Biocontrol of Mushroom Spoilage Fungi andAflatoxin Evaluation During Storage

Ilesanmi Fadahunsi[1], Dayo Ayansina[2], Ayodele Okunrotifa[1]

[1] Department of Microbiology, University of Ibadan, Ibadan, Nigeria
[2] Department of Biological Sciences, Bowen University, Iwo, Nigeria
sanmifadahunsi@yahoo.com

Abstract: The use of chemical substances in the control of pathogens is relatively expensive, it constitutes environmental hazards coupled with high level of toxicity to humans and it is therefore considered unsafe. Studies were carried out to investigate the biocontrol of fungi causing spoilage in mushroom and aflatoxin production during storage. The results obtained revealed that four species of fungi namely *Aspergillus fumigatus, Aspergillus niger, Botryodiplodia theobromae* and *Rhizopus stolonifer* were isolated from the spoilt mushroom samples. The antagonistic activities of four biocontrol agents; *Trichoderma asperellum* CMI T158, *Trichoderma longibrachiatum* CMI T167, *Pseudomonas fluorescence* CMI F113 and *Bacillus subtilis* CMI 22BN against the isolated spoilage fungi were tested using the agar well diffusion method. The optimization of the antagonistic activities of the biocontrol agent metabolites was carried out based on pH and temperature variations. The heat stability of the metabolites produced by the biocontrol agents and the aflatoxin production during storage of the mushroom samples were evaluated. The results obtained revealed that *Bacillus subtilis* did not inhibit *Aspergillus fumigatus,* but inhibited *Aspergillus niger*, *Botryodiplodia theobromae* and *Rhizopus stononifer* with inhibition zones of 25mm ±0.18, 21mm±0.11 and 20mm±0.13 respectively. *Pseudomonas fluorescence* and *Trichoderma longibrachiatum* inhibited all the tested pathogens, while *Trichoderma asperellum* did not inhibit *A. fumigatus* and *A. niger* but inhibited *B. theobromae* and *R. stolonifer* showing inhibition zones of 22mm ±0.15 and 20mm ±014 respectively. The effect of varying pH on the inhibitory activity of the metabolites produced by the biocontrol agents showed that the optimum inhibitory ability of the bacterial biocontrol agents were enhanced at pH 6.0, while the fungal biocontrol agents showed maximum inhibitory activity at pH 5.0. However variations in temperatures revealed that both the bacterial and fungal biocontrol agents were more effective at 30^0C. The monitoring of the thermal stability of the metabolites produced by the biocontrol agents indicated that they were inhibitory at 20^0C and 40^0C but at 60^0C, inhibition was not detected. The assessment of aflatoxin in dried stored mushroom reflected that aflatoxin B1 (4.0ppb±0.1) and B2 (3.88ppb±0.0) were produced in sample A while aflatoxins G1 and G2 were not detected in the same sample. Aflatoxin B1 (3.09ppb±0.02), B2 (2.33ppb ±0.04) and G1 (1.75ppb ±0.0 1) were produced in sample B, while G2 was not detected in the same sample.

[Ilesanmi Fadahunsi, Dayo Ayansina, Ayodele Okunrotifa. **Biocontrol of Mushroom Spoilage Fungi and Aflatoxin Evaluation During Storage.** *Nat Sci* 2013;11(7):7-13]. (ISSN: 1545-0740). http://www.sciencepub.net/nature. 2

Keywords: biocontrol, mushrooms, aflatoxins, spoilage, inhibition.

1. Introduction

Mushroom is a macrofungus with distinct fruiting body and is found on plant bodies, in soil, water, decomposed organic matters and animals in all regions of the world with sufficient moisture to enable it grow (Fasidi *et al.,* 2008). The cultivation of mushroom is a highly efficient method of disposing agricultural wastes and also serves as a method of producing nutritional food from various agro-allied wastes in the world (Akinyele and Akinyosoye, 2005). The production of mushroom is a successful attempt of agro-allied waste recycling (Chiu and Miles, 1993). Documented reports indicated that growing of mushroom started in France in the 17th Century, but today the people's Republic of China is the major producer of edible mushroom in the world producing about 3,918,300M tons yearly or about 64% of the world total mushroom production (Jonathan, 2002).

Mushrooms are distinguishable from higher and lower plants by the absence of green photosynthetic pigment known as chlorophyll (Jonathan and Fasidi, 2001). Structurally mushroom consists of four main parts; the umbrella shaped pileus (cap) lamellae (Gills) anulua (veil) and stipe (stalk). They are cosmopolitan (Dutta, 1994) and grouped into edible e.g. *Pleurotus spp, Agaricus spp, Lentinus spp* etc and the non-edible comprises of *Amanita phalloides, Amanita virosa, Amanita muscaris* and *Cortinarius rubellus* (Lloyd-Davies, 1992). In Nigeria, mushroom eaters depend on the seasonal available and canned imported types (Jonathan and Fasidi, 2001). They are consumed in many parts of the world and there are reports of the utilization of mushroom as food and food supplements for human health, nutrition and disease prevention (Chang, 1996). Nutritionally they are rich in proteins, minerals, vitamins, low fat

and essential amino acids (Sadler, 2003). Presently, global commercial production has increased by 35.9% between 1995 and 2005. It is estimated that the world production of mushroom today is about 5million tonnes fresh weight annually (Omarin *et al.,* 2010).

Previously documented reports had revealed the therapeutic potentials, such as its antioxidant, anti-inflammatory, immuno-suppressant and antibiotic properties (Longvah and Desothale, 1998). In Europe, North America, Japan, China, South East Asia and Australia where adequate technology is available they are cultivated for export trade. In addition, high dietary fibres of mushrooms were reported to function as an antitumor and antiviral agents (Zhang et al., 2004). They contain vitamin B, such as riboflavin, niacin, pantothenic acid and essential minerals such as selemun, coper and potassium

The application of mushroom had been reported since the World War 1 for the dressing of wounds. It is used in dyeing wool and other natural fibres (Musrake, 2000). Currently they are being employed by Ecovative Design LLC to make biodegradable packaging that can directly replace petroleum-based expanded polystyrene packaging. Their application in the development of new biological remediation agents is well documented.

Texture is an important parameter for assessing the quality of fresh mushroom and one of the main changes associated with mushroom deterioration is change in texture, loss of firmness during post harvest storage (Parentelli *et al.,* 2007). Cultivated mushrooms are susceptible to a variety of pests and diseases, such as spoilage by *Pseudomonas tolaasi* and others. In addition mushroom species produce secondary metabolites that are toxic to human beings. Aflatoxin produced by the genus *Aspergillus* have drawn great attention universally, these substances are carcinogenic, mutagenic, and are noticeable during storage of mushrooms

Biological control is the use of natural products or antagonistic microorganisms to control pests and diseases. There are available documented reports about the efficiency of using natural products to control various plant pathogens in many countries (Papavizas and Lumsden, 1980). This approach is not expensive, easy to apply and its application is safe and unhazardous to human health.

This work is therefore focused on the biocontrol of fungi causing spoilage in mushrooms and investigation into the production of aflatoxin during storage.

2. Materials and Methods

Isolation Procedure

The media used for this study were potato dextrose agar (PDA) and broth supplemented with 2% streptomycin sulphate. They were prepared according to the manufacturer's specification and sterilized at 121^0C for 15 minutes in an autoclave. The infected part of the deteriorated sample was removed using sterile surgical blade and 2gm was weighed and transferred to 10ml of sterile distilled water in a test tube. This was vigorously shaken to dislodge the microorganisms present and serially diluted. One ml from 10^4 dilution was inoculated into Petri dishes containing sterile PDA agar. Incubation was carried out at 30^0C aerobically for seven days. The fungi isolates observed on the plates were separately sub-cultured and the pure cultures of the different fungal isolates were stored on different slants in MacCarthney bottles and kept in the refrigerator at 4^0C.

Identification Procedure

The isolates were identified by macroscopic examination on plate and by viewing under the light microscope at x40 objective after staining with lactophenol cotton blue. The final identification of fungal isolates was confirmed with reference to Funders (1961) and Fawole and Oso (1988).

Preparation of Cell-Free Filterate

Five day old cultures of *Trichoderma asperellum* and *Trichoderma longibrachiatum* were inoculated separately into 25ml of sterile PDA broth contained in 150ml Erlenmeyer flasks using a sterile cork borer (5mm in diameter). Incubation was carried out at 30 ± 2^0C for seven days and the resulting broth was filtered with Whatman filter paper No 1 to obtain a mycelia free filterate. The filterates obtained were used to antagonize the fungal pathogens in this study.

One millilitre of 24h old bacterial suspension of *Bacilus subtilis* and *Pseudominas fluorescens* was separately inoculated into 25ml of sterilized nutrient broth contained in 150ml Erlenmeyer flasks. They were incubated at 30 ± 2^0C for 24-48h. The broths obtained were centrifuged at 15,000rpm for 30minutes at 4^0C. The supernatant obtained were used for antagonistic study in this work.

Determination of Antagonistic Activities of the Biocontrol Agents

This was carried out using the agar well diffusion method described by Aslim *et al.* (2004) and the diameter of inhibition zone around the wells was measured in millimeters (control).

Effect of pH on Antagonistic Activities of the Biocontrol Agents

Twenty ml of sterile PDA broths were decanted separately into two 150ml Erlenmeyer flasks. The pH of the medium was adjusted to 3, 4, 5, 6, 7, 8 using 0.1M of NaOH and 0.1M HCl solutions. The flasks were differently inoculated with *Trichoderma asperellum* and *Trichoderma longibrachiatum* using a sterile 5mm cork borer. Another set of two 150ml Erlenmeyer flasks

containing 20ml of sterile nutrient broth were adjusted to pH 3, 4, 5, 6, 7, 8 using 0.1M of NaOH and 0.1M of HCl solutions. The flasks were separately inoculated with 1ml of bacterial cells suspension of *Pseudomonas fluorescens* and *Bacillus subtilis* and incubated at 30^{0}C for 48hr; while the flasks containing PDA broth were incubated at 30^{0}C for seven days. They were all filtered to obtain cell-free filtrates which were used to test the antagonistic activity of the biocontrol agents as earlier described.

Effect of temperature variations on the antagonistic activities of the biocontrol agents

Five millimeter diameter of the fungal biocontrol agents was used to inoculate 20ml of sterilized PDA broth contained in 150 Erlenmeyer flasks separately using a sterile cork borer. The flasks were incubated at 30^{0}C and for 37^{0}C for seven days. One ml of bacterial cell suspension of the two bacterial biocontrol agents was inoculated separately into 20ml of sterile nutrient broth in 150ml Erlenmeyer flask. Incubation was carried out at 30^{0}C and 37^{0}C for 48hr. The broths obtained for the two different biocontrol agents were filtered using Whatman No 1 filter paper and the filterates obtained were used to test for the antagonistic activities of the biocontrol agents as earlier described.

Thermal Stability of the Metabolites Produced by the Biocontrol Agents

Twenty millimeter culture filterate of *Trichoderma asperellum, Trichoderma longibrachiatum, Pseudomonas flourescens* and *Bacillus subtilis* were heated in water bath at 20^{0}C, 40^{0}C and 60^{0}C for 30minutes. Their antagonistic activities were determined as described earlier.

Estimation of Aflatoxin in Dried Stored Mushroom

Fresh *Pleurotus sajor-caju* was oven dried and divided into two parts A and B. Sample A was stored in air tight container at room temperature (28±2^{0}C) while sample B was stored inside sterile nylon and kept in the freezer for six weeks. Aflatoxin estimation was carried out by employing the thin layer chromatography method of Munimbazi and Bullerman (1998) as described by Onilude *et al.,* (2005). The TLC scanner was used to quantify the aflatoxin content.

Statistical Analysis

Results obtained in this study were subjected to analysis of variance using ANOVA and separation of means was carried out by Duncan's Multiple Range Test (Duncan, 1955).

3. Results

Five spoilage fungal isolates obtained from the deteriorated mushroom samples were identified as *Aspergillus niger, Aspergillus fumigatus, Rhizopus stolonifer* and *Botryodiplodia theobromae.*

Table 1: Percentage of occurrence of the fungi in biodeteriorated mushroom

Fungi	% Occurrence
Botryodiplodia theobromae	40
Aspergillus niger	20
Aspergillus fumigatus	20
Rhizopus stolonifer	20

Table 2: Inhibition Zones produced by the Biocontrol Agents against Mushroom Pathogens

Pathogens	Bicontrol Agents			
	Bs	Pf	Ta	Ti
Aspergillus fumigatus	R±0.00a	18.00±0.11a	20.00±0.22a	R.00±0.00a
Aspergillus niger	25.00±0.18d	25.00±0.16c	30.00±0.17c	R.00±0.00a
Botryodiplodia theobromae	21.00±0.11c	29.00±0.10d	30.00±0.19c	22.00±0.15c
Rhizopus stolonifer	20.00±0.13b	23.00±0.00b	25.00±0.15b	20.00±0.14b

Values are means + standard deviation. Values followed by the same alphabets in the same row are not significantly different accor ding to Duncan's Multiple Range Test(P≤0.05)

This result revealed that the biocontrol agents such as *Pseudomonas flourescens* and *Trichoderma asperellum* inhibited all the isolated fungal pathogens with varying inhibition zones. *Pseudomonas fluorescens* produced inhibition zones of 18.0mm±0.11, 25.0mm±0.10, 29.0mm±0.10 and 23.0mm±0.0 against *A. fumigatus, A. niger B. theobromae* and *R. stolonifer* respectively. *T. asperellum* also inhibited all the fungal pathogens producing zones of inhibition on *A. fumigatus* (20.0mm±0.22), *A. niger* (30.0mm±0.17), *B. theobromae* (30.0mm±0.1) and *R. stolonifer* (25.0mm±0.15). While *T. longibrachiatum* did not show any inhibition against *A. fumigatus* and *A. niger* (0mm) but inhibited *B. theobromae* (22.0mm±0.15) and *R. stolonifer* (20.0mm±0.14). This result revealed that the biocontrol agents such as *Pseudomonas flourescens* and *Trichoderma asperellum* inhibited all the isolated fungi pathogens with varying inhibition zones. *Pseudomonas fluorescens* produced inhibition zones of 18.0mm±0.11,

25.0mm±0.10, 29.0mm±0.10 and 23.0mm±0.0 against *A. fumigatus, A. niger B. theobromae* and *R. stolonifer* respectively. *T. asperellum* also inhibited all the fungal pathogens producing zones of inhibition on *A. fumigatus* (20.0mm±0.22), *A. niger* (30.0mm±0.17), *B. theobromae* (30.0mm±0.1) and *R. stolonifer* (25.0mm±0.15). While *T. longibrachiatum* did not show any inhibition against *A. fumigatus* and *A. niger* (0mm) but inhibited *B. theobromae* (22.0mm±0.15) and *R. stolonifer* (20.0mm±0.14).

Table 3: Effect of varying pH on the inhibitory activities of the metabolites produced by the biocontrol agents

Bacillus subtilis							
Pathogens	**Control**	**3**	**4**	**5**	**6**	**7**	**8**
A. funigatus	R±0.000a	R±0.00a	R±0.000a	R±0.00a	5.00±0.10b	3.00±0.20c	R±0.00d
A. niger	25.00±0.10e	R±0.00a	R±0.00a	20.00±0.20d	26.00±0.40f	17.00±0.50c	3.00±0.80b
B. theobromae	21.00±0.10e	R±0.00a	R+0.00a	18.00±0.40d	22.00±0.20f	15.00±0.30c	5.00±0.90b
R. stolonifer	20.00±0.3e	R±0.00a	R±0.00a	14.00±0.60c	23.00±0.10f	17.00±0.70d	4.00±0.10b
Pseudomonas flourescence							
Pathogens	**Control**	**3**	**4**	**5**	**6**	**7**	**8**
A. funigatus	18.00±0.11d	R±0.00a	R±0.00a	5.00±0.00b	21.00±0.18e	14.00±0.25c	5.00±0.35b
A. niger	25.00±0.15e	R±0.00a	R±0.00a	7.00±0.00b	28.00±0.11f	22.00±0.30d	8.00±0.20c
B. theobromae	20.00±0.18e	R±0.00a	R+0.00a	6.30±0.18b	23.00±0.12e	19.00±0.28c	6.00±0.18b
R. stolonifer	23.00±0.14e	R±0.00a	R±0.00a	7.00±1.12b	25±0.00f	20.00±0.21d	8.00±0.11c
Trichoderma asperellum							
Pathogens	**Control**	**3**	**4**	**5**	**6**	**7**	**8**
A. funigatus	20.00±0.20g	R±0.00a	10.00±11e	12.00±0.15f	8.00±0.10d	6.00±0.18c	4.00±0.00b
A. niger	30.00±0.12	R±0.00a	10.00±0.12e	12.00±0.17f	7.00±0.22d	4.00±0.00c	3.00±0.19b
B. theobromae	30.00±0.10f	R±0.00a	20.00±0.16e	33.00±0.00g	18.00±0.18d	12.00±0.18c	7.00±0.11b
R. stolonifer	25.00±0.18f	R±0.00a	15.00±0.22e	27.00±0.11g	14.00±0.10d	10.00±0.16c	6.00±0.00b
Trichoderma longibrachiatum							
Pathogens	**Control**	**3**	**4**	**5**	**6**	**7**	**8**
A. funigatus	R±0.00a	R±0.00a	5.00±0.12d	8.00±0.16e	3.00±0.00c	1.00±0.01b	R±0.00a
A. niger	R±0.00a	R±0.00a	R±0.00a	3.00±0.11b	R±0.00	R±0.00a	R±0.00a
B. theobromae	22.00±0f	R±0.00a	18.00±0.00d	26.00±0.18f	20.00±0.10e	8.00±0.18c	4.00±0.13
R. stolonifer	20.00±0.20e	R±0.00a	15.00±0.20c	24.00±0.12f	17.00±0.12d	6.00±0.13b	2.00±0.19a

Values are means + standard deviation. Values followed by the same alphabets in the same row are not significantly different according to Duncan's Multiple Range Test ($P \leq 0.05$)

The effect of varying pH on the inhibitory activities of the biocontrol agent is seen in table 3. It was observed that the highest inhibitory capability was observed at pH 6 with inhibition zone of 26.00 ±0.4mm, and 28.00 ± 0.11mm produced by *Bacillus subtilis* and *Pseudomonas flourescens* against *A. niger*. However, *Trichoderma asperellum* and *T. longibrachiatum* were highly effective at pH 5 with *T. asperellum* and *T. longibrachiatum* exhibiting inhibition zones of 33 ±000mm and 26.00 ± 0.18mm against *B. theobromae* respectively.

Table 4: Effect of varying temperature on the inhibitory activities of the biocontrol agents

Bacillus subtlis			
Pathogens	**Control**	**30^0C**	**37^0C**
A. funigatus	R.00±0.00a	R.00±0.00a	R.00±0.00a
A. niger	25.00±0.11c	24.00±0.12b	20.00±0.15a
B. theobromae	21.00±0.16c	19.00±0.14b	17.00±0.16a
R. stolonifer	20.00±0.13c	20.00±0.11b	18.00±0.18a
Pseudomonas fluorescence			

Pathogens	Control	30^0C	37^0C
A. funigatus	18.00±0.22c	17.00±0.15b	14.00±0.00a
A. niger	25.00±0.16c	23.00±0.12b	20.00±0.16a
B. theobromae	29.00±0.15c	24.00±0.17b	19.00±0.18a
R. stolonifer	23.00±0.00c	23.00±0.11b	16.00±0.13a
Trichoderma asperellum			
Pathogens	**Control**	**30^0C**	**37^0C**
A. funigatus	20.00±0.01c	20.00±0.07b	17.00±0.16a
A. niger	30.00±0.06c	27.00±0.16b	24.00±0.19a
B. theobromae	30.00±0.18c	26.00±0.18b	22.00±0.12a
R. stolonifer	25.0±0.12c	23.0±0.11b	19.00±0.15a
Trichoderma longibrachiatum			
Pathogens	**Control**	**30^0C**	**37^0C**
A. funigatus	R.00±0.00a	R.00±0.00a	R.00±0.00a
A. niger	R.00±0.00a	R.00±0.00a	R.000±0.00a
B. theobromae	22.00±0.16c	20.00±0.00b	14.00±0.11a
R. stolonifer	20.00±0.11c	16.00±0.18b	10.00±0.18a

Values are means + standard deviation. Values followed by the same alphabets in the same row are not significantly different according to Duncan's Multiple Range Test(P≤0.05)

Table 4 represents the result of varying temperature on the inhibitory activities of the biocontrol agents. This table revealed that the highest inhibitory activities of the biocontrol agents were enhanced at 30^0C.

Table 5: Thermal Stability of the Metabolites Produced by the Biocontrol Agents

Pathogens	**20^0C Zones of Inhibition (mm)**			
	Bs	**Pf**	**Ta**	**Ti**
Aspergillus fumigatus	R.00±0.00a	16.00±0.66a	18.00±0.12a	R.00±0.00a
Aspergillus niger	20.00±0.14c	20.00±0.34b	27.00±0.63c	R.00±0.00a
Botryodiplodia theobromae	18.00±0.11b	25.00±0.38c	22.00±0.10b	20.00±0.00c
Rhizopus stolonifer	17.00±0.17b	20.00±0.67b	21.00±0.43b	18.00±0.11b
Pathogens	**40^0C Zones of Inhibition (mm)**			
	Bs	**Pf**	**Ta**	**Ti**
Aspergillus fumigatus	12.00±0.12a	14.00±0.17a	16.00±0.42a	R±0.00a
Aspergillus niger	16.00±0.15b	20.00±0.12b	20.00±0.28b	R.00±0.00a
Botryodiplodia theobromae	20.00±0.11d	22.00±0.10c	20.00±0.19b	18.00±0.00c
Rhizopus stolonifer	18.00±0.23c	19.00±0.18b	22.00±0.16c	15.00±0.10b
Pathogens	**60^0C Zones of Inhibition (mm)**			
	Bs	**Pf**	**Ta**	**Ti**
Aspergillus fumigatus	R.00±0.00a	R.00±0.00a	R.00±0.00a	R.00±0.00a
Aspergillus niger	R.00±0.00a	R.00±0.00a	R.00±0.00a	R.00±0.00a
Botryodiplodia theobromae	R.00±0.00a	R.00±0.00a	R.00±0.00a	R.00±0.00a
Rhizopus stolonifer	R.00±0.00a	R.00±0.00a	R.00±0.00a	R.00±0.00a

Values are means + standard deviation. Values followed by the same alphabets in the same row are not significantly different according to Duncan's Multiple Range Test (P≤0.05).

The result of the thermal stability of the metabolites produced by the biocontrol agent is presented in Table 5. It can be inferred from the table that the metabolites were inhibitory at 20^0C and 40^0C and at 60^0C the pathogens were all resistant to the metabolites.

Table 6: Estimation of Aflatoxin (ppb) in dried Stored Mushroom

Samples Code	B_1	B_2	G_1	G_2
A	4.00±0.01b	3.88±0.00a	0.00±0.00a	0.00±0.00a
B	3.09±0.02a	2.33±0.04b	17.58±0.01b	0.00±0.00a

Values are means + standard deviation. Values followed by the same alphabets in the same row are not significantly different according to Duncan's Multiple Range Test (P≤0.05)

B_1 = Aflatoxin B_1
B_2 = Aflatoxin B_2
G_1 = Aflatoxin G_1
G_2 = Aflatoxin G_2

The estimation of aflatoxin in dried stored mushroom revealed that aflatoxins B_1 (4.00 ± 0.01 ppb) and B_2 (3.88 ± 0.00ppb) were produced in samples A while aflatoxin G_1 and G_2 were not detected. However aflatoxins B_1 (3.09 ± 0.02ppb) B_2 (2.33 ± 0.04 ppb), G_1 (17.58 ppb) were recorded in samples B while G_2 was not detected.

4. Discussions

The involvement of fungi in the spoilage of mushroom has been earlier reported by Jonathan *et al.,* (2008). The susceptibility of mushroom to fungal spoilage may be due to their rapid respiration rate coupled with inability to protect themselves from excessive loss of water and microbial attack. In addition, microorganisms are ubiquitous thus they can be found in any environment causing biodeterioration. Microorganisms are able to initiate spoilage as a result of their enzymatic browning, dehydration and capability of growing in mushroom.

The ability of these biocontrol agents to inhibit mushroom pathogen is dependent on the production of anti-fungal secondary metabolites that are capable of lysing chitin which is the most important component of fungal cell wall. Moreover the growth inhibition of spoilage fungi could be due to antibiotic or specific cell wall degrading enzymes (Lorito *et al.,* 1993). This occurrence is in conformity with the submission of Ongena *et al* (2009).

The optimum growth of these organisms was observed at pH 6 and 5. Dix and Webster (1995) reported that fungi grow naturally at acidic pH.

The highest inhibitory activities of the metabolites of the biocontrol agents were observed at 30^0C. This occurrence indicates that these organisms are mesophilic in nature.

This observation might be due to the nature of the bioactive natural peptides produced by the biocontrol agents that are denatured by heat or high temperature (Ongena *et al.,* 2009).

The occurrence of aflatoxin had previously been reported in various foods by Pitt (2002). This therefore calls for efficient and safe procedures for preservation of foods against invading fungi as well as safe decontaminantion of aflatoxin contaminated food and feed sources (Onilude, 2005).

In conclusion, this research confirms the effectiveness of the biocontrol agents against the fungal pathogens. Therefore it could be a cheaper alternative method of controlling mushroom fungal pathogens.

Acknowledgement

The authors are grateful to Dr Victor Dania of the Forestry Research Institute of Nigeria, Ibadan, Nigeria for providing the strains of microorganisms used as biocontrol agents in this work.

Correspondence to:
Dr. Fadahunsi, Ilesanmi F.
Department of Microbiology, University of Ibadan, Ibadan, Oyo State, Nigeria
Telephone +234-8062588059
e-mail: sanmifadahunsi@yahoo.com

References

1. Akinyele, B.J. and Akinyosoye, F. A. Effect of Volvacea cultivation on the chemical composition of Agro –wastes. African Journal of Biotechnology 2005 4: 893- 979
2. Aslim, B., Yuksekdag, Z. N., Sarikaya, E. and Beyatli, Y. Determination of the bacteriocin-like substances produced by some lactic acid bacteria isolated from Turkish dairy products, *LWT* - Food Science and Technology 2004. **1**: 1-4.
3. Chang, R. Functional Properties of Mushrooms. Nutr. Rev. 1996. 54: 91-93.
4. Chiu, S.L. and Miles, P.G. Recent trends in world production of cultivated edible mushrooms. Mushrooms J. 1993 504: 15-17
5. Dutta, A.C. Botany for Degree Students, 5th Edition., Oxford University Press, London-1964-P. 524-587.

6. Dix, N. J. and Webster, J. Fungal Ecology 1995. London, Chapman & Hall Publ.
7. Fasiidi I. O., Kadiri, M., Jonathan, S.G., Adenipekun, C.O, Kuforiji, O. O. Cultivation of tropical edible mushroom in Nigeria. 2008, 29-40
8. Funders, S. Practical Mycology (Manual of identification of fungi). 1961. A.W. Btiskers Boktken: A London way: Dolman (1929). Economic Microbiology of Fermented Food.
9. Jonathan, S.G. and Fasidi, I.O. Studies on phytohormone vitamins and mineral elements requirements of *Letinus subnudus* and *Scizophyllum commune* from Nigeria. Food Chem. 2004. 75: 303-307
10. Jonathan, S.G. Vegetative growth requirement and antimicrobial activities of some hjgher fungi in Nigeria ,P.h.D thesis university of Ibadan, Nigeria 2002;268-280.
11. Jonathan, S.G. and Fasidi,I.O. Studies on phytohormone, vitamins and minerial elements requirement of *Lentinus subnudus* and Schizophyllum commune from Nigeria. Food Chem. 2001. 72: 479-483
12. Fawole, M.O. and Oso, B.A.. Laboratory Manual for Microbiology (1 Edn.). Spectrum Book Ltd. Ibadan. 1988. pp: 34-35.
13. Jonathan, S. G., Odebode, A. C. and Bawo, D. D. S. Studies on Collection and Proximate Compositions of *Phallus indusitus* (Vent. Ex. pers), A Nigerian Higher Fungus. World Journal of Agricultural Sciences. 2008 4(1): 18.
14. Llyyd-Davies, V. The Mushroom Cookbook. 1992. Salamander books Ltd. London
15. Longvah, T. and Deosthale, Y.G. Effect of dehulling, cooking and roasting on the protein quality of *Perilla frutescens* seed. Food Chem. 1998 63: 519-523.
16. Lorito, M., Harman, G. E., Hayes, C. K., Broadway, R.M., Troncoso, T., Woo, S.L. and Di Pietro, A. Chitynolytic enzymes produced by *Trichoderma harzianum*: antifungal activity of purified endochitinase and chitobiase. Phytopathol.1993 83: 302-307.
17. Munimbazi, C and Bullerman, L.B. Antifumgal metabolites of *Bacillus pumilus*. 1998. J. of Applied Microbiol. 1998. 84:959-968.
18. Musrake, R.B. Handbook of Natural Colorants. 2000. Wiley Publ., NY. Pp183-200.
19. Omarin, A. Nepote, V., Grosso, N.R., Zygadlo, J.A. and Alberto, E. Sensory analysis and Fruiting bodies characterization of the edible mushroom *Pleurotus ostreatus* and *Polyporus tenuiculus* contained on leaf waste from the essential oil production industry. 2010. International Journal of Food Sci. and Technol. 45: 466-474
20. Ongena, MARC ULg; Henry, Guillaume. 2009. The roles of lipopeptides in the biocontrol activity of Bacillus subtilisThonart, Philippe ULg- in Plant Pathology in the 21st Century, Recent Developments in Disease Management 2009.
21. Onilude, A.A., Fagade, O. E., Bello, M. M. and Fadahunsi, I. F. Inhibition of aflatoxin-producing aspergilli by lactic acid bacteria isolates from indigenously fermented cereal gruels. African Journal of Biotechnology. 2005. 4 (12): 1404-1408.
22. Papavizas, G.C. and Lumsden, R.D. Biological control of soil-borne fungal propagules. Annual Rev. of Phytopathol. 1980. 67: 389-414
23. Parentelli C, Ares G, Corona M, Lareo C, Gámbaro A, Soubes M, Lema P. Sensory and microbiological quality of shiitake mushrooms in modified atmosphere packages. J. Sci. Food Agric. 2007. 87(9): 1645-1652
24. Pitt, J.I. Biology and ecology of toxigenic Penicillium species. In: Mycotoxins and Food Safety. J.W. De Vries. M.W. Trucksess, and L.S. Jaskson ed. Kluver Academy/Plenium Publishers, New York. 2002. Pp. 29-41.
25. Sadler, M. Nutritional Properties of Edible Fungi. Food Nutr. Bull. 2003. 28: 305-308
26. Zhang, M. Chenng, P.K.C. Ooi, V.C.E. and Zhand, L. Evaluation of sulfated fungal B-glucals from the Schlerotium of Plurotus tuberregium as a potential water soluble anti-viral agent. Carbohyd. Res. 2004. 339: 2297-301.

5/16/2013