



# Sensitivity of mycelial growth of mushroom *Agrocybe semiorbicularis* to different concentrations of different disinfectants

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## Abstract

This study was performed to determine the effects of different types of disinfectants (Hypo, Izal, and Dettol) on the mycelial growth of the mushroom *Agrocybe semiorbicularis*. The more evolutionarily advanced mushroom mycelium was expected to show greater resistance to disinfectants than other fungal and bacterial contaminants. Minimal disinfectant concentration was the one at which contaminants were inhibited, while the growth of the desired mushroom mycelia remained unaffected. Different concentrations of different disinfectants were added to the growth media, and the pure mushroom mycelial culture was inoculated on the media and left to grow. The results revealed that the probability of contamination was higher in all the concentrations of Hypo and in lower concentrations of Dettol and Izal. At 5% concentration of the disinfectants (Hypo, Izal, and Dettol), the mean values of contamination were 0.667, 0.417, and 0.00 ( $P < 0.001$ ), respectively. At 10% concentration, the mean contamination values were 0.167, 0.583, and 0.333 ( $P > 0.05$ ), respectively, while at 15% concentration, the mean contamination values were 1.000, 0.417, and 0.250 ( $P < 0.05$ ), respectively. At higher concentrations of the disinfectants, the growth of contaminants was completely suppressed, and the growth of the desired mycelia was also significantly decreased. At 17% concentration of the disinfectants (Hypo, Izal, and Dettol), the mean values of contamination were 0.833, 0.833, and 0.00 ( $P < 0.05$ ), respectively. At 18% disinfectant concentration, the mean contamination values were 0.167, 0.00, and 0.00 ( $P > 0.05$ ), respectively, while at 20% disinfectant concentration, the mean contamination values were 0.583, 0.00, and 0.00 ( $P < 0.05$ ), respectively. The mean values of the mushroom's mycelial growth for the three disinfectants (Hypo, Izal, and Dettol) were 6.26, 15.38, and 21.93 mm ( $P < 0.05$ ), respectively, for 5% concentration; 11.75, 10.08, and 13.90 mm ( $P > 0.05$ ), respectively, for 10% concentration; and 0.00, 12.88, and 18.33 mm ( $P < 0.05$ ), respectively, for 15% concentration. At higher concentrations of the disinfectants (Hypo, Izal, and Dettol), the mean values of the mushroom's mycelial growth were 16.92, 11.90, and 14.33 mm ( $P > 0.05$ ), respectively, for 17% concentration; 18.54, 3.00, and 8.71 mm ( $P > 0.05$ ), respectively, for 18% concentration; and 13.50, 13.25, and 0.00 mm ( $P > 0.05$ ), respectively, for 20% concentration. Disinfectants that yielded 100% (12/12) growth of pure cultures were 18% and 20% concentrations of Izal and 15% concentration of Dettol ( $P < 0.05$ ). Dettol at concentrations of 10%, 17%, and 18% yielded 66.7% (8/12) of pure cultures, but with a significant decrease in growth ( $P < 0.05$ ) and viability; moreover, no contaminants survived at these concentrations. In general, mushroom mycelia were found to exhibit a higher degree of resistance to disinfectants than fungal and bacterial contaminants. Pure mycelial cultures were obtained in almost all the concentrations of all the disinfectants, but there were trade-offs between the levels of contaminations and better growth of the desired mycelia. Dettol was found to have the highest effect on suppressing contaminant growth followed by Izal and the least effective was Hypo. The result of this study will help in the process of mushroom production by reducing the problems of contamination.

**Key words:** *Agrocybe*, contamination, disinfectant, Dettol, mycelia, mushroom

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## Introduction

Mushrooms are spore-bearing distinctive fruiting bodies of macrofungi that are produced above ground or on other growth substrates (Wani et al., 2010; Kumar, 2015). Generally, mushrooms are useful in economic, medicinal, and ecological applications. They have been used as food, medicine, and cosmetics in different parts of the world for centuries. Some mushroom species are sources of bioactive compounds such as ascorbic and organic acids, alkaloids, carotenoids, enzymes, flavonoids, folates, glycosides, lectins, and phenolics, which afford them medicinal properties (Kalac, 2013; Kumar, 2015; Valverde et al., 2015). Some mushrooms are ectomycorrhizal fungi that play important environmental and ecological roles. They form mutualistic relationships between plant roots and specific fungi (Rossi et al., 2007). Ectomycorrhizas provide the plants with efficient water and nutrient uptake, while the fungus obtains photosynthates from the plant (Jha and Kumar, 2011; Klavina, 2015; Torres-Aquino et al., 2017). Hence, they improve agricultural productivity in nutrient-deficient soils (Hryniewicz and Baum, 2011). Additionally, some ectomycorrhizas are found to be efficient in preventing uptake and accumulation of heavy metals in plant tissues and are therefore used for reclaiming soils contaminated with heavy metals (Ingrid, 2011; Nanda and Abraham, 2013). However, for practical and extensive exploitation of the benefits of mushrooms, their artificial production is necessary. The business of mushroom production provides job opportunities, thereby providing an alternative source of income in many countries (Celik and Peker, 2009).

Generally, the cultivation of useful fungi involves obtaining pure mycelia culture of the fungi, followed by the preparation of spawn (Bankole and Salami, 2017). The first critical stage of successful cultivation of any mushroom is the production and maintenance of absolutely pure mycelial culture and spawn. Growth of mushroom mycelial culture is highly affected by several factors such as growth media, pH, temperature, moisture content, high relative humidity, and light intensity. The environmental conditions that are optimal for the growth of mushroom mycelia are also, however, ideal for the growth of microbial contaminants (László et al., 2010; Sardar et al., 2015). Maintaining a suitable environment and controlling the contamination during spawn production are extremely difficult and labor-intensive (Uddin,

2012). The process of suitable spawn production requires several operations that are carefully performed in absolute hygiene conditions to avoid any contamination. These requirements make it a highly technical process that requires specialized scientific facilities and equipment (Sánchez, 2004; Rosmiza et al., 2016).

The minimal requirements for proper spawn production include laboratory equipment such as pressure cooker, autoclave, and inoculation box or laminar air flow cabin, which are commonly available in hospitals, research institutions, and universities (Van Nieuwenhuijzen, 2007; Dias, 2010). Developed countries usually employ computerized environmental control systems that are capable of monitoring environmental parameters and thereby increase the production of mushroom cultures at relatively low cost (Sánchez, 2004). In developing countries, mushroom growers use low technology because the knowledge is too complex, equipment for maintaining spawn production is very expensive, and the process is time-consuming (Kües, 2007; Ndem and Martha, 2016).

Obtaining pure mycelial cultures and the subsequent spawn are the major limiting factors to mushroom cultivation in developing countries (Sardar et al., 2015). The process requires practical exclusion of other contaminant microorganisms, involving strict hygiene conditions that are generally too costly and impractical to operate on a large scale especially in low income countries. Unfortunately, any failure to achieve a satisfactory harvest may often lead to heavy loss in mushroom production (Choi et al., 2010; Belletini et al., 2016; Chang and Wasser, 2017). The contaminants exhibit fast growth and usually out-compete the mycelia of mushrooms for space and nutrients. The contaminants also produce extracellular enzymes and other toxic secondary compounds that can result in a significant decrease in mushroom biomass production or even entirely eliminate it (Mwangi et al., 2017). To prevent contamination, various treatments are used for the preparation of cultures to inhibit competitive fungi, which include disinfection treatments applied to growth substrates at different production phases. For this purpose, the minimal inhibitory concentrations of the disinfectant have to be determined first (László et al., 2010; Colavolpe et al., 2014). Hence, mushroom growers usually obtain their spawn from producers such as universities and other research institu-

tes, which limit their production capacities. These requirements and other technical challenges undoubtedly restrict mushroom production especially in developing countries (Van Nieuwenhuijzen, 2007; Dias, 2010). To boost mushroom production, it is therefore necessary to find cheaper and easier methods of controlling contaminations, so that ordinary small-scale mushroom growers can produce their own spawn (Van Nieuwenhuijzen, 2007).

Mushrooms belong to the phylum *Basidiomycota*, and they are considered to be the most complex and evolutionarily advanced members of the fungi kingdom. Their hyphae are uniquely different from those of all other fungi species, with highly characteristic features and “cellular” compositions (Oberwinkler, 2012; De Mattos-Shipley et al., 2016). The present study aimed to determine the effects of different types of disinfectants on the mycelial growth of the mushroom *Agrocybe semiorbicularis*. Specifically, the study’s objective was to determine the minimal concentration of these disinfectants that can inhibit contaminants and at the same time does not affect the growth of the desired mushroom mycelia. We predicted that the mushroom mycelia, being the most complex and evolutionarily advanced, will also show a higher degree of resistance to disinfectants than other fungal and bacterial contaminants.

## Materials and methods

### Collection of mushrooms

Sporocarps (fruiting bodies) of *A. semiorbicularis* (family: Strophariaceae) were collected from the wild in the rainy season and transported in plastic bags. The specimens were cleaned of debris and then sun-dried for 5 days. After drying, the specimens were put in an airtight polythene bag and stored in a cardboard box at room temperature until use (Fig. 1).

### Obtaining pure mycelial culture

Three different media were initially used to obtain the pure mycelial culture and to ascertain the best media for mycelia growth (Peksen et al., 2013; Mahadevan and Shanmugasundaram, 2018). The media used were malt extract agar (Accumix<sup>TM</sup>), yeast extract agar (Accumix<sup>TM</sup>), and potato dextrose agar (PDA, TM Media<sup>TM</sup>). They were prepared according to the manufacturer’s instructions. The media were autoclaved at 1.1 kg/cm<sup>2</sup> (121 °C) for 20 min. To suppress bacterial growth, chlor



Fig. 1. Mushroom species used in the study

amphenicol (0.03 g/l) was added to the media at 45 °C. Approximately 20 ml of the media was then poured into Petri dishes (90 × 15 mm) inside a sterilized laminar flow hood and subsequently solidified (Mendoza et al., 2014). The mushroom samples were then surface sterilized by spraying 70% ethanol and cut into pieces (approximately 1 cm<sup>2</sup>) with a sterile scalpel. The pieces were then placed with a pair of forceps in the prepared media in a way such that their gills were in direct contact with the media. The mushrooms placed on Petri dishes were then incubated at 28 °C in complete darkness. After 4–7 days, the mycelia were subcultured into new Petri dishes to obtain a pure mycelial culture. Pure mycelia in Petri dishes without contamination were used for the experiment. PDA was finally adopted as the preferred growth media for the subsequent experiments.

### Preparation of media and disinfectants and inoculation of mycelia

The disinfectants used for the experiment were Dettol<sup>®</sup> (chloroxyleneol), Hypo<sup>®</sup> (sodium hypochlorite), and Izal<sup>®</sup>. These are some of the most commonly used disinfectants in this region (Chima et al., 2013). Different concentrations of disinfectants were prepared using distilled water: 5, 10, 15, 18, and 20% and control (distilled water). Ten milliliters of each of the different concentrations of the disinfectants were aseptically added to 200 ml (1 : 20 disinfectant/media ratio) of the prepared PDA growth media in conical flasks at 45 °C and then vigorously shaken to homogenize. Twenty milliliters of the media were poured into Petri dishes and solidified. Actively growing mycelia were taken at 10 days from the

periphery by using a cork borer and placed on two points of each Petri dish (90 × 15 mm). To increase the probability of normal aerial contamination, the inoculation was not performed inside the laminar flow hood. Each treatment was replicated in six Petri dishes. The Petri dishes were incubated in complete darkness at 28 °C for 7 days. The optimal concentrations of disinfectants for mycelia growth were determined by measuring colony diameters (Luangharn et al., 2014; Mendoza et al., 2014).

### **Measurements and data collection**

The growth-colony radius of radial mycelia was recorded using a ruler across the Petri dish (Mahadevan and Shanmugasundaram, 2018; Sharma et al., 2018). The size of the growth-colony radius was estimated by measuring the vertical and horizontal diameters of the mycelia. The sum of the radial measures in millimeter (mm) was divided by four (4) and was regarded as the radial growth for each of the concentrations of the disinfectants (Chima et al., 2013). The level of contamination was taken as the ratio between the numbers of contaminated cultures and the total number of cultures, which were 12. The values ranged from 0.00 to 1.00. Viability of the mushroom mycelia was also taken as the ratio between the number of growing mushroom mycelia inoculums and the total number of inoculums. Here, the values ranged from 0.00 to 1.00.

### **Data analysis**

The data obtained were subjected to analysis of variance (ANOVA) followed by Tukey's Multiple Comparisons of means using Minitab<sup>®</sup> 18.1.

## **Results**

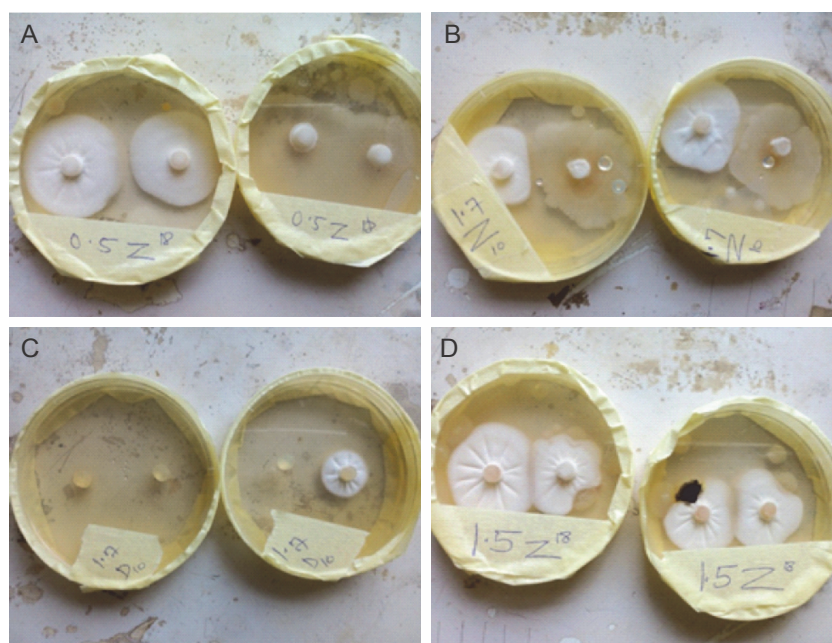
### **Effects of different concentrations of different disinfectants on mycelial growth**

The results of this study show that the rate of the mushroom's mycelial growth, contamination, and the viability of the hyphae from the original mycelia plug inoculums differed depending on concentrations of various disinfectants (Fig. 2). The results also confirmed that mushroom mycelia, being the most complex and evolutionarily advanced, also have a higher degree of resistance to disinfectants than other fungal and bacterial contaminants. At 5% concentration of disinfectants (Hypo, Izal, and Dettol) in standard of 1:20 ml disinfectant/media ratio, the mean radial growth of mycelia co-

lony for Dettol and Izal were significantly ( $P=0.00$ ;  $\alpha=0.05$ ) higher than that for Hypo (Table 1). The mean values of the radial growth for Dettol, Izal, and Hypo were 21.93, 15.38, and 6.26 mm, respectively. All the original mycelia plug inoculums were viable at the time of measurements, but the extent of contamination was significantly higher in Izal and Hypo than in Dettol. All the mycelia cultures in Dettol were pure, i.e., there were no contaminations. This clearly shows that the reduced mycelial growth in Izal and Hypo is due to high rate of the contamination. At 10% concentration of all the three disinfectants, there were no significant differences in contamination and viability of the hyphae (Table 1). The mean contamination values were 0.167, 0.583, and 0.333 ( $P=0.106$ ), respectively. For the mycelial growth, the mean values were 11.75, 10.08, and 13.90 mm ( $P=0.623$ ) for Hypo, Izal, and Dettol, respectively; their mean viability values were 0.583, 0.667, and 0.667 ( $P=0.895$ ), respectively.

Significant differences were observed in the rate of the mushroom's mycelia growth, contamination level, and the viability of the hyphae from the original mycelia plug inoculums across all the different disinfectants at 15% concentration. There was a significant decrease in mycelia growth (0.00 mm) and increase in contamination (1.00) in Hypo ( $P=0.000$ ), and decrease in hyphae viability in Izal, which was 0.583 ( $P=0.002$ ) (Table 1). At 17% concentration of Dettol, there was a significant decrease in contamination (0.00) and viability of hyphae (0.583). Decrease in mycelial growth (14.33 mm) was also observed but not statistically significant ( $P=0.462$ ). This suggests that the decrease in mycelia growth was due to higher concentration of the disinfectants rather than suppression by the competitive contaminants (Table 1).

At 18% concentration of disinfectants, there was a significant decrease in mycelia growth and hyphae viability in Dettol, while contaminations were completely absent (Table 1). The mean values for the growth, viability, and contaminations were 8.71 mm, 0.583, and 0.00, respectively. When using Izal as disinfectant, no contamination has been noted, mycelia growth decreased but viability of hyphae remained significantly high. The mean values for the growth, viability, and contaminations were 3.00 mm, 1.00 and, 0.00, respectively. The usage of Hypo led to insignificant presence of contaminants but significantly high rate of mycelia growth and



**Fig. 2.** A) mushroom mycelial growth showing pure mycelial culture and suppressed growth by bacteria in two different plates, and B) in the same plate; C) complete inhibition of growth (nonviable hyphae) and suppression of growth by disinfectant; D) effect of fungal and bacterial contaminants on the shape of mycelial growth pattern

**Table 1.** Mean values of the effect of 5, 10, 15, 17, 18, and 20% concentrations of disinfectants (Hypo, Izal, and Dettol) on *Agrocybe semiorbicularis* mycelia in 1:20 ml disinfectant/media ratio; significance level ( $\alpha$ ) = 0.05

Factor		5%	10%	15%	17%	18%	20%
Growth radius [mm]	N	mean	mean	mean	mean	mean	mean
Hypo	12	6.26*	11.75	0.00*	16.92	18.542*	13.50*
Izal	12	15.38	10.08	12.88	11.90	3.000	13.250
Dettol	12	21.93	13.90	18.33	14.33	8.71	0.000
Contamination [count]							
Hypo	12	0.667*	0.167	1.000*	0.833*	0.167	0.583*
Izal	12	0.417	0.583	0.417	0.833	0.000	0.000
Dettol	12	0.000	0.333	0.250	0.000	0.000	0.000
Viability [count]							
Hypo			0.583	1.000*	0.92*	1.000*	1.000*
Izal			0.667	0.583	1.000	1.000	1.000
Dettol			0.667	1.000	0.583	0.583	0.000

\* $P < 0.05$

viability of hyphae. Here, the mean values were 18.54 for the growth, 1.00 for viability, and 0.167 for the contaminations. In 20% concentration of Dettol, no contamination, no viability of mycelia and therefore, no growth

were noted, and at the same concentration, there was viability of hyphae and mycelia growth in Hypo and Izal; moreover, there was significant contamination in Hypo but no contamination in Izal ( $P = 0.000$ ) (Table 1). The

**Table 2.** Number of pure mycelial cultures and their growth obtained using different concentrations of disinfectants (Hypo, IZal, and Dettol) in growth media; significance level ( $\alpha$ ) = 0.05

Factor	Pure culture	Percentage	Contamination	Mycelia growth (mm)					
				N	mean	st. dev.	pooled st. dev.	P-value	TPC*
Hypo									
5%	4	33.3	8	12	6.26	9.28	7.362	0.000	BC
10%	5	41.7	2	12	11.75	10.69			AB
15%	0	0.00	12	12	0.000	0.000			C
17%	2	16.7	8	12	16.92	8.94			A
18%	10	83.3	2	12	18.542	2.965			A
20%	5	41.7	5	12	13.50	6.02			AB
IZal									
5%	7	58.3	5	12	15.38	8.03	6.987	0.001	A
10%	5	41.7	7	12	10.08	6.43			AB
15%	7	58.3	5	12	12.88	9.77			A
17%	2	16.7	8	12	11.90	9.50			A
18%	12	100	0	12	3.000	0.798			B
20%	12	100	0	12	13.250	0.892			A
Dettol									
5%	12	100	0	12	21.925	3.340	7.522	0.000	A
10%	8	66.7	4	12	13.90	10.93			AB
15%	9	75	3	12	18.33	4.48			A
17%	8	66.7	0	12	14.33	10.81			AB
18%	8	66.7	0	12	8.71	8.50			BC
20%	0	0.00	0	12	0.000	0.000			C

\* Tukey pairwise comparisons grouping information using the Tukey method and 95% confidence intervals: means that do not share a letter are significantly different

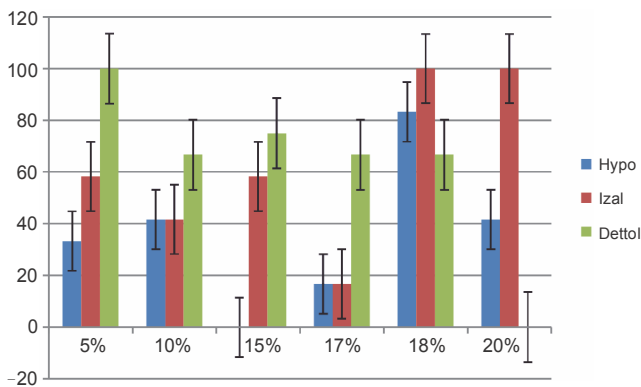
values in both Hypo and IZal were 13.50 and 13.25 mm for growth, 0.58 and 0.00 for contamination, and 1.00 and 1.00 for viability.

#### ***Disinfectants and their concentrations that yield desired mycelia growth***

According to the aim of this study, the desired results were to determine the optimum concentration of a disinfectant that yields pure mycelial culture with higher mycelial radial growth. Pure mycelial cultures were obtained in almost all the concentrations of all the disinfectants tested, although the probabilities of obtaining the pure mycelia culture differed with concentrations of the different disinfectants. There was, however, trade-off between levels of contaminations and the growth of the de-

sired mycelia. Changes in the levels of contaminations were noted to be higher in all the concentrations of Hypo (5–20%) and in the lower concentrations of IZal (5–17%) (Table 1); furthermore, even in lower concentrations of Dettol (5–17%), contaminations were significantly low. When higher concentrations of Dettol and IZal (18–20%) were used for disinfection, contaminants were completely inhibited, but the growth of the desired mycelia was significantly decreased (Table 1). Percentages of growth of pure mushroom cultures obtained at 5% concentration of Hypo, IZal, and Dettol were 33.3, 58.3, and 100%, respectively, as compared to control. For 10% concentration, the percentages were 41.7, 41.7, and 66.7%, respectively. At 15% concentration, the percentages of the growth of pure mushroom cultures were



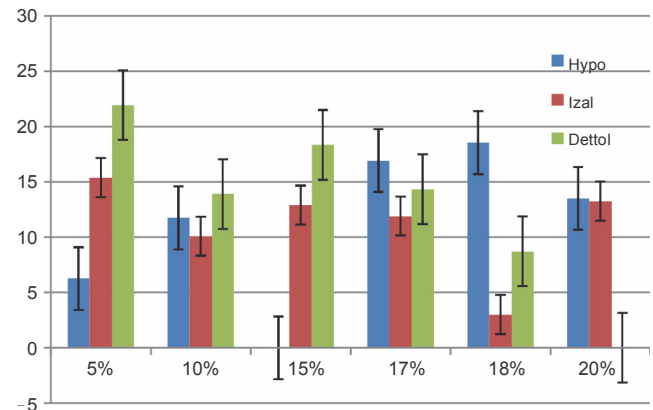


**Fig. 3.** Percentages of pure mycelial cultures obtained in different concentrations of different disinfectants (error bars with standard error)

0.00, 58.3, and 75%, respectively, while at 17% concentration, the growth percentages were 16.7, 16.7, and 66.7%, respectively. For 18% concentration, the percentage growth of the pure mushroom culture was 83.3% for Hypo, 100% for Izal, and 66.7% for Dettol, while at 20% concentration, the percentage growth was 41.7, 100, and 0.00% for Hypo, Izal, and Dettol, respectively. Disinfectant concentrations that yielded 100% of pure cultures were 18% and 20% of Izal and 15% of Dettol (Table 2, Fig. 3). Dettol at concentrations of 17% and 18% yielded 66.7% of pure cultures, although there was a significant decrease in mycelia growth, which was 14.33 and 8.71 mm for 17% and 18% concentrations, respectively (Table 2, Fig. 4). Additionally, no contaminants survived these concentrations, and the viability of the desired mycelia decreased to 0.58. The mycelial growth in Izal decreased significantly at these higher concentrations, but there was no contamination. The values of the mycelial growth in Izal at 17, 18, and 20% concentrations were 11.90, 3.00, and 13.25 mm, while that of contaminants were 0.00, 0.00, and 0.833, respectively.

## Discussion

This study revealed that the rate of the mushroom's mycelia growth, contamination, and the viability of the hyphae from the original mycelia plug inoculums differed with different concentrations of disinfectants used. The results also showed that mushroom mycelia, being the most complex and evolutionarily advanced component, have greater ability to survive higher concentrations of disinfectants than fungal and bacterial contaminants encountered in this study. The mushroom mycelia did



**Fig. 4.** Mycelial radial growth (mean) in different concentrations of different disinfectants (error bars with standard error)

not show any signs of growth due to microbial contamination in the media with no disinfectants, and the growth of the mushroom mycelia could not be measured. High levels of contaminations that suppressed the mycelial growth were observed at lower concentrations (5–15%) of Izal and Hypo, but better mycelial growth and lower contaminations were observed in Dettol (Fig. 4). Similarly, Gargi et al., (2017) found that Dettol even at lower concentration is effective against some clinical pathogens (*Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*) as is in the case of the present study. At higher concentrations (17–18%), Dettol and Izal greatly inhibited the occurrence of contaminants (Table 2), with Dettol being the most effective as it completely inhibited all contaminants and still maintained mycelial growth. The viability of the mycelia was significantly inhibited by Dettol, but it remained almost unaffected when Izal was used at the same concentration (17–18%). The effects of disinfectants on contaminants are known to decrease with a decreasing concentration of all disinfectants (Olasinde et al., 2008; Gargi et al., 2017). The use of Hypo led to better mycelia viability and growth, but cases of contaminations were unfortunately higher. Compared to previous studies, there was no consistency in the effects of the three disinfectants on contaminants. Many similar studies on some pathogenic clinical isolates (*E.coli*, *S. aureus*, *Salmonella typhi*, and *Aspergillus fumigatus*) showed that Hypo has the least effect against the pathogens as compared to Dettol and Izal (Mattei et al., 2013; Okore et al., 2014; Gargi et al., 2017; Soyemi et al., 2018). However, other studies reported that Hypo was more effective than Dettol and Izal on the same orga-

nisms (Al-Dabbagh et al., 2015; Shuaibu et al., 2017). Al-Dabbagh et al. (2015) found that 10% concentration of Hypo killed all the following bacterial isolates: *E. coli*, *Pseudomonas aeruginosa*, *S. aureus*, and *Corynebacterium renali*, while the same isolates showed greater resistance to Dettol. Moreover, some studies on fungi revealed that Hypo at concentrations between 0.03 and 2.4% were effective at killing *Penicillium*, *Cladosporium*, *Mucor*, *Rhizopus*, *Stachybotrys*, *Alternaria*, *Aspergillus*, *Helminthosporium*, *Trichophyton* and *Batrachochytrium* spp. (Reynolds et al., 2004; Becker, and Gratwicke, 2017; Van Rooij et al., 2017).

There are possibly different factors that led to the different results of the effects of the disinfectants on microbial contamination and mushroom mycelia growth obtained in this study. First, disinfectants differ in their compositions and mode of actions against a wide range of microorganisms (McDonnell and Russell, 1999). For example, Dettol inhibits microorganisms by denaturation of the protein components of their cell wall and cytoplasmic membrane, while Hypo (sodium hypochlorite) affects microorganisms by oxidizing their cellular components such as protein, lipid, and nucleic acids (Al-Dabbagh et al., 2015). Second, the nature and composition of the microbial cell surfaces vary from one species to another; consequently, such surfaces could also be changed due to extremes of environmental conditions (McDonnell and Russell, 1999). As a result of differences in cellular structure, composition, and physiology, microbial contaminants can thus respond variedly to different concentrations of disinfectants (McDonnell and Russell, 1999; Singh et al., 2012; Hammond et al., 2014). While some disinfectants kill nearly all microorganisms, others kill only a smaller range of pathogens (Wijesinghe and Weerasinghe, 2010). Third, microorganisms can develop resistance to disinfectants. As Olasehinde et al. (2008) pointed out that dilution of disinfectants renders them less effective and thereby provides the target organisms the likelihood to develop resistance against the disinfectants used. Shuaibu et al. (2017) found that clinical pathogens developed resistance against Dettol, Izal and Hypo, with most resistance observed for Dettol and least resistance to Hypo. Additionally, microorganisms develop increased resistance to antibiotics and can share these disinfectant-resistant markers among themselves (Al-Dabbagh et al., 2015). Fourth, the efficacy of most disinfectants can be reduced by the amount of organic

material that reacts with the available chemical components of the disinfectants, especially chlorine (Boothe, 1998; Shuaibu et al., 2017). More effective disinfection occurs when surfaces to be disinfected are free from organic matter and are smooth and nonporous (Boothe, 1998; Olasehinde et al., 2008). Finally, some disinfectants may fail to exert action as result of spoilage or improper storage (Shuaibu et al., 2017).

It was also observed that there is some level of inconsistencies in the result of this study with regard to Dettol and Hypo. For example, Dettol at 5% concentration showed paradoxically more efficiency in controlling contamination than at both 10% and 15%. There was also more contamination of cultures when Hypo was used at 20% concentration than at 18% concentration. These types of inconsistencies were also noted by Hammond et al. (2014), who reported that differences in contamination of culture can occur by chance. It should be noted that the sources of contamination in this research were not controlled and the contaminants were not identified; hence, the rate of the contamination of a particular culture was by chance. This implies that different microorganisms might have contaminated different cultures in various combinations. Different microorganisms usually have varied degrees of susceptibility to different disinfectants (Singh et al., 2012; Hammond et al., 2014). Additionally, microorganisms interact among themselves (Frey-Klett et al., 2011; Hiscox et al., 2018). These interactions lead to changes in species composition in a culture or natural environment. Interactions can occur for competition for nutrients, space, or inhibition. Fungal mycelia may interfere with the growth of another fungal species by physical contact and block their development of the hyphae through mycoparasitism or depletion of resource and consequently reduce nutrient availability. This may result in morphological changes in the hyphae or elimination of the inferior competitor depending on the fungal species composition (Lahlali and Hijri, 2010; Rogawansamy et al., 2015; Hiscox et al., 2018). Microorganisms that live in proximity compete for the same nutrients. Substrates that are rich in suitable nutrients are usually battlefields for both saprophytic bacteria and fungi (Künzler, 2018). Fungal mycelia may also produce organic compounds that are volatile or diffusible, or have the ability to alter the pH of the media, which have inhibitory effects on spore germination and growth of their competitors (Humphris et al., 2002; Lahlali and Hijri,



2010; Hiscox et al., 2018). Many abiotic factors can also affect the outcomes of interactions between microorganisms, which could be of advantage or disadvantage to one another. However, even under apparently identical conditions, the outcomes of interactions are not always consistent, possibly due to immeasurably tiny differences in conditions (Hiscox et al., 2018). All these interactions might have affected the result of the present study in one way or the other through the environment-bacteria-fungi interactions. Nevertheless, the results of this study can be valuable for mushroom growers and producers of ectomycorrhizas as it show a cheaper and simple method of controlling contamination.

### Conclusions

The present study found that the mycelia of the mushroom *A. semiorbicularis* have higher degree of resistance to disinfectants than other fungi and bacteria that contaminate growth media. This might be because mushrooms are known to be more complex and evolutionarily advanced species among fungi. This study also revealed that the rate at which mushroom's mycelia grow, the level of contamination of the growth media, and the viability of the mushroom's hyphae varied with different concentrations of different disinfectants used in the growth media. Pure mycelial cultures were obtained in almost all the different concentrations of all the disinfectants, although the percentages of these pure mycelial cultures obtained differed with different concentrations of all the disinfectants. However, trade-offs existed between the level of contamination and better growth of the desired mycelia. In general, the result of this study showed that Dettol has the highest effect on suppressing contaminants followed by Izal and the least effective was Hypo. This result will help in mushroom production processes by solving the problems of contamination.

### Acknowledgments

We sincerely appreciate the Department of Ecology, Abubakar Tafawa Balewa University, Bauchi, and TETFUND Institutional Base Research for supporting us to conduct this research.

### Funding

The present study was funded by TETFUND Institutional Base Research

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