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Article

Molecular Characterization of the Disruptive Effects by Two Curcuminoids, Demethoxycurcumin and Bisdemethoxycurcumin, on the Larval Development of *Drosophila melanogaster*

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Simple Summary: Several plant species have diterpene compounds with juvenile hormone (JH) disruptor activity in insects. Demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC) are two curcuminoid components of turmeric, interfere with the JH-mediated formation of the JH receptor complex. *In vitro*, DMC and BDMC also inhibited larval and/or pupal development in *Drosophila melanogaster*. Both results suggest that DMC and BDMC are JH disruptors that affect insect growth and development by regulating JH-mediated gene expression.

Abstract: Juvenile hormones (JH) play a central role in insect development, reproduction and various physiological functions. Given the specificity of JH to arthropods, particularly insects, it has been the focus of extensive research for its potential application in pest management. Accordingly, this study aimed to identify the substances that act as JH disruptors from edible plants. Curcuminoids generally exhibit a wide range of biological activities, such as antioxidant, anti-inflammatory, antibacterial, and insecticidal properties, and they exhibit insect growth inhibitory effects. Demethoxycurcumin and bisdemethoxycurcumin, two curcuminoids from the turmeric plant *Curcuma longa* L. inhibit the formation of a methoprene-tolerant (Met)–Taiman (Tai) heterodimer complex as shown through in vitro yeast two-hybrid assays. An artificial diet containing 1% (w/v) demethoxycurcumin or bisdemethoxycurcumin reduced the number of larvae, leading to no pupal development. Building on our results regarding curcuminoids, researchers can use our study as a reference to develop eco-friendly pesticides.

Keywords: juvenile hormone; methoprene-tolerant; diterpene; juvenile hormone disruptor; curcuminoids; demethoxycurcumin; bisdemethoxycurcumin

1. Introduction

While chemical insecticides with diverse insecticidal effects have long been utilized to control major pests, issues including ecosystem degradation, environmental contamination, and the emergence of resistance in target pests due to the improper use and overuse of these insecticides have arisen [1]. Therefore, eco-friendly insecticides that exhibit effective insecticidal effects without considerably affecting the environment have been actively researched, and several have been characterized from different types of plant extracts [2]. Plants use secondary metabolites as natural defenses against pests such as insects, including nicotine, rotenone, lianas, sabadala, pyrethrum, neem, and turmeric [3]. Turmeric is derived from the root of *Curcuma longa* L., a perennial herb belonging to the Zingiberaceae family [4,5], and contains approximately 60–70% carbohydrates, 6–

8% protein, 5–10% fat, 3–7% minerals, and 6–13% moisture [5–8]. Within turmeric, there are more than 50 structurally related compounds known as curcuminoids, which make up approximately 3–5% of the total composition. The main curcuminoids include three commercially available compounds, curcumin, demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC) [6,9]. Curcuminoids are well-known for their various pharmacological properties, including antidepressant, antioxidant, anti-inflammatory, hepatoprotective, antidiabetic, anticancer, and antibacterial effects [7,10–14]. However, research on insecticidal properties related to curcuminoids has been limited. Some research on insecticidal properties, studies have explored the efficacy of curcuminoids, including ovicidal activity on Helicoverpa armigera eggs [15] In particular, curcuminoids I, II, III inhibited the P-glycoprotein ATPase in *H. armigera* [16] and insecticidal activity against *Aedes aegypti* [17], and other curcuminoids showed high efficacy against *Aedes albopictus* and *Culex pipiens* larvae [18]. In particular, curcumin, demethoxycurcumin, curcumin-BF2 complex, and a monocarbonyl tetramethoxy-curcumin derivative showed remarkable larvicidal activity, indicating their potential as alternative agents for mosquito control strategies [18]. Also found that in *C. pipiens*, curcumin increased mortality in the early larval stages through acetylcholine esterase 1 (AchE1) inhibition [19]. Additionally, research has explored the anti-insect effects of curcuma essential oil, such as feeding inhibition, oviposition deterrence, and reproductive inhibition [20]. However, to our knowledge, studies on the juvenile hormones (JHs) of insects related to curcuminoids have not been conducted.

The intricate interplay between juvenile hormone (JH) and ecdysone in insect development is a finely tuned process. JH, synthesized in the corpus allatum interacts with ecdysone to maintain larval development during molts [21]. Beyond its role in molting, JH plays a multifaceted role in crucial physiological functions, encompassing reproduction, developmental regulation, pheromone production, and caste differentiation, particularly in social insects [22]. Methoprene-tolerant (Met) was identified as a JH receptor in the mutant studies of *D. melanogaster*, accelerating research on JH signaling in *D. melanogaster* [23]. Derived from the germ cell-expressed gene, Met is a member of the basic helix-loop-helix (bHLH) Per-Arnt-Sim (PAS) family [24–26]. Like other members of the bHLH-PAS transcription factor family, Met requires the formation of heterodimeric partner with other bHLH-PAS proteins for its activation [27]. JHs act through a receptor complex consisting of Met and steroid receptor coactivator (SRC; Taiman in *D. melanogaster* or βFtz-F1 Interacting Steroid Receptor Coactivator FISC in *A. aegypti*) to induce the transcription of specific genes [28]. Mets bind to JH with a high affinity, activating transcription [24–26]. Subsequently, Met heterodimerizes with an SRC [23]. To probe the impact of these interactions on the juvenile hormone signaling pathway, in vitro yeast two-hybrid assay systems have been employed, utilizing the JH-dependent heterodimer-binding properties of Met and SRC. These experimental approaches have facilitated the exploration of Juvenile Hormone Disruptor (JHD) activities in various plant extracts, studied not only in *A. aegypti* [29] but also in the Indian meal moth *Plodia interpunctella* [30]. Furthermore, an in vitro assay system capable of quantifying the disruption activity of plant extracts and diterpenes on JH-mediated Met/Taiman (Tai) heterodimer formation in *D. melanogaster* was developed [31]. The outcomes of these studies collectively underscore the prevalence of JHD diterpenes in the plant kingdom, emphasizing their propensity to interfere with JH-mediated endocrine regulation in insects [32].

In this study, we carefully validated the JHD activity of 30 herbal medicines and edible plant extracts, building on previous findings that highlighted their potent JHD effects [31]. Both the plant extracts from *C. longa* L and two curcuminoids, DMC and BDMC, showed strong JHD activity in the in vitro assay system of *D. melanogaster* Met-Tai. DMC and BDMC also blocked larval/pupal development, preventing the formation of pupae and emergence of adults.

2. Materials and Methods

2.1. Chemicals and Insects[29]

JH III and methoprene were purchased from Sigma-Aldrich (St. Louis, MO, USA). Three curcuminoids, curcumin, DMC, and BDMC, were purchased from Aladdin (Pudong, Shanghai,

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China). The plant diterpene methyl lucidone (LE3G) was isolated from *Lindera erythrocarpa*, as described in a previous study [29]. Plant extracts (i.e., methanol extracts of 30 edible plant species) were obtained from the Korean Plant Extracts Bank (Daejeon, South Korea). Each reagent was prepared as a stock solution in either dimethyl sulfoxide (DMSO) or methanol. *D. melanogaster* was grown in an instant fruit fly medium (Hansol Tech, Seoul, South Korea).

2.2. Yeast β-galactosidase Assay

The yeast two-hybrid binding assay, utilizing a quantitative β-galactosidase assay, was conducted with the Y187 strain transformed with the D. melanogaster juvenile hormone (JH) receptor and its interacting partner, Met-Tai, following the established protocol outlined in a prior study [31]. The Y187 strain, which was modified according to the protocol specified in the previous study [31], was grown in culture For the screening of JHD activity, 100 µL of cultured yeast cells with an optical density at 600 nm (OD_{600}) ranging from 0.2 to 0.3 were exposed to a combination of 0.1 ppm JH III and 10 ppm individual plant extracts. This exposure was performed in 96-well plates, with each plate including a positive control comprising 0.1 ppm JH III with 10 ppm methyl lucidone and a negative control involving 0.1 ppm JH III with the control solvent (DMSO). Following a 3-hour incubation period, the cells were subjected to an assay for OD_{420}, providing a quantitative measure of *β*-galactosidase activity. The OD_{420} values were then normalized to an arbitrary unit of JHD. Methyl lucidone served as a positive control due to its strong interference with JH III-mediated Met-Tai binding in the D. melanogaster *β*-galactosidase assay systems tested [31]. A single arbitrary unit of JHD activity was determined based on the binding interference observed with 10 ppm methyl lucidone. To assess the specific JHD activity of each plant extract, the average of duplicate experiments was calculated in arbitrary units using the following formula:

$$A = \frac{OD_{420}\ Control - OD_{420}\ PE}{OD_{420}\ Control - OD_{420}\ ML10'}$$

· A: The activity of the JHD, adjusted to A = 0 if A < 0. This adjustment ensures that negative values do not contribute to the specific JHD activity calculation.

· OD_{420} Control: The absorbance of yeast cells treated with JH III.

· OD_{420} PE: The absorbance of yeast cells treated with 0.1 ppm of JH III and 10 ppm of each plant extract.

· OD_{420} ML10: The absorbance of cells treated with 0.1 ppm of JH III and 10 ppm of methyl lucidone.

The formula essentially compares the observed effects of the plant extract with the positive and negative controls, providing a standardized measure of the specific JHD activity that ensures accurate and meaningful comparisons across different experimental conditions.

2.3. Bioassay

The experimental design commenced by introducing twenty male and twenty female flies separately into individual vials. Each vial was then subjected to a specific dietary regimen. The treatments included 1% (w/v) concentrations of JHD, namely LE3G, DMC, and BDMC, 0.05% (w/v) of the Juvenile Hormone Analog (JHA) methoprene, and a control group treated with 1% (w/v) ethanol. All these compounds were thoroughly incorporated into 3 g of artificial feed. To ensure the proper solidification of the diet and the subsequent evaporation of ethanol, the mixed diets were allowed to stand undisturbed at room temperature overnight. Subsequently, the vials containing the experimental diets were maintained at a controlled breeding temperature of 25 °C. Following a one-day period of oviposition, during which each vial accumulated approximately 150–250 eggs, the adult flies were removed from the vials. After an additional 3–4 days, second-instar larvae were collected from each vial, forming the basis for subsequent analyses.

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2.4. RNA Extraction, Primers, and Quantitative Real Time Polymerase Chain Reaction (qPCR) Analysis

Total RNA was isolated from second instar larvae using the RNeasy isolation kit (Qiagen, Hilden, Germany). Subsequently, cDNA was synthesized for quantitative polymerase chain reaction (qPCR) with the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA) using 1 µg of RNA, quantified with a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific, Waltham, MA, USA). The qRT-PCR primers were designed through NCBI Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/), and the primer sequences can be found in Table 1. For qRT-PCR, the SsoAdvanced Universal SYBR Green Supermix (Bio-Rad) was employed in 96-well plates on a CFX Connect Real-Time Polymerase Chain Reaction System (Bio-Rad). The thermal cycling program consisted of an initial denaturation at 95 °C for 30 s, followed by 40 cycles of 95 °C for 10 s and 60 °C for 30 s. A final melting curve analysis spanned 95 °C for 5 s and 65 °C for 0.5 s. Validation of amplification efficiency and specificity was performed using CFX Maestro Software (Bio-Rad). To normalize the signal intensity of Kr-h1, it was compared to that of rp49. All RNA extractions and qRT-PCR analyses were conducted in triplicate, and the means of each sample were compared.

Table 1. Primer sequences used for qRT-PCR.

Gene	Primer
Krüppel homolog 1	Forward 5′-TCACACATCAAGAAGCCAACT-3′ Reverse 5′-GCTGGTTGGCGGAATAGTAA-3′
Rp49	Forward 5′-ATGCTAAGCTGTCGCACAAATG-3′ Reverse 5′-GTTCGATCCGTAACCGATGT-3′

2.5. Data Analysis

The significance of treatment and control mean differences was assessed using the Student's t-test. Statistical analyses and figure preparations were conducted using the GraphPad Prism 7 software.

3. Results

3.1. JHD Activity of Plant Extracts

In order to evaluate the JHD activities of different edible plant species on the formation of Met-Tai heterodimers in Drosophila melanogaster, 30 plant extracts, previously identified in a comprehensive study and mainly used as herbal medicines and edible plant extracts, were selected for investigation [32]. The selected plant extracts were tested for JHD activity in *D. melanogaster* using yeast-two hybrid β-galactosidase assays (Table 2). Among the extracts tested, 14 showed substantial JHD activity, with the highest activity observed in *C. longa* L. Notably, the curcuminoids present in Curcuma longa L. are linear diarylheptanoids that share similar size and structural characteristics with plant diterpenes and are recognized as well-known secondary metabolites of Curcuma plants. Motivated by these findings, we further investigated the JHD activities of three commercially available curcuminoids-curcumin, demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC)-that are constituents of Curcuma longa L. (Figure 1). Interestingly, two of these curcuminoids, DMC and BDMC, exhibited robust interference with the heterodimer binding of MET-Tai while showing no anti-yeast activity. These results highlight the potential of curcuminoids, particularly DMC and BDMC, as potent modulators of JHD activity and shed light on their intricate role in interfering with juvenile hormone signaling pathways. Further investigations into the

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molecular mechanisms underlying this interference and the specific effects on insect physiology are warranted to fully understand the implications for pest control strategies.

Table 2. Juvenile hormone disrupter (JHD) activity of plant extracts.

Species	Plant part	Family	JHD activity
Curcuma longa L.	Root	Zingiberaceae	1.217175
Pulsatilla koreana Nakai	Root	Ranunculaceae	1.101688
Machilus thunbergii Siebold & Zucc.	Trunk-bark	Lauraceae	1.056931
Echinosophora koreensis Nakai	Root	Fabaceae	0.960778
Pinus densiflora Siebold & Zucc.	Trunk-bark	Pinaceae	0.936298
Alpinia officinarum	Root	Zingiberaceae	0.904591
Smilax sieboldii Miq.	Leaf	Liliaceae	0.886733
Scutellaria baicalensis	Flower	Labiatae	0.825496
Portulaca oleracea L.	Whole	Portulacaceae	0.806894
Magnolia kobus DC	Leaf	Magnoliaceae	0.785417
Broussonetia papyrifera (L.) L'Hér. ex Vent.	Leaf	Moraceae	0.768187
Syringa patula (Palib.) Nakai	Leaf	Oleaceae	0.759335
Agrimonia pilosa Ledeb.	Whole	Rosaceae	0.750968
Cudrania tricuspidata (Carr.) Bureau ex Lavallée	Fruit	Moraceae	0.745534
Psoralea corylifolia (Babchi)	Seed	Fabaceae	0.658419
Phlomis umbrosa Turcz.	Whole	Labiatae	0.65772
Cudrania tricuspidata (Carr.) Bureau ex Lavallée	Trunk	Moraceae	0.613586
Zingiber officinale	Root	Zingiberaceae	0.546682
Myristica fragrans	Seed	Myristicaceae	0.535624
Saururus chinensis (Lour.) Baill.	Whole	Saururaceae	0.493735
Glycyrrhiza uralensis	Root	Fabaceae	0.469728
Picrasma quassioides (D. Don) Benn.	Trunk	Simaroubaceae	0.433324
Sophora flavescens	Root	Fabaceae	0.391224
Morus alba L.	Root	Moraceae	0.390836
Actinostemma lobatum Maxim.	Whole	Cucurbitaceae	0.381283
Salvia miltiorrhiza Bunge	Whole	Labiatae	0.363577
Cudrania tricuspidata (Carr.) Bureau ex Lavallée	Root	Moraceae	0.257315
Eclipta prostrata	Whole	Compositae	0.237714
Magnolia obovata Thunb	Trunk-bark	Lauraceae	0.176234
Angelica keiskei	Leaf	Apiaceae	0.134399

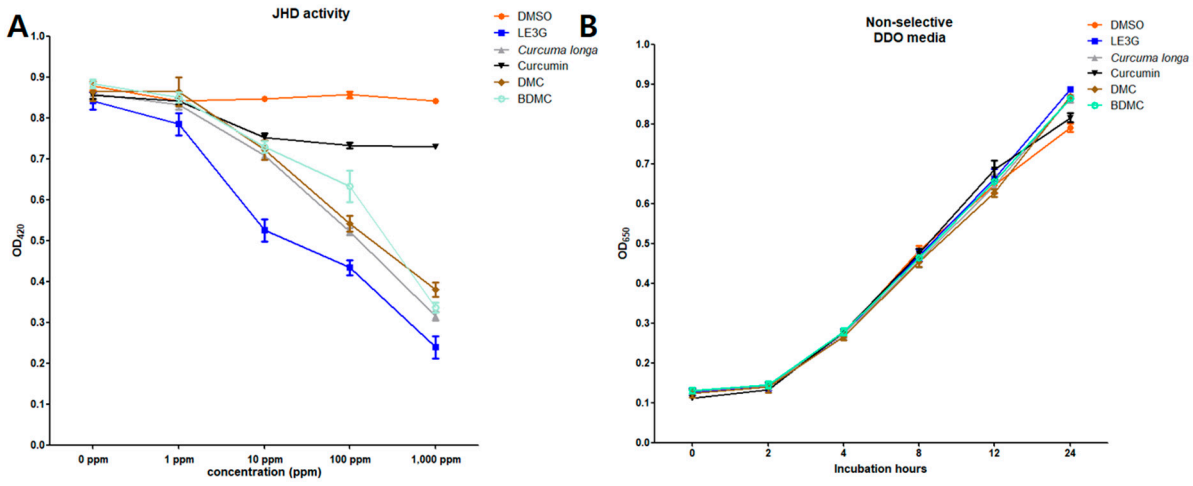

Figure 1. JHD activities of extracts from *Curcuma longa* L and two curcuminoids, demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC). (A) Met-Tai binding triggered by the addition of 0.1

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ppm of JH III and the inhibition of the Met-Tai binding activity by treatment with 10 ppm of methyl lucidone (LE3G) was used as a positive control. The plant extract and compounds were added to the yeast culture at each shown concentration. (B) Anti-yeast activity tests of *C. longa* L with JHD activity. Corresponding compounds were tested for their anti-yeast activity to investigate whether the reduced β-galactosidase activity resulted from JHD activity or anti-yeast toxicity. Mean values with associated error bars are presented as means ± standard deviation (n = 3).

3.2. Changes in the Emergence Rates of D. melanogaster Larvae According to Feeding

LE3G, an isolated diterpene from the extract of *L. erythrocarpa*, has previously been reported to interfere with larval development in mosquitoes, fruit flies, and moths [29,31,32], and this substance was used as a positive control in our study. Building on this, we explored the impact of two commercially available curcuminoids, demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC), on larval development in *D. mlanogaster*. Similar to the larvicidal effects observed with LE3G, the introduction of DMC or BDMC into the diets of D. melanogaster larvae resulted in a concentration-dependent inhibition of larval development. Notably, diets containing 1% (w/v) DMC or BDMC extract exhibited a pronounced disruption in larval development, preventing the progression to pupal stages (Figure 2). Furthermore, in diets supplemented with the JHD (LE3G, DMC, and BDMC at 1%, w/v), no adult flies were observed (Figure 3). This outcome suggests a significant impediment to the normal developmental transitions, emphasizing the potency of these compounds in disrupting the life cycle of D. melanogaster. These findings not only validate the larvicidal activity of LE3G but also extend it to the curcuminoids DMC and BDMC.

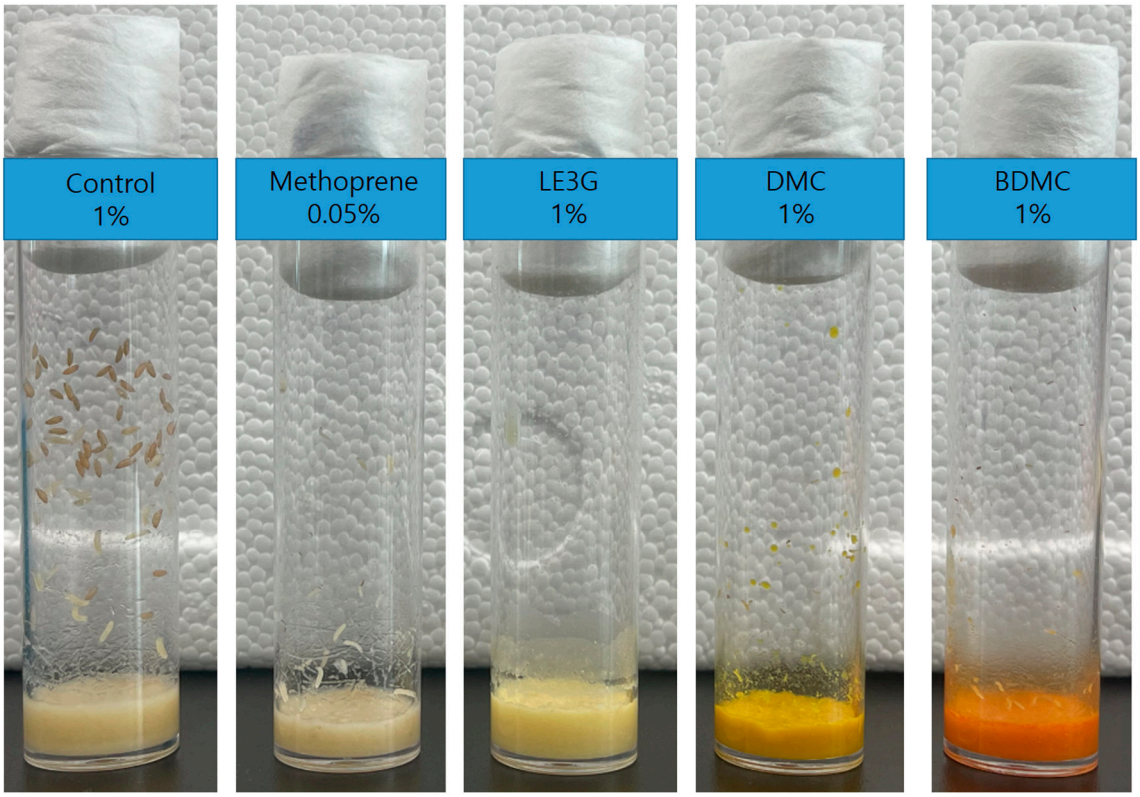

Figure 2. Effect of JHA (methoprene) or JHD (LE3G, DMC, and BDMC) compounds on *Drosophila melanogaster* larval development.

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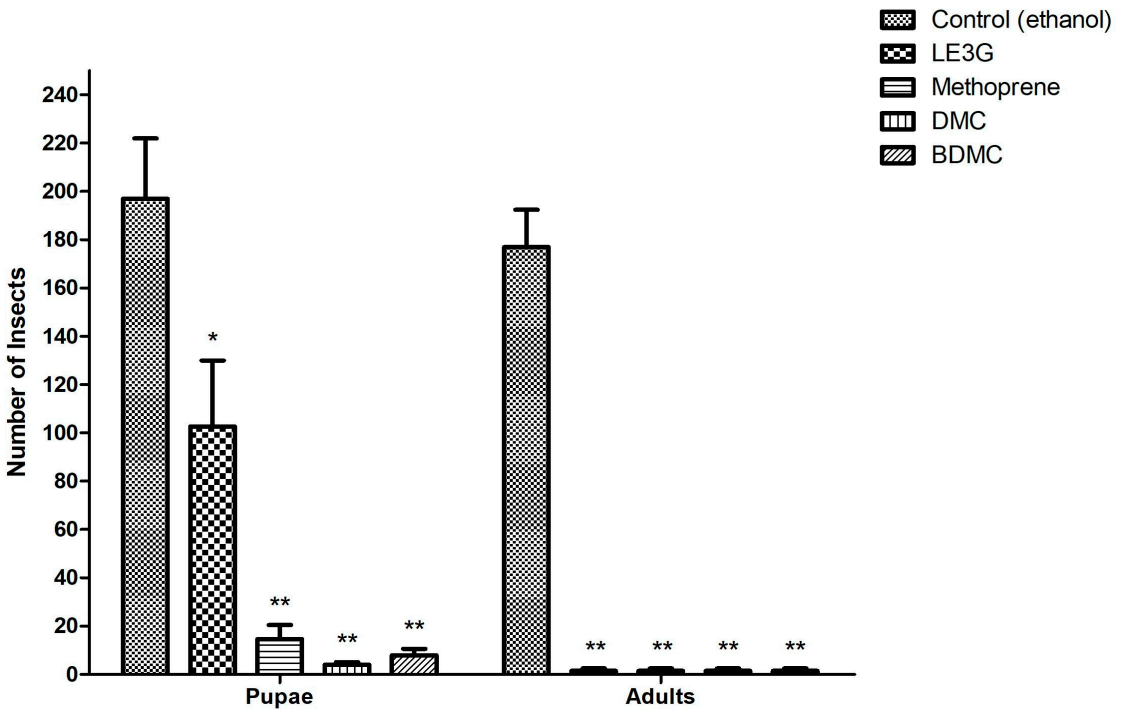

Figure 3. Effect of DMC and BDMC on the growth of *Drosophila melanogaster* larvae. Artificial diets were supplemented with either 1% (w/v) ethanol (control) or JHD compounds (LE3G, DMC, and BDMC], or 0.05% (w/v) JHA (Methoprene). Mean values with associated error bars are presented as means ± standard deviation (n = 3).. * $p < 0.01$ and ** $p < 0.001$ (*t*-test).

3.3. DMC and BDMC Effects on JH-dependent gene expression

Krüppel homolog 1 (*Kr-h1*), identified as an early juvenile hormone (JH) inducible gene, assumes a pivotal role in insect metamorphosis by orchestrating the activation of the JH/Met/Tai complex. In this study, we employed *kr-h1* as a marker gene to elucidate its expression pattern in second-instar *D. melanogaster* larvae under the influence of various treatments. Upon treating *D. melanogaster* larvae with LE3G, demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC), a consistent trend emerged, revealing a decrease in *Kr-h1* expression compared to the control group (Figure 4). This downregulation suggests a potential inhibitory effect of these compounds on the JH/Met/Tai complex, aligning with their observed interference with larval development. In contrast, methoprene-treated flies exhibited an increase in *Kr-h1* expression (Figure 4). Methoprene, a JH analog, is known to mimic the effects of JH in insects, promoting a response akin to natural JH induction. This elevation in *Kr-h1* expression in methoprene-treated flies supports the expected activation of the JH signaling pathway. These findings underscore the modulatory impact of LE3G, DMC, and BDMC on *Kr-h1* expression, indicative of their influence on the JH-regulated processes crucial for insect metamorphosis.

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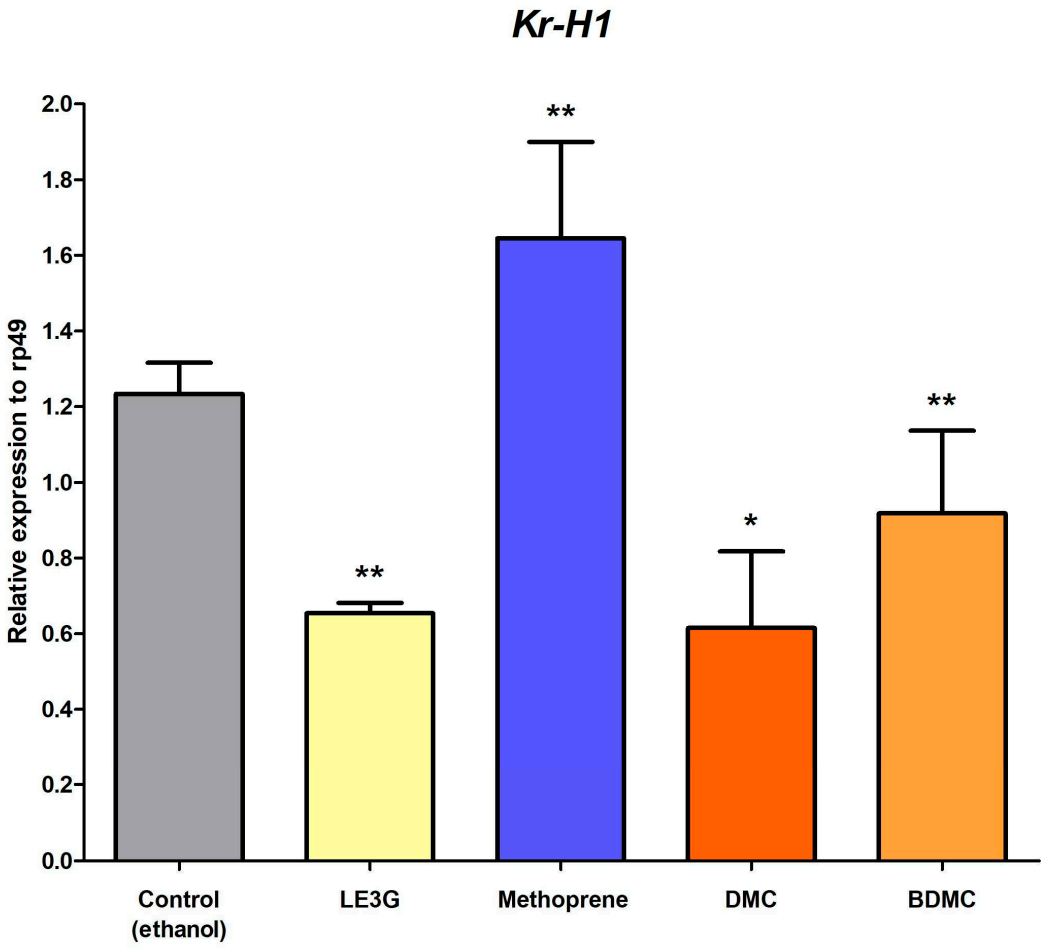

Figure 4. Krüppel homolog 1 (*Kr-h1*) expression after DMC or BDMC treatments. Total RNA was extracted from second instar larvae that were fed with a diet containing each component and analyzed using qRT-PCR. Values and error bars indicate means ± SD (n = 3). *, $p < 0.05$, **, $p < 0.01$ (*t*-test).

4. Discussion

Secondary metabolites in plants are produced as defense mechanisms against insects and other herbivores and have been used in this way throughout their evolutionary history [33,34]. Such secondary metabolites have become a research focus regarding the development of eco-friendly pesticides [35,36]. Plant-derived pesticides are considered ideal defense technologies because of their low toxicity, effectiveness against specific pests, and minimal environmental damage [37,38]. *C. longa* L, possessing insecticidal activity, has been utilized as an insecticide due to its diverse bioactive constituents that disrupt insect behavior and growth, making it possibly applicable for pest control in agriculture [39]. Curcuminoids, major components of *C. longa* rhizome powder, are known to show high insecticidal activity when used against various pests, such as *Culex pipiens* larvae [40]. Additionally, 45–60% growth inhibition and 10–15% mortality were confirmed as a result of insect growth inhibition assay in *Schistocerca gregaria* (Forsskål, 1775) and *Dysdercus koenigii* (Fabricius, 1775) by injecting the nymphs with curcuminoids [41]. In our study, *D. melanogaster* larvae fed with 1% curcuminoids failed to develop normally, suggesting that this substance inhibits larval/pupal development in relation to JH (Figure 3).

Curcuminoids, the yellow polyphenols responsible for the biological activity of turmeric oleoresin [42], are linear diarylheptanoids. Chemically, they are composed of two benzene rings linked by unsaturated chains, and they are a mixture of curcumin [1,7-bis(4-hydroxy-3-methoxy-phenyl)-hepta-1,6-diene-3,5-dione] and two derivatives, DMC and BDMC [43]. Curcumin contains methoxy groups on both ends of the benzene rings, whereas DMC lacks one of these methoxy groups.

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Conversely, BDMC has no methoxy groups and possesses a simpler structure than curcumin and DMC. Considering this structural difference, it is inferred that DMC and BDMC show higher Met-Tai binding inhibitory activity compared to curcumin, which is likely due to the variation in the number of methoxy groups on both ends of the benzene rings (Figure 5). Furthermore, methoprene and pyriproxyfen have similar ring-shaped backbone structures (Figure 5) similar to JH [44]. The cyclopentenedione rings of curcuminoids and LE3G consist of two α,β-unsaturated ketone functional groups [45]. These compounds may interfere with or disrupt the binding of the JH/Met/Tai complex by interacting with the methoxy and hydroxyl groups on both ends. However, explaining the chemical binding with JH as a singular determinant is challenging, as the interaction with JH receptors is regulated by various structural features and chemical properties of different compounds. Considering that curcuminoids have phenolic rings, α- and β-unsaturated carbonyl groups, and multiple substituents, these structural features may influence its interaction with JH receptors.

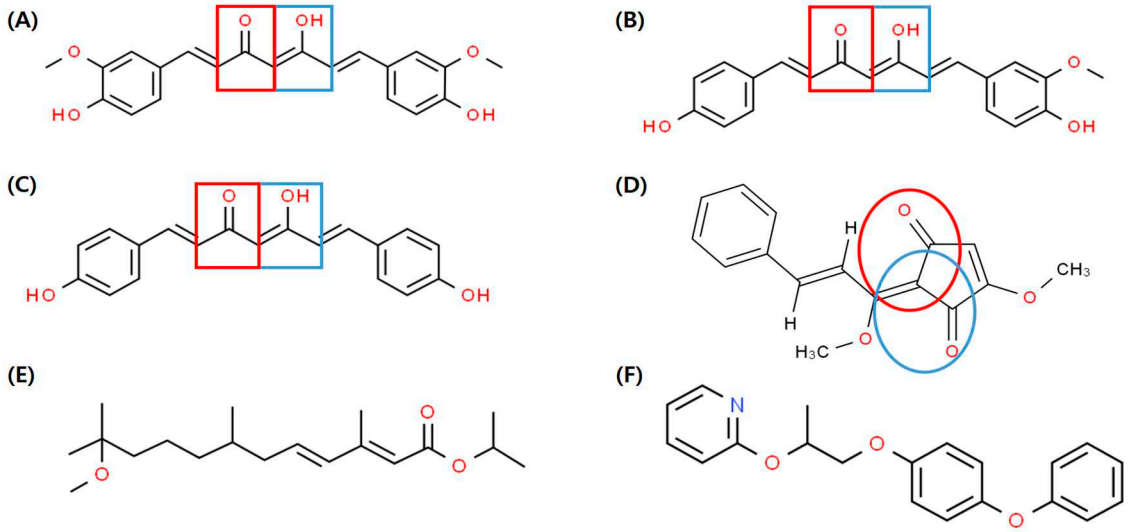

Figure 5. Chemical structures of JHD and JH analog (JHa). JHD: (A) Curcumin, (B) DMC, (C) BDMC, (D) LE3G, JHa: (E) Methoprene, and (F) Pyriproxyfen. Red and blue shapes indicate α,β-unsaturated ketone moiety.

In a previous study, LE3G, an extract of *L. erythrocarpa*, showed the opposite pattern of *Kr-h1* expression than that of JHD and methoprene [31]. JHD hinders larval development by interrupting heterodimer binding between Met and Tai through JH-mediating adjustments, whereas methoprene promotes heterodimer binding between Met and Tai by activating JH-mediating adjustments [46]. DMC and BDMC interfered with the development of *Drosophila* larvae (Figure 2), confirming that these substances had opposite effects compared with methoprene on *Kr-h1* expression (Figure 4). Additionally, it is believed that when insects ingest a diet containing a mixture of DMC or BDMC, it disrupts the regulation of JH-mediated genes while also interfering with normal JH-dependent development. Therefore, the use of insecticidal methods using DMC or BDMC holds great promise for the formulation of novel pest control strategies. The appeal lies in their safety profile, as these substances biodegrade to non-toxic products. In addition, their potential suitability for integration into comprehensive pest management programs enhances their attractiveness as environmentally friendly alternatives. This is expected to serve as a model for the development of new synthetic analogs with favorable biological properties. Furthermore, as both curcuminoids are components of the tumeric, they would be classified as substances harmless to humans and animals. As a result, they can enhance environmental protection and the value of agricultural products. By maintaining the sustainability of insecticide production and preserving a healthy environment, these plant-derived insecticides are considered potential candidates for new insecticidal substances.

5. Conclusions

This study highlights the insecticidal effects of curcuminoids, which are known to be the major constituents of *Curcuma longa* L, particularly against *Drosophila melanogaster* larvae. Components of the curcuminoids, demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC), were shown to affect *D. melanogaster* larval and pupal development, suggesting that they interfere with juvenile hormone (JH)-related processes. Structural differences among curcuminoids, particularly in the number of methoxy groups, likely contribute to variations in their binding affinity with JH/Met/Tai complex. DMC and BDMC, whose effects on *Kr-h1* expression were found to be opposite to those of methoprene, disrupt the heterodimeric bond between Met-taiman, which is important for JH-mediated regulation. More importantly, the use of DMC or BDMC as an eco-friendly pesticide suggests that these substances can be presented as a safe alternative to chemical pesticides with less environmental damage. In addition, the fact that these substances are classified as harmless to humans and animals and are environmentally friendly can contribute to environmental protection and enhancement of agricultural value. The potential applications of the plant-derived pesticides presented in this study are highlighted as sustainable and environmentally friendly alternatives in agriculture, and these substances are considered as potential candidates for new pesticide materials.

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