MATERIALS AND METHODS

### Insects and Diets

The stock colony of *B. dorsalis* was collected in 2008 from Guangzhou in Guangdong province, China. The entire life cycle of *B. dorsalis* was maintained at  $27 \pm 1^\circ C$ ,  $70 \pm 5\%$  RH and 14:10 h L:D. Newly hatched larvae were fed an artificial diet containing corn flour, wheat germ flour, yeast powder, agar, sugar, sorbic acid, vitamin C, linoleic acid, and filter paper. After emergence, the adults were reared in cages and fed an artificial diet consisting of yeast powder, honey, vitamin C, and water (Wang et al. 2013).

### Chemicals and Reagents

Kojic acid (KA, Yuanye Biological Technology, Shanghai, China), catechol (Aladdin, Shanghai, China), L-dopa (Sigma, Sigma, St. Louis, Missouri), Bovine serum albumin (BSA, Shanghai Bio Life Science & Technology Co. Ltd., China), and other biochemical reagents were procured as analytical grade.

### KA Treatment

To determine the optimal mass ratio of KA required to causing a detrimental effect on *B. dorsalis*, newly hatched larvae (1 day old) were fed on artificial diets containing various mass ratios of KA for 6 days followed by monitoring relative mortality. KA was dissolved in 1 mL water and incorporated into artificial diet. Based on such monitoring of mortality relative to dose, the artificial diet containing 0.2% KA was chosen for further experiments. Controls were fed only on artificial diet. Each treatment group was repli-

cated 3 times. Larvae that survived after feeding on KA containing diet for 6 days along with the control larvae were collected, immediately frozen in liquid nitrogen and stored at -80 °C for further analyses.

#### Effect of KA on the Development of *B. dorsalis*

Newly hatched *B. dorsalis* larvae were fed on 0.2% KA-containing artificial diet while control groups were fed on artificial diet only. Then, the third instars which are the last instar of *B. dorsalis* were transferred to a plastic basin containing wet sand for pupation. During this time, larval duration (number of days) and pupation rate (fraction of larvae that pupated) were recorded. One-day-old pupae were weighed and maintained in the same conditions as mentioned above. Pupal period (number of days as pupae) and emergence rate (fraction of pupae that emerged as adults) were recorded every day until adult emergence. Pupae that did not emerge after 15 days were considered as dead. Pupal weights were recorded as the average weight of 50 pupae. Each data point had 3 replicates and each treatment had 50 larvae or pupae.

#### Enzyme Preparation and Assay

Extraction of PO enzyme from *B. dorsalis* was carried out according to the method described by Hu et al (2011) and Chen et al (2012) with slight modifications. Briefly, whole bodies of 15 larvae and the dissected cuticles from 30 larvae were collected from the control and KA treatment groups for enzyme extraction. The samples were homogenized manually on ice with 4 mL sodium phosphate buffer (PBS, 0.2 mol/L, pH 6.8). The homogenate was centrifuged at 5000 × *g* for 5 min at 4 °C. The pellet was discarded and the supernatant was centrifuged again for 30 min at 17500 × *g* and 4 °C. The final supernatant was filtered through a 0.45 µm Millex-HV filter (Millipore, Billerica, Massachusetts) and was used to determine PO activity. Protein content in the supernatant was measured according to Bradford (1976) with bovine serum albumin (BSA) as a standard at 595 nm at 25 °C on a Microplate Spectrophotometer (XMark™, Bio-Rad, Hercules, California, USA).

#### PO Activity Assay

PO activity was determined in a 96-well microplate in a total reaction volume of 300 µL per well with catechol as the substrate, according to the method described by Benjamin & Montgomery (1973). The assay mixture containing 135 µL PBS (0.2 mol/L, pH 6.8), 30 µL of the enzyme extract (supernatant), and 135 µL of 0.01 mol/L catechol, was incubated at 37 °C for 10 min. Changes

in absorbance were measured at 420 nm and 37 °C every 30 sec for 4 min in a Microplate Spectrophotometer (Bio-Rad). A control reaction that contained catechol and PBS without the supernatant was also maintained. One unit of enzyme activity (U) was defined as a change in the absorbance value of 0.001 per min. Each assay had 3 replicates and each experiment was repeated 3 times. The results were expressed as units of enzyme activity per mg protein.

#### Kinetic Analysis of PO

The Michaelis constant ( $K_m$ ) and maximum velocity ( $V_{max}$ ) of PO were determined at different catechol concentrations (0.625, 1.25, 2.5, 5, 10, 20, and 40 mmol/L). PO values were measured at 420 nm in a Microplate Reader and Lineweaver-Burk plots were generated by plotting the reaction rates against substrate concentrations (Chen & Kubo 2002). In addition, L-dopa was also used as the substrate at concentrations of 0.2, 0.4, 0.8, 1.6, 3.2, and 6.4 mmol/L and the absorbance was measured at 475 nm (Wititsuwannakul et al. 2002).

#### *In Vitro* Enzyme Inhibition

Inhibition assays were performed in the absence or presence of various concentrations of KA similar to the PO assay conditions described above. The supernatant (25 µL) was mixed with the same volume of KA solution at the appropriate concentrations and incubated for 10 min at 37 °C and then added to the catechol/PBS substrate mixture, as described above. Reactions without KA were included as control. The median inhibition concentration ( $I_{50}$ ) of KA was determined from a log concentration vs. probit (% inhibition) regression analysis.

#### Effect of pH and Temperature on PO Activity

The effects of pH and temperature on PO activity were determined with 0.01 mol/L catechol as the substrate. The pH in reactions was adjusted from 5.0 to 8.0 with 0.2 mol/L PBS (pH 5.0, 6.0, 6.5, 7.0, 7.5 and 8.0). The effect of temperature on PO activity was determined by incubating the reaction mixture at 25 °C, 30 °C, 34 °C, 37 °C, 40 °C, 43 °C or 45 °C for 10 min, followed by measurement of PO activity.

#### Statistical Analysis

Data were analyzed by independent samples *t*-test ( $P < 0.05$ ) with SPSS version 16.0 for Windows. Regression analyses were performed to calculate kinetic parameters of PO and  $I_{50}$  values of KA.

## RESULTS

Effect of KA on the Development of *B. dorsalis*

Under normal conditions, *B. dorsalis* larval development lasts for 8 days. However, when fed with KA-containing artificial diet, larval developmental period was delayed by 4 days (12 days larval developmental time after KA treatment). Pupal development was also affected by KA, resulting in a 2-day delay (Fig. 1A). KA treatment had a significant negative impact on pupation and adult emergence rates. After larvae were reared on diets containing KA, the pupation rate decreased from 98.00% to 71.33% (Fig. 1B). Similarly, the emergence rate decreased from 74.50%

to 23.33% (Fig. 1B), and pupal weight decreased from 13.03 mg/pupa to 8.86 mg/pupa after treatment with KA (Fig. 1C). The phenotype of larvae from the KA treatment groups looked smaller than the control (Fig. 1D). The newly hatched larvae (1 day old) were fed on artificial diets containing various mass ratios of KA (0.02%, 0.04%, 0.20%, 0.32%, and 0.40%) for 6 days, the relative mortality increased from 5% to 50% (Fig. 1E).

## Specific Activity of PO

The specific activities of PO in the whole larval (day 6) body extracts and cuticle extracts from *B. dorsalis* were  $126.52 \pm 7.58$  and  $301.02 \pm 4.96$  U/

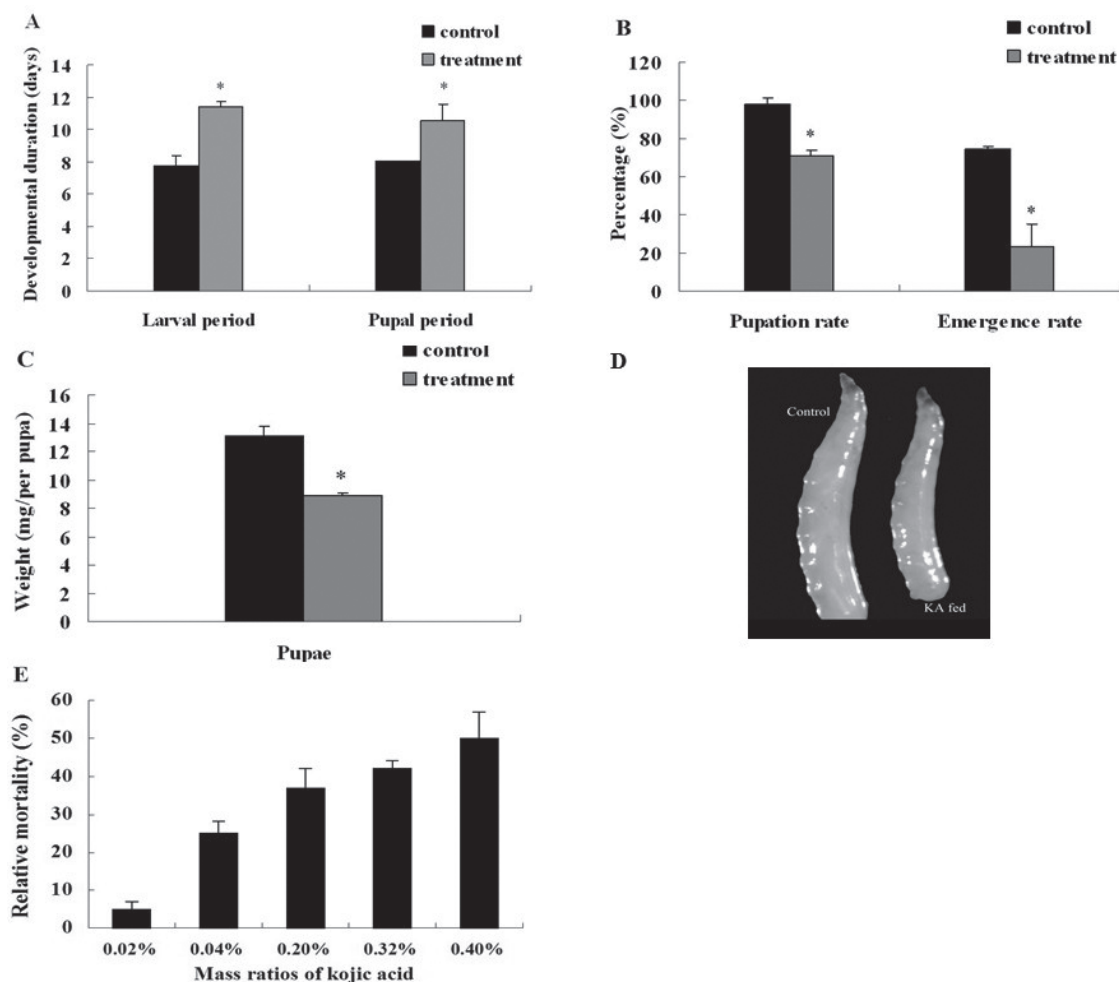


Fig. 1. Larval development of *Bactrocera dorsalis* in control and groups reared on an artificial diet with 0.2% kojic acid (KA). (A) Duration of larval and pupal development. (B) Pupation and adult emergence rates. (C) Pupal weight. (D) These are representatives of the larvae taken from the control (left) and KA fed (right) groups at six days. Left: normal development (control). Right: delayed growth (KA treatment). (E) The effect of different concentration of KA on the relative mortality of larvae. "\*" indicates significantly different (Independent sample *t*-test,  $P < 0.05$ ).

mg, respectively, suggesting that the PO activity per unit mass of cuticle was greater. After treatment with KA, the PO activities in both whole larval body and cuticle extracts decreased to  $29.29 \pm 6.04$  and  $112.76 \pm 14.76$  U/mg (Fig. 2), respectively, which correspond to reductions of 76.85 and 62.54%. This indicates that KA is an effective inhibitor of *B. dorsalis* PO activity.

#### Substrate Specificity and Kinetic Parameters

The  $K_m$  value for the catalytic activity of PO with catechol as the substrate was 2.62 mmol/min for the whole larval body, and 5.51 mmol/min for the cuticle (Table 1). The  $V_{max}$  values of PO in both the larval body (35.54 mmol/L) and the cuticle (75.37 mmol/L) in the KA treated group were significantly lower than the control (larva: 130.8 mmol/L; cuticle: 333.33 mmol/L). When L-dopa was used as the substrate, a similar decrease was observed in the  $K_m$  and  $V_{max}$  of PO between control and KA treatment groups (Table 1).

#### In Vitro Inhibition of PO Activity

We first determined the  $I_{50}$  values (the concentration required to inhibit 50% of PO activity) of KA at the final concentrations of 0.27, 1.10, 4.40, 8.80, and 17.59 mmol/L in the third instar larvae of *B. dorsalis*. The  $I_{50}$  value of KA in the whole larval body was 4.72 mmol/L and in the cuticle was 2.01 mmol/L (Fig. 3). At these concentrations KA significantly inhibited PO activity in both whole larval body extracts and cuticle extracts by 15-84%.

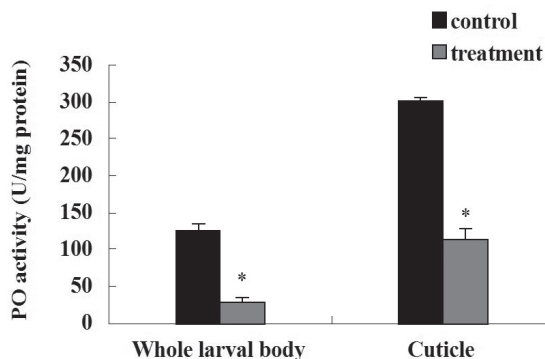


Fig. 2. Phenoloxidase (PO) activity in whole bodies ( $P < 0.001$ ) and cuticles ( $P = 0.001$ ) of *B. dorsalis* third instars fed on artificial diet only (control) or diet containing 0.2% kojic acid (KA) (treated groups) for 6 days. Each value represents the mean ( $M \pm SE$ ) of 3 replications. “\*” indicates significantly different when compared to the respective control (Independent sample  $t$ -test,  $P < 0.05$ ).

#### Effect of pH and Temperature on the PO Activity

The effect of temperature on specific activity of PO was analyzed at a range of temperatures from 25°C to 45°C. The optimal temperature for the specific activity of PO in both the whole larval body and cuticle was 37°C. PO activity increased marginally from 25-37°C and decreased beyond 37°C (Fig. 4A).

PO activity also changed when the pH range varied from 5.0-8.0. The optimal pHs for PO in the whole larval body and in the cuticle were between 7.0 and 7.5. The specific activity of PO was very low below pH 6.5 and above pH 8.0 (Fig. 4B).

#### DISCUSSION

PO is a key factor that plays critical roles in insect development and immunity and involved in multiple processes such as cuticular sclerotization, melanization, and wound repair (Lavine & Strand 2002). In this study, we found that the larval and pupal developmental periods were significantly extended in the KA treated groups. Meanwhile, the sizes of treated larvae were small, and the rates of pupation and emergence were decreased when *B. dorsalis* larvae were fed with KA-containing diet for 6 days. These results suggested that KA resulted in the changes in development of *B. dorsalis*. As a competitive inhibitor of PO, KA has been used to characterize PO activity in several species (Chang 2009; Gokoglu & Yerlikaya 2008). Feeding of *B. dorsalis* larvae for 6 days on KA treated diet markedly inhibited the activity of PO in both the larval body and the cuticle extracts. Because the hardened cuticle is the first line of defense of insects, cuticular sclerotization and melanization are vital for development, and sclerotized cuticles have been reported to effectively block most pathogens (Ashida & Brey 1995). Together with previous findings, our results indicate that the developmental stages of *B. dorsalis* can be compromised by KA treatment.

We found that inhibition of PO activity by KA resulted in delayed larval and pupal development, and decreased rates of pupation and adult emergence, which support the notion that inhibition of PO may lead to changes in the development of *B. dorsalis*. It was reported that KA was capable of reducing *o*-quinones to diphenols to prevent the final pigment (melanin) forming (Chen et al. 1991). So we presume that inhibition of PO may result in the generation of very little quinone, which is required for both sclerotization and melanization. It has been shown that quinones generated by PO polymerize to form melanin and also cross link nucleophilic compounds during wound healing and parasite encapsulation (Lourenço et al. 2005). The essential role of PO in chorion melanization and hardening has been reported in *Aedes aegypti* L. (Diptera: Culicidae) (Kim et al. 2005).

TABLE 1. KINETIC PARAMETERS OF PHENOLOXIDASE (PO) OF 6-DAY-OLD LARVAE AND CUTICLE OF *BACTROCERA DORSALIS*.

Groups		Catechol		L-dopa	
		$K_m$ (mmol/min)	$V_{max}$ (mmol/L)	$K_m$ (mmol/min)	$V_{max}$ (mmol/L)
Larvae	Control	2.62 ± 0.28	130.80 ± 13.04*	0.89 ± 0.05	62.57 ± 12.19*
	KA-treatment	1.21 ± 0.29	35.54 ± 0.98	0.31 ± 0.00	20.87 ± 4.48
Cuticle	Control	5.51 ± 3.28	333.33 ± 0.00*	0.99 ± 0.26	90.73 ± 15.06*
	KA-treatment	1.15 ± 0.31	75.37 ± 21.52	0.47 ± 0.27	40.67 ± 13.18

Each value represents the mean ( $M \pm SE$ ) of three replications.

\*Significant difference (Independent sample  $t$ -test,  $P < 0.05$ ).

Moreover, sclerotization and melanization are linked to insect pupation and emergence. A significant decrease in pupal weight could suggest PO inhibition and lower quinone levels, although other factors could be involved. PO is a critical enzyme involved in insect molting indicating that it could be regulated by ecdysone. The peak of PO expression was reported during the molting stage in *Bombyx mori* (Wang et al. 2012). Therefore, it is likely that the inhibition of PO could affect the molting process leading to the abnormal development of *B. dorsalis* observed in this study.

In *Drosophila melanogaster* Meigen (Diptera: Drosophilidae), PO is responsible for clot hardening by crosslinking and melanization (Bidla et al. 2005). Laccase-type enzymes (EC 1.10.3.2) are a type of PO that oxidize *o*- or *p*-diphenols to quinones during sclerotization and tanning of the cuticle (Dittmer et al. 2004). We also observed that the body color and hardness of *B. dorsalis* changed significantly during metamorphosis from larval to adult stages; body color changed from yellow or dark yellow during larval development, and then transformed to brown or dark brown in pupae and adults. During this

process, the cuticle of *B. dorsalis* became progressively harder. *O*-quinones formed by the catalytic function of PO have been reported to be involved in the changes in color and hardness (Li & Liu 2011). Therefore, inhibition of PO could have resulted in insufficient amounts of quinones that in turn could have led to abnormal body softening. This could presumably reduce the weight of pupae similar to that observed in the KA treated group in this study. Moreover, disrupted cuticular development may be an important factor in the delayed development and decreased rates of pupation and adult emergence.

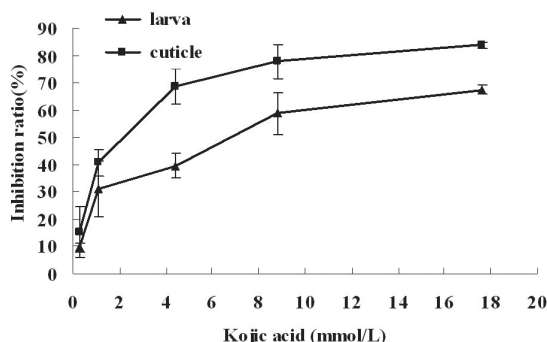


Fig. 3. Inhibition of phenoloxidase (PO) activity by kojic acid in the whole bodies or cuticles of *Bactrocera dorsalis* larvae at 6 days ( $I_{50} = 4.72$  mmol/L) or cuticle ( $I_{50} = 2.01$  mmol/L) of third instars. The kojic acid at 0.2% was provided in the artificial of the *B. dorsalis* larvae. Each value represents the mean ( $M \pm SE$ ) of 3 replicates.

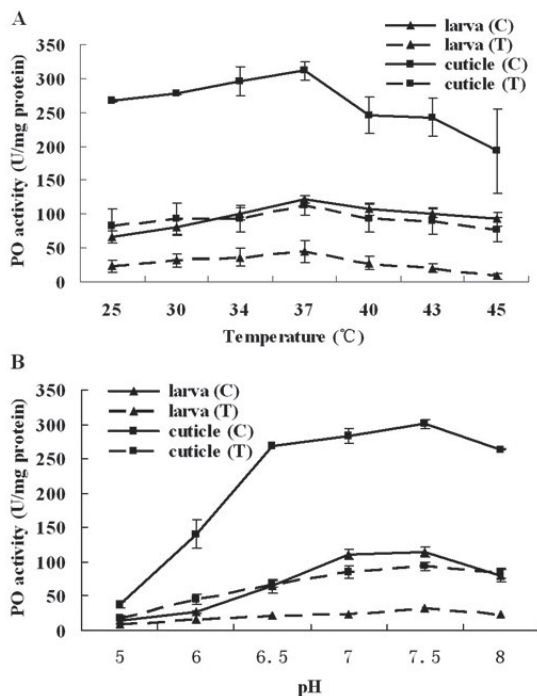


Fig. 4. Effect of temperature (A - upper panel) and pH (B - lower panel) on the phenoloxidase (PO) activity in the whole body (6 days) or cuticle of *B. dorsalis* third instars. The kojic acid at 0.2% was provided in the artificial of the *B. dorsalis* larvae from the time of hatching. Each value represents the mean ( $M \pm SE$ ) of 3 replicates.

In this study, we used Lineweaver-Burk plots to identify the kinetic parameters of the enzyme. Feeding with KA-containing diet significantly decreased the  $V_{\max}$  of PO in *B. dorsalis* larvae. It has been shown that KA binds and blocks the entrance to the active site of copper ions (Sendovski et al. 2011). Therefore, to determine whether KA inhibition of PO activity was specific, we used L-dopa as a second substrate, which lowered the  $V_{\max}$  values. Besides, the affinity of this enzyme toward L-dopa was greater than toward catechol.

Consistent with previous studies that showed the effective inhibition of PO activity by KA in other agricultural pests such as *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) (Gao et al. 2003), we have shown in this study that low levels of KA can effectively inhibit the activity of PO in *B. dorsalis* larvae. The KA  $I_{50}$  values were at millimole levels; 4.72 mM for the whole larval body, and 2.01 mM for the cuticle. It appears that the effect of KA was much stronger in *B. dorsalis* than in *S. exigua* where the  $I_{50}$  was 258.32 mM (Gao et al. 2004) and similar to that of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) ( $I_{50}$  = 1 mM) (Wang et al. 2004).

Temperature and pH are very important in many biochemical reactions; thus a small change in their values may alter enzymatic activity. In this study, the optimal temperature was 37 °C for the catechol catalysis reaction of PO. In other insects, the maximum specific activity of PO was observed as follows: between 30–35 °C in *Locusta migratoria* (L.) (Orthoptera: Acrididae) (Cherqui et al. 1998) and *E. integriceps* (Zibae et al. 2011), 34–37 °C in *B. tau* (Li & Liu 2011), 35–40 °C in *H. cunea* (Ajamhassani et al. 2012), and 45 °C in *Heliothis virescens* F. (Lepidoptera: Noctuidae) (Lockey & Ourth 1992).

The optimal pH of PO activity in *B. dorsalis* was 7.5 and corresponded the values reported for other insects, such as pH 7.5 for *Galleria mellonella* F. (Lepidoptera: Plutellidae) (Dunphy 1991), pH 7.0–7.5 for *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) (Lee & Anstee 1995), and pH 6.5 for *B. tau* (Li & Liu 2011). Higher or lower pH values have also been reported as optimal in some insects; for example, pH 9.0 for *H. virescens* (Lockey & Ourth 1992), pH 10 for *H. cunea* (Ajamhassani et al. 2012), and pH 4.0 for *S. bullata* (Barrett 1987). These differences in the optimal temperature and pH could be associated with the various environmental niches occupied by the different species (Zibae et al. 2011).

In conclusion, we have shown in the present study that KA is an effective inhibitor of PO activity in *B. dorsalis*. When PO was inhibited, the normal development of *B. dorsalis* was disrupted thus correlating the function of PO with larval development, pupation, and adult emergence. These results contribute to a better understanding of the functions of PO in *B. dorsalis* and provide insights to improve management strategies for this pest.

## ACKNOWLEDGMENTS

This research was supported in part by the Natural Science Foundation of Chongqing (CSTC, 2013jjB0176), the Program for Changjiang Scholars and Innovative Research Teams in Universities (IRT0976), the earmarked fund for Modern Agro-industry (Citrus) Technology Research System, and the Fundamental Research Funds for the Central Universities (XDJK2013A017) of China.

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