Available online: www.notulaebiologicae.ro
Print ISSN 2067-3205; Electronic 2067-3264
Not Sci Biol, 2018, 10(3):379-386. DOI: 10.15835/nsb10310284
Original Article

Fungal and Insect Pests of the Edible Mushroom *Pleurotus ostreatus*

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Abstract

Pleurotus ostreatus is an edible mushroom cultivated worldwide, with economic, nutritional and medicinal values, which requires a shorter growing period compared to other edible mushrooms. Fungal and insect pests of cultivated *P. ostreatus* were examined. Fungi from infected mother spawn of *P. ostreatus* were isolated, characterized and identified, while various insect pests on *P. ostreatus* were also collected and analysed. The plates showing micrographs, morphological and cultural characteristics of fungal isolates obtained were recorded. Fungi species isolated from infected mother spawn of *P. ostreatus* were identified as *Aspergillus flavus, A. niger, Cladosporium* sp., *Penicillium oxalicum, Fusarium oxysporium* and *F. verticilliodes*. The growth of *Cladosporium* sp. was slow on Potato Dextrose Agar (PDA) plates compared to *Aspergillus* sp. and *Fusarium* sp. which was very fast. Insect belonging to the orders Collembola, Diptera and Araneae were encountered on the cultivated *P.ostreatus.* These groups of insects were found at adult stage of life. Infestation by order Collembola (springtail) on *P. ostreatus* was found to be higher in incidence with percentage composition of 50.0%, followed by order Diptera (fruit flies) and order Araneae (spider) with 33.3% and 16.67% composition respectively. Distinguishing features of each pest and the features of damage done on mushroom were also observed and recorded.

Keywords: cultural; morphology; mushroom; *Pleurotus ostreatus*

Introduction

Mushroom is the fleshy, spore bearing fruiting body of a fungus, typically produced above ground on soil or on its food source (Jonathan and Adeoyo, 2011). Morphologically, mushroom develops from a nodule, a pinhead, less than two millimetres in diameters, called a primordium, which is typically found on or near the surface of the substrate. It is formed within the mycelium, the mass of thread like hyphae that make up the fungus (Metzler and Metzler, 1992). Among the edible mushrooms produced worldwide, *Agaricus bisporus* is the most cultivated one (38%), followed by the species of the genus *Pleurotus* (25%) and *Lentinula edodes* (10%) (Moda, 2008). There are clearly two mushroom markets: one referring to the most commercialized species, Champignon (*Agaricus bisporus*) and another that gathers exotic mushrooms, including species of *L. edodes, Pleurotus* sp., *Auricularia* sp., *Flamulina velatipes, Grifola frondosa, Hypsizygus marmoreas, Pholiota nameko, Tremella fuciformis* and *Volvariella* sp. (Furlani *et al*., 2005). Mushrooms are the richest source of vegetable proteins, containing 31-40% of protein (Chang and Hayes, 1978). Mushrooms are low-calories food, usually eaten cooked or raw and as garnish to a meal. Dietary mushrooms are good source of B vitamins such as riboflavin, niacin and pantothenic acid and the essential minerals, selenium, copper that help the body to produce red blood cells (Jonathan *et al*., 2008). Fat, carbohydrate and calories content are low, with absence of vitamin C and sodium. Mushrooms have low lipid content, being considered healthy foods (Smiderle *et al*., 2008). However, there are certain abnormalities of several abiotic origins that occur in mushrooms. Such abnormalities include formation of stroma, formation of scales or crocodile skins, changes in colour of fruit bodies, outgrowth on mushroom cap, long stipe, small cap on a normal stipe, rosecomb and scaling (Singh *et al*., 1991; Gbolagade, 2006).

The various insect pest associated with mushrooms include flies such as cecids, phorids, housefly (*Musa domestica*), sciarids and stable fly (*Stomoxys calcitrans*) (Ajayi and Jonathan, 2004). The flies belong to the order Diptera.

Received: 13 Apr 2018. Received in revised form: 30 Aug 2018. Accepted: 05 Sep 2018. Published online: 27 Sep 2018.

sciarid flies also known as fungus gnats belong to the family Sciaridae, while species include: *Sciara multiselta, Sciara agaris, Lyeoriella mali*. Cecid flies also known as gall midges belong to the family *Cecidomydae* and species include: *Mycophila spayeri, Mycophila borresi, Heteropeza pygmaea*. Phorid flies belong to the family Phoridae and species include: *Megaselia nigra*, *Megaselia halterata*. Mites which are found in straw and manure, include small mushroom mites (*Tarsonemas* sp.), straw or hay mites (*Tyrophagus* sp.). The springtails which are tiny insects include species such as *Isotoma simplex, Lepidocrytus* sp., *Cynaneus* sp. (Keil, 1996).

Fungal disease of cultivated mushroom include dry bubble disease caused by *Verticillium fungicola*, wet bubble disease caused by *Mycogone perniciosa*, cobweb or dectylim mildew caused by *Cladobotryum dendroides (Hypomyces rosellus*), green mould caused by *Trichoderma* (Gbolagade, 2005; Fasidi *et al*., 2008).

Pleurotus ostreatus is an edible white rot fungus commonly known as oyster mushroom, which can be cultivated on a variety of organic substrates. It belongs to the kingdom Fungi, Division Basidiomycota, class Agaricomycetes, Order Agaricales, family Pleurotaceae, Genus *Pleurotus,* which include many other species such as *P. flobellotus, P. sojar-caju, P. eryngii, P. osfreafies, P. floride* and *P. sapidus*. (Amuneke *et al*., 2011; Jonathan *et al*., 2012).

The main goal of the present research was to investigate the fungal and insect pest associated with *Pleurotus ostreatus*. The specific aims were (1) to isolate and characterize various fungi associated with the cultivated *P. ostreatus* and (2) to investigate and identify the occurrence of insect pest in cultivated *P. ostreatus*.

Materials and Methods

Study area

The study was conducted in the Mycology laboratory, Department of Botany, University of Ibadan, Oyo State. Ibadan is located in Southwestern Nigeria between latitude 7° N 26' Longitude 3° E 53' at an altitude of 190 m. The city ranges in elevation from 150 m in the valley area to 275 m about sea level (Lyold, 1967). Ibadan has a tropical wet and dry climate with mean monthly temperature and humidity ranging from 23 °C to 30 °C and 55% to 75% respectively (Onibokun and Faniran, 1995).

Sterilization of media and glassware

All glassware used were thoroughly washed with detergent, rinsed with water and air-dried before sterilization in a hot air oven at 180 °C for 3 hours. All media employed were prepared according to the manufacturers' specification and then sterilized by autoclaving at 121 °C for 15 min. Aseptic condition was maintained by swabbing the bench surface with 70% ethanol and flaming of inoculating needles over a spirit lamp before and after inoculation.

Preparation of media

Potato Dextrose Agar (PDA) was used for the isolation of the organisms; it was prepared following the manufacturer's guide (39 g-1,000 ml). Also sterilization at 121 °C for 15 min was done before it was poured in sterile Petri dishes.

Collection of mushroom and insects samples

Mother spawn of *Pleurotus ostreatus* was collected from the mushroom unit of pathology section of Forest Research Institute of Nigeria, Jericho, Ibadan (FRIN).

Isolation of fungi from mushroom samples

The mushrooms (*Pleurotus ostreatus*) were brought to the laboratory for isolation. For the experiment, 2 g of infected region of *P. ostreatus* were plated on Potato Dextrose Agar (PDA). Thereafter, 0.5 mg of Streptomycin was added to the PDA to prevent bacterial contamination. The isolates were plated in triplicates and the plates were incubated at room temperature (25 ± 2 °C) for 7 days according to the procedure described by Gbolagade (2006).

Selection of pure culture

After incubation, the plates observed for fungal growth and colonies were randomly selected. The isolates were sub-cultured 5-6 times on PDA. Pure cultures were prepared on slants and then stored in the refrigerator (Alexopolous *et al*., 1996).

Identification of fungi

Identification of fungi was carried out with the aid of slide cultures, temporary mounts with cover slips before observation under the microscope using ×40 objective lens. The slides were labelled and photographed, and final identification was done using published keys and compendium of soil fungi (Domsh *et al*., 1980).

Morphological and cultural characterization of isolates

Pure cultures of the fungal strains were prepared by sub-culturing them separately on fresh plates containing PDA before incubation for 3-7 days. These organisms were thereafter characterized morphologically using cultural features which include surface (front), reverse (back) colour (Banrnetand Hunter, 1972).

Collection and identification of insects

Insect pests were collected from mushroom by hand picking method. During collection of springtails, the selected mushrooms were covered with polythene bag with dimension (5 x 7 cm) to allow the insects to hop in. Subsequently, the collected springtails were taken to the laboratory for identification. On the other hand, the fruit flies and the spider were collected using bare hand from the mushroom into collecting bottle and taken to the laboratory. The collection was done at Beejay Ventures Odo-ona Kekere. The area coordinate reading was latitude 07N°17' and longitude 03°E51'. The insects were picked from species of *P. ostreatus* and preserved in specimen bottle (50 ml) containing 4% formalin solution (Kim and Hwang, 1996). Insects collected were identified in the Entomology Unit Department of Zoology, University of Ibadan, using basic insect identification key after viewing under an Olympus dissecting microscope (×32). Subsequently, they were recorded photographically using Sams ung 10.2 mega pixel. The collected species were stored in the laboratory for reference purpose.

Results

Morphology, cultural characteristics and microscopic examination of fungal isolates from infected Pleurotus ostreatus

Fungal isolates belonging to the genera *Aspergillus, Cladosporium, Penicillium* and *Fusarium* were isolated from infected *P. ostreatus* (Table 1). The figures showing the micrographs, morphological and cultural characteristics of the fungal isolates obtained are shown below. The isolates were *Aspergillus flavus, Aspergillus niger, Cladosporium* sp., *Penicillium oxalicum, Fusarium oxysporium* and *F. verticillioides*.

Aspergillus flavus

The colonies on PDA at 27 °C attained a diameter of 3-5 cm within 7 days and consisted of a dense felt yellowish-green conidiosphores or mature vesicles bearing phalides over their entire surface with a cream reverse. Rapid growth with usually two sets of sterigmata and with spiny stalks were observed. Some colonies appeared colourless (hyaline) or brightly coloured also termed moniliaceous. Conidiosphores terminated in a vesicle covered with either a single phalides (uniseriate) or a layer of subtending cells (metulae) which bears whorls of phialides known as biseriate structure. Conidia were one celled, smooth-or-rough-walled, hyaline or pigmented and basocatenate, forming long dry chains which maybe divergent (radiate or aggregated in compact columns) up to 1.0 mm, while some isolates were up to 2.5 mm in length. Texture was woolly to cotton or granular. Some species may produce Hulle cells or sclerotia. Sclerotia when present were dark brown. A clear to pale brown exudates may be present in some isolates. Phalides borne directly on the vesicle or metulae, 6x10x4.0-5 µm metulae 6.5-10x3.5 µm. *Coniadia globos*e to subglobose and spiny (Fig. 1).

Aspergillus niger

Colonies on PDA at 27 °C attained a diameter of 4-5 cm within 7days, consisting of a compact white or yellow felt with a dense layer of dark brown to black conidiospores. Mycelial or threadlike hyphae were divided by a septum and transparent conidiosphores (asexually produce fungal spores). *A. niger* usually range from 900-1,600 um in length and contain globose to subglobose, round, radiate head vesicles ranging from 40-60 um in diameter. Each globose vesicle was covered with biseriate phialides which are projections from the conidiosphore of *A. niger*. Conidiospores stipes, long, smooth walled hyaline, but also brown colour phaliades borne on metulae which was the site where conidiogenous cell was created; phialides borne on metulae,7.0-9.5x3.5 um, metulae hyaline, brown often septate,15-25 x 4.5-6.0 um. The phialides go through a process of blastic basipetal conidiogenous to create globose mitospores which have a diameter that ranges from 3 to 5 um. *A. niger* is differenciated from other *Aspergillus* due to the production of carbon black or very dark black spores from biseriate phialides. Conidiospores and spores have conspicuous ridges or spines not arranged in rows (Fig. 2).

Cladosporium sp.

Growth of *Cladosporium* colonies was moderate on PDA at 25 °C and the texture was velvety to powdery. Colonies were rather slow growing species, produced olive-green to brown or blackish brown, but also sometimes grey, buff or brown, suede-like to floccose, often becoming powdery due to the production of abundant conidia.

Table 1. Morphology, cultural characteristics and microscopic examination of fungal isolates from infected *Pleurotus ostreatus*

Isolate code	Isolates	Growth pattern	Surface colour	Reverse colour	Microscopic examination
A	*Aspergillus flavus*	Rapid	Yellowish green	Cream	Phialides borne directly on the vesicle with coniadia head typically radiate and conidiospores coarsely roughened.
B	*Aspergillus niger*	Rapid	Blackish brown	Creamish yellow	Conidia head with metulae and phialides, conidiospores stipes long, smooth walled and hyaline.
C	*Cladosporium* sp.	Slow	Olive green	Blackish brown	Conidiospores tall, dark, upright, branched variously near the apex, conidia ovoid to cylindrical and irregular in shape.
D	*Penicillium oxalicum*	Rapid	Dark green	Yellowish green	Conidiospores branched near the apex, conidia hyaline, one celled, ovoid in dry basipetal chains ending in phialides.
E	*Fusarium oxysporium*	Rapid	Creamy	Yellowish milk	Macroconidia with 3-septate, short to medium length, straight to slightly curved, relatively slender and thin walled. Microconidia oval in shape with 0-septate.
F	*Fusarium verticillioides*	Rapid	Greyish orange	Orange	Macroconidia abundant, single celled, oval to club shape, 3-5 septate and are relatively long, slender and thin walled.

Vegetative hyphae, conidiospheres and conidia were equally pigmented, formed in simple or branching chains. Conidiophores were more or less distinct from vegetable hyphae, erect, tall, straight or flexuous, dark, upright, unbranched or branched only in the apical region, clustered or single, with geniculate sympodial and elongate in some species; conidia (blastospores) smooth, 1-to-4-celled, verrucose or echinulate, with distinct dark hilum were produced in branched acropetal chains, variable in shape and size, ovoid to cylindrical and irregular, some typically lemon-shaped. The term blastocanate is often used to describe chains of conidia where the youngest conidium is at the apical or distal end of the chain. The conidia closest to the conidiophores and where the chains are branch are usually 'shield shape'. The presence of shield shape conidia, distinct helium and chains of conidia that readily disarticulate, are diagnostic for the genius *Cladosporium* (Fig. 3).

Penicillium oxalicum

Colonies were dark green on PDA with a yellow reverse, powdery and compact with rapid growth. Conidiospores arising from a single or less often synemata branched near the apex, penicillate, ending in phalides. The conidiophore was asymmetrical. Conidial were formed in long thin chains which shine like threads of silk under illumination. When an agar plate containing penicillium is tapped, the conidia fall away in crust or sclumps. The conidia were relatively large, relatively elliptical and smooth, round and unicellular; conidial hyaline, 1 celled, mostly globose or ovoid, in dry basipetal chains. *Penicillium oxalicum* is recognised by their dense brush like spore bearing structures. Penicillium species tend to have small hyphae, this makes protoplasmic movement difficult to detect. The small hyphae also tend to smaller peripheral growth (Fig. 4).

Fusarium oxysporium

Colony morphology on PDA varied widely. Mycelia may be floccose, sparse or abundant and range in colour from white to pale violet. Abundant pale orange or pale violet macroconidia were produced in a central spore mass in some isolates. Small pale brown, blue to blue-black or violet sclerotia may be produced abundantly by some isolates. *F. oxysporum* usually produces a pale to dark violet or dark magenta pigment in the agar, but some isolates produce no pigment at all. Some isolates of *F. oxysporum* mutate readily to the pionnotal form or to a flat "wet" mycelial colony with a yellow to orange appearance when cultured on PDA. Macroconidia have 3-septate, short to medium length, straight to slightly curved, relatively slender and thin walled. Its apical cell morphology is tapered and curved, while the basal cell morphology is foot shaped to pointed. It is abundant in sporodochia and occasionally from hyphae growing on the agar surface. The microconidia are oval, elliptical or kidney shaped and usually 0-septate. It is abundant in the aerial mycelium and appears as false heads. The conidiogenous cells have short monophialides (Fig. 5).

Fusarium verticillioides

Initially cultures have white mycelia, but may develop violet pigments with age. Pigmentation in the agar varies, ranging from no pigmentation or grayish orange to violet grey, dark violet or dark magenta (almost black) in others. Blue-black sclerotia may develop in some isolates, but are not diagnostic although they may be indicative of a high level of female fertility.

Sporodochia in the macroconidia may be tan or orange in colour and present as discrete entities or as a pseudo-pionnotal mass. The macroconida which has 3- to 5-septate are relatively long and slender, slightly falcate or straight, and thin walled. The microconidia abundant in the aerial mycelia is oval to club shaped, with a flattened base and usually 0-septate. Long chains are common, aerial mycelium but small aggregates of a few spores occur occasionally. The conidiogenous cells consists monophialides, which are occasionally produced in pairs to give a "rabbit ear" appearance (Fig. 6).

Springtails

Springtails are minute, to medium wingless entognaths insect less than 0.5 cm in length. The springtails fed on mycelium in compost, resulting in disappearance of mycelium from spawn compost. They also affected fruiting bodies of some mushrooms and caused slight pitting or browning at feeding sites. They congregated at base of stipe and ate out mycelia strands (Fig. 7).

Fruit flies

Drosophila melanogaster is a species of Diptera, in the family Drosophilidae. They are about 0.2- 0.5cm in length. They also fed on mushrooms by sucking sap fluxes hence causing damage (Fig. 8).

Spider

Spiders (Arachnida) are air breathing arthropods that have eight legs and chelicerae with fangs that inject venom. They visited the mushroom to sap nutrient, bore hole into the stipe of mushroom and caused mycelium damage (Fig. 9).

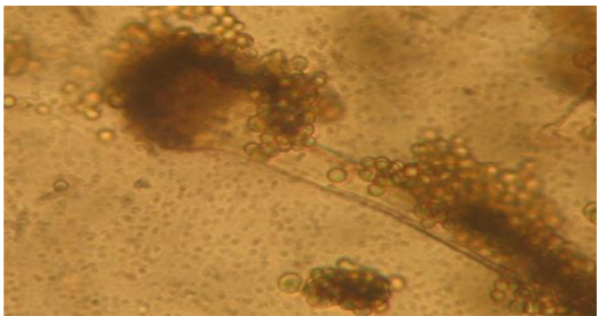

Fig. 1. Micrograph of *Aspergillus flavus*

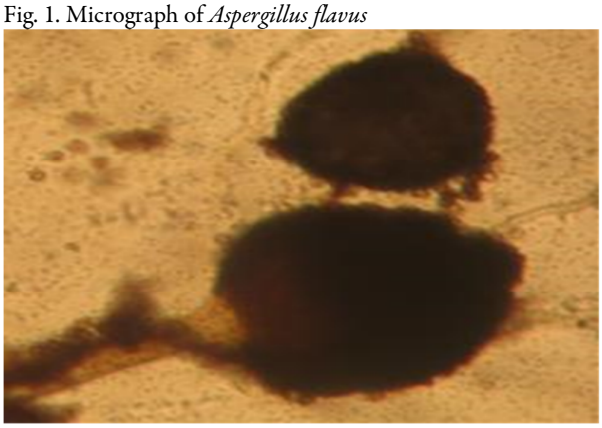

Fig. 2. Micrograph of *Aspergillus niger*

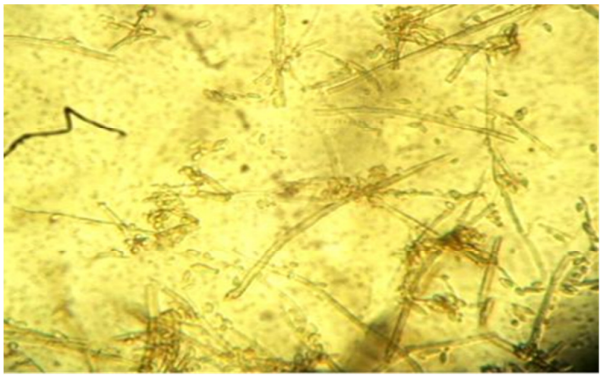

Fig. 3. Micrograph of *Cladosporium* sp.

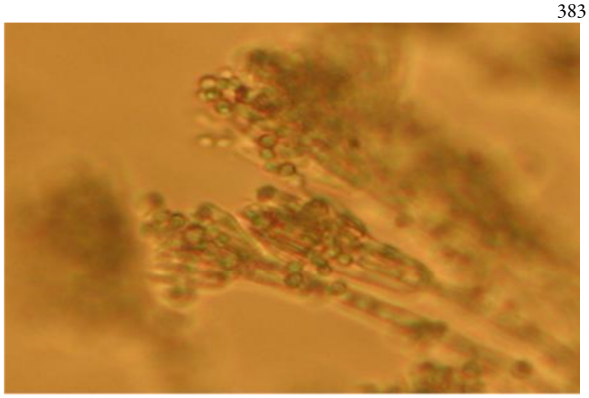

Fig. 4. Micrograph of *Penicillium oxalicum*

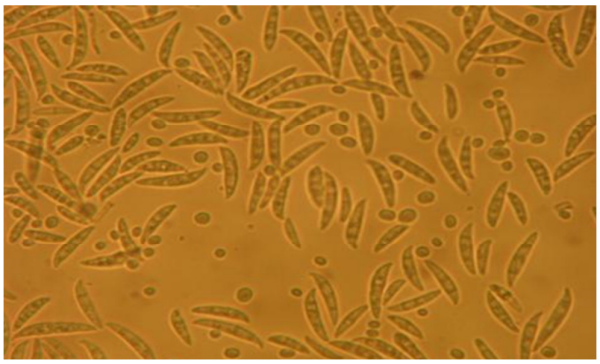

Fig. 5. Micrograph of *Fusarium oxysporium*

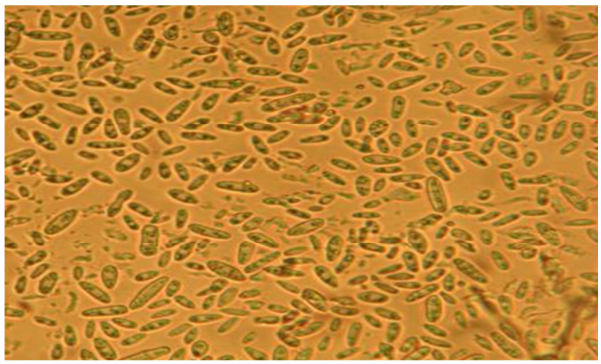

Fig. 6. Micrograph of *Fusarium verticillioides*

Fig. 7. *Lepidocyrtus cyaneus* (Silver springtail), with visible furcular

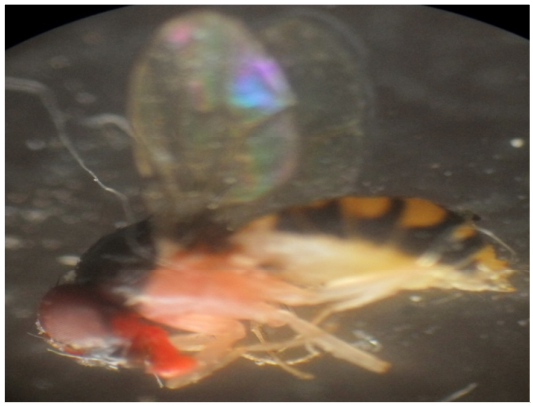

Fig. 8. *Drosophila melanogaster* (fruit fly)

Fig. 9. Arachnida (spider)

Table 2. Number of insect orders encountered on cultured mushroom *(Pleurotus ostreatus)*

	Encountered						
Collection site	Date of collections	Mushroom species studied	No of insects	Insect order	Common name	Scientific name	Life stage
Beejay ventures, Odo ona	20/03/2013	*P. ostreatus*	3	Collembola	Springtail	*Lepidocyrtus cyaneus*	Adult
				Diptera	Fruitfly	*Drosophila melanogaster*	Adult
	10/04/2013			Araneae	Spider	*Arachnida*	Adult
	10/04/2013	*P. ostreatus*	1	Collembola	Springtail	*Lepidocyrtus cyanus*	Adult
	30/04/2013	*P. ostreatus*	2	Collembola	Springtail	*Lepidocyrtus cyanus*	Adult
				Diptera	Fruitfly	*Drosophila melanogaster*	Adult

Discussion

Fungal pathogens isolated from infected mother spawn of *Pleurotus ostreatus* were identified as *Aspergillus niger*, *Aspergillus flavus*, *Cladosporium* sp., *Fusarium oxysporium*, *Fusarium verticilliodes* and *Penicillium oxalicum*. Similar observations were also reported in accordance with the findings of Jonathan *et al*. (2012). The morphology and cultural characteristics of fungal isolates observed from infected mother spawn of *P. ostreatus* also agrees with the observation made by Sigler *et al*. (2003) and Jonathan *et al.* (2012). Many of these fungi are parasite on cultivated plants and on other fungi (mushroom) causing extensive damage to agriculture and pose risk to indoor production during the fruiting stage (Lillian, 2007). Fungal spores can also cause allergies and fungi from different taxonomic groups can evoke allergic reactions (Gryzenhout *et al*., 2006).

The use of sterilized casing soil, proper disposed of spent compost, proper hygiene and sanitation are essential to avoid primary infection (Sharma, 1994). Wuest and Bengtson (1982) suggested that physical treatment for thirty-minute treatment with aerated steam at 60 °C and 82 °C hinder spore germination. Tregoff and Ricard (1976) suggested that trichoderma propagles/litre/m^2 can be sprayed biologically on casing soil to control fungal diseases on naturally infected mushrooms. Geijn (1977) recommended that fungal disease of oyster mushroom (*P. ostreatus*) can be controlled chemically by spraying with carbendazin benomyl or thiophenate methyl at 100, 150 and 200 g/100 m^2 respectively in 100-150 litres of water immediately after casing.

Generally, proper pasteurization and conditioning of compost, sterilizing the supplement before use and using correct concentration of formalin (maximum 2%) was advised to prevent fungi diseases. In order to decide the most effective measures for controlling fungal diseases in mushroom, it is necessary to understand the size of the initial innoculum, density, the rate at which the diseases develop and spreads and the time when the infection takes place (Zhang, 1990). Fungal disease could be best controlled by a complete careful farm management and hygiene and also with the application of the recommended fungicides such as benomyl and chlorothanil (Fasidi *et al*., 2008).

The infestation of *P. ostreatus* with several arthropod pests such as springtails and fruit flies which were found on the mushrooms also conforms to the observations made by Jonathan *et al*. (2012) who encountered Collembola and Diptra orders of insects. On the other hand, spider which belongs to the order Araneae was encountered on *P. ostreatus* during the harvesting period. This could be attributed to high nutritional content of the staple diet of spider which was rotten mushrooms and other microscopic insects.

The increase in number of insects found and the damage done on the mycelia is related to their feeding habit, which could be associated with the high level of sugar alcohol in *P. ostreatus.* Springtails enter into mushroom house along with organic matter, feed on gills resulting in the destruction of gill lining, eat up edges of the pileus and lamella. Also, ingest the mycelium. Furthermore, from this work springtails may be implicated in the damage of mycelium by feeding on their hyphae, also transmitting fungal infection. The observed damages done by the insects and transmission of fungal infection in this study supported the findings of Lillian (2007) and Jonathan *et al*. (2012). Springtails congregate at the base of stipe and cut out mycelia strands due to the cool nature of the environment (Imms, 1965). The Dipterans encountered were moderately sized, with low level of infestation compared to the Collembola. The ability of fruit flies to appear from "nowhere" and the facts that they seem to be everywhere when foods are exposed are source of amazement to most home owners and individuals in food industry. For instance, the predominance of insects in *P. sajor caju* could be attributed to open gilled sporocarps of this mushroom which provided ample hiding space for grubs, larvae and adults of springtail Spiders also visited the mushroom to sap nutrient, bore hole into the stipe of mushroom and cause mycelium damage (Kumar *et al*., 2012).

The present study provides useful information on how the various arthropod pests have caused damage to these mushrooms having the potential to cause economic loss, reduce mushroom yield and quantity. Also, insect have been found to inhabitate mushroom in order to complete their life cycle. In this process, they reduce the growth rate of mushroom, bore holes on different parts of mushroom and thereby reducing the market value of the mushroom.

Measures to minimize insects' infestation can be achieved through maintenance of good hygienic environment for the mushroom, pasteurization of compost and casing material. Others are proper disposal of spent compost, disinfecting the composting yard, empty growing room with 0.05% malathion and mixing diazinon 30 ppm (15 ml diazinon after diluting with water) in 100 kg of compost at the time filling. For controlling infestation during spawn run and cropping periods, malathion or dichlourvos at 0.025-0.05% should be sprayed and observed for a period of 2 to 5 days (Sandhu and Batthall, 1987). According to Cantelo (1980), reducing fly numbers without using insecticides requires a good understanding of fly biology and behaviour. Thereafter, nontoxic chemicals such as Diflubenzuron may be applied in order to arrest the development of insect larvae (Fasidi *et al*., 2008).

Mushroom house vents must have air filters such as screen net. However, to minimize disease problems and favour the growth of mushrooms, it is important to provide optimum growing condition and carry out practices based on the full understanding of biology of mushroom and the pests. This knowledge involves biology and ecology of mushroom, plant pathology, entomology and zoology.

Conclusions

Certain associations were observed between the mushroom *P. ostreatus*, fungi and insect pests. Four different fungi genera and three arthropods orders were encountered in association with the studied mushroom. These fungi and insects exhibited parasitic associations with the mushroom which resulted in the use of the mushroom as food among other uses. Their presence resulted in damages recorded on mushroom due to feeding activities, thus in turn reduce their market values and resulting in financial loss to the growers. Measures to minimize insects' infestation can be achieved through maintenance of good hygienic environment for the mushroom, pasteurization of compost and casing material. Others are proper disposal of spent compost, disinfecting the composting yard. However, to minimize disease problems and favour the growth of mushrooms, it is important to provide optimum growing condition and carry out practises based in combination with knowledge in mushroom pathology and entomology.

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