fermentation

MDPI

Article

Effect and Mechanism of *Lepista nuda* Mycelia Obtained via In Vitro Culture on the Lifespan of *Drosophila melanogaster*

Jinzhe Li, Yaqin Huang *, Dezhi Wang, Nailiang Zhu and Xinrong Qiao

School of Pharmaceutical Engineering, Xinyang Agriculture and Forestry University, Xinyang 464000, China
* Correspondence: hyaqin88@163.com

Abstract: To provide a theoretical basis for biogenic fly-killing pesticides, in this study, we sought to examine the lethal effects of *Lepista nuda* mycelium-supplemented diets in *Drosophila melanogaster*. In doing so, we also studied the effects of *Lepista nuda* mycelium-supplemented diets on lifespan, antioxidant enzyme activity, peroxide content, relative transcript amounts of antioxidant enzyme genes, signaling pathways, and lifespan. Lower *Lepista nuda* mycelium-supplemented diets activated the antioxidant system and prolonged lifespan, while higher mycelium-supplemented diets had a significant toxic effect. After the administration of mycelium-supplemented diets for 24 h, the highest corrected mortality (41.96%) and lifespan inhibition rates (96.50%) were observed. In addition, the antioxidant enzyme activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px); the relative transcript amounts of the key antioxidant enzyme genes *SOD*, *CAT*, and *TrxR-1*; and the lifespan genes *Hep* and *Nrf2* were found to be the lowest, while the contents of the oxidation products malondialdehyde (MDA), protein carbonylation product (PCO), and triglyceride (TG); the relative transcript amounts of the signaling pathway-related genes *S6K*, *TOR*, and *Keap-1*; and the lifespan gene *MTH* were observed to be the highest after 48 h. Higher *Lepista nuda* mycelium-supplemented diets significantly inhibited lifespan, acting via the initiation of oxidative stress systems.

Keywords: *Lepista nuda*; mycelium; biopesticide; *Drosophila melanogaster*; lifespan; longevity gene; mechanism

Citation: Li, J.; Huang, Y.; Wang, D.; Zhu, N.; Qiao, X. Effect and Mechanism of *Lepista nuda* Mycelia Obtained via In Vitro Culture on the Lifespan of *Drosophila melanogaster*. *Fermentation* **2023**, *9*, 212. https://doi.org/10.3390/fermentation9030212

Academic Editor: Xiaofei Tian

Received: 1 February 2023
Revised: 17 February 2023
Accepted: 19 February 2023
Published: 23 February 2023

1. Introduction

Drosophila melanogaster has an extensive host range, reproduces rapidly, and has the capacity to live in a wide range of climatic conditions, all of which have contributed to the global spread of this economically injurious insect [1]. Recent control measures largely rely on chemical insecticide applications, which are toxic to people, livestock, wild animals, soil, water, food, and the human living environment [2]. These are not sustainable strategies due to administrative constraints and the potential for insecticide resistance [3,4]. For these reasons, it is vital to explore novel sources of environmentally friendly biopesticides. Many secondary metabolites of plants and fungal possess remarkable biological activities [5].

Today, edible-mushroom-derived biocides are often used in place of conventional synthetic pesticides. The hydroalcoholic extract of *Pleurotus ostreatus* exhibits contact toxicity and has been shown to impact the propagation and spawning of *Sitophilus zeamais* [6]. *Ganoderma lucidum* contains compounds that are toxic against *Tribolium castaneum* and *D. melanogaster* [7]. Cordycepin from the fruiting body of *Cordyceps militaris* is toxic against the larvae of the Colorado potato beetle *Leptinotarsa decemlineata* [8] and has been shown to kill *Plutella xylostella* [9]. *Amanita muscaria* (Agaricales, Amanitaceae) can be used against the mosquito *Culex quinquefasciatus* (Diptera, Culicidae) [10]. *Amanita muscaria* (L.) Lam. can be used for catching flies when soaked in milk or water [11]. *Trametes odorata* (Wulfen) Fr. powder keeps insects away from clothing, and among the 175 different species of fungi tested, 79 were found to inhibit insect development [12]. The observed results demonstrate

that certain fungi contain repellent, antifeedant, and even toxic compounds that act against insect pests.

Lepista nuda (Bull. ex Fr.) Cooke (*Clitocybe* genus, Tricholomataceae, Agaricomycetes) is an edible mushroom with a rich and subtle flavor [13] and violet coloration. Recent studies indicate that *L. nuda* has a wide range of pharmacological functions, including antitumor [14] and antimicrobial [15] properties. *L. nuda* extracts have been shown to inhibit HIV-1 reverse transcriptase [16] and biofilm production [17] to a certain extent. In fact, extracts of *Clitocybe* genus fruiting bodies have been shown to exhibit potent insecticidal activity against *D. melanogaster* [18]. In addition, *L. nuda* has exhibited insecticidal activity against *D. melanogaster* larvae [19]. To the best of our knowledge, no previous study has investigated the insecticidal mechanism of *L. nuda*.

Studies from around the world show that macrofungi and their secondary metabolites have the advantages of strong activity in natural environments, good compatibility with the environment, mixing easily with other drugs, and safety for humans and animals when used correctly [20]. In China in recent years, wild macrofungi specimens have been extensively collected as their habitats are being continually destroyed [21]. Using in vitro cultures minimizes the overexploitation of endangered, rare, or valuable species, and thus represents a methodology that prioritizes sustainable conservation and the rational utilization of biodiversity [22].

Previous studies have shown that exposure of *D. melanogaster* to 3 Gy electron beam irradiation, 15 mM paraquat (Pq), and the rare-earth element cerium (Ce) can induce oxidative stress, which specifically manifests as a significant increase in malanoldialdehyde (MDA) content. In addition, radiation/Pq/Ce-induced free radicals have been shown to impair antioxidant defense mechanisms, leading to a reduction in superoxide dismutase (SOD) and catalase (CAT) activity and glutathione (GSH) levels. Furthermore, Pq-treated flies exhibited severe locomotor impairments, with 84% of flies unable to fly [23]. There was a significant decrease in mean lifespan, maximum lifespan, and reproductive output with increasing doses of cerium [24]. Recent studies have illustrated that metabolic signaling pathways can mediate age and longevity [25]. For example, target of rapamycin (*TOR*) is a pivotal regulator of cell proliferation and affects senescence, *S6K* is a downstream effector of *TOR* kinase [26], and the Jun kinase (*JNK*) signaling pathway circuitously conveys the cellular oxidative stress response and extends longevity. Moreover, *Hep* is a homolog of *Drosophila* Jun kinase kinase (*JNKK*), and *Drosophila* carrying the *Hep* mutation were shown to be more susceptible to oxidative stress and to have shorter lifespans [27].

At present, the research on *D. melanogaster* biocontrol with macrofungi is limited. In this study, the toxicity of *L. nuda* mycelia obtained via in vitro culture was examined against *D. melanogaster*. Its effects on lifespan, antioxidant enzyme activity, peroxide content, and gene expression related to antioxidant enzymes, signaling pathways, and lifespan were compared, providing a reference for the development and utilization of macrofungi as biological pesticides.

2. Materials and Methods

2.1. Materials

2.1.1. Insects

Adult wild-type *D. melanogaster*, captured in a local cherry orchard, were reared in classic corn yeast medium and propagated in a light incubator at 25 °C.

2.1.2. Macrofungi Strain

L. nuda were collected from among fallen leaves in the summer of 2021 from the forests of the Ta-pieh Mountain region of China.

2.2. Methods

2.2.1. Isolation and Purification of Mycelia

L. nuda were sterilized, cultivated in a PDA culture using tissue isolation, and incubated at 26 °C. The well-grown slant strains were inserted into a PD in vitro medium without agar, placed in a rotating shaker, and incubated for 6 d at 28 °C at 130 r/min. After in vitro culturing, the mycelia were collected via centrifugation at 6000 r/min for 20 min and rinsed 3 times with distilled water, dried to constant weight at 50 °C, and weighed.

2.2.2. Experimental Design and Grouping

The mycelia obtained from the in vitro culture were mixed at a mass ratio of 1:4 with pure water, homogenized via ultrasonic (240 W) crushing for 1 h, bottled at 4 °C, and sealed. The treatment medium was configured on the basis of the corn yeast medium, and the corn yeast medium was used as the control (CK). The corn flour and distilled water in the experimental treatment groups were subtracted from original masses of 2.5, 5.0, 10, 20, and 40%, and the total mass of the two was replaced with the same mass of *L. nuda* mycelia homogenate so that the added mass concentrations of *L. nuda* mycelia were 0.23, 0.46, 0.91, 1.82, and 3.64 g/mL, for a total of five experimental treatment groups (A, B, C, D, and E). Thereafter, the medium was divided into 100 mL triangular flasks and set aside.

2.2.3. Determination of Lethal Effect against *D. melanogaster*

Two female and two male virgin flies that had been starved within the previous 8 h were inserted into bottles. A total of 80 flies were selected for each experimental group and were incubated at 25 °C. They were observed 6, 12, 18, and 24 h after being placed in the bottles. *D. melanogaster* with wings at 45° to the body were considered dead, and the number of dead flies was recorded. Mortality rate (MR, %) and corrected mortality rate (CMR, %) were calculated according to the following formulae:

$$\text{MR} = \text{number of dead flies/number of test flies} \times 100\%, \quad (1)$$

$$\text{CMR} = (\text{treatment mortality} - \text{control mortality})/(1 - \text{control mortality}) \times 100\%. \quad (2)$$

2.2.4. Measurement of *D. melanogaster* Lifespan

From the time the virgin flies were introduced into the treatment medium, they were regularly observed and were counted every 6 h. After 24 h of rearing flies using the macrofungal treatment medium, the remaining surviving flies were transferred to the corn yeast medium for further rearing. When pupae were formed, the flies were again transferred to corn yeast medium until all were dead. Median lethal time (LT_{50}, h), mean lifespan (MLS, h), maximum mean lifespan (MMLS, h), and lifespan inhibition rate (LIR, %) were calculated according to the following formulae:

$$LT_{50} = \text{the time at which half of each group of flies were dead}, \quad (3)$$

$$\text{MLS} = \text{the sum of the survival time of each group of flies/number of flies in the group}, \quad (4)$$

$$\text{MMLS} = \text{the mean of the lifespan of the last 10 dead flies in each group}, \quad (5)$$

$$\text{LIR} = (\text{mean lifespan of control group} - \text{mean lifespan of test group})/\text{mean lifespan of control group} \times 100\%. \quad (6)$$

2.2.5. Determination of Antioxidant Enzyme Activity and Peroxidation Product Content in *D. melanogaster*

In addition, 100 mg of female and male flies reared with the treatment medium for 24 and 48 h were weighed. Each group of flies was combined with 0.9 mL of saline solution and homogenized at 2000 r/min for 10 s in an ice bath with an interval of 10 s. This was repeated three times to produce a homogenate. The activity of SOD, CAT, and glutathione peroxidase (GSH-Px), and the MDA content, protein carbonylation product (PCO), and

triglyceride (TG) were calculated with an enzyme marker according to the kit's instructions (Nanjing Jiancheng Institute of Biological Engineering, Nanjing, China.).

2.2.6. Determination the Relative amounts of Transcripts in *D. melanogaster*

Total mRNA was extracted from *D. melanogaster* using the Trizol method, as described in the Trizol kit's instructions (Invitrogen, Waltham, MA, USA). cDNA synthesis was performed according to the instructions of the PrimeScriptTM RT-PCR Kit. The primer sequences used for the quantitative analysis were synthesized by Shanghai Meiji Biomedical Technology Co. (Shanghai, China). Fluorescence real-time quantitative PCR was performed using a QuantStudio 3 real-time quantitative PCR system, the reagents were used with a DyNAmoTM SYBRR Green qPCR kit, and the experimental operations were performed according to the reagent instructions. Data were collected and processed using CFX-Manager, and the relative expressions of the target genes and internal reference genes were calculated using the Ct ($2^{-\triangle\triangle Ct}$) method. Using ribosomal protein (*RP49*) as an internal reference gene, high-throughput fluorescent quantitative PCR was used to determine the relative amounts of transcripts of antioxidant enzyme genes, including superoxide dismutase (*SOD*), catalase (*CAT*), thioredoxin reductase (*TrxR-1*), and lifespan-related genes, including methuselah (*MTH*), nuclear factor erythroid-2-related factor 2 (*Nrf2*), hemipterus (*Hep*), and signaling-pathway-related genes, including RPS6-p70-protein kinase (*S6K*), target of rapamycin (*TOR*), and Kelch-like ECH-associated protein 1 (*Keap-1*).

2.2.7. Statistical Analysis

All operations in this experiment were repeated three times (n = 3), and data are expressed as the mean ± standard deviation (SD) of each sample. The significance of difference between means was assessed using one-way ANOVA, followed by post hoc Tukey's testing using the software package SPSS v26 (SPSS Inc., Chicago, IL, USA). Different lowercase letters in the same column indicate least significant differences (LSDs) at the 5% level ($p < 0.05$) for each treatment.

3. Results

3.1. Lethal Effects of L. nuda Mycelium-Supplemented Diets on D. melanogaster

The mortality and corrected mortality of flies after receiving *L. nuda* mycelium-supplemented diets are shown in Table 1. It can be seen from the table that after 6, 12, and 18 h, the mortality of treatment groups A, B, and C was not significantly different from that of the control group. As the time spent receiving the mycelium-supplemented diets increased, the mortality of the five treatment groups exhibited a gradually increasing trend. The corrected mortality of the five treatment groups reached its maximum after 24 h, and the corrected mortality of group E was the highest at 41.96%.

Table 1. Mean (±SD) of mortality rate (MR) and corrected mortality rate (CMR) of *Drosophila melanogaster* after *Lepista nuda* mycelium-supplemented diets for 6, 12, 18, and 24 h. Flies with wings at 45° to the body were considered dead. Means with different letters differ significantly at $p < 0.05$ (Tukey's test). CK: control—received basal diet without *L. nuda* mycelium. A, B, C, D, and E—received *L. nuda* mycelium-supplemented diet at 0.23, 0.46, 0.91, 1.82, and 3.64 g/mL, respectively.

Groups	6 h		12 h		18 h		24 h	
	MR/%	CMR/%	MR/%	CMR/%	MR/%	CMR/%	MR/%	CMR/%
CK	0.00 ± 0.00^{c}	–	0.00 ± 0.00^{c}	–	0.00 ± 0.00^{c}	–	1.67 ± 0.72^{d}	–
A	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.83 ± 0.72^{c}	0.83 ± 0.72^{c}	1.67 ± 0.72^{d}	0.00 ± 0.00^{d}
B	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.42 ± 0.72^{c}	0.42 ± 0.72^{c}	2.08 ± 1.44^{d}	0.43 ± 0.74^{d}
C	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	2.08 ± 0.72^{c}	2.08 ± 0.72^{c}	5.83 ± 0.72^{c}	4.24 ± 0.72^{c}
D	5.00 ± 1.25^{b}	5.00 ± 1.25^{b}	8.75 ± 2.50^{b}	8.75 ± 2.50^{b}	11.25 ± 2.50^{b}	11.25 ± 2.50^{b}	17.08 ± 2.60^{b}	15.67 ± 3.11^{b}
E	8.75 ± 1.25^{a}	8.75 ± 1.25^{a}	20.83 ± 1.91^{a}	20.83 ± 1.91^{a}	31.25 ± 2.17^{a}	31.25 ± 2.17^{a}	42.92 ± 3.15^{a}	41.96 ± 2.82^{a}

3.2. Effects of L. nuda Mycelium-Supplemented Diets on the Lifespan of D. melanogaster

The lifespans and inhibition rates of flies after receiving *L. nuda* mycelium-supplemented diets for 24 h are shown in Table 2. It can be seen from Table 2 that, as compared with the control, the LT_{50}, MLS, and MMLS of the A, B, and C groups increased, and the increase in groups B and C was significant as compared with the control. The MLS in group C was the highest (1716 h), i.e., a 21.18% increase as compared with the control. The LT_{50}, MLS, and MMLS in groups D and E were significantly lower than those of the control group, while the MLS in group E was the lowest, i.e., a 96.5% reduction as compared with the control group.

Table 2. Mean (±SD) of median lethal time (LT_{50}, h), mean lifespan (MLS, h), maximum mean lifespan (MMLS, h), lifespan inhibition rate (LIR, %) of *D. melanogaster* after *L. nuda* mycelium-supplemented diets for 24 h. Means with different letters differ significantly at $p < 0.05$ (Tukey's test). CK: control—received basal diet without *L. nuda* mycelium. A, B, C, D, and E—received *L. nuda* mycelium-supplemented diet at 0.23, 0.46, 0.91, 1.82, and 3.64 g/mL, respectively.

Groups	LT_{50}/h	MLS/h	MMLS/h	LIR/%
CK	1360.00 ± 45.83 ^{c}	1418.00 ± 54.11 ^{b}	1956.00 ± 70.74 ^{b}	–
A	1340.00 ± 45.83 ^{c}	1426.00 ± 51.73 ^{b}	1970.00 ± 60.00 ^{b}	−0.61 ± 3.46 ^{d}
B	1492.00 ± 58.28 ^{b}	1660.00 ± 39.04 ^{a}	2096.00 ± 54.11 ^{a}	−17.15 ± 4.29 ^{c}
C	1582.00 ± 40.84 ^{a}	1716.00 ± 26.15 ^{a}	2152.00 ± 58.28 ^{a}	−21.18 ± 6.45 ^{c}
D	518.00 ± 42.14 ^{d}	558.00 ± 30.00 ^{c}	852.00 ± 45.30 ^{c}	60.59 ± 3.03 ^{b}
E	42.00 ± 12.00 ^{e}	50.00 ± 15.10 ^{d}	96.00 ± 18.00 ^{d}	96.50 ± 0.93 ^{a}

3.3. Effects of L. nuda Mycelium-Supplemented Diets on the Antioxidant Activity of D. melanogaster

The activities of the antioxidant enzymes SOD, CAT, and GSH-Px in male and female flies after receiving *L. nuda* mycelium-supplemented diets are shown in Figure 1. It can be seen that the activity of the three antioxidant enzymes in both male and female flies exhibited an increasing and then decreasing trend with the increase in the mycelium-supplemented diets after 48 h. As compared with the control group, the enzyme activities in group C increased significantly, and the antioxidant enzyme activities in the males were higher than in the females, i.e., the SOD, CAT, and GSH-Px activities were higher by 9, 142, and 13 U/mg, respectively. As compared with the control group, the enzyme activity in group E decreased significantly, and the enzyme activity at 48 h was lower than at 24 h.

3.4. Effects of L. nuda Mycelium-Supplemented Diets on Peroxidation Product Content of D. melanogaster

As shown in Figure 2, with the gradient increase in the *L. nuda* mycelium-supplemented diets, the content of the three peroxidation products exhibited a decreasing and then increasing trend in both the male and female flies. The MDA content in treatment group C and the PCO content in groups B and C decreased significantly as compared with the control after 48 h, while the TG contents in groups A, B, and C were not significant. All three peroxisomes in groups D and E significantly increased, and group E exhibited the highest peroxisomal content, with males having higher levels than females.

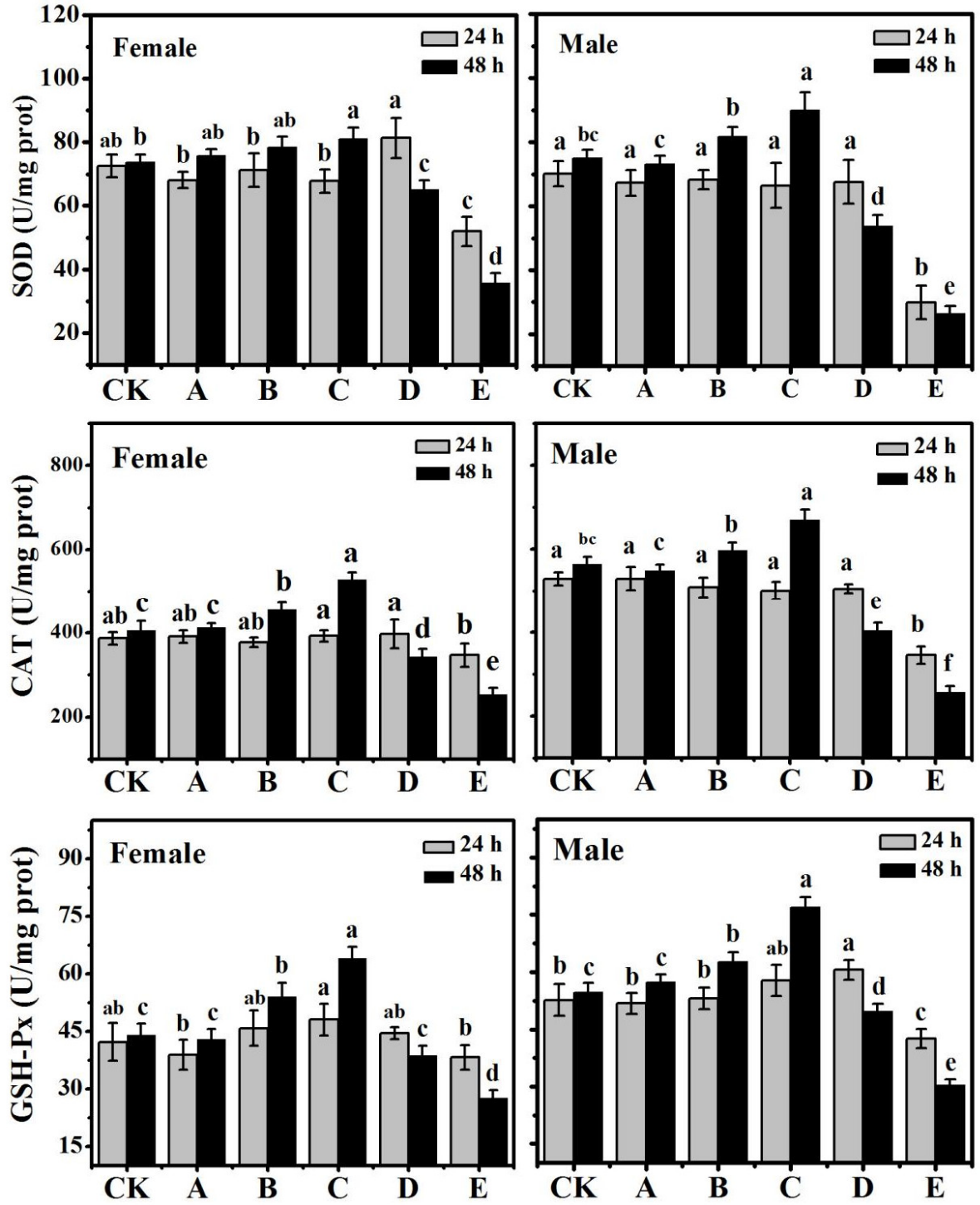

Figure 1. Mean (±SD) of the activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) of *D. melanogaster* after *L. nuda* mycelium-supplemented diets for 24, 48 h. Means with different letters differ significantly at $p < 0.05$ (Tukey's test). CK: control—received basal diet without *L. nuda* mycelium. A, B, C, D, and E—received *L. nuda* mycelium-supplemented diet at 0.23, 0.46, 0.91, 1.82, and 3.64 g/mL, respectively.

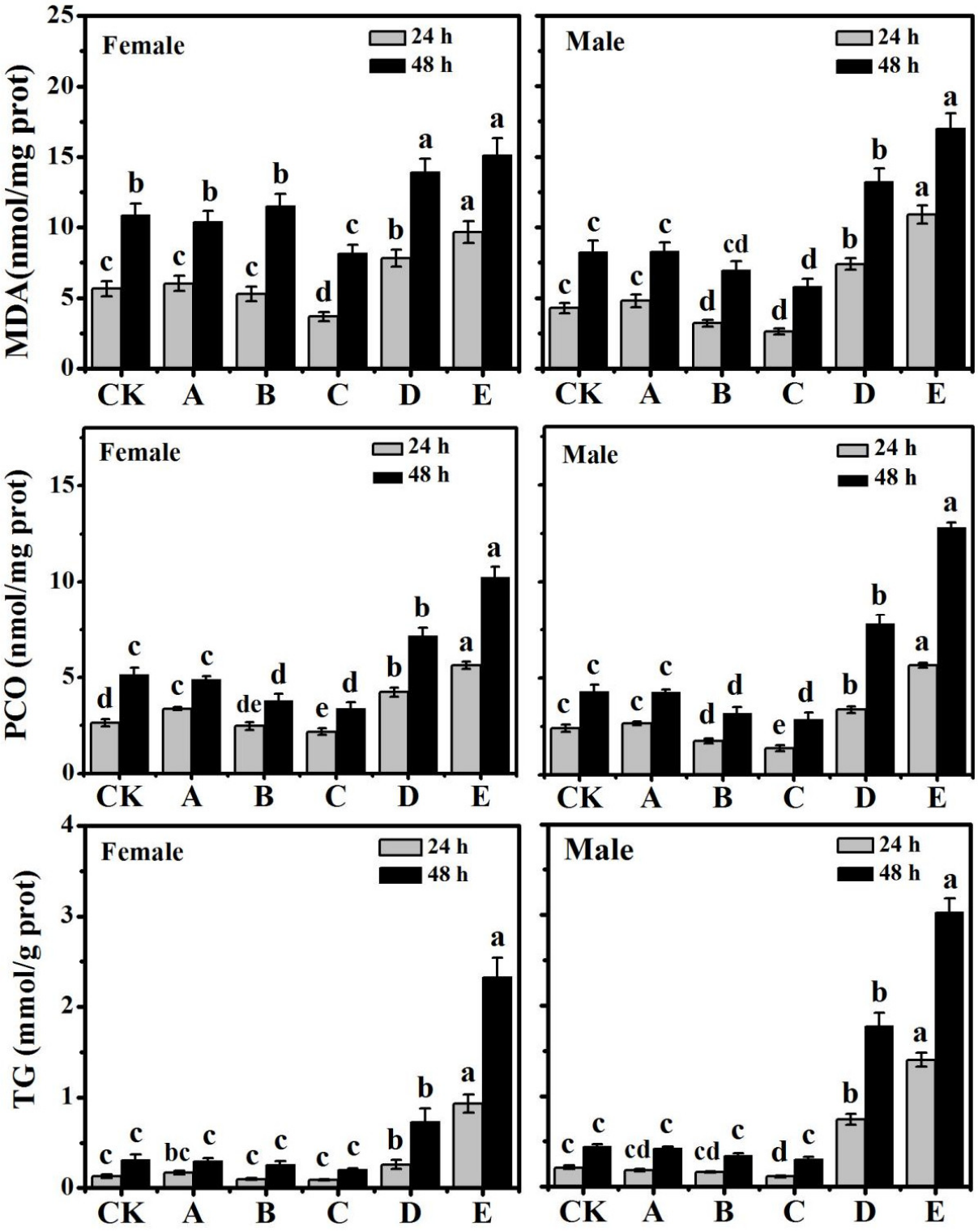

Figure 2. Mean (±SD) of the content of malondialdehyde (MDA), protein carbonylation product (PCO), triglyceride (TG) of *D. melanogaster* after *L. nuda* mycelium-supplemented diets for 24, 48 h. Means with different letters differ significantly at $p < 0.05$ (Tukey's test). CK: control—received basal diet without *L. nuda* mycelium. A, B, C, D, and E—received *L. nuda* mycelium-supplemented diet at 0.23, 0.46, 0.91, 1.82, and 3.64 g/mL, respectively.

3.5. Effects of L. nuda Mycelium-Supplemented Diets on the Levels of Antioxidant-Related Gene Transcripts in D. melanogaster

It can be seen from Figure 3 that with the gradient increase in *L. nuda* mycelium-supplemented diets, the levels of the antioxidant enzymes *SOD*, *CAT*, and *TrxR-1* key gene transcripts in female and male flies exhibited an increasing and then decreasing trend. The gene transcription in group C was the highest, while that of group E was the lowest. As compared with the control, after 24 h and 48 h, the levels of *SOD* and *TrxR-1* key gene transcripts in female and male flies in groups A, B, and C significantly increased, and gene transcription at 48 h was higher than at 24 h. The levels of *SOD*, *CAT*, and *TrxR-1* key gene transcripts in groups D and E were lower at 48 h than at 24 h.

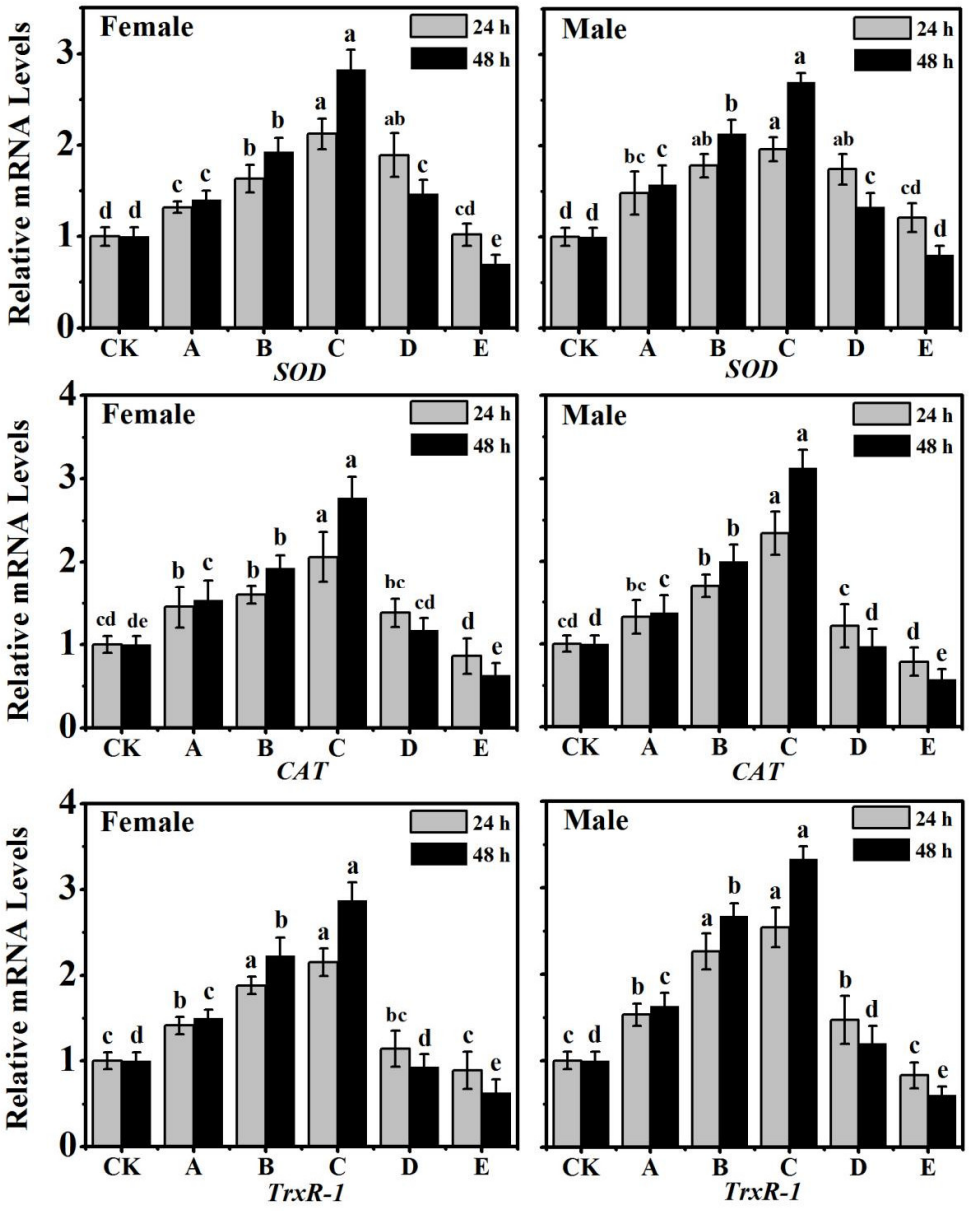

Figure 3. Mean (±SD) of the relative amounts of transcripts of antioxidant enzyme genes, including superoxide dismutase (*SOD*), catalase (*CAT*), thioredoxin reductase (*TrxR-1*) of *D. melanogaster* after *L. nuda* mycelium-supplemented diets for 24, 48 h. Means with different letters differ significantly at $p < 0.05$ (Tukey's test). CK: control—received basal diet without *L. nuda* mycelium. A, B, C, D, and E—received *L. nuda* mycelium-supplemented diet at 0.23, 0.46, 0.91, 1.82, and 3.64 g/mL, respectively.

3.6. Effects of L. nuda Mycelium-Supplemented Diets on Levels of Signaling Pathway Gene Transcripts in D. melanogaster

It can be seen from Figure 4 that with the gradient increase in *L. nuda* mycelium-supplemented diets, the levels of *S6K*, *TOR*, and *Keap-1* signaling pathway gene transcripts all exhibited a decreasing and then increasing trend. The levels of *S6K*, *TOR*, and *Keap-1* transcripts in groups A, B, and C were significantly reduced, and those in group E were significantly increased. Those in group D were not compared with the control after 24 h.

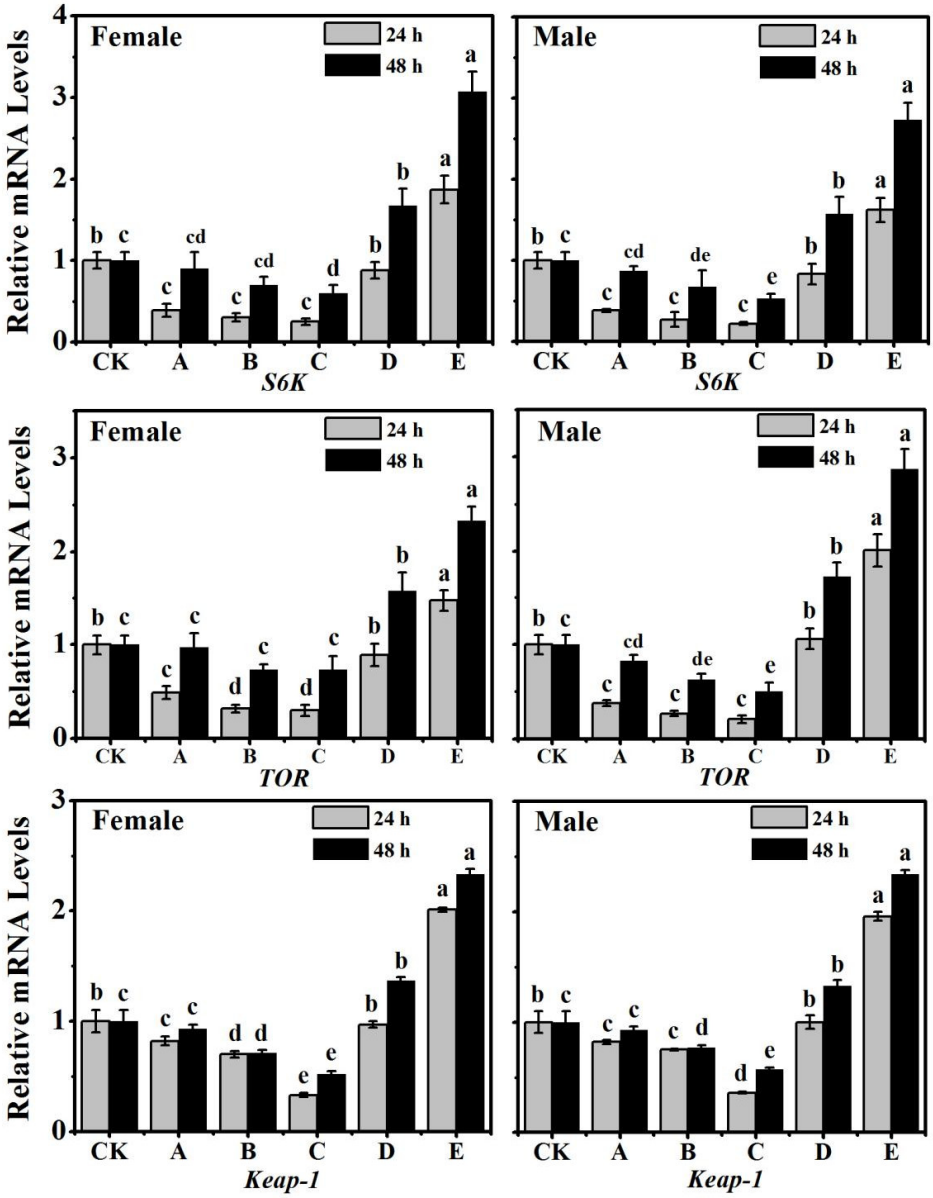

Figure 4. Mean (±SD) of the relative amounts of transcripts of signaling pathway-related genes, including RPS6-p70-protein kinase (*S6K*), target of rapamycin (*TOR*), Kelch-like ECH-associated protein 1 (*Keap-1*) of *D. melanogaster* after *L. nuda* mycelium-supplemented diets for 24, 48 h. Means with different letters differ significantly at $p < 0.05$ (Tukey's test). CK: control—received basal diet without *L. nuda* mycelium. A, B, C, D, and E—received *L. nuda* mycelium-supplemented diet at 0.23, 0.46, 0.91, 1.82, and 3.64 g/mL, respectively.

The levels of *S6K*, *TOR*, and *Keap-1* transcripts in the five treatment groups at 48 h were higher than those at 24 h. As compared with the control, the levels in group D and E significantly increased. Furthermore, the levels of signaling-pathway-related gene

transcripts in group E at 48 h were the highest. The level of *S6K* transcripts in female flies was 3.07 times higher than in male flies, and the levels of *TOR* and *Keap-1* transcripts in male flies were 2.87 and 2.34 times higher than in female flies, respectively.

3.7. Effects of L. nuda Mycelium-Supplemented Diets on the Levels of Lifespan-Related Gene Transcripts in D. melanogaster

It can be seen from Figure 5 that with the gradient increase in *L. nuda* mycelium-supplemented diets, the levels of the *Hep* and *Nrf2* lifespan-related gene transcripts exhibited an increasing and then decreasing trend. In addition, *MTH* expression exhibited a decreasing and then increasing trend. The levels of *Hep* and *Nrf2* transcripts in groups A, B, and C increased significantly as compared with the control after 48 h, while those in group E decreased significantly. The levels of *MTH* transcripts in groups A, B, and C decreased significantly as compared with the control after 24 h. Moreover, *MTH* transcription in group C decreased significantly as compared with the control after 48 h, while that in groups D and E increased significantly. The levels of *MTH* transcripts in group E were the highest, i.e., they were 1.77 times for female flies and 2.37 times for male flies.

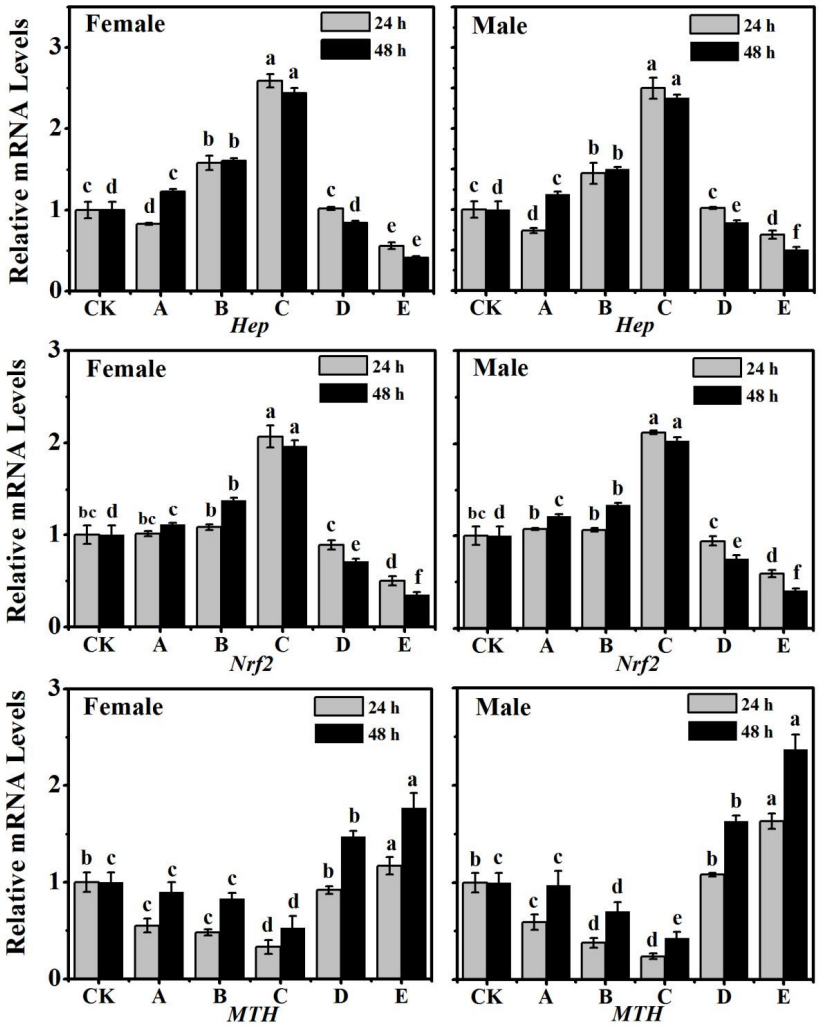

Figure 5. Mean (±SD) of the relative amounts of transcripts of lifespan-related genes, including methuselah (*MTH*), nuclear factor erythroid-2 related factor 2 (*Nrf2*), hemipterus (*Hep*) of *D. melanogaster* after *L. nuda* mycelium-supplemented diets for 24, 48 h. Means with different letters differ significantly at $p < 0.05$ (Tukey's test). CK: control—received basal diet without *L. nuda* mycelium. A, B, C, D, and E—received *L. nuda* mycelium-supplemented diet at 0.23, 0.46, 0.91, 1.82, and 3.64 g/mL, respectively.

4. Discussion

The urgent global need for new pesticides can only be met if we strive to find biologically active secondary metabolites [28]. Various ceramide components [29], sterols, and triterpenoids [30] have been isolated from *L. nuda*. However, existing studies suggest that lucidenic acid O, lactone [31], lectins [32], fungal cyclic peptides [33], and hemolysins are potential fungal insecticides. Clitocine, a novel nucleoside produced by *Clitocybe inversa*, has exhibited strong insecticidal activity against the pink bollworm *Pectinophora gossypiella* [34]. Clitocypin, a fungal cysteine protease inhibitor, exerts an insecticidal effect against Colorado potato beetle larvae by inhibiting their digestive cysteine proteases [35]. Cnispin, a protease inhibitor that inhibits a serine protease called trypsin, is toxic to *D. melanogaster* as they mainly use serine proteases for digestion [36]. It has been shown that the insecticidal biological function of lectins and protease inhibitors is regulated by the b-Trefoil structure, which enables interactions between lectins and protease inhibitors [37].

In this study, *D. melanogaster* was fed with varying gradients of *L. nuda* mycelium-supplemented diets. The results show that *L. nuda* mycelium-supplemented diets had a lethal effect on *D. melanogaster*, and the corrected mortality rate was positively correlated with time and concentration. The corrected mortality rate indicated a dose-dependent decrease as the mycelium-supplemented diets increased (Table 1). This dose-dependence is consistent with the virulence of other insecticides against *D. melanogaster*. Within a certain dose range, the mortality and knockdown times of pyrethroid combined with piperonyl butoxide were higher than those observed for pyrethroid alone, in a dose-dependent manner [38].

Genetic alterations have been documented to interfere with lifespan [39]. To date, a large number of longevity-related genes have been identified in *D. melanogaster* [40]. There are six possible key pathways associated with lifespan, including the longevity regulatory pathway, the peroxisome pathway, the m*TOR* signaling pathway, the FOXO signaling pathway, the diabetic complication AGE-RAGE signaling pathway, and the TGF-b signaling pathway. The expression of six representative key pathway genes, including *Cat*, *Ry*, *S6k*, *Sod*, *Tor*, *Tsc1*, and the predicted genes *Jra*, *Kay*, and *Rheb* were significantly altered in aging *D. melanogaster* as compared to young flies [41]. In this study, after receiving *L. nuda* mycelium-supplemented diets for 48 h, group E, which received the highest dose, exhibited the lowest levels of SOD, CAT, and GSH-Px; the lowest relative transcript amounts of the key antioxidant enzyme genes *SOD*, *CAT*, and *TrxR-1*; and the lowest levels of the lifespan genes *Hep* and *Nrf2* (Figures 1, 3 and 5). In addition, in this group, the contents of the oxidation products MDA, PCO, and TG; the relative transcript amounts of the signaling pathway-related genes *S6K*, *TOR*, *Keap-1*; and the lifespan gene *MTH* were the highest (Figures 2, 4 and 5). At 48 h, this group exhibited the highest corrected mortality rate of 41.96% (Table 1) and the highest lifespan inhibition rate of 96.50% (Table 2). The Methuselah (*MTH*) gene has long been considered to be a determinant of longevity. In addition, various studies have shown that cranberry anthocyanin extract can extend lifespan by downregulating *MTH* in *D. melanogaster* [42]. It has been suggested that the activation of *TOR* [43] and *S6K* [44] expression can reduce lifespan via rapamycin in *D. melanogaster*, which is consistent with the results of the present study.

The overconsumption of *L. nuda* mycelium-supplemented diets by starving *D. melanogaster* led to a large accumulation of chemical components, such as cnispin and clitocine, in the body. This inhibited the transcription of the key antioxidant enzyme genes *SOD*, *CAT*, and *TrxR-1*, resulting in a large accumulation of the oxidation products MDA, PCO, and TG, which activated the transcription of the signaling-pathway-related genes *S6K*, *TOR*, and *Keap-1*. The enrichment of signaling pathway factors downregulated the transcripts of the longevity genes *Hep* and *Nrf2* and upregulated the transcripts of the lifespan gene *MTH*, leading to cellular senescence and apoptosis. This eventually led to the lethal effect of the *L. nuda* mycelium-supplemented diets on *D. melanogaster*, and the shortened lifespan.

Both female and male *D. melanogaster* from group C exhibited significant differences in terms of the observed decreases in MDA and PCO contents as compared to the control

after *L. nuda* mycelium-supplemented diets for 24 h (Figure 2). The SOD, CAT, and GSH-Px activities in group C exhibited significant differences in terms of the observed increases as compared to the control after 48 h (Figure 1). Transcripts of the key antioxidant enzyme genes *SOD*, *CAT*, and *TrxR-1* and the longevity genes *Hep* and *Nrf2* were upregulated at both 24 h and 48 h (Figures 3 and 5).

It has been previously demonstrated that the increased antioxidant enzyme activity of *L. nuda* mycelia [45] can protect against ROS-induced cell damage, reduce lipid peroxidation, stabilize cell membrane integrity, and upregulate antioxidant and lifespan genes. The transcription and regulation of this activity are described above. Recent research has demonstrated that a *Lentinus edodes* mycelia polysaccharide (LEMP) exerts potential antiaging effects in vivo and antioxidant activities in vitro [46]. The addition of LEMP to the diet significantly extended the average lifespan of *D. melanogaster*, reduced MDA levels, and downregulated the expression of the *S6K*, *TOR*, and *MTH* genes [47], which is consistent with the conclusions of this investigation. Thus, *L. nuda* mycelia can be applied as a fly-killing biopesticide and small amounts of pesticide residues on the surface of fruits are harmless to the environment and humans.

5. Conclusions

In summary, in this study, *D. melanogaster* received *L. nuda* mycelium-supplemented diets. Herein, we demonstrated that lower mycelium-supplemented diets activated the antioxidant system and prolonged lifespan, but higher mycelium-supplemented diets had a significant toxic effect on *D. melanogaster*. Excessive consumption of *L. nuda* mycelium inhibited the expression of key antioxidant enzyme genes, resulting in a large accumulation of oxidative products, which activated the expression of genes related to the signaling pathway. The enrichment of signaling pathway factors downregulated the longevity of the *Hep* and *Nrf2* gene transcripts, resulting in a reduced lifespan. These results provide a reference for the development of biogenic fly-killing pesticides using *L. nuda* mycelium. Hence, further studies should consider transcriptome analysis to achieve greater insight into the toxicological mechanisms.

Author Contributions: Resources, data curation, writing—original draft preparation, J.L.; conceptualization, methodology, software, validation, formal analysis, investigation, Y.H.; writing—review and editing, visualization, supervision, D.W.; project administration, N.Z.; funding acquisition, X.Q. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Henan Provincial Science and Technology Research Project, grant number 202102110176, Henan Province Young Backbone Teacher Training Program, grant number 2019GGJS263, Doctoral Start-up Fund of Xinyang Agriculture and Forestry University, and Innovative Research Team of Research on Traditional Chinese Medicine Resources and Series Products Development of Ta-pieh Mountains in Xinyang Agriculture and Forestry University, grant number XNKJTD-009.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors would like to thank Henan Innovation Strategic Alliance of Edible-Medicinal Fungi Industrial Technology and Xinyang Aojite Edible Fungus Development Co., Ltd. for technical assistance and guidance.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Curtsinger, J.W. Reproductive homeostasis and senescence in *Drosophila melanogaster*. *J. Gerontol. Ser. A* **2019**, *74*, 1533–1538. [CrossRef] [PubMed]
2. Wan, N.; Ji, X.; Jiang, J.; Zhang, Y.; Liang, J.; Li, B. An ecological indicator to evaluate the effect of chemical insecticide pollution management on complex ecosystems. *Ecol. Indic.* **2015**, *53*, 11–17. [CrossRef]

3. Van Timmeren, S.; Mota-Sanchez, D.; Wise, J.C.; Isaacs, R. Baseline susceptibility of spotted wing *Drosophila* (*Drosophila suzukii*) to four key insecticide classes. *Pest Manag. Sci.* **2018**, *74*, 78–87. [CrossRef] [PubMed]
4. Deans, C.; Hutchison, W.D. Propensity for resistance development in the invasive berry pest, spotted-wing *drosophila* (*Drosophila suzukii*), under laboratory selection. *Pest Manag. Sci.* **2022**, *78*, 5203–5212. [CrossRef] [PubMed]
5. Khursheed, A.; Rather, M.A.; Jain, V.; Wani, A.R.; Rasool, S.; Nazir, R.; Malik, N.A.; Majid, S.A. Plant based natural products as potential ecofriendly and safer biopesticides: A comprehensive overview of their advantages over conventional pesticides, limitations and regulatory aspects. *Microb. Pathog.* **2022**, *173*, 105854. [CrossRef]
6. Palavecino-De-La-Fuente, F.; Silva-Aguayo, G.; Figueroa-Cares, I.; Gerding, M.; Concepcion Rodriguez-Maciel, J.M.A.L.; Castaneda-Ramirez, G.S.; Sotelo-Leyva, C.; Curkovic, T.; Aguilar-Marcelino, L. Insecticidal effect of hydroalcoholic extracts of *Pleurotus ostreatus* against *Sitophilus zeamais*. *Chil. J. Agric. Res.* **2022**, *82*, 399–406. [CrossRef]
7. Riahi, H.; Sargazi, F.; Sheidai, M. Insecticidal activities of *Ling Zhi* or *Reishi* medicinal mushroom, *Ganoderma lucidum* (W. Curt.: Fr.) P. Karst. (*Aphyllophoromycetideae*) extract against *Tribolium castaneum* and *Drosophila melanogaster*. *Int. J. Med. Mushrooms* **2009**, *11*, 329–334. [CrossRef]
8. Dubovskiy, I.M.; Kryukova, N.A.; Tyurin, M.V.; Yaroslavtseva, O.N.; Kryukov, V.Y.; Glupov, V.V. Insecticidal and immunosuppressive effect of ascomycete *Cordyceps militaris* on the larvae of the Colorado potato beetle *Leptinotarsa decemlineata*. *Biol. Bull.* **2014**, *41*, 276–283. [CrossRef]
9. Kim, J.; Yeon, S.; Kim, H.; Ahn, Y. Larvicidal activity against *Plutella xylostella* of cordycepin from the fruiting body of *Cordyceps militaris*. *Pest Manag. Sci.* **2002**, *58*, 713–717. [CrossRef]
10. Cárcamo, M.C.; Carapeto, L.P.; Duarte, J.P.; Bernardi, E.; Ribeiro, P.B. Larvicidal efficiency of the mushroom *Amanitamuscaria* (Agaricales, Amanitaceae) against the mosquito *Culexquinquefasciatus* (Diptera, Culicidae). *Rev. Soc. Bras. Med. Trop.* **2016**, *49*, 95–98. [CrossRef]
11. Lumpert, M.; Kreft, S. Catching flies with *Amanita muscaria*: Traditional recipes from Slovenia and their efficacy in the extraction of ibotenic acid. *J. Ethnopharmacol.* **2016**, *187*, 1–8. [CrossRef]
12. Mier, N.; Canete, S.; Klaebe, A.; Chavant, L.; Fournier, D. Insecticidal properties of mushroom and toadstool carpophores. *Phytochemistry* **1996**, *41*, 1293–1299. [CrossRef]
13. Noel-Suberville, C.; Cruz, C.; Guinberteau, J.; Montury, M. Correlation between fatty acid content and aromatic compound release in fresh blewit (*Lepista nuda*). *J. Agric. Food Chem.* **1996**, *44*, 1180–1183. [CrossRef]
14. Beattie, K.D.; Ulrich, R.; Grice, I.D.; Uddin, S.J.; Blake, T.B.; Wood, K.A.; Steele, J.; Iu, F.; May, T.W.; Tiralongo, E. Ethanolic and aqueous extracts derived from Australian fungi inhibit cancer cell growth in vitro. *Mycologia* **2011**, *103*, 458–465. [CrossRef]
15. Mercan, N.; Duru, M.E.; Turkoglu, A.; Gezer, K.; Kivrak, I.; Turkoglu, H. Antioxidant and antimicrobial properties of ethanolic extract from *Lepista nuda* (Bull.) Cooke. *Ann. Microbiol.* **2006**, *56*, 339–344. [CrossRef]
16. Zhu, M.; Zhang, G.; Meng, L.; Wang, H.; Gao, K.; Ng, T. Purification and characterization of a white laccase with pronounced dye decolorizing ability and HIV-1 reverse transcriptase inhibitory activity from *Lepista nuda*. *Molecules* **2016**, *21*, 415. [CrossRef]
17. Alves, M.; Ferreira, I.; Lourenço, I.; Costa, E.; Martins, A.; Pintado, M. Wild mushroom extracts as inhibitors of bacterial biofilm formation. *Pathogens* **2014**, *3*, 667–679. [CrossRef]
18. Pohleven, J.; Kos, J.; Sabotic, J. Medicinal Properties of the Genus *Clitocybe* and of Lectins from the *Clouded Funnel Cap* Mushroom, C. nebularis (Agaricomycetes): A Review. *Int. J. Med. Mushrooms* **2016**, *18*, 965–975. [CrossRef]
19. Wang, M.; Trigueros, V.; Paquereau, L.; Chavant, L.; Fournier, D. Proteins as active compounds involved in insecticidal activity of mushroom fruitbodies. *J. Econ. Entomol.* **2002**, *95*, 603–607. [CrossRef]
20. Sheikha, A.F.E. Can mushrooms and their derivatives be efficient bioalternatives to conventional synthetic insecticides? *Int. J. Med. Mushrooms* **2021**, *23*, 1–14. [CrossRef]
21. Liu, J.; Diamond, J. China's environment in a globalizing world. *Nature* **2005**, *435*, 1179–1186. [CrossRef] [PubMed]
22. Hsu, J.; Li, I.; Jiang, Y.; Chen, C. Toxicological evaluation of *Lepista nuda* (Bull. ex Fr) cooke mycelium produced by an in vitro culture methodology. *Int. J. Pharm. Pharm. Sci.* **2015**, *7*, 363–370.
23. Manjula, P.S.; Sarojini, B.K.; Raj, C.G.D.; Sanjeev, G. In vivo antioxidative stress measurement of a 1, 2, 4-Triazole derivative in *Drosophila melanogaster oregon* K flies. *Der Pharma Chem.* **2015**, *11*, 70–78.
24. Huang, S.; Li, Z.; Wang, X.; Wang, Q.; Hu, F. Cerium caused life span shortening and oxidative stress resistance in *Drosophila melanogaster*. Ecotox. *Environ. Safe.* **2010**, *73*, 89–93. [CrossRef] [PubMed]
25. Wei, Y.; Zhang, Y.; Cai, Y.; Xu, M. The role of mitochondria in m*TOR*-regulated longevity. *Biol. Rev.* **2015**, *90*, 167–181. [CrossRef]
26. Soto-Burgos, J.; Zhuang, X.; Jiang, L.; Bassham, D.C. Dynamics of autophagosome formation. *Plant Physiol.* **2018**, *176*, 219–229. [CrossRef]
27. Wang, M.C.; Bohmann, D.; Jasper, H. JNK signaling confers tolerance to oxidative stress and extends lifespan in *Drosophila*. *Dev. Cell* **2003**, *5*, 811–816. [CrossRef]
28. Aly, A.H.; Debbab, A.; Proksch, P. Fifty years of drug discovery from fungi. *Fungal Divers.* **2011**, *50*, 3–19. [CrossRef]
29. Yaoita, Y.; Kohata, R.; Kakuda, R.; Machida, K.; Kikuchi, M. Ceramide constituents from five mushrooms. Chem. *Pharm. Bull.* **2002**, *50*, 681–684. [CrossRef]
30. Yaoita, Y.; Matsuki, K.; Iijima, T.; Nakano, S.; Kakuda, R.; Machida, K.; Kikuchi, M. New sterols and triterpenoids from four edible mushrooms. *Chem. Pharm. Bull.* **2001**, *49*, 589–594. [CrossRef]

31. Mizushina, Y.; Takahashi, N.; Hanashima, L.; Koshino, H.; Esumi, Y.; Uzawa, J.; Sugawara, F.; Sakaguchi, K. Lucidenic acid O and lactone, new terpene inhibitors of eukaryotic DNA polymerases from a basidiomycete, *Ganoderma lucidum*. *Bioorgan. Med. Chem.* **1999**, *7*, 2047–2052. [CrossRef]
32. Pohleven, J.; Brzin, J.; Vrabec, L.; Leonardi, A.; Čokl, A.; Štrukelj, B.; Kos, J.; Sabotič, J. Basidiomycete *Clitocybe* nebularis is rich in lectins with insecticidal activities. *Appl. Microbiol. Biot.* **2011**, *91*, 1141–1148. [CrossRef]
33. Wang, X.; Lin, M.; Xu, D.; Lai, D.; Zhou, L. Structural diversity and biological activities of fungal cyclic peptides, excluding cyclodipeptides. *Molecules* **2017**, *22*, 2069. [CrossRef]
34. Kubo, I.; Kim, M.; Hood, W.F.; Naoki, H. Clitocine, a new insecticidal nucleoside from the mushroom *Clitocybe inversa*. *Tetrahedron Lett.* **1986**, *27*, 4277–4280. [CrossRef]
35. Šmid, I.; Rotter, A.; Gruden, K.; Brzin, J.; Buh Gašparič, M.; Kos, J.; Žel, J.; Sabotič, J. Clitocypin, a fungal cysteine protease inhibitor, exerts its insecticidal effect on Colorado potato beetle larvae by inhibiting their digestive cysteine proteases. *Pestic. Biochem. Phys.* **2015**, *122*, 59–66. [CrossRef]
36. Avanzo, P.; Sabotič, J.; Anžlovar, S.; Popovič, T.; Leonardi, A.; Pain, R.H.; Kos, J.; Brzin, J. Trypsin-specific inhibitors from the basidiomycete *Clitocybe nebularis* with regulatory and defensive functions. *Microbiol.* **2009**, *155*, 3971–3981. [CrossRef]
37. Žurga, S.; Pohleven, J.; Kos, J.; Sabotič, J. β-Trefoil structure enables interactions between lectins and protease inhibitors that regulate their biological functions. *J. Biochem.* **2015**, *158*, 83–90. [CrossRef]
38. Cetin, H.; Demir, E.; Kocaoglu, S.; Koya, B. Insecticidal activity of some synthetic pyrethroids with different rates of piperonyl butoxide (PBO) combinations on *Drosophila melanogaster* (Diptera: Drosophilidae). *Ekoloji* **2010**, *19*, 27–32. [CrossRef]
39. Solovev, I.; Shegoleva, E.; Fedintsev, A.; Shaposhnikov, M.; Moskalev, A. Circadian clock genes' overexpression in *Drosophila* alters diet impact on lifespan. *Biogerontology* **2019**, *20*, 159–170. [CrossRef]
40. Shaposhnikov, M.V.; Zakluta, A.S.; Zemskaya, N.V.; Guvatova, Z.G.; Shilova, V.Y.; Yakovleva, D.V.; Gorbunova, A.A.; Koval, L.A.; Ulyasheva, N.S.; Evgen'ev, M.B.; et al. Deletions of the cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CSE) genes, involved in the control of hydrogen sulfide biosynthesis, significantly affect lifespan and fitness components of *Drosophila melanogaster*. *Mech. Ageing Dev.* **2022**, *203*, 111656. [CrossRef]
41. Li, J.; Duan, D.; Zhang, J.; Zhou, Y.; Qin, X.; Du, G.; Gao, L. Bioinformatic prediction of critical genes and pathways involved in longevity in *Drosophila melanogaster*. *Mol. Genet. Genom.* **2019**, *294*, 1463–1475. [CrossRef] [PubMed]
42. Wang, L.; Li, Y.M.; Lei, L.; Liu, Y.; Wang, X.; Ma, K.Y.; Chen, Z. Cranberry anthocyanin extract prolongs lifespan of fruit flies. *Exp. Gerontol.* **2015**, *69*, 189–195. [CrossRef] [PubMed]
43. Jia, K.; Chen, D.; Riddle, D.L. The TOR pathway interacts with the insulin signaling pathway to regulate C. elegans larval development, metabolism and life span. *Development* **2004**, *131*, 3897–3906. [CrossRef]
44. Min, K.; Tatar, M. *Drosophila* diet restriction in practice: Do flies consume fewer nutrients? *Mech. Ageing Dev.* **2006**, *127*, 93–96. [CrossRef] [PubMed]
45. Shu, X.; Zhang, Y.; Jia, J.; Ren, X.; Wang, Y. Extraction, purification and properties of water-soluble polysaccharides from mushroom *Lepista nuda*. *Int. J. Biol. Macromol.* **2019**, *128*, 858–869. [CrossRef] [PubMed]
46. Wang, L.; Wang, C.; Gao, X.; Xu, N.; Lin, L.; Zhao, H.; Jia, S.; Jia, L. Purification, characterization and anti-aging capacity of mycelia zinc polysaccharide by *Lentinus* edodes SD-08. *BMC Complem. Altern. Med.* **2015**, *15*, 1–11. [CrossRef]
47. Tang, R.; Xiaoyi, C.; Tiantian, D.; Yangni, D.; Zihua, Z.; Qian, L.; Gong, G.; Shuang, S.; Fangli, M.; Linjuan, H.; et al. Lycium barbarum polysaccharides extend the mean lifespan of *Drosophila melanogaster*. *Food Funct.* **2019**, *10*, 4231–4241. [CrossRef]