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WILD PADDY STRAW MUSHROOM (*Volvariella volvacea*) EXHIBIT TOXIC AND TERATOGENIC EFFECTS IN ZEBRAFISH (*Danio rerio*) EMBRYO

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ABSTRACT

This work demonstrated the toxic and teratogenic effects of water extracts of pileus and stipe of wild fruiting bodies of *Volvariella volvacea* collected from the decomposing pile of rice straw. Toxicity assay revealed that coagulation and no heartbeat embryos were the distinct toxic effects of pileus and stipe extracts of *V. volvacea* when embryos were exposed at 3%, 1%, and 0.5% concentrations. No mortality was observed to embryos at 0.10% and lower concentrations of both extracts. A100% hatchability was noted in embryos exposed to 0.01% concentration of both extracts and embryo water. However, embryos at 0.10% and higher concentrations of both pileus and stipe extract showed 100% delayed development. All embryos exposed at 0.10% and some embryos at 0.05% of both extracts showed different malformations such as twisted tail and body, bent tail tip, and hook-like tail. Altogether, *V. volvacea* affects the survival and morphological development of embryo, which are dependent on mushroom part, extract concentration, and time of exposure. The other factors such as substrate and elemental composition of *V. volvacea* that contributed to its toxic and teratogenic effects are currently under investigation.

Keywords: *V. volvacea*, teratogenicity, zebrafish, pileus and stipe, paddy straw mushroom

INTRODUCTION

The Philippines is a nesting ground of rich flora and fauna due to its very fine climatic conditions. Many of these organisms remain in the wild waiting to be discovered and utilized for human benefits. Mushrooms are one of these organisms. They are saprophytic organism that commonly found growing on lignocellulosic substrates. Some of them are edible and medicinal and some are non-edible and non-palatable. Edible mushrooms are very good source proteins, fiber, carbohydrates, lipids, vitamins and minerals. Although vey diverse, only few mushrooms species have been successfully rescued and studies including *Ganoderma, Schizophyllum, Lentinus, Coprinopsis, Auricularia, Collybia, Panaeolus*, *Pleurotus* and *Volvariella,*.

Volvariella volvacea, belongs to family Pluteaceae, is a basidiomycetous fungus that commonly called as *kabuteng dayami* or *kabuteng saging* or paddy straw mushroom. It is the most popular and most cultivated mushroom in the Philippines, which could provide promising nutraceutical values and pharmacological properties **[1]**. The fruiting bodies of *V. volvacea* contain bioactive metabolites and nutritional qualities that contribute not only to its unique and delightful umami taste and aroma but most importantly to its notable functional activities such as anti-coagulant, anti-inflammatory, and anti-hypertensive **[2]**.

Inspite of the valuable attributes of this mushroom, no study was conducted on the toxic and teratogenic effects of *V. volvacea*. Teratogens are agents that cause abnormalities in the growth and development of an organism. However, teratogens are anticancer agents and vice versa **[3]**. In teratogenicity assay, zebrafish (*Danio rerio*) embryo is used as a reliable animal model. Its developmental processes are similar to the higher forms of vertebrate including human. This work highlighted the toxic and teratogenic effects of the water extracts of pileus and stipe of wild *V. volvacea* in zebrafish embryos. The percentage mortality, hatchability, delayed development, malformations were determined and the different morphological endpoints of the exposed embryos were documented.

MATERIALS AND METHODS

Source of mushroom fruiting bodies

The wild fruiting bodies of *V. volvacea* were collected from composted pile of rice straw in Science City of Munoz, Nueva Ecija, Philippines. The stipe and pileus of fruiting bodies were air-dried separately for 5 days,

milled using a blender and prepared for extraction.

Hot water extraction

The extracts of mushroom samples were obtained following the hot water extraction protocol established by Eguchi et al. **[4]**. Five grams of each powdered pileus and stipe were extracted separately in 150 ml hot water at 80 - 90ºC in a water bath for 2 hours. These were filtered using Whatman filter paper No. 2 and the extract filtrates were used to prepare the different treatment concentrations for teratogenicity assay by diluting the extract to embryo water medium **[5]**.

Spawning and fertilization

The procedures on spawning and fertilization were followed after Nagel **[6]**. Mature female and male zebrafish at 1:2 ratio were acclimatized in a glass aquarium with water saturated with oxygen. They were fed two times a day with dry flakes and the quality of water was maintained. In spawning, fish were localized in a plastic mesh and the aquarium was covered with black plastic sheet for 12 hours. After spawning, eggs were exposed to lighted condition for another 12 hours. The eggs were fertilized after 30 minutes of exposure to light. Embryos were collected from the aquarium using a hose and examined for the uniformity of embryos using a microscope. Coagulated and unfertilized eggs were discarded and the normal embryos were used in the assay.

Toxicity and teratogenicity assay

The protocol on the toxicity and teratogenicity using zebrafish embryos was adopted from Dulay et al. **[7]**. Four embryos at segmentation phase were exposed to the different concentrations of each extract. Mortality was determined after 12, 24, 36, and 48 hours of extract exposure. The percentage hatchability, delayed development, and malformation were also recorded. The morphological abnormalities of the treated embryos were based on the parameters established by Nagel **[6]**. The validity of the results was also noted. Analysis of Variance (ANOVA) was used to analyze the data and Least Significant Difference (LSD) was used to compare the means at 5% level of significance.

RESULTS AND DISCUSSION

Toxic effects of *V. volvacea* extracts

V. volvacea is an edible mushroom that usually collected on the decomposing pile of rice paddy by the farmers and mushroom hunters. This mushroom is prepared as food by steaming or combining with other vegetables. Although nutritious and delicious, the toxic effects of this mushroom must be studied using zebrafish embryo, a

reliable animal model in embryo-toxicity assays. Herein, the toxic effects of the different concentrations of water extracts of the pileus and stipe of wild fruiting bodies of *V. volvacea* were evaluated and the percentage mortality of embryos at different periods of observation is presented in Table 1. At 12 hours post treatment exposure, 100% mortality was recorded in embryos exposed to 3% concentration of both extracts and at 1% concentration of stipe extract. A 100% mortality of embryos at 1% concentration of pileus extract was observed after 24 hours of exposure. At 36 hours post treatment exposure, 0.50% concentration of pileus extract had 75% mortality and of stipe extract having 100%. This 75% mortality in pileus extract increased to 100% after 48 hours. Apparently, both extracts exhibited notable toxic effects when embryos were exposed at 3%, 1%, and 0.5%. On the other hand, no mortality was observed to embryos at 0.10% and lower concentrations of both extracts. Considering the time and concentration, stipe extracts showed a significant faster toxic effect in embryos, since lower mortality was noted in embryos at 1% and 0.50% of pileus extract after 12 and 36 hours of exposure, respectively. Coagulation was the most distinct toxic effect of the extracts. However, these significant toxic effects of *V. volvacea* could be equated to other functional properties since toxic substances could possess inhibitory activity against cancer and tumor cells. The antitumor activity of aqueous ethanolic extract of *V. volvacea* cultured mycelium was demonstrated in DLA cell line-induced solid tumor and EAC cell line-induces ascites tumor models in mice and were found to have significant activity **[8]**. In poisonous mushroom, toxophallin, a new highly cytotoxic protein isolated from the fruiting body of *Amanita phalloides* exhibited apoptosis induction via chromatin condensation and DNA and nucleus fragmentation **[9]**.

Hatching process of extract treated embryos

Hatching process is the most important phase of normal embryonic development. In this study, the effect of the different concentrations of the mushroom extracts on the hatching process of zebrafish was also investigated. The percentage hatchability of treated embryos is presented in Table 2. Apparently, 100% hatchability was noted in embryos exposed to 0.01% concentration of both extracts and embryo water. However, at 0.05% concentration of both pileus and stipe extracts recorded 75% and 41.67% hatchability, respectively. Comparing the two

values, stipe had lower percentage hatchability, which indicates to possess a more potent component that interferes hatching process. The stipe could possibly contained immobile nutrients which we need to evaluate such as molybdenum, zinc, manganese, iron, copper, cobalt, and calcium that possibly inhibit of enzymes responsible for the hatching process. No hatched was observed at 0.10% and higher concentrations of the two extracts. The results of the present study strongly suggest that *V. volvacea* extracts could inhibit and delay the hatching process of zebrafish, which is dependent on the part of the fruiting body of this mushroom.

Teratogenic effects of *V. volvacea* extracts

Teratogen is a potential anticancer agent. In the present work, the delayed development and morphological abnormalities were the most distinct teratogenic effects of *V. volvacea* extracts in zebrafish. Table 2 also shows the percentage delayed development after 48 hours and percentage malformations after 72 hours of extract treated embryos. It can be seen that 100% delayed development was significantly recorded in embryos at 0.10% and higher concentrations of both pileus and stipe extracts. However, embryos exposed to 0.05% of pileus extract showed 25% delayed development while 0.05% of stipe extract had 58.33%. Delayed development was not observed to 0.01% concentration of stipe and pileus treated-embryos. These results clearly indicate that the effect of *V. volvacea* on the delayed development of zebrafish was dependent on the parts of the mushroom and concentrations of the extracts.

Morphological abnormalities or malformations are the most important teratogenic parameters in *D. rerio* teratogenicity assay. Apparently, all embryos exposed at 0.10% and some embryos at 0.05% of both extracts showed different malformations (Figure 1). Larvae at 0.10% of both pileus and stipe extract showed twisted tail and body whereas the pileus and stipe extracts at 0.05% caused bent tail tip and hook-like tail, respectively. These malformations of larvae could be associated as effects of the delayed development of embryos at these two concentrations. No malformation was recorded to those embryos at 0.50% concentration of both extracts because embryos at 3% and 1% coagulated after 12 hours of exposure while no visual heartbeat was observed to those at 0.50% after 36 hours of exposure. All embryos at 0.01% of both pileus and stipe extracts and at embryo water were found normal.

Table 1: Mortality of *D. rerio* embryos after 12, 24, 36, and 48 hours of exposure to varying concentrations of the pileus and stipe extracts of *V. volvacea*

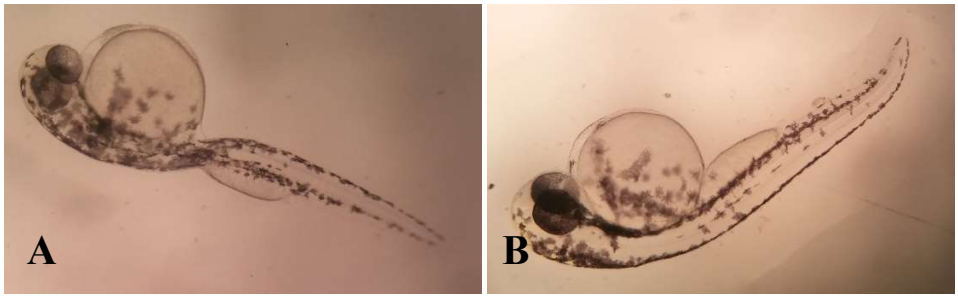
Mushroom Extract	Concentration (%)	Mortality (%)			
		12 hours	24 hours	36 hours	48 hours
Pileus	3.00	100.00[a]	100.00[a]	100.00[a]	100.00[a]
	1.00	0.00[b]	100.00[a]	100.00[a]	100.00[a]
	0.50	0.00[b]	0.00[b]	75.00[b]	100.00[a]
	0.10	0.00[b]	0.00[b]	0.00[c]	0.00[b]
	0.05	0.00[b]	0.00[b]	0.00[c]	0.00[b]
	0.01	0.00[b]	0.00[b]	0.00[c]	0.00[b]
	0.00	0.00[b]	0.00[b]	0.00[c]	0.00[b]
Stipe	3.00	100.00[a]	100.00[a]	100.00[a]	100.00[a]
	1.00	100.00[a]	100.00[a]	100.00[a]	100.00[a]
	0.50	0.00[b]	0.00[b]	100.00[a]	100.00[a]
	0.10	0.00[b]	0.00[b]	0.00[b]	0.00[b]
	0.05	0.00[b]	0.00[b]	0.00[b]	0.00[b]
	0.01	0.00[b]	0.00[b]	0.00[b]	0.00[b]
	0.00	0.00[b]	0.00[b]	0.00[b]	0.00[b]

Treatment means of each extract having the same letter of superscript are not significantly different from each other at 5% level of significance using LSD

Table 2. Percentage hatchability and delayed growth after 48 hours and percentage malformation after 72 hours of *D. rerio* exposed to varying concentrations of the pileus and stipe extracts of *V. volvacea*

Mushroom Extract	Concentration (%)	Hatchability (%)	Delayed development (%)	Malformation (%)
Pileus	3.00	0.00[c]	100.00[a]	Coagulated*
	1.00	0.00[c]	100.00[a]	Coagulated*
	0.50	0.00[c]	100.00[a]	Dead*
	0.10	0.00[c]	100.00[a]	100.00[a]
	0.05	75.00[b]	25.00[b]	16.67[b]
	0.01	100.00[a]	0.00[c]	0.00[c]
	0.00	100.00[a]	0.00[c]	0.00[c]
Stipe	3.00	0.00[c]	100.00[a]	Coagulated*
	1.00	0.00[c]	100.00[a]	Coagulated*
	0.50	0.00[c]	100.00[a]	Dead*
	0.10	0.00[c]	100.00[a]	100.00[a]
	0.05	41.67[b]	58.33[b]	33.33[b]
	0.01	100.00[a]	0.00[c]	0.00[c]
	0.00	100.00[a]	0.00[c]	0.00[c]

Treatment means of each extract having the same letter of superscript are not significantly different from each other at 5% level of significance using LSD. *No malformation was recorded due to the coagulated and dead embryos at earlier period of observation.



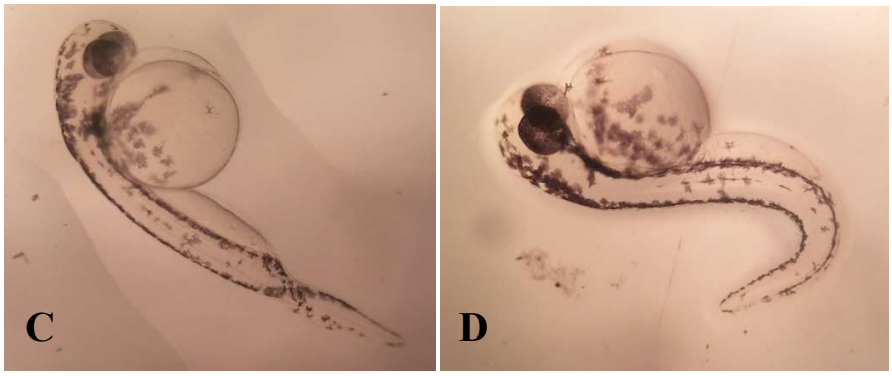


Figure 1: Morphological abnormalities of zebrafish larvae at 0.1% (A) and 0.05% (B) of pileus extract and at 0.1% (C) and 0.05% (D) stipe extract of *V. volvacea* after 72 hours of exposure

CONCLUSION

Although edible, nutritious and medicinal, *V. volvacea* fruiting bodies collected from the wild could exhibit toxic and teratogenic properties at concentration, exposure time, and mushroom part dependent manner. This important activity of *V. volvacea* could also be possibly dependent on the substrate and/or origin of fruiting bodies, which we need to comfirm in the next study. Fruiting bodies collected from the wild probably contain elements or agents that are absorbed by the mushroom from their natural habitat, which may contribute to the toxic and teratogenic effects of mushroom. This interesting matter is currently under investigation.

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