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Bioaccumulation of insecticide in *Agaricus subrufescens*

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ABSTRACT

Agaricus subrufescens, known as Sun Mushroom, is a high-value mushroom because of its medicinal properties, used as nutraceutical food to stimulate the immune system and to prevent some diseases, including cancer. Mushrooms are generally characterized by their great ability to bioaccumulate heavy metals and other toxic substances from the mushroom compost. Sugarcane bagasse is a common raw material used in mushroom compost production for *Agaricus subrufescens* cultivation, whereas insecticides, such as fipronil, can be applied to combat several pests in sugarcane crops. For this reason, we aimed to assess mushroom yield and fipronil bioaccumulation in Sun Mushroom, regarding different concentrations added to the mushroom compost (0, 8, 16 and 32 mg kg^{-1}) and casing layer (0, 2, 4 and 8 mg kg^{-1}). Each experiment was arranged in a completely randomized design with four replicates. Regression analysis from mushroom production data was applied using SISVAR 5.1 program. Fipronil was quantified using gas-liquid chromatography (HP 6890) with thermionic detector (NPD). Mushroom yield was affected when fipronil was added, decreasing from 12% (compost with 0 mg kg^{-1} of fipronil) to 4.8% (compost with 32 mg kg^{-1}). However, bioaccumulation was not detected. In contrast, insecticide bioaccumulation was detected when adding fipronil to casing layer, increasing from <0.01 mg kg^{-1} (casing layer with 2 mg kg^{-1}) to 0.26 mg kg^{-1} (casing layer with 8 mg kg^{-1}), however, mushroom yield was not affected.

Keywords: Sun mushroom, edible mushroom, fipronil, pest control.

RESUMO

Bioacumulação de inseticida em *Agaricus subrufescens*

O *Agaricus subrufescens*, conhecido popularmente como cogumelo do sol, é muito consumido como nutricêutico devido ao seu valor medicinal, sendo usado como um estimulante do sistema imunológico e no tratamento de várias doenças, incluindo o câncer. Cogumelos comestíveis, de uma forma geral, apresentam capacidade de bioacumular metais pesados e outras substâncias tóxicas a partir do substrato de cultivo. O bagaço de cana-de-açúcar é um dos resíduos mais utilizados na produção de composto para o cultivo de *Agaricus subrufescens*. Entretanto, no cultivo da cana-de-açúcar, diversos inseticidas como o fipronil, podem ser aplicados para combater pragas. Em função do exposto, objetivou-se avaliar a bioacumulação de fipronil pelo cogumelo do sol, a partir da adição de diferentes concentrações do inseticida no composto e na camada de cobertura. Para avaliar a produtividade deste cogumelo, fipronil foi adicionado ao substrato de cultivo nas concentrações de 0, 8, 16 e 32 mg/kg. Outro experimento foi realizado para testar a capacidade do cogumelo de bioacumulação do fipronil, adicionando-o à camada de solo de cobertura nas concentrações de 0, 2, 4 e 8 mg/kg. Foi feito um delineamento inteiramente casualizado com quatro repetições. Os dados de produção de cogumelos foram submetidos à análise de regressão pelo SISVAR (Sisvar 5.1). Fipronil foi quantificado utilizando-se cromatografia gás-líquido (HP 6890) com detector termiônico (NPD). A adição de fipronil ao composto afetou a produtividade, com redução de 12% (composto sem fipronil) e para 4,8% (composto com 32 mg kg^{-1}). No entanto, a bioacumulação não foi detectada. Em contraste, a bioacumulação do inseticida foi detectada quando o fipronil foi adicionado à camada de cobertura, aumentando de <0,01 mg kg^{-1} (camada de cobertura sem fipronil) para 0,26 mg kg^{-1} (camada de cobertura com 8 mg kg^{-1}), no entanto, a produtividade do cogumelo não foi afetada.

Palavras-chave: Cogumelo do sol, cogumelo comestível, fipronil, controle de pragas.

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Brazil, due to its climatic conditions, stands out as the largest world producer of sun mushroom (*Agaricus subrufescens*), exporting approximately 95% of its total production, mainly to the Japanese market (Tomizawa *et al*., 2007). This makes it a viable economic alternative based on the value paid, R$189,00 for the dehydrated form (Gern *et al*., 2010).

The growing interest in cultivation and commercialization of this mushroom is due to its nutritional and therapeutic properties reported in the scientific literature (Delmanto *et al*., 2001). The sun mushroom is considered a functional food and nutraceutical product (Soares *et al.,* 2009) containing β-glucan with antitumor, immunomodulatory, and antibacterial activity that have been previously reported (Delmanto *et al.*, 2001; Wasser, 2002; Mantovani *et al.*, 2008) in basidiocarps grown in different substrates. Currently, in addition to its medicinal properties, this mushroom has also aroused interest for its culinary qualities (Escouto *et al.*, 2005; Dias, 2010).

Several agro-industrial residues, such as corn, sugarcane bagasse, coffee pulp, banana leaves, soybean pulp, cereal straw, among others, are used for mushroom compost production (Bonatti *et al.*, 2004; Salmones *et al.*, 2005). In Brazil, fipronil 5-amino-1-[2.6-dichloro-4-(trifluoromethyl)

phenyl]–4-(trifluoromethylsulfinyl)-1H-pyrazole-3-carbonitrile is an insecticide used for pest control in crops (Scharf & Siegfried, 1999; Wilde *et al.*, 2001). This insecticide effectively controls insects in rice, citrus, cotton, corn, mango, sugarcane, and sunflower, among others (Hadjmohammadi *et al.*, 2006). However, using this insecticide may lead to formation of toxic metabolites in the environment (Gunasekara *et al.*, 2007), such as sulfone and desulfinyl, which are photodegradation products reported as toxic for insects, mammals, fishes, and birds (Das *et al.*, 2006).

In addition, it is also known that exposure to pesticides is neurotoxic to rodents and other mammals, including humans (Terçariol & Godinho, 2011) due to their wide commercial and domestic uses (Tingle *et al.*, 2003).

Furthermore, fipronil, is also indicated for pest control during *Agaricus bisporus* cultivation (Shamshad *et al.*, 2009; Erler *et al.*, 2011) and could also be used for the same purpose in sun mushroom cultivation. It can be mixed into the casing layer for *Agaricus bisporus* cultivation for pest control (Shamshad *et al*., 2009; Erler *et al*., 2011).

Sun mushroom may bioaccumulate fipronil from the compost if it is present in the straw. It is known that edible mushrooms are characterized by the ability to accumulate heavy metals (Vetter & Berta, 2005; Garcia *et al.*, 2009) and other toxic substances from the substrate or environments where they reproduce (Kalac, 2010). Thus, this attribute makes the quality of mushroom compost a concern for sun mushroom cultivation, since a natural and functional food is expected to also be free of contamination by heavy metals and pesticides.

Therefore, we aimed to assess the effect of fipronil insecticide on sun mushroom yield and to test the mushroom's bioaccumulation of this insecticide at different concentrations in compost and soil casing layer.

MATERIAL AND METHODS

The sun mushroom [*A. subrufescens* (CS1)] was provided by the laboratory of the Federal University of Lavras, Brazil. Inoculum spawn was prepared using rice husks enriched with 10% wheat bran, autoclaved successively for two hours. For the compost, were used sugarcane bagasse (*Saccharum officinarum*), coast-cross hay (*Cynodon dactylon*), and wheat bran as raw material. The compost was prepared as described by Siqueira *et al.* (2009), except for composting interval, which was only four weeks.

To assess sun mushroom yield, fipronil was added to the compost at concentrations of 0, 8, 16, and 32 mg kg^{-1}, considering the dry matter of the colonized substrate. Another experiment was conducted to test the mushroom's ability to bioaccumulate fipronil from casing layer, so it was added to the casing soil at concentrations of 0, 2, 4, and 8 mg kg^{-1}, and then added to the mushroom compost at the same concentrations used to test yield. The experiment was a completely randomized design with four replicates.

The mushroom samples were lyophilized, ground, and weighed. Next, 4 g were transferred to Erlenmeyer flasks to extract the insecticide by adding 100 mL of acetone and agitation for 4 hours. The extract was purified by filtering through a funnel with Büchner flask, coupled to a vacuum pump. A 20 mL aliquot of extract was transferred to a round-bottomed flask (50 mL) and placed inside a rotaevaporator with 56°C bath to evaporate the acetone (and water from the sample). Next, the extract was transferred to a separation funnel and subjected to successive partitions with 20 mL of dichloromethane. After agitating the funnel for one minute and subsequent rest for five minutes, the organic phase (bottom) was collected. Next, the dichloromethane was removed using a round-bottomed flask in a rotaevaporator. Partition was repeated by adding 20 mL of dichloromethane, which was then eliminated in a rotaevaporator and the residue was transferred into a centrifuge tube containing 2 mL of acetone. This tube was placed in a freezer (-20ºC) until purification.

Purification was carried out by thin layer chromatography (TLC) technique. An aliquot of 2 mL was transferred to a 50 mL round-bottomed flask and acetone was removed using a rotaevaporator with 56ºC bath. Next, the residue was successively washed three times with 0.25 mL of acetone and then transferred to a glass chromatoplate (20x10 cm) containing 0.75 mm of silica-gel (60 GF_{254}), used as stationary phase. The sample was distributed along a 3 cm line on the inside of the plate, where standard fipronil solution was added to the margins along the same line where samples were applied. The margins were isolated by removing the silica along the vertical lines with a pencil.

A chromatoplate was placed inside a mobile, glass container containing a mixture of hexane and acetone (175:75), leaving only the base of the plate submerged. Next, the solvent mixture reached a height of approximately 2 cm below the top edge of the chromatoplate. The plate was removed from the container and placed to dry under laminar flow with the hood turned on. Under ultraviolet light, the fipronil band was identified and transferred the silica to a glass funnel containing cotton. The glass funnel was suspended in a round-bottomed flask and the sample was washed three times using 10 mL of acetone to remove the fipronil absorbed in the silica-gel. The acetone was eliminated in a rotaevaporator with 56ºC bath. The residue was dissolved in 1 mL of acetone and stored in a freezer (-20ºC) to quantitatively determine the fipronil insecticide.

After extracting and purifying, the fipronil was quantified using a gas-liquid chromatography system (HP 6890) with thermionic detector (NPD). A HP-5 capillary column (0.25 μm film thick, 30 m long, 0.32 mm internal diameter) was used. The operating conditions were: oven temperature: 100ºC (2 min), increasing 20ºC/minute until reaching 280ºC; injector temperature: 260ºC; detector temperature: 300ºC, carrier gas (N_2) flow at 2.3 mL min^{-1} ("make-up" 30 mL min^{-1}); synthetic air flow: 60 mL min^{-1}; H_2 flow: 30 mL min^{-1}; injection mode: splitless; purge time: 2 minutes; injection volume: 4 μL.

Fipronil with 98.2% purity was used as analytical standard. Efficiency of the analytical methods was determined by analyzing the fipronil samples at concentrations of 0.1 and 1.0 μg g^{-1}. Recovery percentages were found to be above 90%.

The mushroom production data were subjected to regression analysis by SISVAR (Sisvar 5.1).

RESULTS AND DISCUSSION

Sun mushroom yield was affected by adding fipronil to the mushroom compost (Figure 1) with increasing concentrations. The mushroom yield was affected by the presence of fipronil in the compost at all pesticide concentrations. Lower mushroom yield tended to occur at higher fipronil concentration in the compost, but with insignificant differences. Shamshad *et al.* (2009) reported that adding insecticides into casing layer or compost has been associated with reduced mushroom yield. Therefore, although not being fungicides, they show some kind of toxicity against *Agaricus bisporus.*

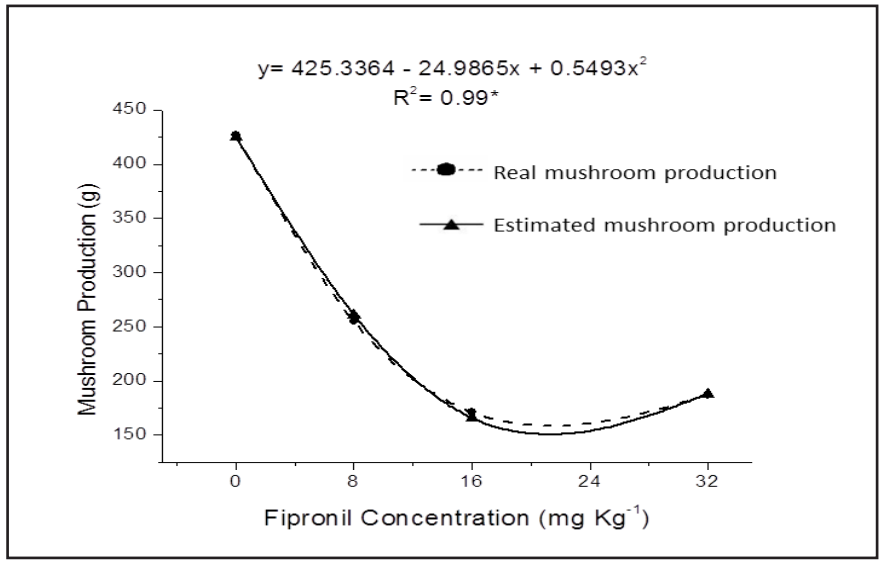

Figure 1. Productivity of the sun mushroom (*Agaricus subrufescens*) using mushroom compost with fipronil at increasing concentrations (produtividade do cogumelo do sol (*Agaricus subrufescens*) utilizando composto com concentrações crescentes de fipronil). Lavras, UFLA, 2008.

However, the fipronil was not detected in mushrooms produced on pesticide-contaminated compost (Table 1). This information is noteworthy because the presence of fipronil in the compost affected mushroom yield, but the pesticide was not translocated to the fruiting bodies.

Based on these results, another study was conducted to test the effect of fipronil accumulation in sun mushroom when added to soil casing layer (Table 1). Unlike the first experiment, the results showed that fipronil bioaccumulated in the mushrooms, however there was no decreased yield due to fipronil added to casing. According to the results, there is a difference of bioaccumulation between the two environments (compost and soil). According to Forgarty & Touvinen (1991), pesticide may be degraded during the composting process, however, in the present study, fipronil was only added when the compost was ready. Another important aspect is that although bioaccumulation did not occur, fipronil present in the compost drastically reduced mushroom yield. Therefore, it is unlikely that fipronil degradation in the compost is a response to non-bioaccumulation in mushrooms. A possible explanation may be fipronil's strong adsorption into organic matter present in compost, which probably occurs more intensely than in soil.

According to that reported by Bobé *et al.* (1997) and Gunasekara *et al.* (2007), fipronil adsorption is higher in soils containing higher percentages of organic matter. This demonstrates that the organic fraction in soil is primarily responsible for fipronil adsorption, and consequently, adsorption is expected to be more intense in compost than in any other soil type. Therefore, it is possible that although affecting yield, fipronil adsorption by the organic fraction impairs the insecticide from being translocated to the fruiting body. Thus, it can be inferred that the differences in bioaccumulation are more related to adsorption intensity, which would be more intense in compost than in soil. Studies indicate that fipronil is relatively mobile in soil (Dpr, 2001; Gunasekara *et al.*, 2007), which corroborates this hypothesis and would explain its easy translocation to fruiting bodies when the pesticide was added to casing layer.

However, one cannot also rule out the possibility that, although still toxic, the insecticide has been partially

Table 1. Bioaccumulation of fipronil added to mushroom compost (*Agaricus subrufescens*) at concentrations of 0, 8, 16, and 32 mg kg^{-1} and bioaccumulation of fipronil in mushrooms cultivated on casing layer soil contaminated with increasing concentrations of the pesticide (bioacumulação de fipronil adicionado ao composto de cultivo (*Agaricus subrufescens*) em concentrações de 0, 8, 16, e 32 mg kg^{-1} e bioacumulação de fipronil no fungo cultivado com solo da camada de cobertura com concentrações crescentes de pesticida). Lavras, UFLA, 2008.

Fipronil concentration on mushroom compost	Fipronil content in mushrooms	Fipronil concentration at casing layer	Fipronil content in mushrooms
		(mg kg^{-1})	
0	<0.01	0	<0.01 c
8	<0.01	2	0.03 c
16	<0.01	4	0.08 b
32	<0.01	8	0.26 a

Means followed by the same letter do not differ by Scott-Knott test at 5% probability [medias seguidas por letras iguais não diferem pelo teste de Scott-Knott (5%)].

degraded to an intermediate form (Connelly, 2001; Fenet *et al.*, 2001; Demchek & Skrobialowski, 2003). Similarly, this intermediate form may be present in the mushrooms even without being detected, since the method was performed to detect only the original form (Forgarty & Touvinen, 1991). In contrast, degradation could have also occurred in the casing layer since the process is common in soil. However, observing the pattern of mushroom yield, there was no effect of possible fipronil degradation during the cultivation cycle.

According to Erler *et al.* (2011), the use of synthetic chloropyrific insecticides exhibited a mycotoxic effect against *Agaricus bisporus*, causing reduced yield when applied to the casing layer. This is unlike the insect growth regulators used as insecticides, which promoted higher yield compared to the negative control. Therefore, the mycotoxic effect of insecticides has already been reported; however, in the present study this effect was observed in compost but not in casing layer.

Considering the technological aspect of using pesticide for pest control, we may suggest that fipronil can be used only in casing layer, since under these conditions yield is uncompromised. However, considering the quality of natural and functional foods, use of fipronil resulted in pesticide-contaminated mushrooms, thus it is not recommended for consumers.

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