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Effects of Sublethal Doses of Methyl Benzoate on the Life History Traits and Acetylcholinesterase (AChE) Activity of *Aphis gossypii*

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Abstract: Safer alternatives to synthetic pesticides are essential for sustainable agriculture. Methyl benzoate (MB) is a volatile essential oil found in several plants. Recent reports of the toxicity of MB to arthropod pests suggest that MB may be a useful alternative insecticide. The present study assessed the effects of a sublethal concentration of MB (LC_{30}, 0.22%) on the life history and reproductive characteristics of the cotton aphid, *Aphis gossypii* Glover, in both a treated parental generation (F_0) and untreated progeny (F_1). MB treatment significantly decreased longevity and fecundity in both the F_0 and F_1 generations, and prolonged the developmental duration of each immature instar of the F_1 generations, compared with controls. The intrinsic rate of increase (r), finite rate of increase (λ), and net reproductive rate (R_0) of the F_1 generation were significantly reduced, compared to controls. The mode of action of MB is not known, but in aphids treated with LC_{30} MB, the activity of the enzyme acetylcholinesterase (AChE) decreased by more than 65%, compared with untreated controls. AChE activity was rapidly inhibited within 1 h, and remained inhibited for 6 h after in vivo exposure to MB. Moreover, molecular docking analysis revealed that MB had a strong affinity with the catalytic site of AChE, with a binding energy value of −6.2 kcal/mole. Our results suggest that MB targets AChE, and that a sublethal dose of MB can have adverse transgenerational effects on cotton aphids.

Keywords: natural pesticide; sublethal effects; two-sex life table; population parameters; enzyme activity; molecular target

1. Introduction

The cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), is a common agricultural insect pest that attacks crops worldwide [1]. The cotton aphid damages plants directly by consuming

sap, and indirectly by transmitting viral diseases and contaminating plants with honeydew [2,3]. Cotton aphids are carriers of at least 76 viral diseases, threatening 900 known host plants [1]. Synthetic chemical insecticides are the most common current control methods for cotton aphids [4,5]. However, cotton aphids have developed resistance to many pesticides, due to frequent use [6–8]. In addition, the side effects of synthetic pesticide use include environmental hazards, residue problems, and negative impacts on natural enemies, reiterating the importance of finding alternative control methods.

Pesticides cause insect pest mortality when a lethal dose is applied; however, sublethal effects should also be considered to gain a comprehensive understanding of the effect of insecticides. Insects are often exposed to sublethal concentrations of pesticides because the chemicals naturally degrade following application to crops [9]. Sublethal pesticide doses do not kill the whole pest population, but may result in behavioral or physiological effects, such as altering the development, longevity, and reproduction of individual insects [10–12]. Additionally, certain effects of sublethal insecticide exposure in one insect generation can be transferred to the unexposed offspring of the next generation [13,14]. Most research investigating the transgenerational effects of insecticides has focused on egg development and hatching success [13–16]. Apart from these short-term variables, the transgenerational effects of insecticides on longer-term life-cycle traits require further research [14].

Life table analysis is a key method for the study of insect population dynamics [17]. In this study, we evaluate the effects of a sublethal concentration of methyl benzoate (MB) on selected biological parameters of cotton aphids. We compare the effects of MB on an exposed parental population with their unexposed offspring generation. Although the cotton aphid mostly reproduces parthenogenetically, the age-stage, two-sex life table approach takes into consideration different growth rates between individuals, as well as stage differentiation.

Recently, MB was demonstrated to be an effective insecticide against several insect pests [18]. MB is a naturally occurring compound found in various plant species [19] that degrades slowly in the atmosphere [20]. Our recently published results show that MB was acutely toxic to several arthropods, namely *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae), *Tetranychus urticae* Koch (Acari: Tetranychidae), and *A. gossypii*. However, MB was much less toxic to the non-target organisms, *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) and *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae) [21–24]. Based on the above results, MB has potential as a safe, or "green", pesticide that can be applied in integrated pest management.

Agricultural insecticides control pests through several "modes of action". The majority of insecticides affect the nervous system, typically by inhibiting acetylcholinesterase (AChE), an important enzyme that catalyzes the breakdown of the neurotransmitter acetylcholine, resulting in the termination of nerve impulse transmissions [25]. The AChE system is targeted by organophosphate (OP) and carbamate insecticides [26,27]. To date, no literature has been published on the effects of MB on the AChE system in cotton aphids, nor on possible transgenerational effects of MB. Thus, we investigated the mechanism of action of MB and evaluated the effects of a sublethal dose of MB on aphid growth and reproductive traits, while also considering the transgenerational effects. An investigation of AChE activity inhibition by MB and a molecular docking study were undertaken to evaluate the possibility of molecular interaction between MB and AChE in the cotton aphid. The results of our research provide a benchmark for understanding the efficacy of MB for cotton aphid control, as well as important aspects of the physiological and biochemical responses of cotton aphids to MB treatment.

2. Materials and Methods

2.1. Insects and Reagents

A laboratory cotton aphid colony was sourced from the Rural Development Administration (RDA), Jeonju, Korea. The colony was established on cucumber plants (*Cucumis sativus* L.) in a laboratory

maintained at 25 ± 1 °C and 60 ± 10% relative humidity (RH), with a 16:8 h light/dark photoperiod. The colony has been maintained without any exposure to insecticides since 2017.

Commercially available MB (99% purity) and emulsifiers Tween 20 and Tween 80 were purchased from Sigma-Aldrich (St. Louis, MO, USA). The sublethal dose of MB was the LC_{30} concentration (0.22%) determined in our previous study [22]. The 0.22% MB solution and the control solution were prepared in distilled water, according to our published procedure [21].

2.2. Sublethal Effects of Methyl Benzoate on the Cotton Aphid F0 Generation

The toxicity of MB against cotton aphid adults (≤24-h-old) was evaluated, according to the procedure published by Cui et al. [8]. Cucumber leaf discs (20 mm in diameter) were dipped into the control or MB solution (LC_{30}) for 5 s, then allowed to air dry for ~30 min. After drying, the treated leaf discs were placed into Petri dishes (5 cm D × 1.5 cm H) containing 1% agar (Junsei Chemical Co., Ltd., Tokyo, Japan), with the leaf underside resting on the agar bed. The agar provided moisture to prevent desiccation of the leaf discs. Ventilation was provided in the Petri dishes by cutting a 2 cm diameter hole in each lid and covering the hole with nylon mesh. For each treatment, including controls, 75 apterous adult aphids were used. Mortality data were collected 24 h after the aphids were placed in the treatments. Any surviving aphids were transferred from treated leaves onto fresh untreated leaf discs using a camel hair brush. The untreated leaf discs were replaced every 3 d and mortality data were collected every 24 h until all of the remaining aphids were dead. These bioassays were conducted under laboratory conditions at 25 ± 1 °C, 60 ± 10% RH, and 16:8 h light/dark cycle.

2.3. Transgenerational Effects of Methyl Benzoate on the Cotton Aphid F1 Generation

Newly born nymphs from the MB-treated F_0 female adults were collected within 24 h and placed individually on untreated cucumber leaf discs in Petri dishes (as described in Section 2.2) to observe the transgenerational effects of MB. A total of 75 newborn F_1 nymphs were collected from each treatment (MB and control). Life-cycle characteristics, such as growth stage, fecundity, mortality, and longevity, were monitored daily. At the time of reproduction, the newly born nymphs were counted and transferred daily to fresh cucumber leaf discs until the reproducing adult died. Throughout the experiment, leaf discs were replaced every three days. All bioassays were conducted under the laboratory conditions described in the previous section.

2.4. Preparation of Cotton Aphid Proteins

Cotton aphid adults were treated with the LC_{30} dose of MB (0.22%). Aphids (n = 60) were exposed to MB for 0, 1, 3, and 6 h. After the exposures, the surviving aphids were collected and homogenized using 300 µL of ice-cold phosphate buffer (PBS) (0.1 M, pH 7.4). Next, the homogenate material was centrifuged at 12,000 rpm for 15 min at 4 °C, and the supernatant was collected for the protein source. The protein concentration of the enzyme extract was evaluated using the Bradford method [28], with bovine serum albumin as the standard.

2.5. Determination of Acetylcholinesterase Activity

To measure AChE activity, acetylthiocholine iodide (ATChI, Sigma-Aldrich, St. Louis, MO, USA) was used as a substrate, following the methods described by Ellman et al. [29]. Next, 20 µL of enzyme solution and 160 µL of PBS (0.1 M, pH 7.4) were added to each well of a 96-well microplate (SPL, Pocheon, Korea). The mixtures were then incubated in a shaker for 15 min at 27 °C. After this procedure, 10 µL of 75 mM ATChI and 10 µL of 0.1 M dithionitrobenzoic acid (DTNB, Sigma-Aldrich, St. Louis, MO, USA) were added to each well. The absorbance was measured at 412 nm using a microplate reader (Victor3, PerkinElmer, Waltham, MA, USA). The change in absorbance was recorded every 30 s for 20 min. All treatments were performed in triplicate. AChE activity was indicated as nmol of ATChI hydrolyzed/min/mg protein using an extinction coefficient of 1.36×10^4 M^{-1} cm^{-1}.

2.6. Molecular Docking Analysis of the Protein-Ligand

The crystal structure of AChE (PDB ID 1QON) was obtained from the Protein Data Bank (PDB) (http://www.rcsb.org/pdb/home/home.do) [30]. To investigate whether MB has the ability to inhibit AChE, a molecular docking test was performed using AutoDock Vina. The protein structures were prepared using UCSF Chimera 1.13.1 (http://www.cgl.ucsf.edu/chimera) to remove all non-receptor atoms, including water, ions, and miscellaneous compounds. The ligand (MB) was prepared using ChemBio3D Ultra, version 12.0 (PerkinElmer, Waltham, MA, USA), then an MMFF94 energy minimization was performed. AutoDock Vina (The Scripps Research Institute, La Jolla, CA, USA) was used for the molecular docking simulations. The ligand was removed, and a site sphere was specified to define the active site of AChE. The center of the grid box was 33, 67, and 10 Å on the x, y, and z axes, respectively, whereas the dimension (Å) was 16.02, 15.98, and 16.25 on the x, y, and z axes, respectively. The best confirmation with the lowest binding energy was selected. After the docking search was completed, it was visualized using a PyMOL Molecular Graphics System (version 1.7.4, Schrödinger, Inc., New York, NY, USA). The binding results were visualized as 3D and 2D diagrams using Discovery Studio Visualization, version 4.5 (Accelrys, Inc., San Diego, CA 92121, CA, USA) and LigPlot viewer (v.2.2, EMBL-EBI, Wellcome Genome Campus, Hinxton, Cambridgeshire, CB10 1SD, UK, http://www.ebi.ac.uk/thornton-srv/software/LIGPLOT), respectively.

2.7. Statistical Analyses

The population parameters, including intrinsic rate of increase (r), finite rate of increase (λ), net reproductive rate (R_0), mean generation time (T), age-stage specific survival rate (s_{xj}), age-specific survival rate (l_x), age-specific fecundity (m_x), adult pre-reproductive period (APRP), total pre-reproductive period (TPRP), reproductive days (R_d), age-specific maternity ($l_x m_x$), age-stage life expectancy (e_{xj}), and reproductive value (v_{xj}), were calculated, according to the age-stage, two-sex life table theory [31–34] using TWOSEX-MSChart (ver. 2019.08.01; http://140.120.197.173/Ecology/Download/Twosex-MSChart-exe-B100000.rar) [35]. The population parameter variances and standard errors were calculated using the 100,000 random resampling bootstrap technique [36]. The differences in population parameters between the control and MB treatment groups were measured using the paired bootstrap test, based on the confidence intervals implemented in TWOSEX-MSChart [34,35,37]. One-way ANOVA was used to assess differences in enzyme activity, followed by post-hoc Tukey HSD test ($p < 0.05$) (SAS version 9.4) [38]. SigmaPlot 12.5 [39] was used to create all graphs.

3. Results

3.1. Sublethal Effects of Methyl Benzoate on the F_0 Generation

Treatment of adult cotton aphids with 0.22% MB using the leaf disc method significantly affected longevity and fecundity (Figure 1). Compared with the untreated control group, the adult longevity of F_0 individuals treated with MB was significantly reduced from 20.37 d to 8.56 d ($p < 0.0001$; Figure 1). Fecundity of the F_0 generation also significantly decreased from 27.88 offspring/female in the control to 9.63 offspring/female in the MB treatment ($p < 0.0001$; Figure 1).

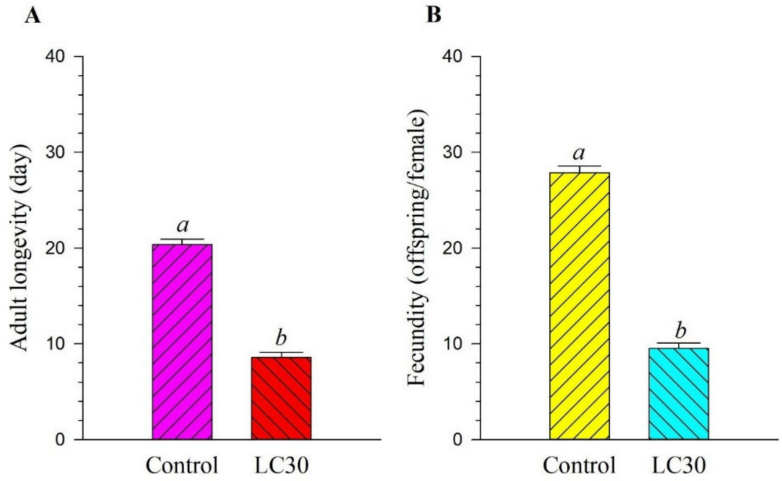

Figure 1. The initial (F_0) adult longevity (**A**) and fecundity (**B**) of *Aphis gossypii* treated with LC_{30} methyl benzoate (MB) or control for 24 h. Bars labelled with different letters are significantly different based on a paired bootstrap test ($p < 0.05$). (**A**) and (**B**) indicates the adult survival and offspring/individual of *A. gossypii*, respectively.

3.2. Sublethal Effects of Methyl Benzoate on the F_1 Generation

The effects on the F_1 generation of treating the F_0 generation of aphids with LC_{30} MB are presented in Table 1. Compared with the untreated control group, the mean longevity and fecundity of the F_1 generation significantly decreased, due to the treatment of the parental generation with LC_{30} of MB ($p < 0.0001$). Furthermore, after exposing the F_0 individuals to MB, the growth period of each immature stage of the F_1 generation significantly increased ($p < 0.0001$). This shows that F_0 MB treatment significantly increased the pre-adult duration ($p < 0.0001$), APRP ($p < 0.0001$), and TPRP ($p < 0.0001$) of the F_1 progeny, compared to the control. A significant difference in the number of reproductive days of the F_1 generation was also observed between the MB and control treatment groups ($p < 0.0001$; Table 1).

Table 1. Transgenerational effects of methyl benzoate on developmental times, longevity, adult pre-reproductive period (APRP), total pre-reproductive period (TPRP), and mean fecundity of F_1 progeny *Aphis gossypii* when parental individuals (F_0) were exposed to the sublethal concentration (LC_{30}) of methyl benzoate.

Biological Parameters	Developmental Time (Days) of F1 Progeny				*p*
	Control		Methyl Benzoate (LC_{30})		
	N	Mean ± SE [a,b]	N	Mean ± SE [a,b]	
First instar (d)	68	2.10 ± 0.05a	67	2.31 ± 0.06b	0.0108
Second instar (d)	66	1.55 ± 0.06a	65	2.14 ± 0.06b	<0.0001
Third instar (d)	66	1.41 ± 0.06a	65	2.15 ± 0.07b	<0.0001
Fourth instar (d)	65	1.14 ± 0.04a	65	2.05 ± 0.06b	<0.0001
Pre-adult (d)	65	6.17 ± 0.08a	65	8.68 ± 0.09b	<0.0001
Pre-adult survival rate (s_a)	75	0.87 ± 0.039a	75	0.87 ± 0.039a	1.000
Adult longevity (d)	65	23.94 ± 0.45a	65	12.0 ± 0.43b	<0.0001
Total longevity (d)	75	26.51 ± 1.14a	75	18.21 ± 0.82b	<0.0001
APRP (d)	65	0.08 ± 0.04a	65	0.65 ± 0.14b	<0.0001
TPRP (d)	65	6.25 ± 0.08a	65	9.32 ± 0.16b	<0.0001

Table 1. *Cont.*

Biological Parameters	Developmental Time (Days) of F1 Progeny				
	Control		Methyl Benzoate (LC_{30})		*p*
	N	Mean ± SE [a,b]	N	Mean ± SE [a,b]	
Reproductive days (d)	65	18.55 ± 0.31a	65	8.69 ± 0.2b	<0.0001
Fecundity (offspring/individual)	65	35.2 ± 0.45a	65	13.49 ± 0.26b	<0.0001

[a] Standard errors (SE) were estimated using the bootstrap technique with 100,000 re-samplings; [b] differences between two treatments were compared using a paired bootstrap test implemented in TWOSEX-MSChart. The means in the same rows followed by different lower-case letters indicate significant differences between treatments ($p < 0.05$).

3.3. Transgenerational Effects of Methyl Benzoate on Population Parameters

The transgenerational effects of MB (LC_{30}) on population parameters of the F1 generation were assessed using a life table-based bootstrap technique. Compared to the control group, the intrinsic rate of increase (r), finite rate of increase (λ), the net reproductive rate (R_0), and gross reproductive rate (GRR) significantly decreased ($p < 0.0001$), while the mean generation time (T) (13.71 ± 0.24) significantly increased in the MB treatment group, compared with the control group (12.42 ± 0.23) ($p < 0.0001$; Table 2).

Table 2. Transgenerational effects of methyl benzoate on population parameters of the F_1 generation of *Aphis gossypii*.

Population Parameter [a]	Bootstrap (Mean ± SE [b,c])		*p*
	Control	MB (LC_{30})	
r (d^{-1})	0.2752 ± 0.0069a	0.1793 ± 0.0048b	<0.0001
λ (d^{-1})	1.3169 ± 0.0092a	1.1964 ± 0.0058b	<0.0001
R_0 (offspring/individual)	30.51 ± 1.43a	11.69 ± 0.57b	<0.0001
T (d)	12.42 ± 0.23a	13.71 ± 0.24b	<0.0001
GRR (offspring/individual)	44.83 ± 1.63a	21.92 ± 1.51b	<0.0001

[a] r: intrinsic rate of increase; λ: finite rate of increase; R_0: net reproductive rate; T: mean generation time (offspring/individual); GRR: gross reproduction rate; [b] standard errors (SE) were estimated using the bootstrap technique with 100,000 re-samplings; [c] differences between two treatments were compared using a paired bootstrap test implemented in TWOSEX-MSChart. The means in the same rows followed by different lower-case letters indicate significant differences between treatments ($p < 0.05$).

3.4. Transgenerational Effects of Methyl Benzoate on Age-Stage Specific Rate of Survival and Fecundity

The age-stage specific rate of survival (s_{xj}) is the probability that a newly-born individual will survive to age x and stage j (Figure 2). Due to variable growth rates between individuals, considerable overlaps were observed between the life stages in the control and MB treatment groups (Figure 2). The pre-adult survival rate (s_a) was not significantly different between treatments (Table 1).

The age-survival rate (l_x) provides a simplistic description of the rate of survival without considering the stage distinction (Figure 3). In this study, the l_x curve of MB-treated groups significantly decreased from 16-days-old, while the l_x in the control group dropped from 22-days-old (Figure 3). The l_x value for LC_{30} treatment with MB declined earlier, compared to the control. Based on the curve m_x (age-specific fecundity) the control group's maximum age-specific fecundity rate (2.2 offspring) occurred at the age of 7 d. Additionally, the MB-treated group had peak fecundity at 14-days-old (1.51 offspring) (Figure 3). Based on both l_x and m_x, the maximum net maternity $l_x m_x$ value of 1.93 offspring occurred in the control group at 7-days-old, while the $l_x m_x$ 1.31 in the MB-treated groups occurred at 14-days-old (Figure 3).

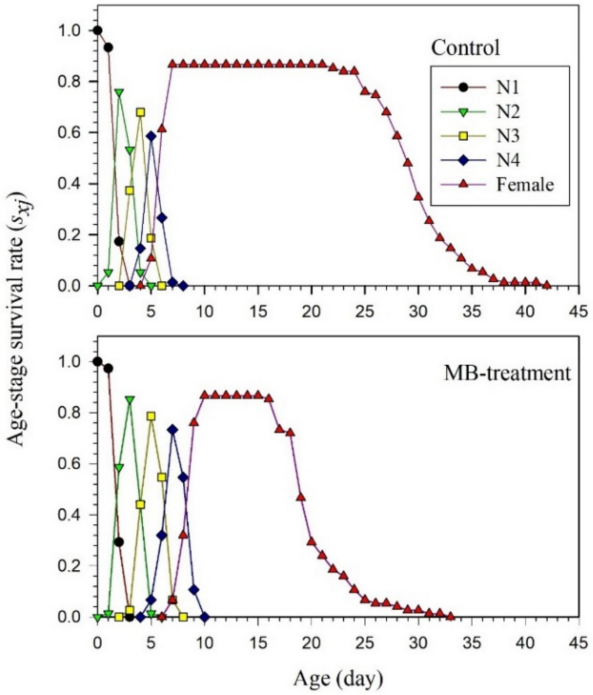

Figure 2. Age-stage-specific survival rates (s_{xj}) for F_1 *Aphis gossypii* descended from the parental generation (F_0) under control and MB-treatment (LC_{30}) condition. N1–N4 indicates the first to fourth instars of *A. gossypii*.

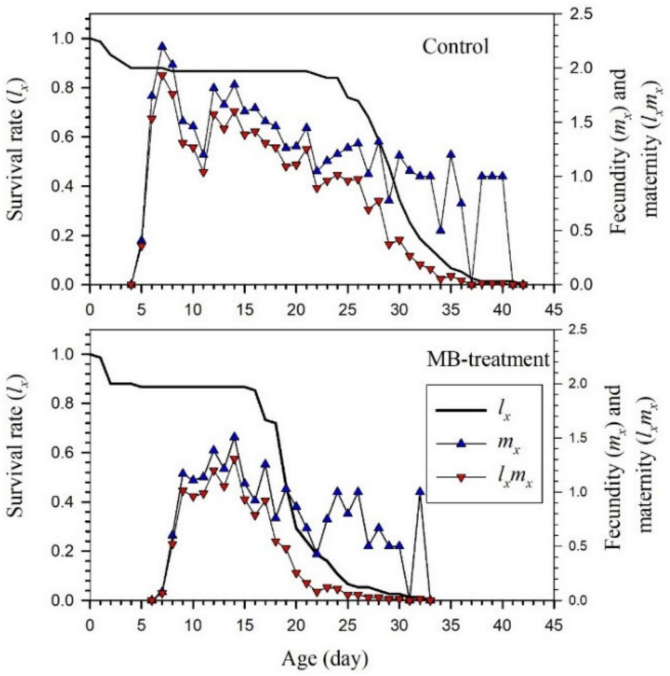

Figure 3. Age-specific survival rate (l_x), age-specific fecundity of total population (m_x), and age-specific maternity (l_xm_x) for F_1 *Aphis gossypii* descended from F_0 exposed to LC_{30} methyl benzoate (MB).

The age-specific life expectancy (e_{xj}) is the length of time that an individual of age x and stage j is expected to survive after age x (Figure 4). The life expectancy (e_{xj}) of cotton aphids treated with MB was lower than the control group (Figure 4).

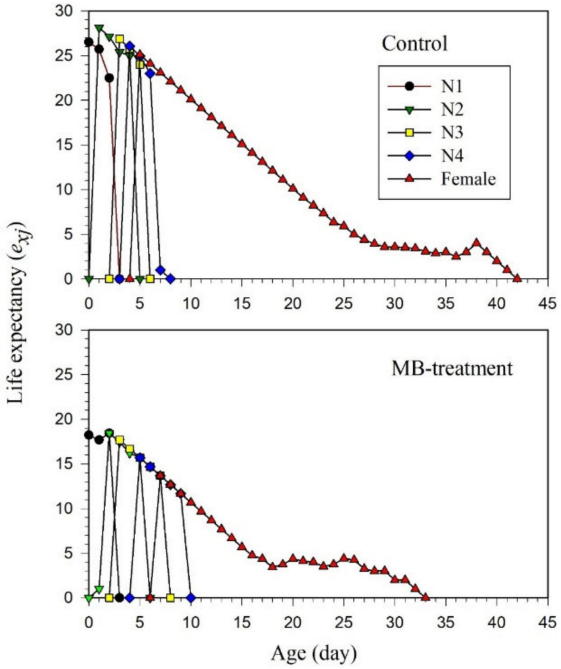

Figure 4. Age-stage-specific life expectancy (e_{xj}) for F_1 *Aphis gossypii* descended from F_0 exposed to LC_{30} methyl benzoate.

In addition, the age-stage reproductive value (v_{xj}) is an indicator of an individual's contribution to the future population at various ages and stages (Figure 5). In the MB-treated group, the maximum v_{xj} value occurred significantly later (6.86/d at 8-days-old), compared to that in the control group (9.53/d at 5-days-old) (Figure 5).

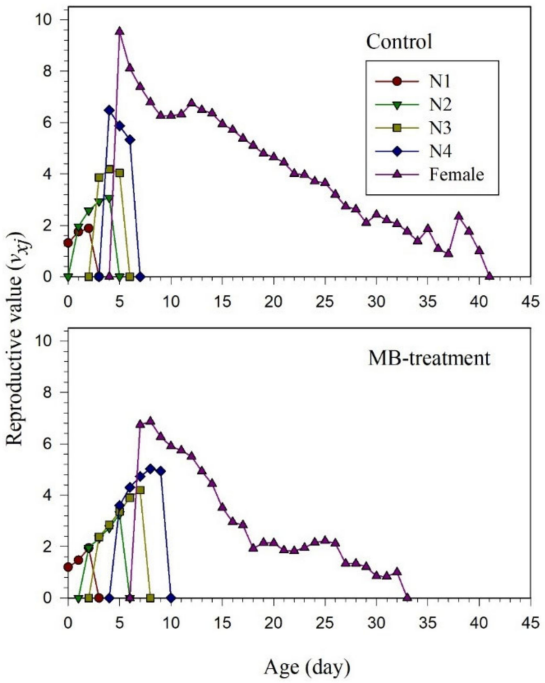

Figure 5. Age-stage reproductive value (v_{xj}) of F_1 *Aphis gossypii* descended from F_0 exposed to LC_{30} methyl benzoate.

3.5. Effect of Methyl Benzoate on AChE Activity

A direct correlation was observed between AChE activity and MB exposure period, thereby emphasizing the time-dependence of MB activity ($F = 7.99$; df = 3, 23; $p < 0.0001$) (Figure 6). In the time-course inhibition studies, AChE activity of *A. gossypii* was quickly inhibited by MB treatment, and remained inhibited following in vivo exposure (Figure 6). The high level of AChE inhibition by 1 h bioactivation occurred rapidly after exposure to MB. AChE activity decreased by more than 65% in MB-exposed aphids, compared to the control ($p < 0.0001$; Figure 6).

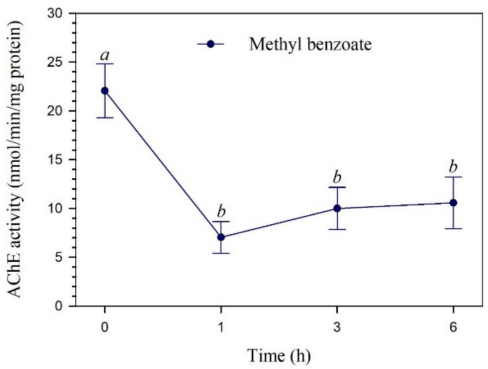

Figure 6. AChE activity of *Aphis gossypii* treated with LC_{30} (0.22%) methyl benzoate. The results are presented as mean ± SE. Different letters above the bars indicate a significant difference between the times at $f < 0.05$.

3.6. Protein–Ligand Molecular Docking Analysis

The molecular docking protocol must be validated before performing molecular docking studies. Therefore, to validate the protocols used in this analysis, the ligand crystallographic information was subjected to the development of docking until the spatial conformation was found by comparison with the original crystallographic structure of the AChE inhibitors (PDB ID 1QON). The validation was performed by retrieving the structure of an AChE inhibitor (I40) by calculating the root-mean-square deviation (RMSD) of 0.98 Å. The binding mode predicted using docking indicates that when the RMSD is less than 2.0 Å regarding the crystallographic pose of a respective ligand, the validation is considered satisfactory [40,41]. The satisfactory results are illustrated in Figure 7a.

The binding affinity between MB and the AChE protein was expressed as a binding energy value of −6.2 kcal/mol, while the actual ligand I40 (9-(3-iodobenzylamino)-1,2,3,4-tetrahydroacridine) binding with AChE had a value of −12.5 kcal/mol. As shown in Figure 7b–d, the individually-observed interaction after MB docking was identical to I40 in the active site of AChE, found around the α-helix between amino acid residues Tyr-370, Tyr-374, and Tyr-71, as well as the β-sheet with amino acid residue Trp-83. Quantitative data on residues, distances, and types between MB and the insect AChE are presented in Table S1 and Figure S1.

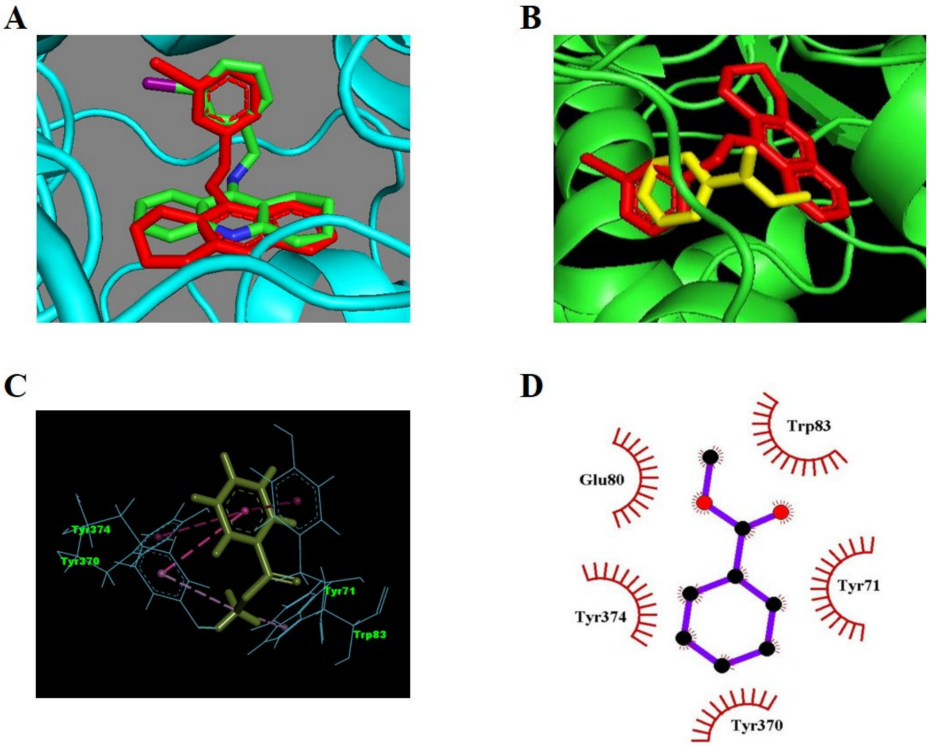

Figure 7. Molecular docking of MB with acetylcholinesterase (AChE); Protein Data Bank (PDB): 1QON. Overlays of crystallographic ligand positions of I40 (in red), with the calculated pose (in green) (**A**); co-localization of the ligand I40 and MB (**B**); interactions of the active site of AChE of *Drosophila melanogaster* with the molecule MB in 3D (**C**) and 2D (**D**).

4. Discussion

Natural products can often provide a more environmentally-friendly approach to managing insect pests and plant diseases [42,43]. MB, which is a naturally occurring compound found in many plants, exhibits potent insecticidal activity against a variety of insect pests [18,21–24,44]. Therefore, MB could provide an alternative to synthetic insecticides for agricultural control of cotton aphids in the near future. However, the sublethal and transgenerational effects of MB on cotton aphids were previously unknown.

This study showed the effects of a sublethal dose of MB on the biological traits and AChE activity of the cotton aphid. We observed a significant decrease in adult survival and fecundity of *A. gossypii* directly exposed to the previously determined LC_{30} of MB. Similar effects were documented in an earlier study, in which the fecundity and longevity of *A. gossypii* significantly declined with exposure to sublethal doses of buprofezin [45]. Additionally, adult survival and fertility were significantly reduced when cotton aphids were treated with LC_{10} and LC_{40} cycloxaprid [46]. Aphid longevity and fertility in the aforementioned studies substantially decreased, but similar decreases were not observed in *B. tabaci* exposed to sublethal doses of imidacloprid [47]. Likewise, no decrease in longevity or fecundity was observed in *Apolygus lucorum* Meyer-Dür (Hemiptera: Miridae) exposed to a sublethal concentration of cycloxapride [16]. In addition, the reduced longevity and fecundity patterns observed in cotton aphids suggest a lack of hormesis, which is a common sublethal effect of insecticide exposure. Hormesis is a concentration–response biphasic process, which is normally characterized by stimulation at low concentrations and inhibition at high concentrations [48,49]. Insecticide-induced hormesis has been recorded in several insect species, for example, higher fecundity in *Myzus persicae* Sulzer (Hemiptera: Aphididae) subjected to low imidacloprid concentration [50] and *Oligonychus ilicis* McGregor (Acari: Tetranychidae) outbreaks caused by low doses of pyrethroid [51]. However, hormesis effects on the longevity and fecundity of the parental generation (F_0) of *A. gossypii* exposed to a sublethal concentration of MB were not observed in this study.

Transgenerational effects of MB on cotton aphid progeny (F_1) were identified when the parental generation (F_0) was exposed to LC_{30} MB. The length of each nymphal stage and the pre-adult duration of the F_1 progeny of MB-exposed F_0 were significantly extended. The delayed development rate we observed is comparable to earlier reports, such as the delayed development of *Plutella xylostella* L. (Lepidoptera: Plutellidae) exposed to LC_{25} chlorantraniliprole [13]. Similarly, the duration of each developmental stage in *Rhopalosiphum padi* L. (Hemiptera: Aphididae) increased when exposed to sublethal concentrations of beta-cypermethrin or indoxacarb [52]. We found that the demographic parameters in the F_1 generation were substantially reduced, relative to the control, when the F_0 generation was subjected to MB exposure. Similar transgenerational effects on the offspring of white-backed planthopper, *Sogatella furcifera* Horváth (Hemiptera: Delphacidae) [53], and brown planthopper, *Laodelphax striatellus* Fallén (Hemiptera: Delphacidae) [54], were recorded after parental exposure to sublethal concentrations of buprofezin and sulfoxaflor. Although we have reported the GRR in this study, it is necessary to point out that because GRR excludes the survival rate, a higher GRR does not necessarily represent higher fitness; thus, it should be interpreted with caution.

Plotting the s_{xj}, l_x, m_x, $l_x m_x$, and e_{xj} curves revealed the adverse effects of a sublethal dose of MB on cotton aphid population growth parameters. In the control treatment, the reproduction began on age 5 day, the peak of reproduction was on age 7 day, and the reproductive period extended from age 5 to 40 day. Consequently, the intrinsic rate of increase and finite rate of increase of the control treatment are significantly higher than that of MB treatment. Due to various physical and chemical processes, the pre-adult and mean generation time (T) increased after exposure to MB. In a few other studies on *A. gossypii*, similar effects were reported at the demographic level [12,55].

MB has been demonstrated as an excellent organic pesticide; however, the insecticidal mechanism of action of MB was not previously elucidated. Few studies have been conducted on the role of MB in human and animal subjects. A related compound methyl hydroxybenzoate has been shown to act on nervous conduction in the spinal root fibers of cats [56]. In rabbits, a sublethal dose of MB (500 mg/kg) increased blood cell counts but reduced cholinesterase activity; frequent application of high doses resulted in damage to the central nervous system [57]. AChE in the central nervous system of insects is an important target molecule for organophosphate and carbamate insecticides [58]. Our study showed that *A. gossypii* AChE activity decreased following treatment with MB. This finding indicates that MB works directly or indirectly to suppress AChE activity, and therefore, AChE inhibition may be the mechanism for cotton aphid mortality. Furthermore, we simulated the molecular interaction between MB and AChE using a molecular docking program. Our results showed that MB docked at the catalytic site of the AChE molecule. Additionally, MB exhibited hydrophobic interactions with at least five AChE amino acids.

5. Conclusions

The results of this study demonstrate that the application of a sublethal concentration of MB adversely affects cotton aphid growth by extending the pre-adult period and suppressing the population growth of the progeny. In addition, this study is the first to explore the constructed atomistic AChE model for *A. gossypii* and its potential binding of MB through molecular docking analyses. Further research on the mode of action of MB on insects is recommended. Importantly, biopesticides such as MB could become key components of crop protection programs, reducing the use of synthetic and harmful chemical products. Adopting a more sustainable approach to pest management will benefit human health, the environment, and biodiversity.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/9/1313/s1: Figure S1: The crystallographic structure of the *Drosophila melanogaster* acetylcholinesterase (AChE) complexed with tacrine derivative 9-(3-iodobenzylamino)-1,2,3,4-tetrahydroacridine (I40). The structure was downloaded from the Protein Data Bank (PDB), with PDB ID 1QON. Interactions between the active site of acetylcholinesterase of *Drosophila melanogaster* with the ligand I40; Table S1: Comparison of the molecular interaction of acetylcholinesterase with the reported inhibitor, I40 and MB.

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