Larvicidal efficiency of the fungus *Amanita muscaria* (Agaricales, Amanitaceae) against *Musca domestica* (Diptera, Muscidae)

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Resumo

Ação larvicida do fungo *Amanita muscaria* (Agaricales, Amanitaceae) em *Musca domestica* (Diptera, Muscidae). Foi relatada a ação larvicida de duas formulações de *Amanita muscaria* em *Musca domestica*. Dois métodos de extração foram testados: extrato aquoso de basidioma desidratado e triturado (BDT), e extrato de basidioma fresco liquefeito em água (BFL). A mortalidade causada pelo extrato BDT variou de 14,67% a 100%. A eficácia do extrato BFL variou de 10,67 a 89,33%. A concentração letal média (CL_{50}) do extrato BDT foi de, aproximadamente, 1.931,02 ppm, enquanto que a CL_{50} do extrato BFL foi em torno de 30%. As substâncias extraídas por esses métodos de extração não interferiram no período de desenvolvimento dos imaturos e não influenciaram no peso pupal dos indivíduos. Esses resultados mostram o potencial de extratos de *A. muscaria* para o controle de *M. domestica*.

Palavras-chave: Cogumelo; Extrato aquoso; Inseticida; Moscas; Vetor

Abstract

This work reports the larvicidal action of two formulations of *Amanita muscaria* against *Musca domestica*. Two methods of extraction were tested: an aqueous extract from dried, powdered basidiomes (DPB); and an extract from fresh basidiomes liquefied in water (FLB). The mortality caused by the DPB extract varied from 14.67% to 100%. The efficiency of the FLB extract varied from 10.67% to 89.33%. The mean lethal concentration (LC_{50}) of the DPB extract was approximately 1,931.02 ppm, whereas the LC_{50} for the FLB extract was about 30%. The extracted substances from these methods did not interfere with the development period of immatures and did not influence pupal weight. These results show the potential of *A. muscaria* extracts for controlling *M. domestica*.

Key words: Aqueous extract; Flies; Insecticide; Mushroom; Vector

Musca domestica Linnaeus, 1758 (Diptera, Muscidae) plays an important role in the health of humans and domestic animals because it can act as a mechanical vector of bacteria, viruses, fungi, protozoans, and helminths (FÖRSTER et al., 2007; MALIK et al., 2007; BARIN et al., 2010). In addition, this species can be a biological vector of *Habronema muscae* (Carter, 1861) (Spirurida, Habronematidae) and *Habronema megastoma* (Rudolphi, 1819) (Spirurida, Habronematidae), nematodes that cause gastric and cutaneous injuries in equids (JOHNSTON; BANCROFT, 1920).

Due to its vectorial ability and large populations, this species often needs to be controlled. Control is usually done with synthetic chemical insecticides, which cause many problems, such as environmental contamination, human and domestic animal poisoning, and the appearance of resistance in populations (ACEVEDO et al., 2009; KOZAKI et al., 2009; WANG et al., 2012).

As an alternative to synthetic insecticides, natural products have been studied (CABRAL et al., 2008). The most important ones are obtained from plants and, although poorly understood, some fungal species show insecticidal activity (MIER et al., 1996). *Amanita muscaria* (Linnaeus, 1758) (Agaricales, Amanitaceae) is popularly known as fly agaric because some traditional communities use solutions of this fungus to control flies (CORDIER, 1876; MOYEN, 1888 apud MIER et al., 1996). Insecticidal properties of *Amanita muscaria* are mainly associated with the action of ibotenic acid and muscimol (TAKEMOTO et al., 1964).

Although the use of this fungus by traditional communities is known (MICHELOT; MELENDEZ-HOWELL, 2003), there are no reports of its use against *M. domestica* immatures. Thus, this study aimed to evaluate the efficiency of aqueous extracts of *A. muscaria* against *M. domestica* immatures.

Adults of *M. domestica* were collected in Pelotas, Rio Grande do Sul, Brazil (31°48'S, 52°25'W), identified with the help of a dichotomous key (CARVALHO et al., 2002), and maintained in cages (30 x 30 x 30 cm). The *M. domestica* specimens were maintained according to Ribeiro et al. (2000) for rearing *Ophyra aenescens* (Diptera, Muscidae), differing only in the diet of the larvae, which in this work was composed of meat meal and sawdust (proportion 2:1). Throughout the experimental period, stock and test colonies were maintained in a climatic chamber (25 ± 2°C, RH 70 ± 10%, 12L: 12D).

Amanita muscaria specimens were collected in July in a grove of *Pinus elliottii* Engelmann, 1880 (Pinales, Pinaceae), in Capão do Leão, Brazil (31°48'08.7"S, 52°24'51.2"W). Basidiomes were collected with the pileus completely opened and were used to make two types of extracts: an extract from dried, powdered basidiomes (DPB); and an extract from fresh basidiomes liquefied in water (FLB).

For the first test (DPB extract), as soon as the basidiomes were collected they were placed in an incubator at 45°C for 96 h, and then milled with an electric mill with 0.5 mm mesh. The crude extract was obtained from the fungal powder added to distilled sterile water in the proportion of 1:10 (w/v). The resulting mixture was placed in a shaking incubator at 120 rpm and 25°C for 48 h. Then, the product was centrifuged for 10 min at 3,000 rpm and the supernatant was filtered using filter paper.

For this test, we prepared six concentrations: 0 ppm (control group), 928 ppm, 1,856 ppm, 2,784 ppm, 3,712 ppm and 4,640 ppm. We used 75 first-instar larvae divided in three replicates of 25 larvae for each concentration, totaling 450 individuals. Replicates were formed with 25 g of diet (composed of meat meal and sawdust in the proportion of 2:1) moistened with 25 mL of extract or distilled sterile water (control group). The container with the diet and larvae was placed in a larger container with moistened sawdust so the post-feeding larvae could abandon the substrate and pupariate in the sawdust. The larger container was closed with voile.

We observed the treatments daily and the post-feeding larvae found outside of the substrate were placed in containers with slightly moistened sawdust. We weighed the pupae after 48 hours and watched them until adult emergence.

The second test (FLB extract) followed the same procedure as the first test, differing only in the solution that was used to moisten the diet that, in this test, was obtained from the liquefaction of fresh basidiomes in water.

We prepared six concentrations with the fresh basidiomes and distilled sterile water (w/v): 0% (control group), 10%, 20%, 30%, 40%, and 50%. The mixture was filtered with filter paper, the solid portion was discarded, and the liquid was used to moisten the diet where the larvae were placed.

For both tests, total mortality (larval and pupal), development period, and pupal weight were evaluated using an analysis of variance (ANOVA) and means compared by Tukey's test ($p < 0.05$). LC_{50} and LC_{90} of each test were estimated with a Probit analysis. All analyses were performed using the software SPSS for Windows, version 22.0 (SPSS INC., 2013).

The DPB extract caused significant mortality of *M. domestica* immatures ($F = 185.07$; $DF = 5$; $p < 0.001$).

The mean mortality varied from 44.00% to 100%, whereas the control group showed a mean mortality of 10.67% (Table 1).

The FLB extract also caused significant mortality ($F = 178.87$; $DF = 5$; $p < 0.001$). The mean mortality varied from 25.33% to 89.33%, whereas the control group showed a mean mortality of about 6.67% (Table 1). For the first concentration of both extracts, we did not observe an insecticidal effect.

The mean lethal concentration (LC_{50}) of the DPB extract was 1,931.02 ppm and the LC_{90} was 3,174.30 ppm. For the FLB extract, the LC_{50} was 29.77% and the LC_{90} was 49.42% (Table 2).

The development period (larva to adult) of *M. domestica* was not influenced by the DPB extract ($F = 3.73$; $DF = 3$; $p = 0.1$) or the FLB extract ($F = 0.37$; $DF = 5$; $p = 0.86$) of *A. muscaria*. The mean period varied from 14.50 (928 ppm) to 14.98 days (control) for the DPB extract, and from 14.38 (40%) to 14.85 days (20%) for the FLB extract (Table 3).

TABLE 1: Mean mortality (%) of *Musca domestica* subjected to different concentrations of *Amanita muscaria* aqueous extracts from dried, powdered basidiomes (DPB), and fresh basidiomes liquefied in water (FLB).

DPB extract		FLB extract	
Concentration (ppm)	Mean mortality (%) ± SD *	Concentration (mushroom: water) (w: v)	Mean mortality (%) ± SD *
Control	10.67 ± 2.31 A	Control	6.67 ± 2.31 A
928	14.67 ± 6.11 A	10%	10.67 ± 8.33 A
1,856	44.00 ± 4.00 B	20%	25.33 ± 6.93 B
2,784	88.00 ± 8.00 C	30%	48.00 ± 2.31 C
3,712	93.33 ± 6.11 C	40%	78.67 ± 6.11 D
4,640	100 C	50%	89.33 ± 4.00 D

* In the columns, values followed by different letters represent a significant difference between mean mortalities of each concentration (Tukey's test – $p < 0.05$).

TABLE 2: Lethal concentrations of *Amanita muscaria* aqueous extracts (from dried, powdered basidiomes – DPB, and fresh basidiomes liquefied in water – FLB) required to cause the death of 50% and 90% of *Musca domestica* immatures.

Extracts	LC_{50} (CI)	LC_{90} (CI)	Slope ± SE	X^2
DPB	1,931.02 (1,751.62 – 2,098.03) ppm	3,174.30 (2,949.71 – 3,469.87) ppm	0.01 ± 0.001	15.12
FLB	29.77 (27.48 – 32.05)%	49.42 (45.82 – 54.34)%	0.06 ± 0.006	3.74

TABLE 3: Development period (larva to adult) (days) and pupal weight (mg) of *Musca domestica* originating from larvae subjected to different concentrations of aqueous extracts of *Amanita muscaria*: from dried, powdered basidiomes (DPB), and from fresh basidiomes liquefied in water (FLB).

DPB extract			FLB extract		
Concentrations (ppm)	Period (larva to adult) (days) ± SD	Pupal weight (mg) ± SD	Concentrations (mushroom: water) (w: v)	Period (larva to adult) (days) ± SD	Pupal weight (mg) ± SD
Control	14.98 ± 0.95	27 ± 2	Control	14.42 ± 1.15	25 ± 2
928	14.50 ± 0.92	25 ± 1	10%	14.55 ± 0.78	27 ± 1
1,856	14.57 ± 0.77	25 ± 2	20%	14.85 ± 0.69	23 ± 2
2,784	14.56 ± 0.72	24 ± 2	30%	14.41 ± 0.81	25 ± 3
3,712	*	*	40%	14.38 ± 0.50	26 ± 1
4,640	*	*	50%	14.63 ± 0.74	*

* Groups that were not evaluated because they did not have the minimum number of individuals.

The substances of *A. muscaria* extracted using the methods did not influence the pupal weight of *M. domestica*: DPB extract (F = 2.07; DF = 3; p = 0.12) and FLB extract (F = 0.87; DF = 4; p = 0.08). The mean pupal weight varied from 24 mg (2,784 ppm) to 27 mg (control) for the DPB extract, and from 23 mg (20%) to 27 mg (10%) for the FLB extract (Table 3).

Our study shows, for the first time, that aqueous formulations of *A. muscaria* exhibit larvicidal activity against *M. domestica*. Due to resistance of this insect species to synthetic chemical insecticides, similar studies had already been described as important in the 1960s (PETZSCH, 1960); however, little was known beyond reports from traditional communities.

We observed that the larvicidal effect was dose-dependent, as reported by Cárcamo et al. (2016) for *Culex quinquefasciatus* (Diptera, Culicidae) larvae subjected to the same fungal species. However, the efficiency that we observed was lower than observed by these authors. They reported an LC_{50} of about 50 ppm, and in the present study we estimated a concentration of 1,931 ppm to kill the same percentage of larvae. These differences are probably associated with specific features of these species, since the method of extraction was the same for both experiments.

The larvicidal effect of *A. muscaria* was not observed by Mier et al. (1996) for *Drosophila melanogaster* (Diptera, Drosophilidae) larvae. For this fly, other *Amanita* species were considered toxic: *A. phalloides*, *A. citrina* and *A. rubescens*. Larvicidal action can be associated with different factors, such as the method used to obtain the active ingredient, since the way fungal compounds are extracted influences larvicidal activity (THONGWAT et al., 2015).

The two tested formulations killed immatures of *M. domestica*, and the FLB extract was easier to produce. Nevertheless, it can only be produced in colder seasons when fresh basidiomes of *A. muscaria* grow in Rio Grande do Sul, Brazil (BERNARDI, E.: personal communication, 2015). On the other hand, the DPB extract can be stored up to six months without losing efficiency (CÁRCAMO et al., 2016).

The inability of these two extracts to alter the development period and pupal weight is probably due to the mode of action of the toxic compounds. The main compound found in *A. muscaria* is muscimol (MICHELOT; MELENDEZ-HOWELL, 2003), which is a potent agonist of $GABA_A$ (FRØLUND et al., 2002) that acts as a neurotoxic insecticide.

We obtained promising results with two methods of application of *A. muscaria* as a larvicidal insecticide. In addition, this is the first scientific report of the use of aqueous extracts as an insecticide against *M. domestica* larvae, which is an important species in urban and animal production environments. Nonetheless, other aspects of its use, such as the impact of this insecticide against

non-target populations and its efficiency under field conditions, must be thoroughly evaluated.

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