Universidade Federal do Pampa

SIBELE MARQUES BOLSON

Efeito da suplementação na ração de *Drosophila melanogaster* (MEIGEM, 1830) com o fungo *Pleurotus citrinopileatus* (SINGER, 1942) e *Lentinus sajor-caju* (Fr.) Fr. e a sua relação com a reprodução de moscas das frutas.

Dissertação de Mestrado

São Gabriel

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Dissertação apresentada ao programa de Pós-graduação Stricto sensu em Ciências biológicas da Universidade Federal do Pampa, como requisito parcial para obtenção do Título de Mestre em Ciências biológicas.

Orientador: Filipe de Carvalho Victória

Co-orientador: Margéli Pereira de Albuquerque

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RESUMO

Efeito da suplementação na ração de *Drosophila melanogaster* (MEIGEM, 1830) com o fungo *Pleurotus citrinopileatus* (SINGER, 1942) e *Lentinus sajor-caju* (Fr.) Fr. e a sua relação com a reprodução de moscas das frutas.

Os cogumelos do gênero *Pleurotus* são muito utilizados na alimentação humana, ricos em proteínas e antioxidantes, quando agregados a alimentação animal eleva o seu valor nutritivo. Os fungos deste gênero destacam-se por crescerem facilmente em uma ampla variedade de substratos o que facilita o seu cultivo e amplia o seu uso biotecnológico. A espécie de mosca, *Drosophila melanogaster* (MEIGEN, 1830) é referência em estudos biológicos e genéticos, destacando-se pela fácil manutenção em laboratório e principalmente por possuírem reações metabólicas similares a dos mamíferos. Na natureza essa espécie utiliza como alimentação leveduras que colonizam frutos, flores e fungos em estágio de putrefação. O presente trabalho objetiva identificar o efeito *de Pleurotus citrinopileatus* e *Lentinus sajor-caju* no organismo de *Drosophila melanogaster*. Primeiramente os fungos foram cultivados em palha de arroz, as frutificações foram pesadas em 0,5-1,0-1,5-2,0-2,5-3,0(g) que foram homogeneizadas a ração composta de farinha de milho (Klein *et Al*, 1999) e fornecida como alimento para *D. melanogaster*, estas foram mantidas em BOD a 25ºC ± 1. A contagem de larvas e pupas foi realizada a cada vinte quatro horas e o numero final de moscas foi quantificado no fim do tratamento. Como analise das possíveis alterações genotípicas fez-se a extração do DNA e PCR, esses dados foram analisados com o programa NTSYS 2.1. Os resultados foram testados quanto à análise de variância com o auxilio do programa Statistix8. Foi observado que o micélio de *P. citrinopileatus* estimulou a reprodução em *D. melanogaster,* acelerando o ciclo reprodutivo e o número de indivíduos quando comparado com o controle e com a outra espécie *L. sajor-caju*. A concentração de 1,5g de micélio no substrato foi a que melhor expressou essa característica e a única que demonstrou alteração genotípica. O experimento sugere que o uso do micélio de *P. citrinopileatus* agregado a ração para *D. melanogaster* cultivadas em laboratório, pode ser utilizado como estimulante reprodutivo para este organismo, porém seu efeito em alterações genéticas deve ser melhor investigado.

Palavras chave: *Pleurotus*, reprodução *D. melanogaster*, estímulo reprodutivo

ABSTRACT

Effects of supplementation in the diet of *Drosophila melanogaster* (MEIGEM, 1830) with the fungus *Pleurotus Citrinopileatus* (SINGER, 1942) and *Lentinus sajor-cashew* (Fr.) Fr. and its relationship with the reproduction of fruit flies.

The mushrooms of genus *Pleurotus* are widely used as food, rich in protein and antioxidants when added to animal feed increases its nutritional value. The fungi of this genus are noted for easily grow in a wide variety of substrates which facilitates cultivation and increases its biotechnological use. The species of fly, *Drosophila melanogaster* (Meigen, 1830) is a reference in biological and genetic studies, especially because it is easy to maintain in the laboratory and mainly have similar metabolic reactions to mammals. In nature uses as food yeasts that colonize fruits, flowers and fungi putrefying stage. This study aims to identify the effect of *Pleurotus Citrinopileatus* and *Lentinus sajor-caju* in *Drosophila melanogaster* body. First fungi were grown on rice straw, the fruiting bodies were weighed into 0,5-1,0-1,5-2,0-2,5-3,0 (g) were homogenized ration composed of flour maize (Klein et al, 1999) and supplied as feed for *D. melanogaster*, they were kept in chamber at 25 ° C ± 1. The counting larvae and pupae every twenty-four hours and the final number of flies. How to analyze the possible genotypic changes made to DNA extraction and PCR, these data were analyzed with the NTSYS 2.1 program. The results were tested for analysis of variance with the aid of Statistix8 program. It was observed that the mycelium of *P. citrinopileatus* stimulated reproduction in *D. melanogaster*, accelerating the reproductive cycle and the number of individuals compared with control and other species *L. sajor-caju*. The concentration of 1.5g of mycelium in the substrate was best expressed this feature and the one that showed genotypic change. The experiment suggests that the use of mycelium of *P. citrinopileatus* added to feed for *D. melanogaster* grown in the laboratory, it can be used as a reproductive stimulant for this organism, but its effect on genetic changes should be better investigated.

Keywords: *Pleurotus*, reproduction *D. melanogaster*, reproductive stimulant

LISTA DE FIGURAS

LISTA DE ABREVIATURAS E SIGLAS

PSC – *Lentinus sajor-caju*

PAM – *Pleurotus citrinopileatus*

LISTA DE TABELAS

SUMÁRIO

INTRODUÇÃO GERAL

Os fungos possuem potencial diverso, como na produção de remédios, tratamentos ambientais, bioindicadores e bioremediadores. Nem todos os benefícios dos fungos foram estudados porém uma das aplicações bem difundida é o uso dos fungos na alimentação. Entre os cogumelos comestíveis estão os fungos do gênero *Pleurotus* (HANSKI, 1989; KOMONEN, 2003; AMAT-GARCÍA *et al.*, 2004). Este gênero compreende espécies comestíveis difundidas na gastronomia por ser facilmente cultivado, por suas propriedades organolépticas favoráveis além de propriedades medicinais.

Cogumelos são alimentos de alto valor nutritivo, com baixo teor de carboidrato e gorduras e com significativas quantidades de proteínas e vitaminas (PUTZKE; PUTZKE, 1998), variando a composição química de acordo com a espécie (BONONI; TRUFEN, 1985)

As espécies de *Pleurotus* estão entre os principais cogumelos cultivados no Brasil (EIRA; MINHONI, 1997). *P. citrinopileatus* é considerado um cogumelo de boa, palatabilidade rico em nutrientes (GHOSH *et al* 1991). Com base em pesquisas sobre modelos animais sugere-se que o cogumelo desta espécie pode ter alguns efeitos fisiológicos, incluindo antitumoral, aprimoramento imunológico e anti-hiperglicemia. (WANG *et al*. 2005; SHU *et al* 2006).

Além da importância gastronômica, *L. sajor-caju*, pode ser utilizado na fungicultura, também pode ser considerado espécie importante para a prospecção de substâncias anticancerígenas. Segundo Pramanik *et al*, (2005) o extrato aquoso de *Lentinus sajor-caju*, contem vitaminas B1, B2 e C e pode reduzir o nível de colesterol no sangue. Como citado por Gregori *et al*. (2007), esses polissacarídeos induzem apoptose, causando o fim do ciclo celular. São reconhecidos por apresentarem glicanos como constituintes da parede celular tanto do basidioma como do micélio. (BOBEK *et al*., 1991a; BOBEK *et al*., 1991b; ZHANG *et al*., 1994; NOSÁL OVÁ, *et al*., 2001; HOSSAIN *et al*., 2003; PRAMANIK, 2005). A medicina tradicional atribui propriedades medicinais para *Pleurotus* spp, várias substâncias deste fungo têm sido caracterizadas por exibir atividades antibióticas, antiviral, antitumoral e atividades anticolesterolêmica.

Diferentes substâncias farmacêuticas foram purificadas e caracterizadas (WASSER; WEIS, 1999a), tornando-os biotecnologicamente importantes.

Os princípios ativos considerados benéficos à saúde foram popularizados e no mercado estes cogumelos são comercializados com diferentes nomes categóricos, como suplementos

alimentares, alimentos funcionais, alimento de desempenho, entre outros (WASSER *et al.*, 2000; NASCIMENTO, 2003).

Dos polissacarídeos extraído do cogumelo *Pleurotus*, cinco destes apresentam atividades antitumorais contra o Sarcoma 180 em camundongos (MIZUMO; ZHUANG, 1995).

Apesar da maioria dos estudos concentrarem-se no cultivo de *P. citrinopileatus* usando diferentes substratos ou misturas de resíduos (AYYAPPAN, CHANDRASEHAR, GNANASAMBANDAN, KUMARAN, 2000; PANI, PATRA, 1994; RAGUNATHAN, GURUSAMY, PALANISWAMY, SWAMINATHAN, 1996; RAGUNATHAN, PALANISWAMY, GURUSAMY, SWAMINATHAN, 1996; RAJARATHINAM, VEERASAMY, MEHALINGAM, JAYABALAN, 1994). O valor nutricional e componentes do *P. citrinopileatus* do corpo de frutificação e do micélio também foram estudados (HUANG, 2003).

A composição centesimal (umidade, energia, fibra, gordura bruta, cinzas, minerais e proteínas) aminoácidos e vitaminas de *P. citrinopileatus Indigena* cultivado na instalação Planta Piloto do Instituto de Pesquisa e Desenvolvimento Kenya Industrial (Kirdi) foram determinados, revelando que ele contem o percentual de proteína de 22,10%, 1,32% de lípidos em bruto e 20,78% de fibra (FREDRICK *et al* 2013).

O cogumelo *P. citrinopileatus* também contém quantidades variáveis de sais minerais, sendo o potássio o mineral mais predominante encontrado além cobre, zinco e ferro. Oito aminoácidos essenciais foram encontrados Leucina> Valina> Treonina> Lisina> fenilalanina> isoleucina> Metionina> triptofano. Ácido glutâmico em proporção elevada. Vitamina B_3 (ácido nicotínico), vitamina B_5 (ácido pantotênico) e vitamina B_2 (Riboflavina) em maior abundancia e a vitamina B_{12} (cianocobalamina) e vitamina A (retinol) em menor quantidade (MUSIEBA *et al* 2013).

Essas características nutricionais presentes nos fungos vem despertando interesse em diversas áreas entre elas a biotecnológica. O processo pelo qual avançamos em conhecimentos ligados a nutrição, saúde, mecanismos de ação de determinadas doenças, inclui o uso de modelos experimentais animais, como camundongos, porco da índia, baratas e *Drosophilas* entre outros.

A espécie *Drosophila melanogaster* é muito utilizada na técnica de bioensaio, um método de triagem utilizado na determinação de resíduos (ALMEIDA; REYES, 1999; ALMEIDA et al., 2001; BAGDONAS et al., 1988; JOSEPH JR.; KNOBEL, 1980; PAULINO et al., 1992).

As moscas desta espécie são organismos intensamente estudados, servem como um sistema modelo para a investigação de muitos processos do desenvolvimento celular e comuns aos eucariotas superiores, incluindo seres humanos. (MARK et al, 2000).

Drosophila melanogaster (MEIGEN, 1830) pertence á ordem *Díptera*, e ao subgênero *Drosophila* é conhecida popularmente como mosca-da-fruta, por encontrar-se próxima a frutas em estágio de decomposição. Esta espécie utiliza uma ampla variedade de substrato para sua reprodução (SHORROCKS, 1982), os quais estão relacionados á alimentação e á oviposição (CUNHA; MAGALHÃES, 1965; CARSON, 1971; STARMER, 1981; TIDON *et al* 2005).

Os insetos de modo geral, necessitam de uma dieta que lhes ofereçam carboidratos, proteínas, vitaminas e alguns sais minerais. Esses nutrientes são encontrados na natureza e desempenham uma série de funções para os organismos como fonte de energia, manutenção e construção de tecidos, precursores de hormônios entre outras. As proteínas tem um importante papel nos processos metabólicos; são os constituintes alimentares que mais influenciam no crescimento, fecundidade e longevidade de diversos insetos (DADD, 1985). Alguns trabalhos, (SOUZA *et al.,* 1978; JONES, 1988; F ERRAZ, 1994; SIMPSON *et al.,* 1994; CANGUSSU E ZUCOLOTO, 1997), mostram que a ingestão de fontes proteicas aumenta a longevidade dos insetos inclusive espécies de moscas-da-fruta. Já em moscas na fase adulta a ingestão de proteínas interfere na produção de ovos (BRAGA E ZUCOLOTO, 1981; TSITSPIS, 1989). Dentre os recursos alimentares da espécie *D. melanogaster* estão as leveduras, fonte de proteínas, que colonizam organismos vegetais em decomposição (FREIRE-MAIA; PAVAN, 1949).

Kiriacou *et al* (1990); Sheeaba *et al* (2000) demonstram que mutações nos genes relacionados ao relógio circadiano e condições fotoperiódica afetam o tempo de desenvolvimento em *D. melanogaster*. Sendo o papel do relógio circadiano em fitness definido como uma medida do sucesso reprodutivo (BEAVER *et al* 2001). O número de ovos esta ligado ao acasalamento, mas para que ele ocorra com sucesso são levados em conta fatores como condições climáticas comportamento e principalmente como descrito por BANGHAM *et al* 2002 o tamanho do corpo dos individuos (PAVKOVIC-LUAIC; KEKIC, 2011). Organismos em populações naturais geralmente apresentam diferenciado sucesso reprodutivo em relação a organismos mantidos em laboratório (MARKOW, 1988). Sensível em termos de mecanismos o acasalamento pode ser baseado em fatores morfológicos (EBERHARD, 1985) e comportamentais (THORNHILL; ALCOCK, 1983).

A *D. melanogaster*, é um organismo eucarionte, da ordem Díptera, com 2n = 8 cromossomos sendo 3 pares de autossomos e 1 par sexual, tem sido material biológico largamente utilizado pelos pesquisadores, por ser de fácil manutenção em laboratório e principalmente, como citado por Graf, (1994) apresentam reações metabólicas semelhantes às dos mamíferos, o que permite um certo grau de extrapolação para humanos. (FONSECA; PEREIRA, 2004).

De acordo com Savalli, (2001), a ecologia comportamental é um dos temas centrais da biologia evolutiva, varia devido à variedade de comportamentos e táticas associados com acasalamento, bem como as consequências de aptidão ligados a essas estratégias. Good e Tatar, (2001), reporta que uma das consequências de *fitness* nas *Drosophilas* é um "*trade-off*" envolvendo reprodução e longevidade, que tem sido apoiada por modelos evolutivos, bem como por evidências empírica.

Kokko e Jennions, (2008), relatam que as fêmeas fazem um investimento maior do que os machos na reprodução, como nos insetos, há uma tendência das fêmeas para se tornar mais seletivo na escolha do companheiro, o que implica uma seleção sobre a capacidade masculina de competir por oportunidades de acasalamento (COSTA *et al*, 2010).

Drosophila melanogaster tem uma Relação Sexual Operacional (OSR) tendenciosa em condições naturais (MARKOW, 2000) e em experimentos de laboratório, responde a essa condição com o aumento da exposição da corte masculino e taxa de cruzamento. (WHIGBY; CHAPMAN, 2004). Como consequência do "*trade-off"* envolvendo reprodução e longevidade, o aumento da exposição a corte e a taxa de acasalamento, acabam reduzindo a expectativa de vida (CORDTS; PARTRIDGE, 1996), embora a aptidão global está positivamente relacionada com a taxa de reprodução. (LESSELLS, 2006).

É possível utilizar os cogumelos como enriquecimento na ração para moscas *D. melanogaster* mantidas em laboratório, pois são fontes de proteínas e sais minerais. Seria satisfatório se além de fonte alimentar para as moscas o fungo agregasse na aceleração do ciclo reprodutivo, permitindo que obtivesse maior quantidade de indivíduos em menor período de tempo?

OBJETIVOS

Objetivo Geral

- Identificar o uso potencial dos fungos comestíveis, *P. citrinopileatus* Singer e *L. sajor-caju* (Fr.) Fr. como suplemento na alimentação de *D. melanogaster* e verificando a influência destes dois cogumelos nas taxas reprodutivas das moscas das frutas.

Objetivos Específicos

- Determinar a melhor concentração dos cogumelos escolhidos para otimização das taxas de reprodução de *D. melanogaster*;

- Comparar *Pleurotus citrinopileatus* e *Lentinus sajor-caju* (Fr.) Fr. a fim de avaliar as diferentes influências destas espécies na reprodução das moscas das frutas.

EFEITO DE *Pleurotus citrinopileatus* SINGER E *Lentinus sajor-caju* (Fr.) Fr. COMO SUPLEMENTO ALIMENTAR PARA *Drosophila melanogaster* (MEIGEM, 1830) E SUA RELAÇÃO COM A CAPACIDADE REPRODUTIVA DA MOSCA DA FRUTA

(Artigo submetido à revista International Journal of Medicinal Mushrooms)

EFFECT OF *Pleurotus citrinopileatus* SINGER E *Lentinus sajor-caju* (FR.) FR. AS A FEED SUPPLEMENT IN *Drosophila melanogaster* AND THEIR RELATIONSHIP ON THE REPRODUCTIVE FITNESS OF FRUIT FLIES.

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Abstract

Several fungal species are used by human as food sources, thus favoring nutritional benefits to be consumed. *Pleurotus* spp were considered as extremely rich mushroom under the nutritional point of view, having physiological effects when consumed, including anti-tumor effect and immunological enhancement. However few mushrooms this group were tested for phenotypic and genotypic responses in animal models, thereby ensuring the proper dosage for use by these fungi. *Drosophila melanogaster* is used as a model in biological and genetic studies highlighting to be easy to maintain in laboratory conditions and mainly because they have similar metabolic reactions within mammals. Aiming to evaluate the effect of two species of mushrooms on the reproductive capacity of *D. melanogaster*, *Pleurotus citrineopilleatus* and *Lentinus sajor-caju* were added as feed supplement to the fruit flies in distinct concentration of both mushrooms. The total number of larvae, pupae and adults were measure in each treatment. ISSR markers analysis were carried to infer about genotipical changes in the flies fed with mushrooms. Our results suggests that using any of the fungal species as a supplement in feed fruit flies, we have a positive change in the reproductive capacity of *D. melanogaster*, being the use of PAM generates an acceleration in the life cycle of flies, meanwhile showing a higher genetic dissimilarity compared to controls and other tested treatments.

Introduction

Several fungal species are used in human nutrition as sources of proteins and glycans, primarily because these substances are constituents of cell wall of basidiomata (Bobek et al., 1991a; Bobek et al., 1991b, Zhang et al., 1994 ; Nosál Roe, et al., 2001; Hossain et al., 2003; Pramanik, 2005), thus favoring nutritional benefits to be consumed. Traditional medicine attributes medicinal properties to *Pleurotus* spp because various substances of these fungi were characterized by displaying antibiotic, antiviral and antitumor activities, many of these already used as pharmaceuticals (Wasser et al, 2000).

Pleurotus citrinopileatus Singer is considered an extremely rich mushroom under the nutritional point of view (Breene, 1990; Ghosh et al 1991). Research approaches using animal models suggest that this species of mushroom can have physiological effects when consumed, including anti-tumor effect and immunological enhancement (Wang et al 2005;. Shu et al 2006).

Drosophila melanogaster (Meigen, 1830) is popularly known as fruit fly is used as a model in biological and genetic studies highlighting to be easy to maintain in laboratory conditions, have low nutritional requirements, short life cycle and mainly because they have similar metabolic reactions within mammals (Graf, 1994). This species uses a wide variety of substrate for reproduction (Shorrocks, 1982) which are related to feeding and oviposition (Da cunha e Magalhães,1965; Carson, 1971; Starmer, 1981; Tidon et al 2005).

Among the food resources used for the growth of fruit flies are the yeasts that colonize fruits and flowers on stage of decomposition (Freire-Maia and Pavan, 1949), and observed that females of *D. melanogaster* when exposed to a diet rich in yeast increase the number of eggs (Min and Tatar, 2005). Previous studies had identified members of the phylum Ascomycota as a important food resouce to *Drosophila* survival and fitness (Anagnostou et al., 2010). However, Basidiomycota fungi are not rare as a food resource for fruit flies, such mainly agaricales that have been detected, by metagenomic strategies, in guts from *Drosophila* spp. (Chandler et al., 2012).

Thus, this resorces may be altering the reprodution rate in fruit flies? In addition, Is ther some change in genotypic point of view associated with feeding these flies with mushrooms? These are questions far from being completely understood, but there are some indications in the literature that can guide to clarify this issue.

Woods et al. (1998) reports that most species of vertebrates and invertebrates, feeding thresholds are rapidly modulated during the course of a meal before systemic homeostasis is restored, indicating that feeding thresholds are partly set by signals emanating from the digestive tract (Murphy and Bloom, 2006). Recent studies have found genetic responses associated with quantitative trait loci of large effects on phenotypic plasticity of fruit flies (Gutteling et al., 2007), being assigned a part of this response to forragind genes (Kent et al., 2009).

The aim of this study is to evaluate the effect of two kinds of mushrooms on the reproductive capacity, both phenotypical as genytopical, of fruit flies when these fungi are added to the diet of this animal model.

Methods

The *P. citrinopileatus* e *L. Sajor-caju* mycelium stored in mineral oil (Castelani, 1967) were reactivated in PDA culture medium (Potato Dextrose Agar).

Culture media were poured into a 90x60 mm Petri dishes, always in a laminar flow chamber. On each plate were accommodated 38mm discs inoculum and the plates were incubated at 27 ± 10 °C in a incubator until the mycelial growth, which occurred in seven days.

The spawn was obtained using as rice grains growing substrates previously boiled for 15 minutes. Rice grains were drained and packed in glass jars of 8.6 x 14cm, which are closed with foil and plastic film. The autoclaving was performed at 121 °C (1 atm) for 15 minutes in two stages, at intervals of 24 hours after they were cooled to room temperature. The vials containing the spawn was inoculated in a laminar flow hood using 10 mm diameter discs with the fungal culture (primary matrix) obtained anteriorly. This material was incubated at 28 °C to colonization by fungi of the grains, thus obtaining the secondary matrix.

The fruiting bodies of the mushroom were obtained by Jun Cao technique (Eira and Minhoni, 1997; Urben and Uriartt, 2001) using rice straw as substrate, which was dried at room temperature and fragmented straw into pieces of 7 cm and immersed in water for 24 hours, thereafter, drained to remove excess water and packed in polyethylene bags with 2 L capacity.

Polyethylene bags have their openings closed with cotton caps and were identified for each species used in this study. The substrates were subjected to heat treatment: pasteurization

at 80-90ºC for 30 minutes and cooled to room temperature until reach the 25 ±1°C. The bags with pasteurized substrate were inoculated with 3% of spaw produced previously.

The bags were kept at a temperature of 25°C until the appearance of the basidiomata. The basidiomata were manually collected and frozen in Ultrafreezer to about -50 ±1 °C

The bags were kept at 25 ±1°C in the growth chamber until the appearance of the basidiomata. The mushrooms were manually collected and frozen in Ultrafreezer to about -50 ±1°C, after freezing these were lyophilized and milled a powder to be added to the *D. melanocaster* feed. The flies were kept in photoperiod chamber at 25 ± 1° C durring all experiment.

To verify the effect of both mushrooms on *D. Melanogaster* a base médium was prepared (Klein et al, 1999) and mixed with the fungi powder of both fungi species tested. For preparation of flies feed were used: 1 kg thick cornflour; 800g medium cornflour, 250g wheat germ, 160g sugar, 12g of powdered milk, 10g of salt, 7.5g of soy flour and 5g milled rye. To this preparation was added water at a ratio of 1:2, then the mixture was boiled in a beaker for 15 min. After cooled the mushroom powder were added different concentrations into 15g of the base medium. This mixture was distributed in flasks with a cotton cover, occupying 1/3 of capacity. After cooling the mixture, each flask received 15 flies in BOD at 25 ± 10°C over a period of eight days.

Strains of *Pleurotus Citrinopileatus* Singer (WFP) and *Lentinus sajor-caju* (Fr.) Fr. (PSC) were obtained from mushrooms module FCA / UNESP / BOTUCATU and flies were donated by GPEOSC laboratory (Research Group on Stress oxidative and Cell Signaling) at the Federal University of Pampa.

Experiments to test the effect of PSC and PAM on reprodutive fitness of *Drosophila melanogaster*

The experiment was conducted considering the effect of different concentrations of each mushroom species in the reproduction capacity of the flies during eight days.

The following concentrations were evaluated 0.5g, 1.0g, 1.5g, 2.0g, 2.5g and 3.0g of the mushroom powder added to the basal feed. The control consisted of 15g tubes containing basal culture feed free from mushrooms. In each tube was placed fifteen flies, and thus were kept in phtoperiod chamber at 25 ± 1°C, an eight day time period. The larvae and pupae were

counted every 24 hours after incubation. For each treatment 10 replicates were made for a total of 70 flasks.

Results were submitted to ANOVA, with completely randomized design, and Tukey's test were made to evaluate statistical significance of the means. Statistical analyzes were performed using the Statistix 8 program.

DNA extraction and PCR assays to evaluate the genotypic differentiation caused by treatments.

At the end of treatment the flies were sacrificed to extract the DNA. 20 flies per treatment without the head were macerated in separated microtubes with 300 μL of TirD (Tris, EDTA, NaCl), 12 μL of SDS (20%) and 1,5μL proteinase K. The microtubes were kept in water bath at 65 °C for 20 minutes. After bath were added 300 uL of phenol-chloroform-isoamyl alcohol for each tube and centrifuging for 5 minutes at 12000rpm. After centrifugation 300 uL of supernatant was removed and add 600 uL of absolute ethanol, centrifugating again for 5 minutes at 12000rpm. The liquid were discarded and 200 uL 70% ethanol were added to a new centrifugation at 12000 rpm 5 minutes. After this prcedure the microtubes were allow to dry for 1 hour and the resultant DNA were re-suspended in 40 uL TE.

For PCR assays, we used 16 ISSR markers (Table 1), since these markers are considered ideal for several studies on genetic variation/diversity (Wang *et al*., 2012; Shafiei-Astani *et al.*, 2015). The amplification reaction was performed with 6.25 uL of Gotag PCR mix (Promega®), 1.25 uL of ISSR primer, 2.00 uL DNA primer and 2.75 uL H2O in a final volume of 12,75 μL. The amplification cycles were performed in a thermocycler (Eppendorf®) with a 30 seconds of denaturation step in 95 °C and 30 cycles of 95 °C and 60 °C (2 min both), followed by a final enlogation step at 60 °C for 10 min.

The amplicons evaluation were done using 3% agarose gels from the PCR product and 50 bp ladder. The presence/absence of bands obtained in ISSR analysis of flies feeded with both mushroons in different concentration and also the control mushroom free allowed to evaluate the change in genotypic variability in each flies tested. For the evaluation of amplicons associated with response to treatment was created an array and built one phenogram by UPGMA method (*Unweighted pair group method with arithmetic mean*) with help of computer program NTSYS 2.1 pc (Rohlf, 1998). The Cophenetic Correlation

Coefficient (CP) was used for the evaluation of differences in genetic dissimilarities between the treatments tested.

Table 1 - Primers used for amplification ISSR regions in *D. melanogaster*

Locus	**Sequence 5' - 3'**
ISSR1	GAGAGAGAGAGAGAGAGAGAA
ISSR2	GAGAGAGAGAGAGAGAGAGAC
ISSR3	GAGAGAGAGAGAGAGAGAGAG
ISSR4	GAGAGAGAGAGAGAGAGAGAT
ISSR5	CTCTCTCTCTCTCTCTCTCTA
ISSR6	CTCTCTCTCTCTCTCTCTCTC
ISSR7	CTCTCTCTCTCTCTCTCTCTG
ISSR8	CTCTCTCTCTCTCTCTCTCTT
ISSR9	AGAGAGAGAGAGAGAGAGAGA
ISSR10	AGAGAGAGAGAGAGAGAGAGC
ISSR11	AGAGAGAGAGAGAGAGAGAGG
ISSR12	AGAGAGAGAGAGAGAGAGAGT
ISSR13	ATATATATATATATATATATA
ISSR14	ATATATATATATATATATATC
ISSR15	ATATATATATATATATATATG
ISSR16	ATATATATATATATATATATT

Results

Effect of PSC and PAM on reprodutive fitness of *Drosophila melanogaster*

When flies were exposed to different concentrations, there was a variation in the reproduction period, the amount of larvae and pupae and especially in the final number of flies. These variations are observed on the confrontation of flies control, treatment with PAM and PSC.

The flies were fed MBP have different results on the number of larvae, pupae, and the flies to the end of the treatment compared with the control and flies fed with PSC. The values

of larvae and pupae were summed across all replicas and divided by 10 resulting in the average number of individuals per flask during the treatment (Table 1 and 2).

First larvae were observed on the third day after incubation for PAM and fourth day for PSC, the same were occurred in both control tubes. Larvae counting was carried out until the fifth day, due to the large number of larvae observed in the PAM treatment.

Tabela 2 – Average* of the quantitative larvae, pupae and adult flies after the treatment at different concentrations *P. citrinopileatus* (PAM) added on the *Drosophila melanogaster* diet.

PAM Concentration (g)	**Initial number of fruit flies**	**Number of larvae***	**Number of pupae**	**Final number of fruit flies**
0	15	$40,7^{A}$	12.5^{C}	$15,0^{A}$
0,5	15	$93,6^{AB}$	$109,6^{AB}$	$50,6^{A}$
1,0	15	$110,1^{A}$	$144,2^{A}$	$49,8^{A}$
1,5	15	$134,9^{AB}$	$149,0^{A}$	$52,1^{A}$
2,0	15	$37,8^{BC}$	$138,5^{AB}$	$40,0^{A}$
2,5	15	$35,6^{BC}$	$59,1^{BC}$	$19,7^{A}$
3,0	15	$1,2^{C}$	$26,7^{C}$	$20,4^{A}$

* Different letters in the columns differ from each other values of significance levels in the Tukey test ($\alpha = 0,05$).

The pupa were viewed since the fourth day in PAM. For PSC and the controls the first pupa were observed only on the seventh day.

Within treatment with PAM concentration of 1.5g (0.1g of basiomata / per gram of substrate) had a higher elevation in the number of larvae (134.9), pupae (149.0), and adult flies (52.1) (Table 2).

Treatment with PAM accelerated the reproduction cycle of fruitflies, anticipated and increased the number of larvae and pupae differing from the control and treatment with PSC (Figure 1). When PAM concentrations exceeded the 1.5g per flask, was observed an decrease of the number of larvae and pupae signaling the concentration 1.5g or 0.5g of fungi

powder/gram of substrate as the best concentration to accelerate the reproductive cycle of *D. melanogaster*.

Table 3 –Average* of the quantitative larvae, pupae and adult flies after the treatment at different concentrations *P. sajor-caju* (PSC) added on the *Drosophila melanogaster* diet.

PSC Concentration (g)	Initial number of fruit flies	Number of larvae*	Number of pupae	Final number of fruit flies
0	15	$4,9^{A}$	$42,3^{A}$	$15,2^{A}$
0,5	15	$8,8^{A}$	$61,1^{A}$	$15,4^{A}$
1,0	15	$10,9^{A}$	$38,5^{A}$	$15,0^{A}$
1,5	15	$8,3^{A}$	$56,4^{A}$	$15,3^{A}$
2,0	15	$10,9^{A}$	$85,5^{A}$	$15,5^{A}$
2,5	15	$14,1^{A}$	$66,6^{A}$	$15,4^{A}$
3,0	15	$5,17^{A}$	$96,0^{A}$	$15,6^{A}$

* Different letters in the columns differ from each other values of significance levels in the Tukey test ($\alpha = 0,05$).

Genotypic analysis of fruit flies feeded with mushroom supplement

Twelve to sixteen ISSR primers amplify in one or more treatments, which are recruited to the analysis of genetic dissimilarity. The ISSR-PCR analysis of the mushroom flies fed with MBP showed genetic alterations between different treatments (Figure 2). The 1.5 g concentration of the fungus as a supplement, considered the best performance in the reproductive capacity of fruit flies, had the highest genotypic dissimilarity between the concentrations tested for this mushroom species, based in the Cophenetic Correlation Coefficient (CP=0,77). Nevertheless for PSP, the data from phenotypic characterization are corroborated by the genotypic analysis by ISSR markers, which were not observed differences between treatments (Figure 3).

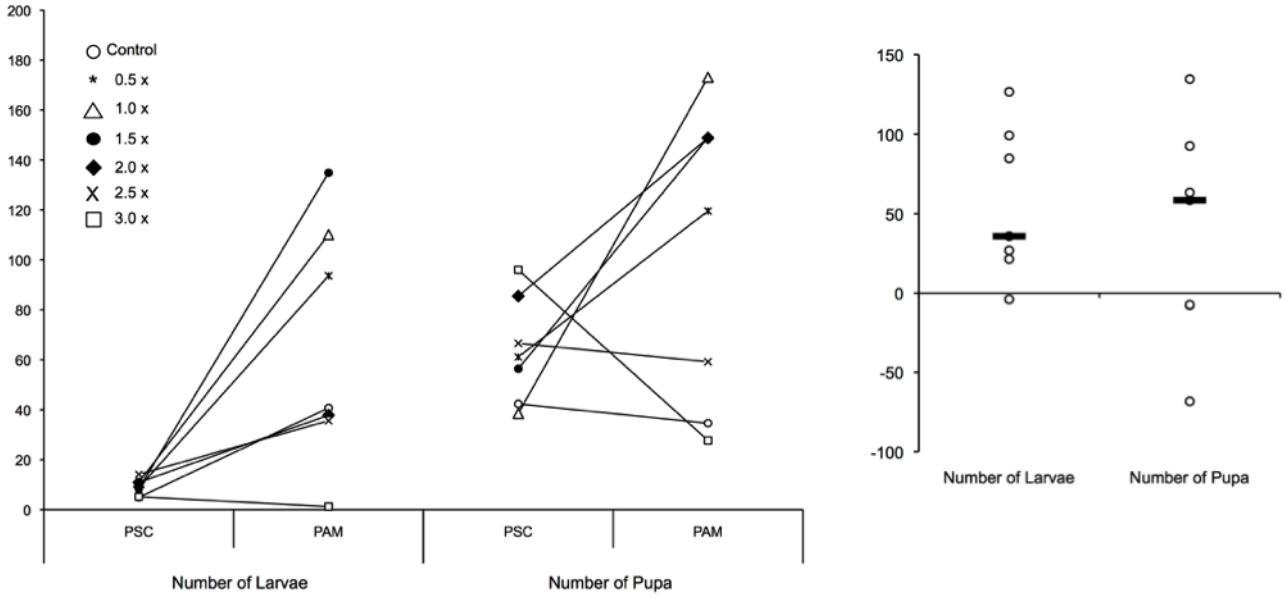


Figure 1 – Comparative of larvae and pupae quantities in the distinct treatments using PSC and PAM as feed supplement. The median observed of different measurements (dark bar).

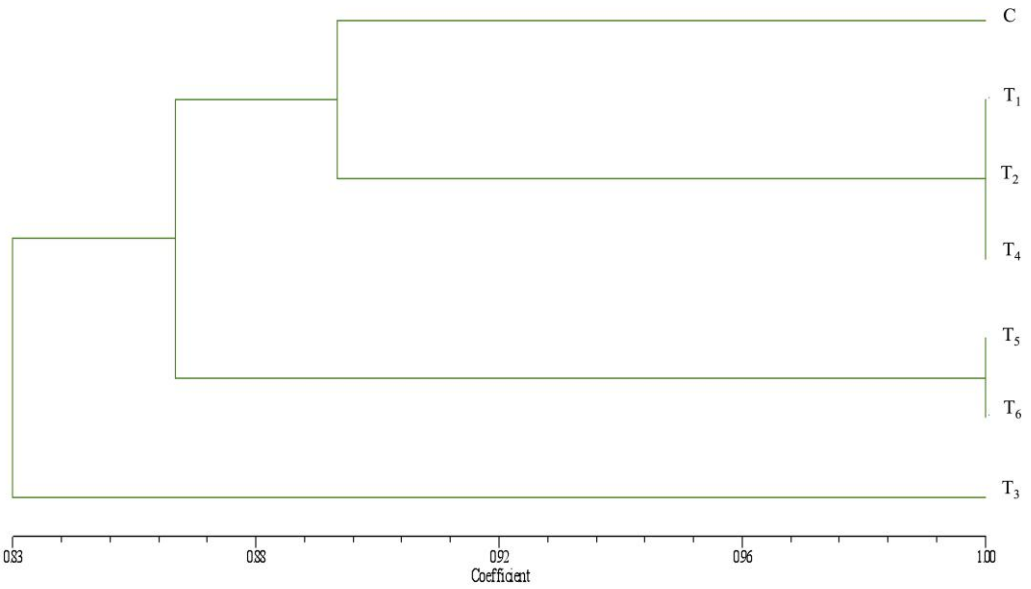


Figure 2 – Dendrogram of fruit flies fed with PAM in different concentrations (C = control, T1 = 0.5 g, 1.0 g = T2, T3 = 1.5 g, 2.0 g = T4, T5 = 2.5G and T6 = 3.0 g). In the comparison it is observed that the treatment 3 (1.5 g of PAM powder) has the highest genotypic dissimilarity when compared to other treatment, indicated by the Cophenetic Correlation Coefficient (CP =0,77).

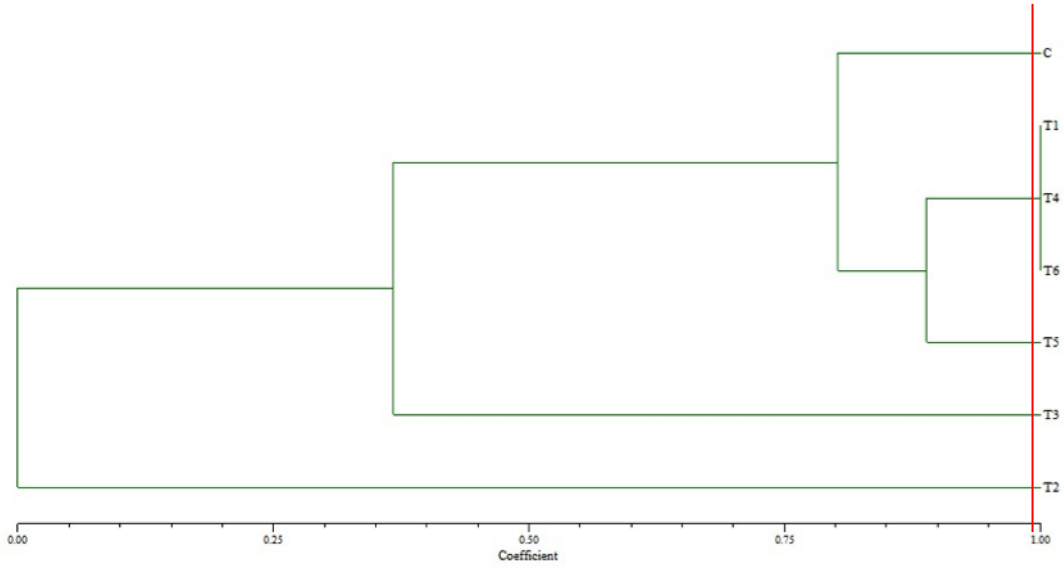


Figure 3 – Dendrogram of fruit flies fed with PSC in different concentrations (C = control, T1 = 0.5 g, 1.0 g = T2, T3 = 1.5 g, 2.0 g = T4, T5 = 2.5G and T6 = 3.0 g). Comparing all treatments are not observed genotypic dissimilarities, Comparing all treatments are not observed genotypic dissimilarities, indicates by the red line wich represent the Cophenetic Correlation Coefficient (CP =0,99).

Discussion

It was observed that *Drosophila melanogaster* when supplied with the fungus *P. citrinopileatus* presented an higher development of larvae and pupae and increase the number of individuals in a shorter period. Despite treatment with 3.01,5 g of PSC have increased the number of pupae during the experiment, the total cycle time of flies in the treatments with this mushroom were all late in relation to treatment with PAM.

Flies fed with *P. citrinopileatus* have demanded a reduction in the time for development. The flies control and treatment with PSC take a period of 4 days in the appearance of the first larvae, while the flies were treated with PAM took 3 days. Several factors may affect the cycle, such as temperature, light availability and food. To eliminate these potential interference to the flies were fed with other fungal species (PSC) which can be observed that the changes in the cycle are not due to environmental induced changes since both treatments were subjected to the same conditions. This may be due to the large amount of protein that is found in *P. citrinopileatus* fruiting body (Musieba et al., 2013) which is essential to the diet of insects and adults influence the egg production (Plácido-Silva et al., 2005).

According to the life cycle of *D. melanogaster* (Griffiths et al, 2000) the first pupae come five days after deposition of eggs, as can be observed in the controls and treatment with

PSC. In flies treated with PAM this stage is anticipated on a day other treatments. For the complete cycle would take a period of 9 to 11 days (Griffiths et al., 2000), but our results showed that in the course of 8 days from the initial amount was increased from 15 to 52 individuals. These changes may also be due to protein concentration in *Pleurotus citrinopileatus*, up to 22.10%, when compared with other *Pleurotus* spp. (Musieba et al., 2013; Phan et al., 2014).

Despite insects such as fruit flies, consider species-strategists "r", exploit empty ecological niches or available resources temporarily and produce large numbers of offspring with each reproductive cycle (Osborne, 2000), the greater availability of mushrooms as a supplement in feed did not generate exponential increase in the fitness of the treated flies. It was also shown no hint of any change of the genotypes of flies submitted the largest mushroom concentrations as a food supplement. Only for PAM, it was evidenced phenotypic (increase of reprodutional behavior) and genotypic changes when the flies were fed a 3.0 g this mushroom.

Chippindale et al. (1993), reports that the dietary restriction can greatly reduce fecundity through the early and middle part of the life span, as well as reducing the total egg output. Other studies, shows a significant increases in longevity as a result of a shift resources from reprodutive activity to adults survival, suggesting the possibility that genetically-mediated allocation of energetic reserves in *D. melanogaster* (Holehan and Merry, 1985; Rose, 1991). It is actually known that both genes and neuromodulators can contribute in response to changes in nutrient availability to adjust discrete aspects of feeding, as forragin genes and allatostatin, hugin, corazonin (Melcher et al., 2005; Kent et al., 2009; Hergarden et al., 2012). Therefore, it was not evidenced restrictive effect of fungi in feeding the flies treated in this work, as in all treatments was observed higher response or at least similar to controls (without the addition of mushroom supplement). This was expected because naturally these insects use musmroom resource recurrently, to the point that in the case of cultivated species, fruit flies are considered pests to mushroom cultivators (Gnaneswaran and Wijayagunasekara, 1999).

The present findings with PAM feeding, suggest that genotypic changes occurring in a positive effect on fruit flies reproduction when them are fed with this mushroom, while for PSC its was not observed. Both mushroom species are reported in the literature as highly nutritious species, presenting antioxydant and scavenging effect when consumed (Musieba et al., 2013; Phan et al., 2014), however the lack of studies to evaluate the effects of these mushrooms experimentally in animal models, much less its effects on fertility and fitness,

prevents us from making major assumptions on the results. There may be even discarded the population fluctuations in fruit flies due to food availability (Beaver, 1984). The higher PAM concentrations might have caused an increase in competition between larvae, since insects that breed in discrete unequal resources often experience intense larval competition for food. This competition has been found in natural populations of mushroom-breeding species of *Drosophila* (Jaenike, 1990).

Thus, the effects observed when using PAM, especially under the genotypic point of view, should be studied to elucidate the actual relation between the increase of reproduction in animal models when fed with this species of mushroom.

Conclusion

Our finds suggests that using any of the fungal species as a supplement in feed fruit flies, we have a positive change in the reproductive capacity of *D. melanogaster*, being the use of PAM generates an acceleration in the life cycle of flies. The exposure of *Drosophila melanogaster* to *Pleurotus citrinopileatus* altered the reproductive rate of flies, increasing the number of larvae, pupae and adults. The different concentrations of this mushroom showed that the best values for the reproductive stimulus is the concentration of 1.5g (0.1g of mushroom powder per gram of substrate). The concentration greater than 1.5g reduced the reproductive fitness, suggesting the existence of a maximum dosage for use in this species of mushroom as a food supplement. High concentrations inhibit changes in reproductive capacity, equaling the rates observed when the mushrooms is not used as a supplement.

These results highlight the potential of mushroom species as a stimulant of reproduction in animal model in laboratory conditions, as the fruit flies.

References

Anagnostou C, Dorsch M, Rohlfs M. Influence of dietary yeasts on Drosophila melanogaster life-history traits. Entomol. Exp. Appl. 2010;136:1–11

Beaver RA. Insect exploitation of ephemeral habitats. *South Pacific* Journal of Natural Science. 1984;6:3-47.

Bobek P, Ginter E, Kuniak L, Babala MD, Jurcovicová M, Ozdín L, Cerven J. Effect of mushroom *Pleurotus ostreatus* and isolated fungal polysaccharide on serum and liver lipids in Syrian hamsters with hyperlipoproteinemia. Nutrition. 1991a; 7:105-108.

Bobek P, Ginter E, Jurcovicová M, Kuniak L. Cholesterol-lowering effect of the mushorrom *Pleurotus ostreatus* in hereditary hypercholesterolemic rats. Annual Nutritional Metabolism. 1991b; 35:191-195.

Breene WM. Nutritional and medicinal value of speciality mushrooms. Journal of food protection. Des moines. 1990; 53:883-94,

Castellani A.A Maintenance and cultivation of the common pathogenic fungi of man in sterile distilled water: further researches. Journal of Tropical Medicine & Hygiene,1967; 70: 181-184.

Carson HL. The ecology of *Drosophila* breeding sites. University of Hawaii. Harold L-Lyon Arboretum Lecture, Honolulu. 1971;2:1-28.

Chandler JA, Eisen JA, Kopp A. Yeast Communities of Diverse *Drosophila* Species: Comparison of Two Symbiont Groups in the Same Hosts. Appl. Environ. Microbiol. 2012;78(20): 7327-7336.

Chippindale AK, Leroi AM, Kim SB, Rose MR. Phenotypic plasticity and selection in *Drosophila* life-history evolution. I. Nutrition and the cost od reprodution. J. Evol. Biol. 1993;6:171-193.

Da Cunha AB, Magalhães LE. A ecologia e a genética de populações de *Drosófila* no Brasil. Ciência e Cultura, São Paulo, 1965; 17(14):525-527.

Eira AF, Minhoni MTA. Manual teórico-prático do cultivo de cogumelos comestíveis. módulo de cogumelos – FEPAF. 2.ed. Botucatu: Unesp, 1997. 115 p.

Freire-Maia N, C. Pavan. Introdução ao estudo da *Drosophila.* Cultus, São Paulo.1949; 1:1-171.

Ghosh N, Mitra DK, Chakravarty DK. Composition analysis of tropical white oyster mushroom (*Pleurotus citrinopileatus*). Ann. Appl. Biol. 1991; 118:527-231.

Gnaneswaran R , Wijayagunasekara HNP. Survey and identification of insect pests of oyster mushrooms (*Pleurotus ostreatus*) cultures in central province of Sri Lanka. Tropical Agricultural Research and Extension. 1999;2(1): 21-25

Graf U. The actual situation of SMART (Somatic Mutstion And Recombination Test) in *D. melanogaster*. Ver. Int. Contam. Ambient.1994;1:5-7.

Griffiths, A. J., Miller, J. H., Suzuki, D. T., Lewontin, R. C. & Gelbart, W. M. . Sex Chromosomes and Sex-Linked Inheritance. In: An Introduction to Genetic Analysis (7th ed.). New York: W. H. Freeman. 2000, p.

Gutteling EW, Riksen JA, Bakker J, Kammenga JE. Mapping phenotypic plasticity and genotype-environment interactions affecting life-history traits in *Caenorhabditis elegans*. Heredity, 2007;98: 28.

Hergarden AC, Tayler TD, Anderson DJ. Allatostatin-A neurons inhibit feeding behavior in adult *Drosophila*. Proc. Natl. Acad. Sci. 2012;109:3967–3972.

Holehan, AM, Merry BJ. Lifetime breeding studies in fully fed and dietary restricted CFY Sprague-Dawley rats. I Effects of age, housing conditions and diet on fecundity. Mech Ageing Dev. 1985;33:19-28.

Hossain S, Hashimoto M, Choudhury EK, Nuhu Alam, Hussain S, hasan M, Choudhury SK Mahmud I. Dietaty mushroom (*Pleurotus ostreatus*) ameliorates atherigenic lipid in hypercholestrolameic rats. Clinical and Experimental Pharmacology. 2003; 30:470-475.

Jaenike, J. Factors maintaining genetic variation for host preference in *Drosophila*. In: Barker JSF, Starmer WT, MacIntyre R, editors. Ecological and Evolutionary Genetics of *Drosophila*. Plenum Press, New York.1990, p. 195-207.

Kent CF, Daskalchuk T, Cook L, Sokolowski MB, Greenspan RJ. The Drosophila foraging Gene Mediates Adult Plasticity and Gene–Environment Interactions in Behaviour, Metabolites, and Gene Expression in Response to Food Deprivation. PLoS Genet. 2009;5(8): e1000609. doi:10.1371/journal.pgen.1000609.

Klein F. A central role for cohesins in sister chromatid cohesion, formation of axial elements, and recombination during yeast meiosis. 1999; 98(1):91-103.

Melcher C, Pankratz MJ. Candidate gustatory interneurons modulating feeding behavior in the *Drosophila* brain. PLoS Biol. 2005; 3:e305.

Min KJ, Tatar M. *Drosophila* diet restriction in practice: Do flies consume fewer nutrients? 2005. Mech Ageing Dev. 2006; 127(1):93-6.

Murphy KG, Bloom SR. Gut hormones and the regulation of energy homeostasis. Nature. 2006; 444:854–859.

Musieba F, Sheila O, Mibey RK, Wanjiku S, Moraa K. Proximate composition, amino acids and vitamins profile of Pleurotus citrinopileatus Singer: An indigenous mushroom in Kenya. American Journal of Food Technology. 2013;8:200-206.

Nosal'ová V, Bobek P, Cerna S, Galbavy S, Stvrtinas S. Effects of pleuran (beta-glucan isolated from *Pleurotus ostreatus*) on experimental colitis in rats. Physiology Research. 2001; 50(6):575-581.

Osborne PL. Tropical ecosystems and ecological concepts. Cambridge: University Press, 2000.

Plácido-Silva MC, Zucoloto FS, Joachim-Bravo IS. Infuence of protein on feeding behavior of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae): comparison between immature males and females. Neotropical Entomology, 2005; 34:539-545.

Phan CW, David P, Tan Y, Naidu M, Wong KH, Kuppusamy UR, et al. Intrastrain comparison of the chemical composition and antioxidant activity of an edible mushroom, *Pleurotus giganteus*, and its potent neuritogenic properties. Scientific World Journal. 2014;378651:10.1155/2014/378651

Pramanik M, Mondal S, Chakraborty I, Rout D, Islam SS. Sttructural investigation of a polysaccharide (Fr.II) isolated from the aqueous extract of an edible mushroom, *Pleurotus sajor-caju*.Carbohydrate Research, 2005;340(4)629-639.

Rohlf FJ. NTSYS-pc: numerical taxonomy and multivariate analysis system (Version 2.0), State University of New York, USA, 1998.

Rose MR. *Evolutionary Biology of Aging*. Oxford University Press, New York, 1991.

Shafiei-Astani B, Ong AHK, Valdiani A, Tan SG, Yong CSY, Ahmady F, Alitheen NB, Ng WL, Kaur T. Molecular genetic variation and structure of Southeast Asian crocodile (*Tomistoma schlegelii*): comparative potentials of SSRs versus ISSRs. Gene. 2015;571:107–116.

Shorrocks B. Os locais de reprodução de *Drosophilas* em florestas temperadas. Genética e Biologia de Drosophila. EdsA.Ashburner, HL Carson e JNJr Thompson, Academic Press, Londres. 1982;3B:385-428.

Starmer WT, A comparison of *Drosophila* habitats according to the physiological attributes of the associated yeast communities. Evolution, Lancaster. 1981; 35:35-52.

Shu Y, Hasenstaub A, Duque A, Yu Y, McCormick DA. Modulation of intracortical synaptic potentials by presynaptic somatic membrane potential. Nature.2005 441:761-

Tidon R. Leite DF, Ferreira EB, Leão FD, drosofilídeos (Diptera Insecta) do Cerrado. Ecologia e biodiversidade do cerrado.2005;(1)412.

Urben AF, Oliveira HCB, Vieira W, Correia MJ, Uriartt AH, Produção de cogumelos por meio de tecnologia chinesa modificada. Brasília : Embrapa, 2001; 151

Wang JC, Hu SH, Liang ZC, Yeh CJ. Optimization for the production of water-soluble polysaccharide from *Pleurotus citrinopileatus* in submerged culture and its antitumor effect. Appl Microbiol Biotechnol. 2005, 67:759–766

Wang X, Yang R, Feng S, Hou X, Zhang Y, Li Y, Ren Y. Genetic variation in *Rheum palmatum* and *Rheum tanguticum* (Polygonaceae), two medicinally and endemic species in China using ISSR markers. PLOS One. 2012;7: e51667.

Wasser S, Nevo E, Sokolov D, Reshetnikosv SV, Timor-Tismenetsky M. Dietary supplements from medicinal mushrooms: diversity of types and variety of regulation. International Journal of Medicinal Mushorooms. 2000;2:1-19,

Woods SC, Seeley RJ, Porte D Jr, Schwartz MW. Signals that regulate food intake and energy homeostasis. Science. 1998; 280:1378–1383.

Zhang J, Wang G, Li H, Zhuang C, Minuzo T, Ito H, Suzuki C, Okamoto H, Li J. Antitumor polysaccharides from a chinese mushroom, "yuhuangmo", the fruiting body of *Pleurotus citrinopileatus.*Biosci, Biotechnol, Biochem, 1994: 58:1195-1201.

CONSIDERAÇÕES GERAIS

Moscas da espécie *D. melanogaster* quando alimentadas com o fungo *Pleurotus Citrinopileatus* alteram seu ciclo reprodutivo, acelerando o surgimento de larvas e pupas, além de elevar a quantidade de indivíduos adultos em um curto período. Kyung-jin (2005), descreve que fêmeas de *D. melanogaster* quando expostas a uma dieta rica em levedura elevam o número de ovos. Esse relato ainda não havia sido observado com a utilização de cogumelos na alimentação dessa espécie. Esse fato devesse a espécie *Pleurotus citrinopileatus* uma vez que quando as moscas foram tratadas com outra espécie de fungo, não observou-se alterações reprodutivas.

Sheeba *et al*., (2000), fala que moscas expostas a um ciclo de claro e escuro por 24 horas tiveram menor número de postura de ovos em comparação com moscas expostas a luz constante, sendo que esta situação reduziu o tempo de vida dos indivíduos (VERMA, 2014).

Os insetos foram mantidos num fotoperíodo padrão, tanto para os controles quanto para os tratamentos, não sendo possível atribuir as alterações ao estresse causado pela presença ou ausência excessiva de luz. Da mesma forma para a temperatura, que foi a mesma para todos os tratamentos.

Não foi possível determinar a forma de atuação do fungo, mas observou-se que na concentração 1,5g, melhor resultado observado, houve alterações genotípicas. De modo que nos outros tratamentos, tanto para a mesma espécie com diferentes concentrações, quanto para a espécie diferente, não houve alterações similares.

Acredita-se que as alterações sejam positivas e que a espécie *P. citrinopileatus* possa ser usada como reforço na alimentação de *D. melanogaster* e assim servir como um facilitador para a reprodução rápida desses indivíduos em pesquisas.

REFERÊNCIAS BIBLIOGRÁFICAS

ALMEIDA, G.R. & REYES, F.G.R. ***Drosophila melanogaster*** **Meigen: 1. Sensibilidade Ao Endosulfan E Biomonitoramento De Seus Resíduos Em Couve-Manteiga**. Rev. Inst. Adolfo Lutz, v.58, n.2, p.15-24, 1999.

ALMEIDA, G.R.; REYES, F.G.R.; RATH, S**.** ***Drosophila melanogaster*** **Meigen: 3. Sensibilidade Ao Carbofuran E Biomonitoramento De Seus Resíduos Em Repolho**. Quím. Nova, v.24, n.6, p.768-772, 2001.

AMAT-GARCÍA, E.C., AMAT-GARCÍA, G.D. HENAO-M., L.G**. Diversidad taxonómica y ecológica de la entomofauna micófaga en un bosque altoandino de la cordillera oriental de Colombia**. Ecología, 28, 223-231, 2004.

AYYAPPAN, S., CHANDRASEHAR, G., GNANASAMBANDAN, S., & KUMARAN, K. **Utilization of sugarcane trash and coir waste for the cultivationof oyster mushroom (*Pleurotus spp*.).** Journal of Ecobiology, 12,317–319. 2000.

BAGDONAS, M.; MELLO, M.H.S. DE; UNGARO, M.T.S.; GUINDANI, C.M.A.; FERREIRA, M.S.; GAETA, R**. Ensaios Biológicos Como Testes Preliminares Na Detecção De Resíduos De Inseticidas Em Frutas E Hortaliças.** Rev. Bras. Toxicol., v.1, n.1, p.3-5, 1988**.**

BANGHAM, J.; T. CHAPMAN L. PARTRIDGE. **Effects of body size, accessory gland and testis size on pre- and postcopulatory success in *Drosophila melanogaster***. Animal Behaviour.2002 64: 915-921

BEAVER L. M., GVAKHARIA B. O., VOLLINTINE T. S., HEGE D. M., STANEWSKY R., GIEBULTOWICZ J. M. **Loss of circadian clock function decreases reproductive fitness in males of Drosophila melanogaster**. 2001

BOBEK P, GINTER E, KUNIAK L, BABALA MD, JURCOVICOVÁ M, OZDÍN L, CERVEN J. **Effect of mushroom *Pleurotus ostreatus* and isolated fungal polysaccharide on serum and liver lipids in Syrian hamsters with hyperlipoproteinemia**. Nutrition. 1991a; 7:105-108.

BOBEK P, GINTER E, JURCOVICOVÁ M, KUNIAK L. **Cholesterol-lowering effect of the mushorrom *Pleurotus ostreatus* in hereditary hypercholesterolemic rats**. Annual Nutritional Metabolism. 1991b; 35:191-195.

BONONI, V.R.L E TRUFEM, S.F.B. **COGUMELOS COMESTÌVEIS.** 2a ed., São Paulo: Ícone 1985. 83p.

BRAGA, M.A.S. & ZUCOLOTO, F.S**. Estudos sobre a melhor concentração de aminoácidos para as moscas-das-frutas adultas de Anastrepha obliqua (Diptera: Tephritidae).** Revista Brasileira de Biologia, v. 41: p. 75-79, 1981**.**

CANGUSSU, J.A. & ZUCOLOTO, F.S. **Effect of protein sources on fecundity, food acceptance and sexual choice by Ceratitis capitata (Diptera: Tephritidae).** Revista Brasileira de Biologia, v. 57: p. 611-618, 1997.

CARSON HL. **The ecology of *Drosophila* breeding sites**. University of Hawaii. Harold L-Lyon Arboretum Lecture, Honolulu. 1971;2:1-28.

CORDTS, R., L. PARTRIDGE. **Courtship reduces longevity of male *Drosophila melanogaster***. Animal Behaviour 52: 269–178, 1996.

COSTA M., MATEUS R. P., MOURA M. O., MACHADO L. P. B., **Adult Sex Ratio Effects On Male Survivorship Of Drosophila melanogaster Meigen (Diptera, Drosophilidae)**, Revista Brasileira de Entomologia, 2010.

DADD, R. H. **Nutrition: Organisms**, In G. A. Kerkut L. I. Gilbert [eds.], Comprehensive insect physiology, biochemistry and pharmacology, pp. 313- 390. vol. 4:1985

DA CUNHA AB, MAGALHÃES LE. **A ecologia e a genética de populações de *Drosófila* no Brasil**. Ciência e Cultura, São Paulo; 17(14):525-527. 1965.

EBERHARD, W. G. **Sexual selection and animal genitalia**. Cambridge, MA: Harvard University Press.1985.

EIRA, A.F.; MINHONI, M. T. A. 1997. **Manual teórico prático do cultivo de cogumelos comestíveis**. Botucatu: Fundação de Estudos e Pesquisas Agrícolas Florestais, 1997. p 34-46.

FERRAZ, M.V. **Longevity of adults of Peckia chrysostoma and Adiscochaeta ingens (Diptera: Tephritidae) reared with and without protein**. Memórias do Instituto Oswaldo Cruz, v. 89: p. 421-424, 1994.

FREDRICK MUSIEBA, SHEILA OKOTH, RICHARD K. MIBEY, STELLA WANJIKU E CAVALEIRO MORAA. **Composição centesimal, aminoácidos e vitaminas Perfil de *Pleurotus Citrinopileatus Singer***:. Um cogumelo Indígena no Quênia American Journal of Food Technology, 8:. 200-206: 2013.

FONSECA C. A., PEREIRA. D. G., **Aplicações Da Genética Toxicológica Em Planta Com Atividade Medicinal**, Informa, v.16, nº 7-8, 2004.

GHOSH N, MITRA DK, CHAKRAVARTY DK**. Composition analysis of tropical white oyster mushroom (*Pleurotus citrinopileatus*)**. Ann. Appl. Biol. 1991; 118:527-231.

GOOD T,TATAR M, **Age-specific mortality and reproduction respond to aduet dietary restriction in *Drosophila melanogaster*** J. Insect Physiol.. 2001;47:1467-1473.

GRAF U. **The actual situation of SMART (Somatic Mutstion And Recombination Test) in *D. melanogaster*.** Ver. Int. Contam. Ambient.1994;1:5-7.

HANSKI**, Metapopulation dynamics does it help to have more of the same**? Trends ecology and evolution 4: 113-114, 1989.

HOSSAIN S, HASHIMOTO M, CHOUDHURY EK, NUHU ALAM, HUSSAIN S, HASAN M, CHOUDHURY SK MAHMUD I. **Dietaty mushroom (*Pleurotus ostreatus*) ameliorates atherigenic lipid in hypercholestrolameic rats**. Clinical and Experimental Pharmacology. 2003; 30:470-475.

HUANG, G.-W. **Taste quality and antioxidant and antimutagenicproperties of *Pleurotus citrinopileatus*.** Master's thesis, National Chung-Hsing University, Taichung, Taiwan. 2003.

JONES, V.P. **Longevity of apple maggot (Diptera: Tephritidae) lures under laboratory and field conditions in Utah**. Environmental Entomology, v. 17: p. 704- 708, 1988.

JOSEPH JR., H. & KNOBEL, M.G**. Estudo Preliminar Da Sensibilidade Da Mosca *Drosophila melanogaster* A Diversos Pesticidas Organoclorados.** Rev. Inst. Adolfo Lutz, v.40, n.1, p.43-47, 1980.

KYRIACOU C. P., OLDROYD M., WOOD J., SHARP M., HILL M. **Clock mutations alter developmental timing in *Drosophila***. Heredity 64, 395–401 10.1038.1990

KOKKO, H. & M. D. JENNIONS. **Parental Investment, Sexual Selection And Sex Ratios**. Journal of Evolutionary Biology 21: 919–948, 2008.

KOMONEN, A. **Hotsposts of insect diversity in Boreal forests.** Conservation Biology, 17, 976-981, 2003.

KYUNG-JIN MIN, MARC TATAR, **Drosophila diet restriction in practice: Do flies consume fewer nutrients?**,2005.

LESSELLS, C. M. **The Evolutionary Outcome Of Sexual Conflict. Philosophical Transactions** of the Royal Society B 361: 301–317, 2006.

MARK, D. ADANS**. The Genome Sequence of *Drosophila melanogaster.*** Science 24 March 2000, Vol. 287, 5461 pg. 2185-2195.

MARKOW, T. A. 2000**. Forced matings of *Drosophila* Females In Natural Populations**. American Naturalist 156: 100–103, 2000.

MARKOW, T. A. 2000**. Forced matings of *Drosophila* Females In Natural Populations**. American Naturalist 156: 100–103, 1988.

MEIGEN, J.W. **Systematische Beschreibung der bekannten europäischen zweiflügeligen Insekten.** 6 Theil. 401 pg. Schulzische Buchhandlung, Hamm, 1830.

MIZUNO, T.; ZHUANG, C**. *Maitak, Grifola* frondosa: farmacologia effects.** Food Reviews International, v.11, p. 135-149. 1995.

MUSIEBA F, OKOTH S, MIBEY RK, WANJIKU S, MORAA K**. Proximate composition, amino acids and vitamins profile of *Pleurotus citrinopiletus* Singer: An indigenous Mushroom in Kenia**. American Journal of Food Technology, 2013;18(3):p200,10.3923/ajft.2013.

NASCIMENTO, J.S. **Etiologia, controle e demanda de energia na prevenção da falsa trufa em cultivos de *Agaricus blazei.*** 2003. 111p. Tese (Doutorado em Agronomia) – Universidade Estadual Paulista, Botucatu.

NOSAL'OVÁ V, BOBEK P, CERNA S, GALBAVY S, STVRTINAS S. **Effects of pleuran (beta-glucan isolated from *Pleurotus ostreatus*) on experimental colitis in rats**. Physiology Research. 2001; 50(6):575-581.

PANI, B. K., & PATRA, A. K. **Growth of some species of Pleurotus(oyster mushroom) in different liquid media.** Orissa Journal ofAgricultural Research, 7(Suppl.), 66–68. 1994.

PAULINO, C.A.; MAZANTI, M.T.; GAETA, R. **Método De Triagem Para Detecção De Intoxicações Pelo Carbofuran Usando Moscas *Drosophila melanogaster***. Pesqui. Agropecu. Bras., v.27, n.1, p.209-212, 1992.

PAVKOVIC-LUCIC S. KEKIC V. **Are larger and/or more symmetrical Drosophila melanogaster (Diptera, Drosophilidae) males more successful in mating in nature? —** Rev. Bras. Entomol. 55: 583–588. 2011.

PRAMANIK M, MONDAL S. CHAKRABORTY I, ROUT D, ISLAM SS**, Structural investigation of a polysaccharide (FR. II) isolated from the aqueous extract of an edible mushroom, *Pleurotus sajor-Caju*.** Carbohydrate Research, Oxford, v.340, n.4, p.629-636, 2005.

PUTZKE, J.; PUTZKE, M. T. L. **OS REINOS DOS FUNGOS**. Vol. 1. Editora da Universidade de Santa Cruz do Sul, 1998. 606p.

RAGUNATHAN, R., GURUSAMY, R., PALANISWAMY, M., & SWAMINATHAN, K**. Cultivation of Pleurotus spp. on various agro-residues.** Food Chemistry, 55, 139–144.1996.

RAGUNATHAN, R., PALANISWAMY, M., GURUSAMY, R., & SWAMINATHAN, K**. Bioconversion of agricultural and agro industrial wastes by *Pleurotus spp*.** Cultivated Mushroom Research CMR Newsletter, 3, 17–21. 1996.

RAJARATHINAM, K., VEERASAMY, S., MEHALINGAM, P., & JAYABALAN, M. **Cultivation of *Pleurotus citrinopileatus* on paddy straw–coir waste mixtures.** Journal of Ecobiology, 6, 77–79.1994.

SHORROCKS B. **Os locais de reprodução de *Drosophilas* em florestas temperadas**. **Genética e Biologia de Drosophila**. EdsA.Ashburner, HL Carson e JNJr Thompson, Academic Press, Londres. 1982;3B:385-428.

SAVALI, U. M. **SEXUAL SELECTION**, p. 207–221. In: C. W. Fox; 2001.

SHEEBA V., SHARMA V. K., SHUBHA K., CHANDRASHEKARAN M. K., JOSHI A. **The effect of different light regimes on adult life span in Drosophila melanogaster is partly mediated through reproductive output**. J. Biol. Rhythms 15, 380–392. 2000

SHU Y, HASENSTAUB A, DUQUE A, YU Y, MCCORMICK DA. **Modulation of intracortical synaptic potentials by presynaptic somatic membrane potential**. Nature.2005 441:761-

SIMPSON, S.J. ABISGOLD, J.D. AND DOUGLAS, A.E. **Response of pea aphid (Acyrthosiphon pisum) to variation in dietary levels of sugar and amino acids: Significance of amino acid quality.** Journal of Insect Physiology, v. 45: p. 71-75, 1994.

SOUZA, H.M.L.; PIEDBRABUENA, A.E. AND PAVAN, O.H.O. **Biologia de C. capitata (Wiedmann) (Diptera: Tephritidae). Um novo meio artificial de criação para a produção em massa**. Papéis avulsos Zoologia, v. 31: p. 213-219, 1978.

STARMER WT, **A comparison of *Drosophila* habitats according to the physiological attributes of the associated yeast communities**. Evolution, Lancaster. 1981; 35:35-52.

THORNHILL, R., & ALCOCK, J. **The evolution of insect mating systems. Cambridge, MA**: Harvard University Press. 1983

TIDON R. LEITE DF, FERREIRA EB, LEÃO FD, **drosofilídeos (Diptera Insecta) do Cerrado.** Ecologia e biodiversidade do cerrado.2005;(1)412.

TSITSIPIS, J.A. **Nutrition Requeriments. In: Fruit Flies, their biology, natural enemies and control, Robinson**, World Crop Pest, Vol. 3B, Elsevier, 289p. 1989.

VERMA A., SENGUPTA S., LAKHOTIA S. C. **DNApol-(gene is indispensable for the survival and growth of *Drosophila melanogaster***. *Genesis* 50 86–101. 2012.

WANG JC, HU SH, LIANG ZC, YEH CJ. **Optimization for the production of water-soluble polysaccharide from *Pleurotus citrinopileatus* in submerged culture and its antitumor effect**. Appl Microbiol Biotechnol. 2005, 67:759–766

WASSER, S. NEVO, E., SOKOLOV, D., RESHETNIKOSV, S. V. e TIMOR-TISMENETSKY, M. **Dietary supplements from medicinal mushrooms:diversity of types and variety of regulation.** International Journal of Medicinal Mushorooms**,** v. 2, p. 1-19, 2000.

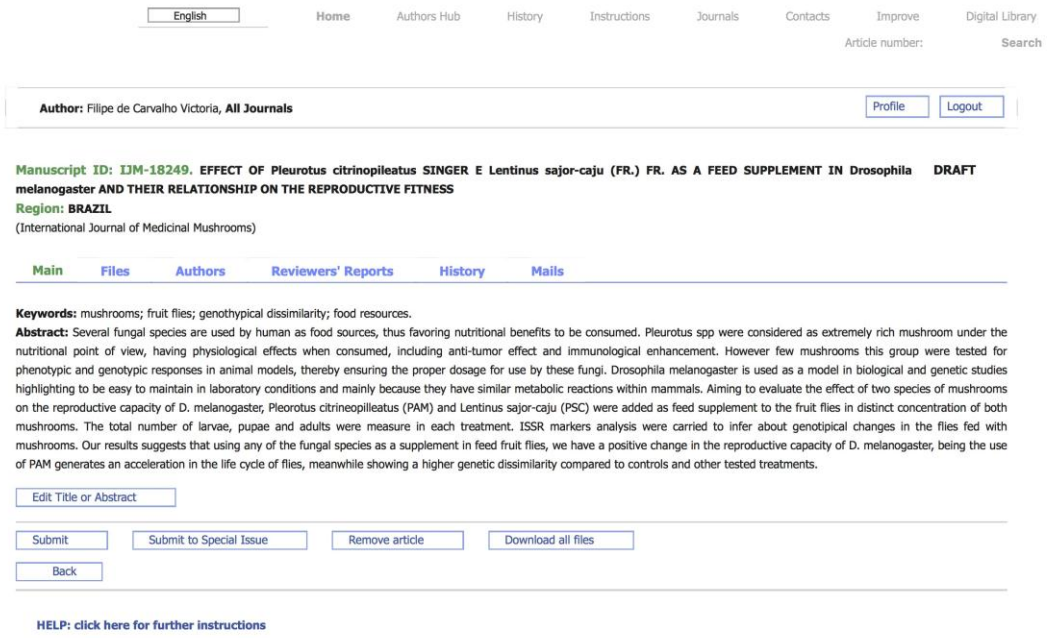
WASSER, S. P. **Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides.** Applied Microbiology and Biotechnology, 60, 258–274, 2002.

WASSER SP, WEIS AL **Medicinal properties of substances occurring in Higher Basidiomycetes mushrooms: current perspectives**. Int J Med Mushrooms 1:31–62. 1999a

WHIGBY, S. & T. CHAPMAN. **Female Resistance To Male Harm Evolves In Response To Manipulation Of Sexual Conflict.** Evolution 58: 1028–1037, 2004.

ZHANG J, WANG G, LI H, ZHUANG C, MINUZO T, ITO H, SUZUKI C, OKAMOTO H, LI J. **Antitumor polysaccharides from a chinese mushroom, "yuhuangmo", the fruiting body of *Pleurotus citrinopileatus***.Biosci, Biotechnol, Biochem, 1994: 58:1195-1201.

ANEXOS



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Manuscript ID: IJM-18249. EFFECT OF Pleurotus citrinopileatus SINGER E Lentinus sajor-caju (FR.) FR. AS A FEED SUPPLEMENT IN Drosophila melanogaster AND THEIR RELATIONSHIP ON THE REPRODUCTIVE FITNESS **DRAFT**
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Keywords: mushrooms; fruit flies; genothypical dissimilarity; food resources.
Abstract: Several fungal species are used by human as food sources, thus favoring nutritional benefits to be consumed. Pleurotus spp were considered as extremely rich mushroom under the nutritional point of view, having physiological effects when consumed, including anti-tumor effect and immunological enhancement. However few mushrooms this group were tested for phenotypic and genotypic responses in animal models, thereby ensuring the proper dosage for use by these fungi. Drosophila melanogaster is used as a model in biological and genetic studies highlighting to be easy to maintain in laboratory conditions and mainly because they have similar metabolic reactions within mammals. Aiming to evaluate the effect of two species of mushrooms on the reproductive capacity of D. melanogaster, Pleorotus citrineopilleatus (PAM) and Lentinus sajor-caju (PSC) were added as feed supplement to the fruit flies in distinct concentration of both mushrooms. The total number of larvae, pupae and adults were measure in each treatment. ISSR markers analysis were carried to infer about genotipical changes in the flies fed with mushrooms. Our results suggests that using any of the fungal species as a supplement in feed fruit flies, we have a positive change in the reproductive capacity of D. melanogaster, being the use of PAM generates an acceleration in the life cycle of flies, meanwhile showing a higher genetic dissimilarity compared to controls and other tested treatments.

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