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Research

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Comparison of toxic effects of 5 macrofungi against *Drosophila melanogaster*

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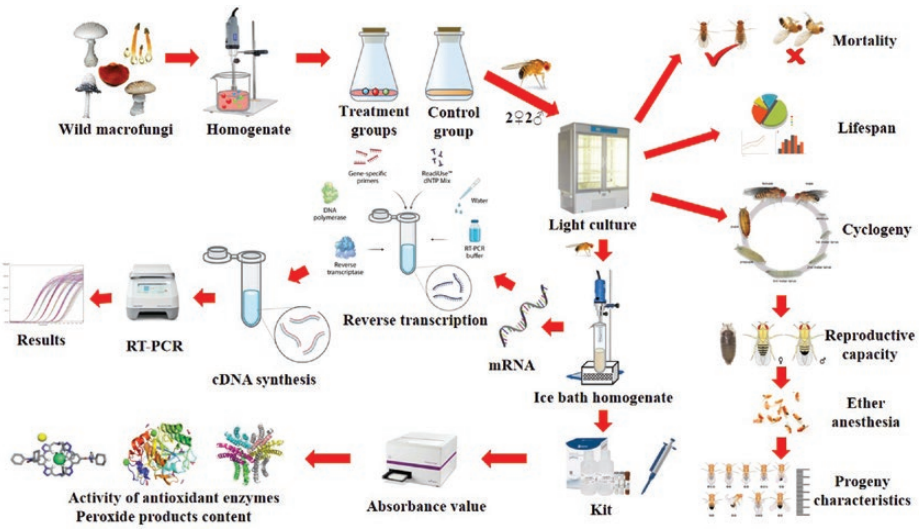
Traditional chemical pesticides pose potential threats to human health, the environment, and food safety, and there is an urgent need to develop botanical pesticides that are easily degradable, renewable, and environmentally compatible. This research serves to detect the lethal impacts of Amanita pantherina(DC.:Fr) Schrmm.(Agaricales, Amanitaceae, Amanita), Amanita virgineoides Bas (Agaricales, Amanitaceae, Amanita), Coprinus comatus (O.F.Müll.) Pers. (Agaricales, Psathyrellaceae, Coprinus), Pycnoporus cinnabarinus(Jacq.:Fr) Karst (Polyporales, Polyporaceae, Polyporus) and Phallus rubicundus (Bosc) Fr. (Phallales, Phallaceae, Phallus) on Drosophila melanogaster(Diptera, Drosophilidae, Drosophila), including their effects on lifespan, fecundity, offspring growth and developmental characteristics, antioxidant enzyme activity, peroxide content, and the gene transcription associated with signaling pathways and lifespan of D. melanogaster. The results demonstrated that they all produced lethal effects on D. melanogaster. Female flies were more sensitive to the addition of macrofungi to their diet and have a shorter survival time than male flies. The toxic activity of A. pantherina-supplemented diet was the strongest, so that the D. melanogaster in this group had no offspring. The macrofungal-supplemented diets were able to significantly reduce the activity of antioxidant enzymes, accumulate peroxidation products, up-regulatd the transcription of genes related to signaling pathways, inhibit the expression of longevity genes, reduce the lifespan and fertility of D. melanogaster. Consequently, we hypothetically suggest that medicinal C. comatus, P. cinnabarinus and P. rubicundus hold the potential to be developed into an environmentally friendly biopesticide for fly killing.

Graphical Abstract



Key words: macrofungi, biopesticide, *Drosophila melanogaster*, poisoning, longevity gene

Background

The invasive insect pest, *Drosophila suzukii* Matsumura (Diptera, Drosophilidae), owns a wide host scope, reproduces speedily, and owns the capability of surviving in numerous climatic situations, all of which have contributed to the fast global distribution of this economically crucial injurious insect (Curtsinger 2019). The chemical fly pesticides utilization aren't the sustainable strategies on account of the administrative limitations and the potential for resistance of the insecticide (Van Timmeren et al. 2018, Deans and Hutchison 2022). Many plants and fungal compounds possess remarkable biological activities related to the presence of secondary metabolites (Cespedes et al. 2015, Damalas and Koutroubas 2018). *Amanita muscaria* (L.) Lam. (Agaricales, Amanitaceae) is capable of being adopted for catching flies while being soaked in water or milk (Lumpert and Kreft 2016). *Amanita muscaria* is capable of being adopted against the mosquito *Culex quinquefasciatus* (Diptera, Culicidae) (Cárcamo et al. 2016). Additionally in 175 tested disparate species of fungi, 79 were discovered to impede the insect progression (Mier et al. 1996). The observed consequences prove that some fungi include antifeedant and repellent even poisonous compounds that act against *Sitophilus zeamais* (Palavecino-De-La-Fuente et al. 2022), *Drosophila melanogaster, Tribolium castaneum* (Riahi et al. 2009), *Leptinotarsa decemlineata* (Kryukov et al. 2014), and *Plutella xylostella* (Kim et al. 2002).

Amanita pantherina (DC.:Fr) Schrmm. can produce symptoms that central nervous system (CNS) depression, ataxia, waxing and waning obtundation, religious hallucinations, and hyperkinetic behavior when eaten by humans by mistake (Satora et al. 2006). Although Korean people consume *Amanita virgineoides* Bas (Phat et al. 2016), some studies indicate that it can be poisonous (Asano et al. 2013). *Coprinus comatus* (O.F.Müll.) Pers. have antiplatelet, anticoagulant (Poniedziałek et al. 2019), anti-glioma(Nowakowski et al. 2021), antidiabetic, antioxidant (Ratnaningtyas et al. 2022) activities, and can relieve alcohol-induced liver injury (Zhao et al. 2018), inhibit adipocyte differentiation (Park et al. 2014), and reduce the incidence of chronic inflammatory disorders (Van de velde et al. 2015). Studies have shown that of the pigments of *Pycnoporus* strains; cinnabarin, cinnabarinic acid, and tramesanguin are the main components, have anti-inflammatory activity and can poison cancer cells, *Aedes*, *Anopheles* larvae (Tellez-Tellez et al. 2016), and *Diatraea magnifactella* (Díaz-Godínez et al. 2016). The secondary metabolites of *Phallaceae* have anti-inflammatory, immune-enhancing, and anticancer activity, as well as α-glucosidase inhibitory potential (Ker et al. 2011, Chaiyama et al. 2020, Lee et al. 2020, Jianhua et al. 2022). As far as we know, no previous research has investigated fly-killing activity of *A. pantherina*, *A. virgineoides*, *C. comatus*, *Pycnoporus cinnabarinus* (Jacq.:Fr) Karst, and *Phallus rubicundus* (Bosc) Fr.

Prior researches have indicated that *D. melanogaster* exposure to 3Gy electron beam irradiation, the rare-earth element cerium (Ce) as well as 15 mM paraquat (Pq) could trigger the oxygenated stress, which particularly proves as an obvious growth inside the content of malanoldialdehyde (MDA). Furthermore, the radiation/Pq/Ce-induced free radicals can in turn impair the antioxidant defense systems, bringing about a decrease in catalase (CAT) and superoxide dismutase (SOD) activity as well as glutathione (GSH) levels. What's more, Pq-treated flies showed serious locomotor impairments, with 84% of flies uncapable of flying (Manjula et al. 2015). There existed an obvious reduction in maximum lifespan, mean lifespan, and reproductive output as doses of cerium increased (Huang et al. 2010). Present researches have illuminated that the metabolic signaling pathways is capable of mediating longevity and age (Wei et al. 2015). Target of rapamycin *(TOR)* is an essential regulator of cellular proliferation and impacts the senescence, *S6K* is just one downstream *TOR* kinase effector (Soto-Burgos et al. 2018), the Jun kinase (*JNK*) signaling pathway indirectly expresses the cell oxygenated stress reaction and enlarge the longevity. *Hep* is one sort of homolog of Jun kinase kinase (*JNKK*), and *D. melanogaster* having the *Hep* mutation were exhibited to be more sensitive to the oxidized stress and possess the shorter longevity (Wang et al. 2003).

At present, the molecular mechanism of *Amanita* to kill flies is not sufficiently studied, and *Amanita*, although highly toxic, pose a threat to the environment and humans. It is worth exploring whether it is possible to find macrofungi from edible or medicinal mushrooms which are beneficial to humans, that have the same fly-killing activity as *Amanita*. In this study, *A. pantherina*, *A. virgineoides*, *C. comatus*, *P. cinnabarinus*, and *P. rubicundus* were selected to check for their toxicity against *D. melanogaster*. Additionally, their influences upon the lifespan, fecundity, the growth and development characteristics of offspring, peroxide content, antioxidant enzyme activity; additionally, the gene transcription relating to signaling pathways and lifespan of *D. melanogaster* were contrasted. The genetic relationship between *D. melanogaster* and *D. suzukii* is close. This was performed in order to provide a reference for biological control of *D. suzukii* using macrofungi.

Materials and Methods

Materials

Captured in one local orchard of cherry (114°E, 31°N), adult wild-type *D. melanogaster* were fostered inside typical yeast medium of corns and breeded inside one light hatcher at 25 °C. Early harvested *A. pantherina*, *A. virgineoides*, *C. comatus*, *P. cinnabarinus*, and *P. rubicundus* originated in fallen leaves on 11 July 2021 in the forests of Chinese Ta-pieh Mountain.

Flies Stock and Treatment

The macrofungal fruiting bodies were respectively germfree, mixed at a mass proportion of 1:4 with the purified water, homogenized by means of ultrasonic (240 W) crushing for 1 h, sealed, being bottled at 4°C. The therapy medium was set based upon the yeast medium of corns, which was regarded as the control (CK). A (*C. comatus*), B (*P. rubicundus*), C (*P. cinnabarinus*), D (*A. pantherina*), and E (*A. virgineoides*) were set up in 5 experiment therapy teams in all. In the experiments, 30% of the original mass of distilled water and corn flour in the foundational medium were subduced; additionally, the entire 2 mass was put with the identical mass of macrofungal homogenate. During the course of configuration, the homogenate was added together with yeast powder; the medium was split into 100-ml triangular flasks and subsequently put apart. Two male and 2 female virgin flies which were hungry in prior 8 h were inserted in the bottles. Eighty flies in total were chosen for every experiment team and were hatched at 25 °C.

Longevity Analysis

Since virgin flies were placed in therapy medium, they were termly surveyed and enumerated each 6 h. Behind rearing flies for 24 h with the macrofungal therapy medium, the subsisting flies were switched to the yeast medium of corns for rearing deeply. As the pupae were shaped, those flies were transferred again to the yeast medium of corn. *Drosophila melanogaster* possessing wings at 45° to bodies

were thought to pass away. Count the number of fruit fly deaths every 6 h until all flies die. Draw a survival curve based on the results.

Measurement of *D. melanogaster* Reproductive Capacity

The numbers of newly formed adult pupae and adult flies per bottle per day were recorded from the day the offspring of adult flies previously treated with the fungal compounds had pupae formed on the bottle wall until there were no more pupae or flies being formed. The quantity of flies and pupae was put down; besides, the rate of flies per female was figured out in light of the formulae: The rate of flies = number of flies/number of pupae × 100% (Kharat et al. 2020).

Measurement of Growth and Developmental Characteristics of *D. melanogaster* Offspring

The offspring of *D. melanogaster* were collected at 24 h after fledging, and their phenotypes were observed under a microscope after anesthesia with ether. The number of aberrations, female and male flies, were recorded, and their body lengths were measured using an eyepiece micrometer. Two hours after awakening, the flies were released outdoors and those that could take off normally were counted as "flyable". Finally, the sex ratio, body length, aberration rate, and flyability were counted.

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

What`s more, 100 mg of male and female flies fostered by means of the therapy medium lasting for 3 and 6 h were weighed. Every team of the flies was blended with 0.9 ml of saline solution and homogenized at 2,000 r/min, lasting for 10 s in 1 ice bath during an interval of 10 s. That was redone 3 times to generate a homogenate. The activities of SOD, CAT, as well as the content of malondialdehyde (MDA) were figured out using 1 enzyme marker.

Determination of the Corresponding Quantity of Transcriptions in *D. melanogaster*

The whole mRNA originating in *D. melanogaster* by the Trizol approaches, as depicted inside the Trizol kit's guidance (Invitrogen). The synthesis of cDNA was implemented in light of the guidance for the PrimeScriptTM RT-PCR Kit. The primer sequencing adopted for the sake of the quantitative dissection were compounded by Shanghai Meiji Biomedical Technology Co. Fluorescence real-timely quantitative PCR was carried out by means of one system of QuantStudio 3 real-timely quantitative PCR, these reagents were employed by means of 1 DyNAmoTM SYBRR Green qPCR kit; additionally, experiment operations were implemented in light of the guidance of the reagent. Data was gathered and managed with CFX-Manager; besides, corresponding expressions of internal reference and target genes were figured out with the Ct ($2^{-\Delta\Delta Ct}$) approach. By means of the ribosomal protein (*RP49*) as 1 internal gene for reference, large-throughput fluorescent quantitative PCR was adopted with a view to deciding the corresponding quantities of transcriptions of lifespan-related genes, like hemipterus (*Hep*), nuclear factor erythroid-2-related factor 2 (*Nrf2*), methuselah (*MTH*) and signaling-pathway-related genes.

Statistical Analysis

The whole manipulations in the test were redone 3 times and a total of 240 flies were used in each experimental group (2 couples per bottle). Survival curve was performed using the Kaplan-Meier survival plot and calculated by the log-rank test (Schissel et al. 2021). Fecundity and offspring characteristics were tested for specific differences in more detail using ANOVA or Kruskal Wallis tests, depending on whether the data were normally distributed (Mason et al. 2018). As for the distinction between means, its importance was evaluated by means of one-way ANOVA, then post hoc Tukey's experiment using SPSS v26, which is a software package (SPSS Inc., Chicago, USA). Disparate lowercase letters inside the identical column illustrate the least significant differences (LSDs) at the 5% level ($P < 0.05$) for treatment every time.

Results

Effects of Macrofungal-Supplemented Diets Upon the Longevity of *D. melanogaster*

Longevity assay was performed to know the influence of macrofungi on lifespan extension of flies. Male and female flies were reared on diet containing *C. comatus* (A), *P. rubicundus* (B), *P. cinnabarinus* (C), *A. pantherina* (D), and *A. virgineoides* (E). From the survival curve (Fig. 1), it can be seen that the control group of flies had the longest lifespan, and all 5 groups of flies in the diet supplemented with macrofungi died. The Log rank test results showed a significant difference of $P < 0.0001$. The maximum lifespan of female and male flies is the same, with groups A, B, C, D, and E having a maximum lifespan of 48, 60, 84, 24, and 36 h, respectively. Group D has the shortest lifespan and survival analysis ratios were 0.05, 0.04, and 0.03 at 6, 12, and 18 h, respectively. The paired comparative analysis results between 6 groups showed that in regard to female flies, $P < 0.01$ was observed between group A and B, $P < 0.05$ was observed between groups A and E, and $P < 0.001$ was observed among other groups; In regard to male flies, $P < 0.01$ was observed between groups A and B, groups B and C, $P < 0.05$ was observed between groups A and E, and $P < 0.001$ was observed among all other groups.

The survival rate was analyzed using Kaplan-Meier, and the results are shown in Table 1. From Table 1, it can be seen that the average survival time of flies in the 5 macrofungal diet supplemented groups was C > B > A > E > D for both female and male flies. The half survival time of female flies in groups A, B, C, and E was lower than that of male flies. The LT_{25}, LT_{50}, and LT_{75} of female flies in group C were 54, 30, and 18 h, respectively, lower than those of male flies. It can be seen that female flies were more sensitive to the addition of macrofungi to their diet and have a shorter survival time than male flies.

Influences of Macrofungal-Supplemented Diets Upon the Fecundity of *D. melanogaster*

Reproduction assay was performed to know the influence of macrofungi on fertility of flies. Male and female flies were reared on diet containing *C. comatus* (A), *P. rubicundus* (B), *P. cinnabarinus* (C), *A. pantherina* (D), and *A. virgineoides* (E). The pupa number, adult fly number, and adult fly rate of parent fly decreased significantly after receiving macrofungal-supplemented diets as compared with the control (Fig. 2). It can be seen from Fig. 2 that the toxic activity of group D was the strongest, so that the *D. melanogaster* in this group had no offspring. In contrast, *D. melanogaster* were able to produce offspring after receiving other 4 macrofungal-supplemented diets. As compared with the control group, fecundity decreased significantly in group A ($P < 0.05$), extremely significantly in group E ($P < 0.01$), and had no significant in group B and C

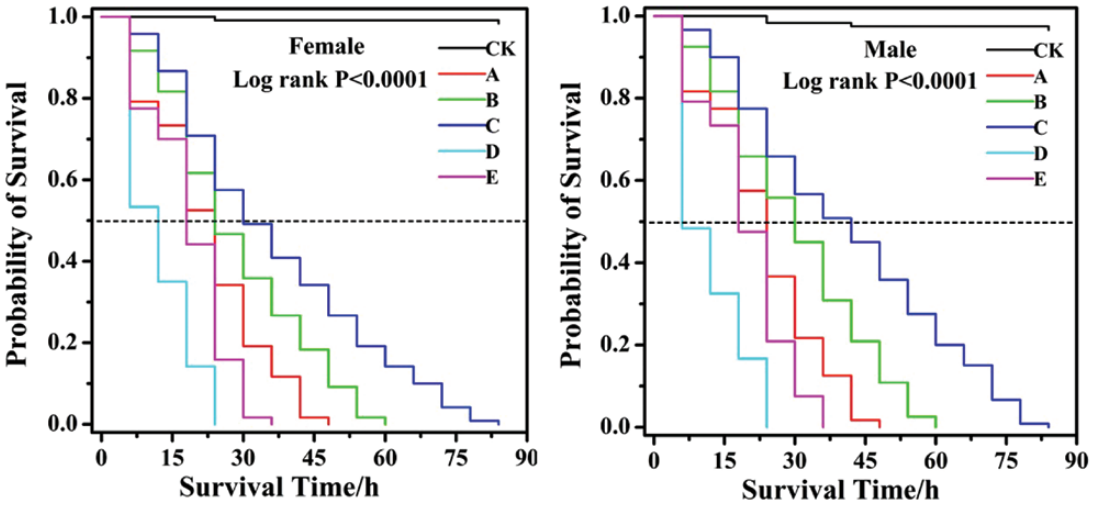


Fig. 1. Kaplan-Meier survival curves of *Drosophila melanogaster* (Diptera, Drosophilidae), having received combinational macrofungal-supplemented diets. The flies having wings at 45° to bodies were thought to pass away. CK—the control team—obtained an ordinary diet with no macrofungal. Groups A, B, C, D, and E received a macrofungal-supplemented diet with *Coprinus comatus* (O.F.Müll.) Pers., *Phallus rubicundus* (Bosc) Fr., *Pycnoporus cinnabarinus* (Jacq.:Fr) Karst, *Amanita pantherina* (DC.:Fr) Schrmm., and *Amanita virgineoides* Bas, separately.

Table 1. Mean (±SD) of survival time (h), median (h), LT_{25} (h), LT_{50} (h), LT_{75} (h) of *D. melanogaster*, having received macrofungal-supplemented diets. Groups A, B, C, D, and E received a macrofungal-supplemented diet with *C. comatus*, *P. rubicundus*, *P. cinnabarinus*, *A. pantherina*, and *A. virgineoides*, separately

Gender	Groups	Survival time/h	Median/h	LT_{25}/h	LT_{50}/h	LT_{75}/h
Female	A	22.40 ± 1.09	24.85	30.00 ± 1.43	24.00 ± 1.36	12.00 ± 0.00
	B	28.40 ± 1.35	28.67	42.00 ± 2.42	24.00 ± 2.12	18.00 ± 1.33
	C	36.60 ± 1.88	35.40	54.00 ± 2.88	30.00 ± 3.29	18.00 ± 1.71
	D	12.15 ± 0.61	13.09	18.00 ± 0.92	12.00 ± 0.00	6.00 ± 0.00
	E	18.55 ± 1.00	22.65	24.00 ± 0.71	18.00 ± 1.05	12.00 ± 0.00
Male	A	23.35 ± 1.07	26.16	30.00 ± 1.87	24.00 ± 1.27	18.00 ± 2.24
	B	30.35 ± 1.38	33.23	42.00 ± 2.22	30.00 ± 2.52	18.00 ± 1.64
	C	41.30 ± 1.97	42.86	60.00 ± 2.77	42.00 ± 4.67	24.00 ± 2.15
	D	11.95 ± 0.64	11.88	18.00 ± 1.29	6.00 ± 0.00	6.00 ± 0.00
	E	19.70 ± 0.83	23.42	24.00 ± 1.11	18.00 ± 1.04	12.00 ± 0.00

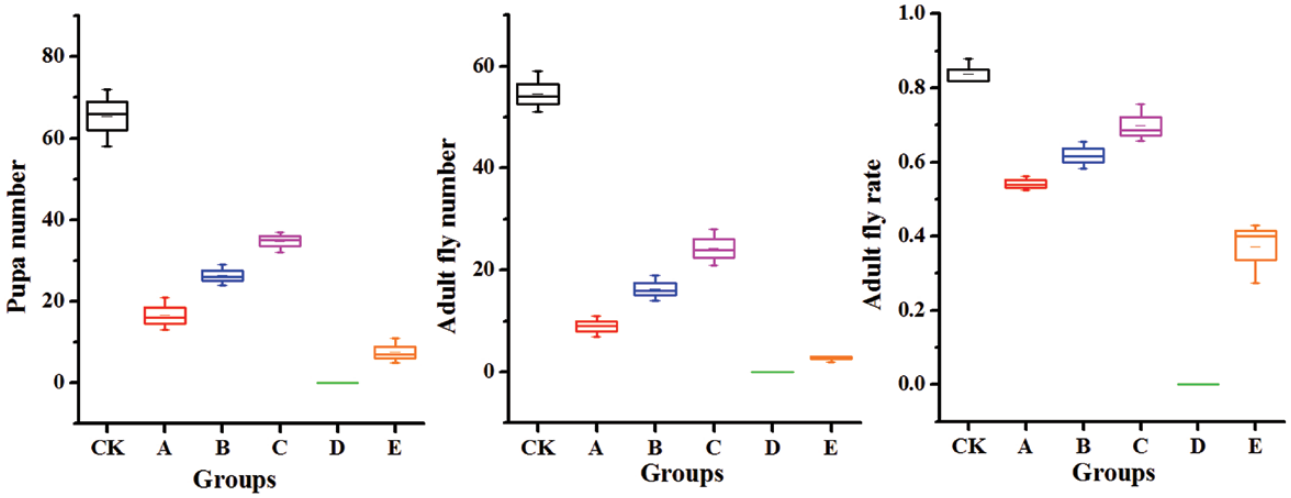


Fig. 2. The pupa number, adult fly number, and adult fly rate of *D. melanogaster*, having received macrofungal-supplemented diets. Fecundity was tested for specific differences in more detail using ANOVA or Kruskal Wallis tests, depending on whether the data were normally distributed. CK—the control team—obtained an ordinary diet with no macrofungal. Groups A, B, C, D, and E received a macrofungal-supplemented diet with *C. comatus*, *P. rubicundus*, *P. cinnabarinus*, *A. pantherina*, and *A. virgineoides*, separately.

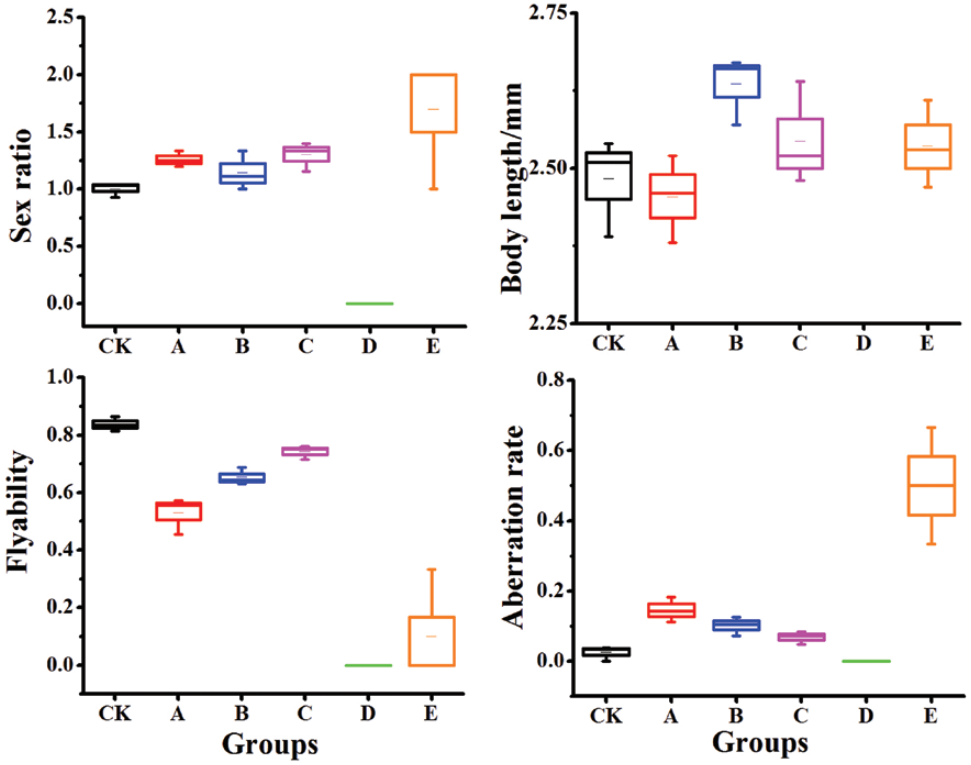


Fig. 3. The sex ratio, body length, flyability, aberration rate of *D. melanogaster*, after receiving macrofungal-supplemented diets. Offspring characteristics were tested for specific differences in more detail using ANOVA or Kruskal Wallis tests, depending on whether the data were normally distributed. CK—the control team—obtained an ordinary diet with no macrofungal. Groups A, B, C, D, and E received a macrofungal-supplemented diet with *C. comatus, P. rubicundus, P. cinnabarinus, A. pantherina*, and *A. virgineoides*, separately.

($P > 0.05$). There was a significant difference in fecundity between groups E and C ($P < 0.05$). Group C had the least inhibition on the fecundity of flies, with an adult fly rate of 69.96%. The number of pupae was 34.67, which was 53.07% that of the control, and the number of adult flies was 24.33, which was 44.50% that of the control. In all, the toxicity to *D. melanogaster* fecundity was E > A > B > C.

Influences of Macrofungal-supplemented Diets Upon the Growth and Development Characteristics of *D. melanogaster* Offspring

Microscopy and behavioral assay was performed to know the influence of macrofungi on the growth and development characteristics of offspring. It can be seen from Fig. 3 that, except for the most toxic activity of *A. pantherina* to *D. melanogaster*, which did not produce offspring, the other 4 kinds of macrofungi-supplemented diets had different effects on the growth and development characteristics of offspring. When compared with the control, the sex ratio of group E was as high as 1.67, and all 4 groups saw no significant difference as compared with the control ($P > 0.05$). There was a significant difference in body length between groups A and B ($P < 0.05$). As compared with the control group, flyability decreased and aberration rate increased significantly in group A ($P < 0.05$), extremely significantly in group E ($P < 0.01$), and had no significant in groups B and C ($P > 0.05$). It is worth noting that the aberration rate of the offspring in group E was as high as 50.00%, 21.19 times than that of the control. It is concluded that different macrofungal-supplemented diets have different toxicity to fly offspring.

Influences of Macrofungal-supplemented Diets Upon the Activity of the Antioxidant Enzymes and the Content of Peroxides in *D. melanogaster*

Antioxidant assay was performed to know the influence of macrofungi on the oxidative level of flies. It can be seen from Fig. 4 that the CAT activity of groups D and E decreased significantly compared with the control after receiving macrofungal-supplemented diets for 3 and 6 h. The SOD activity in groups A, B and C significantly increased at 3 h, while groups D and E significantly decreased. The SOD activity in the 5 groups decreased significantly at 6 h, and the SOD activity of group D was the lowest, with the value of 22.45 U/mg for female flies and 20.41 U/mg for male flies.

The MDA content of 5 treatment groups increased significantly at 6 h. The MDA content of group D increased the most at 6 h as compared with the values found at 3 h, with a 53.61% increase to 35.13 nmol/mg in female flies and an 82.00% increase to 39.73 nmol/mg in male flies.

Influences of the Macrofungal-Supplemented Diets Upon the Levels of Genes Transcriptions Related to the Signaling Pathway in *D. melanogaster*

Real time PCR assay was performed to know the influence of macrofungi on the levels of genes transcriptions related to the signaling pathway of flies. It can be seen from Fig. 5 that the levels of signal pathway related genes *S6K*, *TOR* and *Keap-1* transcripts in the 5 groups raised significantly at 6 h as compared with the control. The level of *S6K* transcripts increased most significantly at 6 h as compared with those found at 3 h, especially the level of *S6K* transcripts of female flies in groups D and E increased 2.16 and 2.31

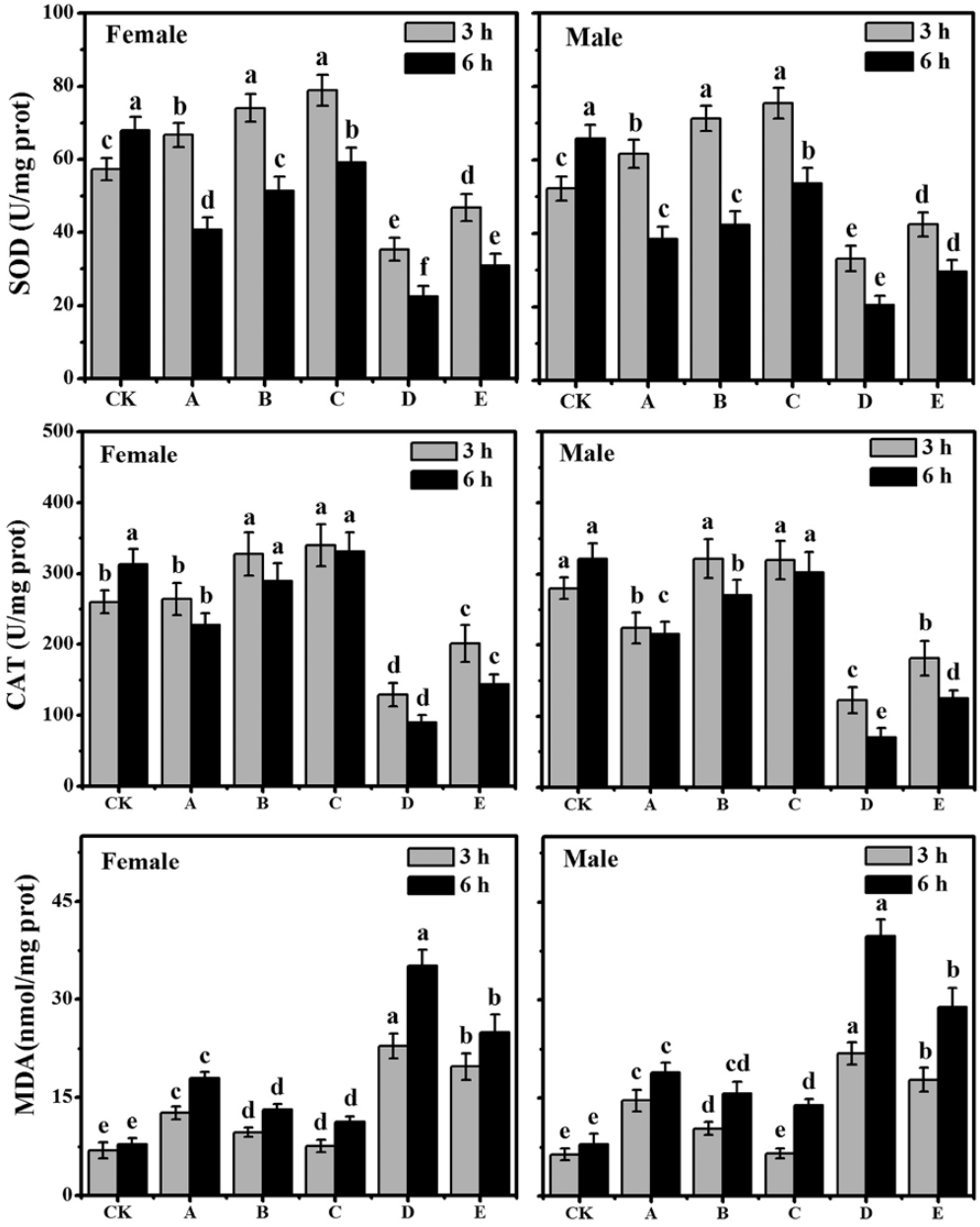


Fig. 4. Mean (±SD) of the activity of catalase (CAT) and superoxide dismutase (SOD), the content of malondialdehyde (MDA) of *D. melanogaster*, after receiving macrofungal-supplemented diets for 3, 6 h. The means blessed with disparate letters are different obviously at $P < 0.05$ (Tukey's trial). CK—the control team—obtained an ordinary diet with no macrofungal. Groups A, B, C, D, and E received a macrofungal-supplemented diet with *C. comatus*, *P. rubicundus*, *P. cinnabarinus*, *A. pantherina*, and *A. virgineoides*, separately.

times, respectively, and that of male flies increased 2.22 and 2.44 times respectively. The level of *S6K*, *TOR*, and *Keap-1* transcripts in female and male flies of group D was the highest at 6 h, and male flies were 0.66, 0.42, and 0.33 higher than female flies, respectively. It can be seen that the signaling pathway genes can respond to the macrofungal-supplemented diets.

Influences of the Macrofungal-Supplemented Diets Upon These Levels of Lifespan-Related Gene Transcriptions in *D. melanogaster*

Real time PCR assay was performed to know the influence of macrofungi on the levels of genes transcriptions related to the lifespan of flies. It can be seen from Fig. 6 that the levels of the lifespan-related *Hep* and *Nrf2* genes transcripts decreased significantly in groups D and E at 3 and 6 h as compared with the control. The levels of *Hep* and *Nrf2* transcripts reduced at 6 h as compared with those observed at 3 h. At this time, the 5 groups of female flies showed significant differences, while the groups D and E of male flies showed no significant differences. The level of *MTH* transcripts increased at 6 h as compared with those observed at 3 h, and the 5 groups showed significant increase as compared with the control. The level of *MTH* transcripts in group D was the highest, 2.62 times in female flies and 2.69 times in male flies, while that of group C was the lowest, 1.3 times in female flies and 1.44 times in male flies. It can be seen that the lifespan-related genes can respond to the macrofungal-supplemented diets.

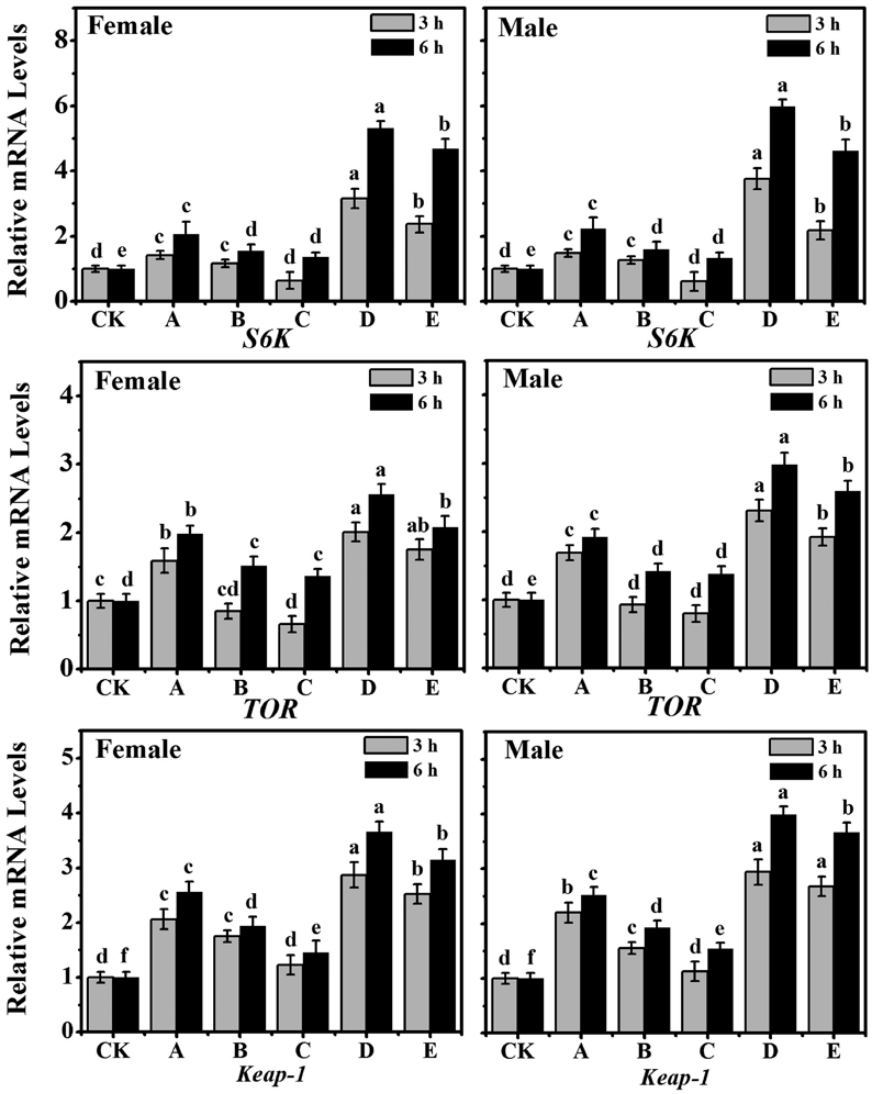


Fig. 5. Mean (±SD) of the corresponding quantities of the transcriptions of the signaling pathway-related genes, after receiving macrofungal-supplemented diets for 3, 6 h. The means blessed with disparate letters are different obviously at $P < 0.05$ (Tukey's trial). CK—the control team—obtained an ordinary diet with no macrofungal. Groups A, B, C, D, and E received a macrofungal-supplemented diet with *C. comatus*, *P. rubicundus*, *P. cinnabarinus*, *A. pantherina*, and *A. virgineoides*, separately.

Discussion

In this study, *A. pantherina* and *A. virgineoides*, which are common local species, were used to configure the treatment medium and then fed to *D. melanogaster*. The toxic effect of *A. pantherina* was the most obvious. Adding *Amanita* species to the diet can disrupt the antioxidant system, upregulate gene transcription related to signaling pathways, inhibit gene expression of longevity, reduce the useful life and fertility of *D. melanogaster*. The toxic peptides, amanitin, and phalloidins identified in a phalloides syndrome case, due to poisoning caused by mushrooms (Ramchiun et al. 2018), were found not only in *A. phalloides*, but in 3 other *Amanita* species, 8 species of *Galerina*, and 11 *Lepiota* (Vetter 1998). Our results are similar to previous studies that showed that low α-amanitin concentrations negatively impact larva-to-adult progression time, pre-adult viability, body size of adult, along with adult longevity (Mitchell et al. 2015). These results may be due to the fact that pantheric acids A–C in poisonous mushrooms promote lipid accumulation in adipocytes (Lee et al. 2019). It has already been supported that activation of *TOR* (Jia et al. 2004) and *S6K* (Min and Tatar 2006) expression is capable of lessening the lifespan by means of rapamycin in *D. melanogaster*, which corresponds to these consequences of the current research. The *Methuselah* (*MTH*) gene has been thought as one key longevity element for long. What`s more, diverse researches have exhibited that overexpression of *MTH* gene in *D. melanogaster* can shorten lifespan (Wang et al. 2015).

It is evident that the macrofungi of the *Amanita* species possess promising applications for the development of biogenic insecticides. However, *D. melanogaster* has developed ibotenic acid (Tuno et al. 2007) and a-amanitin resistance, which act by binding to RNA polymerase II and inhibiting RNA transcription, over a long period of natural evolution (Scott Chialvo and Werner 2018). These characteristics are associated with cytochrome P450 activity (Stump et al. 2011) and not with gut microbiota (Griffin and Reed 2020). It was considered in a former study that toxin entry blockage by means of the cuticle, stage I and II detoxification, sequestration inside particles of lipid, along with the proteolytic cleavage of α-amanitin contribute, to the quantitative trait (Mitchell et al. 2014). *Widerborst* and *Tequila*, which were candidate genes underlying the α-amanitin

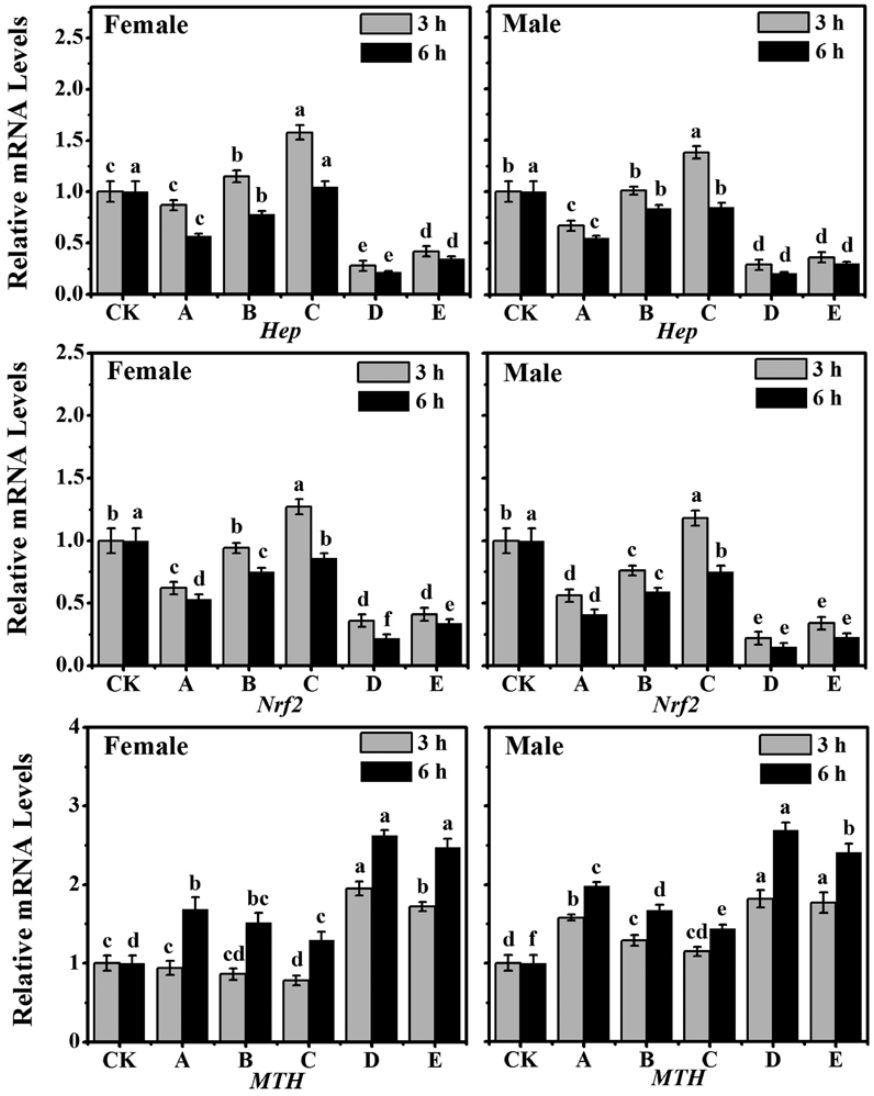


Fig. 6. Mean (±SD) of corresponding quantities of lifespan-related genes transcriptions, like hemipterus (*Hep*) of *D. melanogaster*, nuclear factor erythroid-2 related factor 2 (*Nrf2*), methuselah (*MTH*), after receiving macrofungal-supplemented diets for 3, 6 h. The means blessed with disparate letters are different obviously at $P < 0.05$ (Tukey's trial). CK—the control team—obtained an ordinary diet with no macrofungal. Groups A, B, C, D, and E received a macrofungal-supplemented diet with *C. comatus*, *P. rubicundus*, *P. cinnabarinus*, *A. pantherina*, and *A. virgineoides*, separately.

resistance phenotype, are upstream regulators of *TOR*, which is a key regulator of autophagy and *Megalin*-mediated endocytosis (Mitchell et al. 2017).

In order to find more medicinal fungal resources that can be used to kill flies and thus solve the problem of α-amanitin resistance in *D. melanogaster* and environmental pollution caused by fungal toxins, this study also used the common local treatment media of *C. comatus*, *P. cinnabarinus*, *P. rubicundus* to feed *D. melanogaster*. The results showed that they can have a toxic effect on *D. melanogaster*, reduce the activity of antioxidant enzymes, accumulate peroxide products, inhibit the expression of longevity genes, and reduce the lifespan and fecundity. Present researches claimed that lactone (Mizushina et al. 1999), lucidenic acid O, lectins (Pohleven et al. 2011), fungal cyclic peptides (Wang et al. 2017), and hemolysins in mushroom are potential natural insecticides. Cnispin, 1 protease inhibitor which impedes one kind of serine protease named trypsin, is poisonous to *D. melanogaster*. Clitocine, one sort of new nucleoside has shown the powerful insecticidal activation against the pink bollworm *Pectinophora gossypiella* (Kubo et al. 1986). If the insecticidal active ingredients in macrofungi can be isolated and identified in the future, it will help to reveal the molecular mechanism of several key molecules acting synergistically to kill flies at a deeper level and provide reference for the development of fly killing pesticides.

After receiving a *C. comatus*-supplemented diet for 3 h, the SOD activity significantly increased as compared with the control, which may be related to the higher antioxidant enzyme activity of *C. comatus* itself, which could protect from the damage of ROS-induced cell, lessen the peroxidation of the lipid, and make the cell membrane integrity stable. Similar results also were discovered in the *P. cinnabarinus* and *P. rubicundus* groups. It can be seen that consuming a small amount of these 3 mushrooms does not pose a threat to human health.

The resources of macrofungi in the wild are always limited, not only by time and place, but also wild specimens of macrofungi have been extensively collected, and the habitat of the species has been continually demolished in recent years in China (Jianguo and Jared 2005). Adopting in vitro cultures decreases the over-exploitation of rare at the lowest degree, valuable, or endangered

species, presenting a methodology which gives a priority to the sustainable safeguard and the reasoning application to biodiversity (Hsu et al. 2015). It is an inevitable trend to use fresh fruiting bodies for tissue separation and in vitro liquid culture to obtain mycelia for the development and utilization of environment-friendly biological insecticides, which is also worth further exploration.

Conclusion

In summary, the findings of this research, which examined the effects on *D. melanogaster* in diets supplemented with *A. pantherina*, *A. virgineoides*, *C. comatus*, *P. cinnabarinus*, and *P. rubicundus*, demonstrated that they all exerted lethal effects on *D. melanogaster*. The medicinal *C. comatus*, *P. cinnabarinus*, and *P. rubicundus* were able to significantly reduce the activity of antioxidant enzymes, accumulate peroxidation products, up-regulatd the transcription of genes related to signaling pathways, inhibit the expression of longevity genes, reduce the lifespan and fertility of *D. melanogaster*. These achievements provide a reference for the application of macrofungi for the discovery of biogenic fly-killing pesticides.

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Author Contributions

Jinzhe Li (Data curation-Equal, Investigation-Equal, Visualization-Equal, Writing – original draft-Equal), Yaqin Huang (Data curation-Equal, Investigation-Equal, Software-Equal, Visualization-Equal), Dezhi Wang (Supervision-Equal, Validation-Equal), Nailiang Zhu (Conceptualization-Equal, Writing – review & editing-Equal), Xinrong Qiao (Conceptualization-Equal, Funding acquisition-Equal, Methodology-Equal, Supervision-Equal).

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Data Availability

All data generated or analyzed during this study are included in this published article.

References

Asano M, Yamada K, Tanaka T, Matsuo Y, Kouno I. New bisabolane sesquiterpene from the mycelia of *Amanita virgineoides*. *Chem Pharm Bull (Tokyo)*. **2013**:61(3):366–369. https://doi.org/10.1248/cpb.c12-01070

Cárcamo MC, Carapeto LP, Duarte JP, Bernardi E, Ribeiro PB. Larvicidal efficiency of the mushroom *Amanita muscaria* (Agaricales, Amanitaceae) against the *mosquito Culexquinquefasciatus* (Diptera, Culicidae). *Rev Soc Bras Med Trop*. **2016**:49(1):95–98. https://doi.org/10.1590/0037-8682-0175-2015

Cespedes CL, Alarcon J, Aqueveque PM, Lobo T, Becerra J, Balbontin C, Avila JG, Kubo I, Seigler DS. New environmentally-friendly antimicrobials and biocides from Andean and Mexican biodiversity. *Environ Res*. **2015**:142:549–562. https://doi.org/10.1016/j.envres.2015.08.004

Chaiyama V, Keawsompong S, LeBlanc JG, Moreno De LeBlanc A. de, Chatel J, Chanput W. Action modes of the immune modulating activities of crude mushroom polysaccharide from *Phallus atrovolvatus*. *Bioact Carbohydr Diet Fibre*. **2020**:23:100216.

Curtsinger JW. Reproductive homeostasis and senescence in *Drosophila melanogaster*. *J Gerontol Ser A*. **2019**:74:1533–1538.

Damalas C, Koutroubas S. Current status and recent developments in biopesticide use. *Agriculture*. **2018**:8(1):13. https://doi.org/10.3390/agriculture8010013

Deans C, Hutchison WD. Propensity for resistance development in the invasive berry pest, spotted-wing *drosophila* (*Drosophila suzukii*), under laboratory selection. *Pest Manag Sci*. **2022**:78(12):5203–5212. https://doi.org/10.1002/ps.7139

Díaz-Godínez G, Téllez-Téllez M, Rodríguez A, Obregón-Barbosa V, de Lourdes Acosta-Urdapilleta M, Villegas E. Enzymatic, antioxidant, antimicrobial, and insecticidal activities of *Pleurotus pulmonarius* and *Pycnoporus cinnabarinus* grown separately in an airlift reactor. *Bioresources*. **2016**:11:4186–4200.

Griffin LH, Reed LK. Effect of gut microbiota on α-amanitin tolerance in *Drosophila tripunctata*. *Ecol Evol*. **2020**:10(17):9419–9427. https://doi.org/10.1002/ece3.6630

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.

Huang S, Li Z, Wang X, Wang Q, Hu F. Cerium caused life span shortening and oxidative stress resistance in *Drosophila melanogaster*. *Ecotoxicol Environ Saf*. **2010**:73(1):89–93. https://doi.org/10.1016/j.ecoenv.2009.09.017

Jia K, Chen D, Riddle DL. The *TOR* pathway interacts with the insulin signaling pathway to regulate *C. elegans* larval development, metabolism and life span. *Development*. **2004**:131(16):3897–3906. https://doi.org/10.1242/dev.01255

Jianguo L, Jared D. China's environment in a globalizing world. *Nature*. **2005**:435:1179–1186.

Jianhua L, Lan Y, Shiyu L, Jing D, Mingyu Y, Dongyu F, Changtian L, Fenghua T, Yu L. New aniline derivatives from the volva of *Phallus rubrovolvatus* and their anti-inflammatory activity. *Bioorg Chem*. **2022**:119:105577.

Ker Y, Chen K, Peng C, Hsieh C, Peng RY. Structural characteristics and antioxidative capability of the soluble polysaccharides present in *Dictyophora Indusiata* (Vent. Ex Pers.) Fish Phallaceae. *Evid-Based Complement Altern Med*. **2011**:2011:1–9. https://doi.org/10.1093/ecam/neq041

Kharat P, Sarkar P, Mouliganesh S, Tiwary V, Priya VBR, Sree NY, Annapoorna HV, Saikia DK, Mahanta K, Thirumurugan K. Ellagic acid prolongs the lifespan of *Drosophila melanogaster*. *Geroscience*. **2020**:42(1): 271–285. https://doi.org/10.1007/s11357-019-00135-6

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(7):713–717. https://doi.org/10.1002/ps.508.

Kryukov VY, Yaroslavtseva ON, Dubovskiy IM, Tyurin MV, Kryukova NA, Glupov VV. Insecticidal and immunosuppressive effect of ascomycete *Cordyceps militaris* on the larvae of the Colorado potato beetle *Leptinotarsa decemlineata*. *Biol Bull*. **2014**:41(3): 276–283. https://doi.org/10.1134/s1062359014020046

Kubo I, Kim M, Hood WF, Naoki H. Clitocine, a new insecticidal nucleoside from the mushroom *Clitocybe inversa*. *Tetrahedron Lett*. **1986**:27(36):4277–4280. https://doi.org/10.1016/s0040-4039(00)94251-5

Lee SR, Lee D, Lee BS, Ryoo R, Pang C, Kang KS, Kim KH. Phallac acids A and B, new sesquiterpenes from the fruiting bodies of *Phallus luteus*. *J Antibiot (Tokyo)*. **2020**:73(10):729–732. https://doi.org/10.1038/s41429-020-0328-z

Lee SR, Yi SA, Nam KH, Ryoo R, Lee J, Kim KH. Pantheric acids A-C from a poisonous mushroom, *Amanita pantherina*, promote lipid accumulation in adipocytes. *J Nat Prod*. **2019**:82(12):3489–3493. https://doi.org/10.1021/acs.jnatprod.9b00782

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. https://doi.org/10.1016/j.jep.2016.04.009

Manjula PS, Sarojini BK, Darshan Raj CG, Sanjeev G. In vivo antioxidative stress measurement of a 1, 2, 4-Triazole derivative in *Drosophila melanogaster* oregon K flies. *Der Pharma Chem*. **2015**:7:70–78.

Mason JS, Wileman T, Chapman T. Lifespan extension without fertility reduction following dietary addition of the autophagy activator Torin1 in *Drosophila melanogaster*. *PLoS One*. **2018**:13(1):e0190105. https://doi.org/10.1371/journal.pone.0190105

Mier N, Canete S, Klaebe A, Chavant L, Fournier D. Insecticidal properties of mushroom and toadstool carpophores. *Phytochemistry*. **1996**:41(5):1293–1299. https://doi.org/10.1016/0031-9422(95)00773-3

Min K, Tatar M. *Drosophila* diet restriction in practice: do flies consume fewer nutrients?. *Mech Ageing Dev*. **2006**:127(1):93–96. https://doi.org/10.1016/j.mad.2005.09.004

Mitchell CL, Latuszek CE, Vogel KR, Greenlund IM, Hobmeier RE, Ingram OK, Dufek SR, Pecore JL, Nip FR, Johnson ZJ, *et al*. Alpha-amanitin resistance in *Drosophila melanogaster*: a genome-wide association approach. *PLoS One*. **2017**:12(2):e0173162. https://doi.org/10.1371/journal.pone.0173162

Mitchell CL, Saul MC, Lei L, Wei H, Werner T. The mechanisms underlying alpha -amanitin resistance in *Drosophila melanogaster*: a microarray analysis. *PLoS One*. **2014**:9(4):e934891–e934816. https://doi.org/10.1371/journal.pone.0093489

Mitchell CL, Yeager RD, Johnson ZJ, Annunzio SED, Vogel KR, Werner T. Long-term resistance of *Drosophila melanogaster* to the mushroom toxin alpha-amanitin. *PLoS One*. **2015**:10:e0127569.

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*. *Bioorg Med Chem*. **1999**:7(9):2047–2052. https://doi.org/10.1016/s0968-0896(99)00121-2

Nowakowski P, Markiewicz-Żukowska R, Gromkowska-Kępka K, Naliwajko SK, Moskwa J, Bielecka J, Grabia M, Borawska M, Socha K. Mushrooms as potential therapeutic agents in the treatment of cancer: evaluation of antiglioma effects of *Coprinus comatus*, *Cantharellus cibarius*, *Lycoperdon perlatum* and *Lactarius deliciosus* extracts. *Biomed Pharmacother*. **2021**:133:111090.

Palavecino-De-La-Fuente F, Silva-Aguayo G, Figueroa-Cares I, Gerding M, Rodriguez-Maciel JC, Lagunes-Tejeda A, Castaneda-Ramirez GS, 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(3):399–406. https://doi.org/10.4067/s0718-58392022000300399

Park HJ, Yun J, Jang S, Kang SN, Jeon B, Ko Y, Kim H, Won C, Kim G, Cho J. *Coprinus comatus* cap inhibits adipocyte differentiation via regulation of PPAR gamma and Akt signaling pathway. *PLoS One*. **2014**:9(9):e105809. https://doi.org/10.1371/journal.pone.0105809

Phat C, Moon B, Lee C. Evaluation of umami taste in mushroom extracts by chemical analysis, sensory evaluation, and an electronic tongue system. *Food Chem*. **2016**:192:1068–1077. https://doi.org/10.1016/j.foodchem.2015.07.113

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 Biotechnol*. **2011**:91(4):1141–1148. https://doi.org/10.1007/s00253-011-3236-0

Poniedziałek B, Siwulski M, Wiater A, Komaniecka I, Komosa A, Gąsecka M, Magdziak Z, Mleczek M, Niedzielski P, Proch J, *et al*. The effect of mushroom extracts on human platelet and blood coagulation: in vitro screening of eight edible species. *Nutrients*. **2019**:11(12):3040. https://doi.org/10.3390/nu11123040

Ramchiun S, Sikaphan S, Leudang S, Polputpisatkul D, Nantachaiphong N, Khaentaw T, Nooron N, Uttawichai C, Parnmen S. Molecular characterization and liquid chromatography-mass spectrometric multiple reaction monitoring-based detection in case of suspected phalloides syndrome poisoning. *J Assoc Med Sci*. **2018**:52:48–55.

Ratnaningtyas NI, Husen F, Sukmawati D, Wibowo ES, Hikam AR, Aksoy A. Antidiabetic effects and enzymatic antioxidant activity of chicken drumstick mushroom (*Coprinus comatus*) extract in diabetic rats model. *J Pure Appl Microbiol*. **2022**:16(4):2764–2774. https://doi.org/10.22207/jpam.16.4.48

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.

Satora L, Pach D, Ciszowski K, Winnik L. Panther cap *Amanita pantherina* poisoning case report and review. *Toxicon*. **2006**:47(5):605–607. https://doi.org/10.1016/j.toxicon.2006.01.008.

Schissel M, Best R, Liesemeyer S, Tan Y, Carlson DJ, Shaffer JJ, Avuthu N, Guda C, Carlson KA. Effect of Nora virus infection on native gut bacterial communities of *Drosophila melanogaster*. *AIMS Microbiol*. **2021**:7(2):216–237. https://doi.org/10.3934/microbiol.2021014

Scott Chialvo CH, Werner T. *Drosophila*, destroying angels, and deathcaps! Oh my! A review of mycotoxin tolerance in the genus *Drosophila*. *Front Biol*. **2018**:13(2):91–102. https://doi.org/10.1007/s11515-018-1487-1.

Soto-Burgos J, Zhuang X, Jiang L, Bassham DC. Dynamics of autophagosome formation. *Plant Physiol*. **2018**:176(1):219–229. https://doi.org/10.1104/pp.17.01236

Stump AD, Jablonski SE, Bouton L, Wilder JA. Distribution and mechanism of α-Amanitin tolerance in mycophagous *Drosophila* (Diptera: Drosophilidae). *Environ Entomol*. **2011**:40(6):1604–1612. https://doi.org/10.1603/EN11136

Tellez-Tellez M, Villegas E, Rodriguez A, Acosta-Urdapilleta ML, O'Donovan A, Diaz-Godinez G. Mycosphere essay 11: fungi of *Pycnoporus*: morphological and molecular identification, worldwide distribution and biotechnological potential. *Mycosphere*. **2016**:7:1500–1525.

Tuno N, Takahashi KH, Yamashita H, Osawa N, Tanaka C. Tolerance of *Drosophila* flies to ibotenic acid poisons in mushrooms. *J Chem Ecol*. **2007**:33(2):311–317. https://doi.org/10.1007/s10886-006-9228-3

Van de velde J, Wilbers RH, Westerhof LB, van Raaij DR, Stavrakaki I, Sonnenberg AS, Bakker J, Schots A. Assessing the immunomodulatory potential of high-molecular-weight extracts from mushrooms; an assay based on THP-1 macrophages. *J Sci Food Agric*. **2015**:95(2):344–350. https://doi.org/10.1002/jsfa.6726

Van Timmeren S, Mota-Sanchez D, Wise JC, Isaacs R. Baseline susceptibility of spotted wing *Drosophila* (*Drosophila suzukii*) to four key insecticide classes. *Pest Manag Sci*. **2018**:74(1):78–87. https://doi.org/10.1002/ps.4702

Vetter J. Toxins of *Amanita phalloides*. *Toxicon*. **1998**:36(1):13–24. https://doi.org/10.1016/s0041-0101(97)00074-3

Wang L, Li YM, Lei L, Liu Y, Wang X, Ma KY, Chen Z. Cranberry anthocyanin extract prolongs lifespan of fruit flies. *Exp Gerontol*. **2015**:69:189–195. https://doi.org/10.1016/j.exger.2015.06.021

Wang MC, Bohmann D, Jasper H. *JNK* signaling confers tolerance to oxidative stress and extends lifespan in *Drosophila*. *Dev Cell*. **2003**:5(5):811–816. https://doi.org/10.1016/s1534-5807(03)00323-x

Wang X, Lin M, Xu D, Lai D, Zhou L. Structural diversity and biological activities of fungal cyclic peptides, excluding cyclodipeptides. *Molecules*. **2017**:22(12):2069. https://doi.org/10.3390/molecules22122069

Wei Y, Zhang Y, Cai Y, Xu M. The role of mitochondria in *mTOR*-regulated longevity. *Biol Rev*. **2015**:90:167–181.

Zhao H, Zhang J, Liu X, Yang Q, Dong Y, Jia L. The antioxidant activities of alkalic-extractable polysaccharides from *Coprinus comatus* on alcohol-induced liver injury in mice. *Sci Rep*. **2018**:8(1):11695. https://doi.org/10.1038/s41598-018-30104-6