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Short Communication

Larvicidal efficiency of the mushroom *Amanita muscaria* (Agaricales, Amanitaceae) against the mosquito *Culex quinquefasciatus* (Diptera, Culicidae)

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ABSTRACT

Introduction: We report the larvicidal activity of two formulations from *Amanita muscaria* against *Culex quinquefasciatus*, as well as the viability of the aqueous extract after storage. **Methods**: The larvicidal activity of aqueous extract and powder from *A. muscaria*, and the viability of the aqueous extract after storage, were evaluated. **Results**: The aqueous extract caused larval deaths, which varied from 16.4% to 88.4%. The efficiency of the powder varied from 29.2% to 82.8%. Storage did not interfere with the larvicidal efficiency of the aqueous extract of *A. muscaria*. **Conclusions**: These results show the potential of *A. muscaria* to control *C. quinquefasciatus*.

Keywords: Bioinsecticide. Fly agaric. Filariasis vector.

The mosquito *Culex quinquefasciatus* Say, 1823 (Diptera, Culicidae), is the main vector of the human parasitic roundworm, *Wuchereria bancrofti* (Cobbold, 1877) (Spirurida, Onchocercidae) on the American continent[1]. Reisen[2] reported that this mosquito can also act as a vector of arboviruses, such as West Nile and St. Louis encephalitis.

This species is usually controlled by the use of synthetic chemical insecticides. The use of these products can cause problems, such as human and environmental contamination, as well as the development of resistance in insect populations[3].

Plant-based products are the most recently researched alternatives, showing great results in mosquito control[4] [5]. However, although there are few studies that investigate the use of fungi as a control measure, some fungal species have shown promising results against insects[6] [7].

Among the fungal substances known to be toxic to insects, ibotenic acid and muscimol are both found in the ectomycorrhizal fungus *Amanita muscaria* (Linnaeus, 1758) (Agaricales, Amanitaceae)[8]. This fungus is generally found in temperate areas, and in Brazil, has been reported from São Paulo to the southern part of the country in association with plantations of *Pinus* spp. or *Eucalyptus* spp.[9]. Although *A. muscaria* possesses substances that are toxic to insects, there are no studies regarding the impact of this fungal species against culicids. Thus, this work aims to report the toxicity of two formulations, an aqueous extract, and a powder, from *A. muscaria* against *C. quinquefasciatus* larvae, as well as to evaluate the ability to store the aqueous extract of this fungal species.

The larvae of *C. quinquefasciatus* used in this study were obtained from a laboratory colony, and raised according to the methodology proposed by Gerber[10], differing only in the diet offered to the larvae. In this investigation, the larvae were fed with minced fish feed.

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). Basidiomata were collected when their rings were completely opened, and were subsequently put in an incubator at 45°C for 96h. After dehydration, basidiomata were milled in an electric mill with a mesh of 0.5mm.

Crude extract was obtained from the fungal powder and added to sterile distilled water at a concentration of 1:10 (w/v). The resulting mixture was put in a shaker (120rpm) at 50°C for 48h. Subsequently, the product was centrifuged for 10 min at 3,000rpm and the supernatant was filtrated using a paper filter. A portion of the extract was used immediately and the remainder was either stored in refrigerator (3°C ± 2°C) or freezer (-20°C) for six months.

The experiment to detect larvicidal activity was performed following the standard protocol from the World Health Organization[11], with some modifications. Insects were maintained in a climatic chamber (25°C ± 2°C, 70% ± 10% RH, and a photoperiod of 12:12h) for all experiments.

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We evaluated the efficiency of *Amanita muscaria* against *Culex quinquefasciatus* larvae using two formulations, an aqueous extract and a fungal powder. We used five concentrations of each formulation. To evaluate the aqueous extract, we diluted the crude extract in water to the following concentrations: 14.5ppm, 29.0ppm, 58.0ppm, 116.0ppm, and 232.0ppm. The preparation of the powder formulation was created by adding varied concentrations of powder directly to water and shaking gently for 1 minute. The final concentrations of the fungal powder formulations were 0.2mg/ml, 0.4mg/ml, 0.8mg/ml, 1.6mg/ml, and 3.2mg/ml. Each concentration was composed of five replicates, each with 50ml of liquid (extract or water) and 50 third-instar larvae of *C. quinquefasciatus*. For each formulation, we established a control group, composed in the same way as larvicidal treatment groups, differing only in that there was no *Amanita muscaria* added. The groups were evaluated after 24h and larvae that did not show any motility after being lightly touched were considered dead.

After six months of chilling or freezing, the larvicidal activity of stored extracts were evaluated against *C. quinquefasciatus* larvae using a final concentration of 232.0ppm. For each group, five replicates were created using 50ml of extract and 50 third-instar larvae. Two treatments served as control conditions: a negative control (water only plus larvae) and a positive control (aqueous extract that had not been stored plus larvae). The evaluation of larvicidal activity was performed as previously described.

Mortality was evaluated using an analysis of variance (ANOVA) ($p = 0.05$), in which means were compared using Tukey's test ($p = 0.05$). The median lethal concentration (LC_{50}) and 90% lethal concentration (LC_{90}) of each formulation were analyzed using probit analysis. All analyses were performed using SPSS version 22.0 for Windows (IBM, Armonk, NY, USA)[12].

The aqueous extract of *Amanita muscaria* caused significant mortality in *C. quinquefasciatus* larvae ($F = 156.11$; $DF = 5$; $p < 0.001$). The mean mortalities caused by extracts varied from 16.4% to 88.4%, while in control groups, the mean mortality was approximately 3.2% **(Figure 1)**.

The powder of *Amanita muscaria* caused significant mortality of *C. quinquefasciatus* larvae ($F = 101.01$; $DF = 5$; $p < 0.001$). The mean mortalities caused by the powder preparation varied from 29.2% to 82.8%, whereas, in the control group, the mean mortality was approximately 3.2% **(Figure 2)**.

The mean lethal concentration (LC_{50}) of the aqueous extract of *A. muscaria* was approximately 51.46ppm and the LC_{90} was approximately 248.07ppm. The powder formulation resulted in an LC_{50} of approximately 1.19mg/ml and an LC_{90} of approximately 3.98mg/ml **(Table 1)**.

The mortalities of *C. quinquefasciatus* larvae caused by different methods of storing the aqueous extract of *A. muscaria* did not show significant differences between groups ($F = 0.9$; $DF = 2$; $p = 0.43$), which varied from 83% to 86.8%. The mortalities observed were significantly higher than those observed

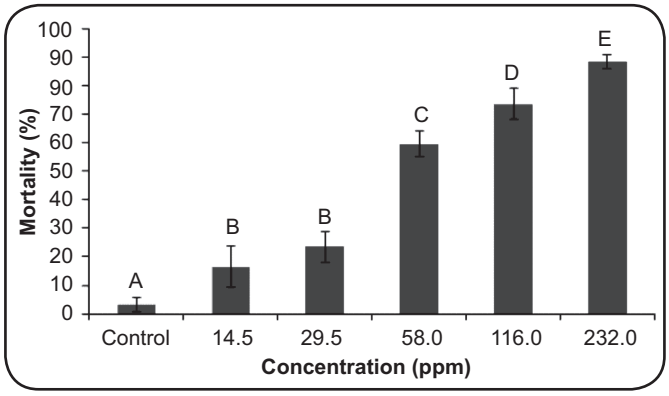

FIGURE 1 - Mean mortality (%) of *Culex quinquefasciatus* larvae subjected to different concentrations of aqueous extract from *Amanita muscaria*. Different letters represent significant difference between groups (Tukey's test; p = 0.05). Error bars represent the standard deviation of the mean.

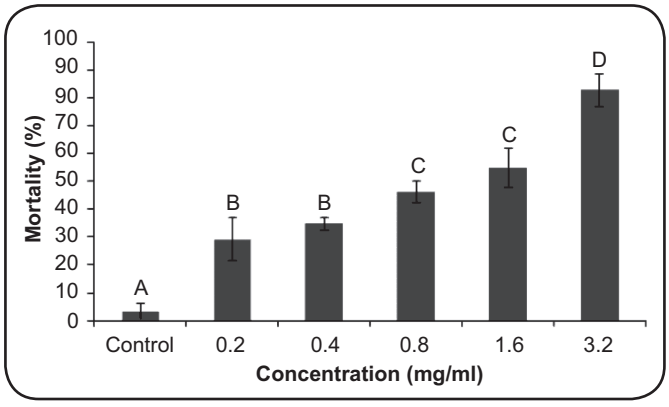

FIGURE 2 - Mean mortality (%) of *Culex quinquefasciatus* larvae subjected to different concentrations of powder from *Amanita muscaria*. Different letters represent significant difference between groups (Tukey's test; p = 0.05). Error bars represent the standard deviation of the mean.

TABLE 1 - Lethal concentrations of aqueous extract and powder from Amanita muscaria needed to cause death in 50% (LC_{50}) and 90% (LC_{90}) of Culex quinquefasciatus larvae in 24 hours.

Group	LC_{50}	LC_{90}	Slope ± SE	χ^2	N
Extract	51.46 (39.87-64.92) ppm	248.07 (170.17-452.09) ppm	0.81 ± 0.04	6.98	250
Powder	1.19 (1.03-1.35) mg/ml	3.98 (3.57-4.45) mg/ml	4.58 ± 0.36	2.15	250

LC: lethal concentration; **SE:** standard error; **χ^2:** Chi-square.

for the negative control ($F = 261.31$; $DF = 3$; $p < 0.001$), which was approximately 2.8%.

Both aqueous and powder formulations from *A. muscaria* displayed larvicidal activity against *C. quinquefasciatus* larvae in a dose-dependent manner. The aqueous extract possessed toxicity classified between moderate and high according to the categories proposed by Komalamisra[13].

The larvicidal efficiency of the aqueous extract of *A. muscaria* observed in this study was higher than the efficiencies of different fungal formulations from *Pycnoporus* sp. (Linnaeus, 1758) (Polyporales, Polyporaceae) and *Pestalotiopsis* sp. Steyaert, 1949 (Xylariales, Amphisphaeriaceae) against the yellow fever mosquito, *Aedes aegypti* (Linnaeus, 1762) (Diptera, Culicidae), as observed by Bucker[7]. The observed differences could be related to species-specific differences or to the methods of extraction, as the author observed different impacts according to tested formulations. This illustrates the importance of evaluating other methods of extraction for *A. muscaria* in order to maximize the efficiency of this fungus against *C. quinquefasciatus* larvae. However, we consider the use of water as a solvent for extraction to be an advantageous alternative, as it is a low-cost method and does not result in environmental problems as do other solvents.

The extract of *A. muscaria* had a higher larvicidal efficiency against *C. quinquefasciatus* than extracts from four other fungal species tested by Chelela[14]. The highly toxic activity of *A. muscaria* is likely a result of ibotenic acid and muscimol, toxic substances found in *A. muscaria* that are known to be responsible for causing death in flies[8].

The toxicity of *Amanita muscaria* powder against *Culex quinquefasciatus* larvae was different than the effect observed by Mier[6] against *Drosophila* Fallen, 1823 (Diptera, Drosophilidae) larvae. According to this author, although *A. muscaria* is not toxic to *Drosophila*, other species of the genus *Amanita* were shown to be toxic to this dipteran, such as *A. phalloides* (LC_{100}=0.1mg/ml), *A. citrina* (LC_{100}=30mg/ml), and *A. rubescens* (LC_{100} = 53mg/ml). In the present study, the LC_{90} was approximately 3.98mg/ml in *A. muscaria*. Direct comparisons between the results of various investigations are difficult because of differences in the dipteran species tested, highlighting the importance of evaluating the fungal specificity against various targeted insect species.

Even after six months of chilling or freezing, the extracts maintained their larvicidal efficiency against *C. quinquefasciatus*. This result is important considering the time disparity between the appearance of *A. muscaria* and the period of abundance of *C. quinquefasciatus*. *C. quinquefasciatus* usually reaches its highest numbers during the warmest period of the year[15], while *A. muscaria* usually occurs during months that have lower mean temperatures (such as July and August) in Rio Grande do Sul, Brazil (E Bernardi: personal communication, 2015).

In conclusion, we obtained promising results with the two methods of application of *A. muscaria* as a larvicide, even following long-term storage of the toxic extract, illustrating that this mushroom can be used as a source of bioinsecticide compounds. Despite these results, other aspects of its use, such as the impact of this insecticide against non-targeted populations and its efficiency under field conditions, require further evaluation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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