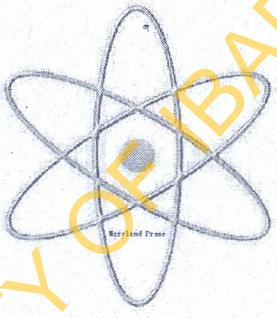
An International Journal

Nature and Science

ISSN 1545-0740

Volume 10 - Number 09 (Cumulated No. 66), September 25, 2012



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Nature and Science

ISSN 1545-0740

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ISSN 1545-0740

9 771545 074382

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Insect and fungal pests of some mushrooms collected from university of Ibadan,Nigeria campus

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Abstract: Ten mushrooms species collected within the premises of University of Ibadan were examined for infestation of various insect and fungal pests .Insects belonging to the orders; Coleoptera, Hymenoptera, Diptera, and Collembolla were encountered both at the larval and adult stages of life on the collected mushroom samples. Infestation by the order Coleoptera (adult beetle) on *Pleurotus squar-rosulus* was found to be higher in incidence, with a total number of 17species which were found at the adult stage of life; but the larva stage were found on *Lycoperdon gigantum* . Fungal species identified to be *Aspergillus niger, Aspergillus terreus*, *Fusarium redolens*, *Trichoderma viride*, *Rhizopus stolonifer* and *Mucor piriformis* were found to be associated with several species of mushrooms.

[Jonathan S.G., Popoola K.O.K., Olawuyi OJ ,Ajiboye M. and Oyelakan A. O. **Insect and fungal pests of some mushrooms collected from university of Ibadan,Nigeria campus.** Nature and Science 2012; 10(9): 142-147]. (ISSN: 1545-0740). http://www.sciencepub.net. 20

Keywords: Mushrooms, fungal pathogens, insects, collection, pollution

1. Introduction

Morphologically, mushrooms have a fruiting body which can be easily distinguished by the sporocarps. A typical mushroom is made up of the pileus or cap; it is an expanded portion which may be thick, fleshy, membranous and also with varied shape (Zoberi,1973;Jonathan and Adeoyo,2011a). The lamellae or gill which is leaf-like radiating from the edge inward towards the stem and the stipe or stalk supporting the pileus. (Atkin, 1982, Jonathan,2002). Mushrooms are the richest source of vegetable proteins. They contain 31-40% of protein. Mushrooms contain minerals like calcium, potassium, sodium, phosphorus and vitamins like B, C, D and K. mushroom contains niacin which is ten times higher than other vegetables (Jonathan *et al.,*2012. The fruit bodies of mushrooms are used to produce suede-like material from which hand bags, hats, clothing, and picture frames are made. (Chang and Hayes 1978) .

Mushrooms have very less calories and contain approximately 80 to 90 percent water(Aina *et al*,2012). At the same time, they have low sodium, carbohydrate and fat content and high fibre content. This is the reason why mushrooms are considered good for those aiming to loose weight . Mushrooms are valuable health foods that is low in calories, high in vegetable proteins chitin iron zinc fibre essential amino acids, vitamins, and minerals, such as copper that help the body to produce red blood cells (Esminger and Esminger 1986,Jonathan *et al,*2006;Aina *et al*., 2012).

Mushrooms are excellent source of potassium. In fact, it is said that there is more potassium in a mushrooms than in banana. Since potassium helps lower blood pressure and diminish the risk of stroke, mushrooms are recommended to people suffering from hypertension (Chang *et al*., 1989; Gbolagade, 2005). Mushrooms are rich in copper, a mineral that has cardio-protective properties. A single serving of mushrooms is said to provide about 20 to 40 percent of the daily needs of copper. They are excellent source of selenium, an antioxidant that works with vitamin E to protect cells from the damaging effects of free radicals. Researchers have suggested that white button mushrooms could reduce the risk of breast and prostate cancer. In fact, extract of white button mushrooms has been found to help in diminishing cell proliferation as well as tumour size. It has been found that mushroom extract helps stop migraine headaches and is beneficial for people suffering from mental illnesses, like obsessive-compulsive disorder (Jonathan, 2002;Aina *et al.*,2012) .Oyster mushrooms are said to be useful in strengthening of veins and relaxation of the tendons.

Despite nutritional and medicinal importance of mushrooms, they are being face with many pests and diseases. The various insects pests associated with mushrooms include; flies such as sciarids, phorids and cecids (Ajayi and Jonathan,2004;Fasidi *et al*, 2008). The flies belong to the order Diptera. Sciarid flies also known as fungus gnats belong to the family: Sciaridae and Species include*; Sciara multiselta, Sciara agaris.* Cecid flies also known as gall midges belong to the family: *Cecidomydae* and

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Species include; *Mycophila speyeri*, *Mycophila borresi*. 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 (*Tarsonemus sp*), straw or hay mites (*Tyrophagus sp)*, Red pepper mites/pygmy mites (*Pygmephorus sp*). Eelworms or nematodes, they are tiny and transparent, they include the parasitic eelworms which are directly harmful such as; *Composticola, Ditylenchus muceliophagus*, and also the saprophytic nematodes which are indirectly harmful such as the Rhabilit types. The springtails which are also tiny insects include species such as *Isotoma simplex, Lepidocrytus cyaneus*. (Keil, 1996). Fungal diseases of cultivated mushrooms include; Dry bubble disease caused by *Verticillium fungicola*, wet bubble disease caused by *Mycogone perniciosa*, Cobweb or Dactylium mildew caused by *Cladobotryum dendroides (Hypomyces rosellus),* Green mould caused by *Trichoderma. (*Gbolagade, 2005, Fasidi *et al*., 2008*)*.There are certain abnormalities that occur in mushrooms and these disorders have several abiotic origins. Such abnormalities include; formation of stroma, formation of scales or crocodile skins, changes in the colour of fruit bodies, outgrowth on mushroom cap, long stipe, small cap on a normal stipe, rosecomb and scaling. (Singh et *al*,1991;Ajayi and Jonathan,2004;Gbolagade, 2006). The objectives of this research work were to Identify various insect and fungal pests found on wild edible mushrooms and their features of damage and suggest possible control measure for the insect and fungal pests of mushrooms.

2. Materials and Methods

2.1 Study area

This study was conducted at the University of Ibadan, Oyo state. Ibadan is located in the South western Nigeria approximately between Latitude N 7^{o} 26^{1} Longitude E 3^{o} 53^{1} and an Altitude of 190m. The city ranges in elevation from 150m in the valley area to 275m above sea level . Ibadan has a tropical wet and dry climate with mean monthly temperatures fluctuating between 23^{o} C to 30^{o} C and humidity is usually from 55% to 75% .

2.2 Mushroom collection

The sample collection site for this research work was the University of Ibadan premises including Ibadan University Botanical Gardens. Between the month of April and August 2011. Survey trips and inventory of mushrooms in these areas were taken at seven days intervals. Ten species of mushrooms were collected from the sample areas and each of the specie was replicated ten times. Collections were made in the morning. Mushrooms were collected using a shovel for obtaining part of the substratum (wood) on which mushrooms were growing, following the procedure of Jonathan and Adeoyo (2011b).They were identified using the standard procedures of Zoberi (1973).

2.3 Insect collection

Insect pests were removed from the mushroom samples by hand picking method. Insects were picked from each species of mushrooms at the point of collection and kept in specimen bottles. After collection, mushrooms were brought to the laboratory and part of the sporophores were carefully opened up using a dissecting knife in order to bring out the insects that had bored into the mushroom tissues. Pests were brought out and placed in labelled specimen bottles; insects were then preserved in 4% formalin. (Kim and Hwang, 1996).

2.3 Identification of insects

They were identified using the procedures of Kim and Hwang (1996).Accuracy of identification were were carried out using the method and Bartlett. (1996).They were authenticated by Dr K.O.K Poopoola an Entomologist in the Department of Zoology, University of Ibadan. The identified arthropod species were stored in the Entomology laboratory, Department of Zoology,University of Ibadan,Ibadan,Nigeria for reference purpose.

2.4. Fungal isolation and characterization

Fungal infected mushrooms were collected and brought to the laboratory for isolation. Excised portions of the infected portion of *Pleurotus squarrosulus, Pleurotus pulmonarius* and *Pleurotus tuber-regium* were plated using potato dextrose agar (Oxoid). Streptomycin sulphate (0.05g/1000cm^{-3}) was added to prevent bacterial contamination (Jonathan and Fsidi,2001). The isolates were plated in triplicates and incubated at room temperature (25 $\pm$ 2^{0}C) for 7 days. At the end of the incubation period, the plates were observed for fungal growth and different colonies were sub-cultured on fresh plates of potato dextrose agar. Wet mount was done on greese free slides using 0.1% lactophenol cotton blue and were observed under the microscope (Domsh *et al*., 1980). Cultural features observed on isolated fungi and characterization were carried out using the descriptions of Alexopolous(1996).

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3. Results and Discussion

Results from preliminary studies revealed that the species of mushrooms (*Pleurotus squarrosulus, Volvariella esculenta, Termitomyces robustus, Pleurotus tuber-regium, Coprinus commatus, Lycoperdon gigantum, Boletus edulis, Macrolepiota sp, Agaricus campestris* and *Psathyrella hydrophila*) were infested with various arthropod pests. *Boletus edulis* was found with the highest number, having a percentage composition of 10.1% and *Polyporous melanopus* was found with the lowest number and a percentage composition of 1.4%.

Table 1. List of Mushrooms by Families on University of Ibadan Campus.

Families	Mushrooms	Numbers	%Composition
Agaricaceae	*Agaricus campestris*	12	8.1
	Pleurotus tuber-regium	10	6.8
	Pholiota terrestris	5	3.4
	Pleurotus squarrosulus	11	7.4
Polyporaceae	*Polyporous melanopus*	2	1.4
	Ganoderrma lucidium	7	4.7
Coprinaceae	*Coprinus commatus*	8	5.4
	Psathyrella hydrophila	13	8.8
Amanitaceae	*Amanita verna*	5	3.4
Lycoperdaceae	*Lycoperdon germinatum*	5	3.4
Clavariaceae	*Clavaria vermicluris*	3	2.0
Boletaceae	*Boletus edulis*	15	10.1
Tricholomalaceae	*Tricholoma aurantum*	6	4.1
	Chlorophyllum molybdbtis	10	6.8
	Macrolepiota sp	7	4.7
	Volvariella esculenta	10	6.8
	Termitomyces robustus	9	6.1
	Cylocybe dilate	10	6.8

Table2. Mean number of Edible mushrooms from sample areas in the University of Ibadan

Sample Areas	Numbers	Means
Botanical garden		
Agaricus campestris	12	1.2
Volvariella esculenta	10	1.0
Bolestus edulis	15	1.5
Nursery (Botany dept.)		
Termitomyces robustus	9	0.9
Macrolepiota sp	7	0.7
Pleurotus tuberigium	10	1.0
Jaja		
Coprinus commatus	8	0.8
Lycoperdon germinatum	5	O.5
Balewa road		
Pleurotus squarrosulus	11	1.1
Psathyrella hydrophila	13	1.3

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Table 3. Insect Pests Encountered on Mushrooms, order, common name, life stage and number collected.

Sample areas	Mushroom	Insect order	Common name	Life stage	Number collected
Botanical garden	*Agaricus campestris* *Volvariella esculenta* *Boletus edulis*	Coleoptera	Beetle	Larval Adult	4 11
Nursery	*Termitomyces robustus* *Macrolepiota sp* *Pleurotus tuberigium*	Coleoptera Coleoptera Hymenoptera Collembola Diptera	Beetle Beetle Ant Springtail True fly	Larva Adult Adult Adult Adult	4 1 1 5 1
Jaja	*Coprinus commatus* *Lycoperdon germinatum*	Hymenoptera Coleoptera	Ant Beetle	Adult Larva	12 1
Balewa	*Pleurotus squarrosulus Psathyrella hydrophila*	Coleoptera	Adult Beetle	Larval Adult	17 2

Table 4. Distinguishing features of pests and damages done on different part of mushroom.

Order	Distinguishing features	Damages done on Mushroom
Collembola	Moderate sized, elongated body which is slivery in colour	Eats up the edges of the pileus and lamella, ingestion of mycelium
Diptera	Presence of a shiny black head capsule usually elongated and vermiform in shape	Feeds on lamellae, loss of mycelium and pileus, reduction of stipe.
Hymenoptera	Comparatively large, with a pair of antennae of 4-13 segments, abdomen distinctly constructed at the base, No wings.	Loss of pileus, lamella and reduction of stipe.
Coleoptera	Elongated body with dark elliptical body, covered with setae	Bore hole into the stipe of the mushrooms

Table 5- Identified isolates from infected mushroom samples

Isolate code	Isolate	Surface colour	Reverse colour
SQ1	*Aspergillus niger*	Blackish brown	Creamish yellow
FL1	*Aspergillus niger*	Blackish brown	Creamish yellow
SAJ1	*Mucor piriformis*	Black	Milky
SQ2	*Fusarium redolens*	Orange	Creamish
FL2	*Aspergillus terreus*	Cinnamon(brownish)	Yellowish brown
SAJ2	*Rhizopus stolonifer*	Reddish brown	Milky
FL3	*Trichoderma viride*	Green	Creamy

Table 6. Morphology and cultural characteristics of fungal isolates obtained from infected mushroom samples

Isolates	Mycelia colour	Reverse colour	Growth pattern	Microscopic examination
Aspergillus terreus	Cinnamon (Brownish)	Yellowish brown	Rapid	Conidial head showing metulae and phialides
Aspergillus niger	Blackish brown	Creamish yellow	Rapid	Conidial head with metulae and phialides
Mucor piriformis	Whitish mycelia with blackish sporangia	Milky	Grows very fast	Sporangiophore tips with columellae
Rhizopus stolonifer	Reddish brown	Milky	Rapid	straight dark brown sporangiophore with collumellae
Fusarium redolens	Orange	Creamish	Rapid	Macro conidia formed with chlamydospores arising in the mycelium and conidia
Trichoderma viride	Green	Creamy	rapid	Conidiophore pyramidically branched, phialides slender and irregularly bent.
Aspergillus niger	Blackish brown	Creamish yellow	Rapid	Conidial head with metulae and phialides

Insect pests such as ants, beetles and true flies were encountered on the mushrooms, they were found at the larval and adult stages. Insect orders such as Coleoptera, Hymenoptera, Collembola and Diptera were present. Infestation by Coleoptera (Adult beetle) in *Pleurotus squarrosulus* was found

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to be high, with a total number of 17 which were found at the adult stage of life and the number found in *Lycoperdon gigantum* was low also found at its larval stage. Their population was observed to be high in the lamellae of *Pleurotus squarrosulus* due to their feeding habits and protection derived from the lamell. The distinguishing features of the Coleopterans were also recorded; they have elongated body with dark elliptical body which are covered with setae. Their features of damage were also observed, they bore holes into the stipe of the mushroom.

In this study, beetle larvae also caused damages to the mushrooms, this type of damage was found to be related to those reported by Jonathan (2008). This work also showed that they were responsible for mycelium damage by feeding on the hypha and also transmits fungal infection which can be related to the report of (Fasidi *et al.,* 2008). The Collembola (Springtails) were found present at the Adult stage, they are moderately sized, with elongated body which is silvery in colour. They eat up the edges of the pileus, lamella and also ingest the mycelium. The Diptera (True fly) were also found on *Termitomyces robustus at* the Adult stage, they posses a shiny black head capsule usually elongated and vermiform in shape, they damage the mushroom by feeding on the lamella, they cause loss of mycelium and pileus, they also reduce the stipe. The Hymenoptera (Ants) were present on *Pleurotus tuber-regium* and *Coprinus commatus* at the Adult stages of life. The Ants having a blue-black with brown stripes, the abdomen distinctly constricted at the base, they also lack wings. The damages caused includes; loss of pileus, lamella and reduction of stipe. Since it is generally known that ants eat almost anything sugary and the major constituent of mushroom is sugar-alcohol mannitol. This justifies their presence in mushrooms. However, this study provides useful information on how the various Arthropod pests have caused damage to these mushrooms. Also, insects have been found to infest mushrooms for them to be able to complete their life cycle and, in this process, they reduce the growth rate of mushroom; they nibble holes on different parts of the mushroom thereby reducing the market value of the mushroom. As described by Cantelo (1980), reducing fly numbers without using insecticides require a good understanding of fly biology and behaviour, therefore non-toxic chemicals such as Diflubenzuron may be applied in order to arrest the development of insect larvae (Fasidi *et al.,* 2008)

Fungal isolates such as *Aspergillus niger, Fusarium redolens, Mucor piriformis, Aspergillus terreus, Rhizopus stolonifer and Trichoderma viride* were isolated from the fungal infected mushrooms (*Pleurotus pulmonarius, Pleurotus tuber-regium* and *Pleurotus squar-rosulos).* Similar fungal species were reported by Ajayi and Jonathan (2004).The morphology and cultural characteristics of fungal isolates obtained from infected mushroom samples were observed. Identification of each genus was based on morphological and cultural characteristics compared to compendium of soil fungi (Domsh et al., 1980).They were further characterised using the standard descriptions of Alexopolous *et al*(1996).Confirmed identification were carried out using illustrated manual of Singh *et al*., (1991).

Aspergillus niger was a fast growing fungus which appeared dark brown at first and later turned black, with conidia heads which were globose and later spilled to conidia chain which were brownish and smooth. *Rhizopus stolonifer,* was reddish brown in colour had a rapid growth with straight dark brown sporangiophore and collumellae. *Mucor piriformis* which had whitish mycelia with blackish sporangia covered the plates after 48 hours, produced sporangiophore tips with collumellae. *Aspergillus terreus* had colonies with cinnamon (brownish) colour, the conidia was globose, conidia head showed metulae and phialides. *Fusarium redolens,* appeared orange in colour, formed macro conidia with chlamydospores arising in the mycelium and conidia. *Trichoderma viride,* the growth greenish in colour, and the growth was much after 48 hours. Microscopically, the conidiophores was pyramidically branched, phialides slender and irregularly bent.

Banrnet and Hunter 1972,suggested that fungal diseases can be managed physically by steaming at 54.4^{0}C for 15 minutes which will eliminate the disease from casing soil. Kim and Hwang (1996), suggested three methods of prevention of fungal diseases which includes; steam sterilization of mushroom beds, formaldehyde fumigation and fungicidal application. Jonathan (2008) suggested that fungal diseases could be best controlled by a complete careful farm management and hygiene, also recommended fungicides such .He also suggested application of benomyl and chlorothanil at a recommended dosage. It should be noted that these control measures could only be applied to cultivated or domesticated mushrooms. The diseases in wild mushrooms may be difficult for treatment and control unless if they grow together in a specific habitat.

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