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Insects Associated with an Edible Mushroom *Pleurotus tuberregium* (Basidiomycota): First fact-finding Approach in Benin City, Nigeria

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ABSTRACT: Mushroom growers are confronted with insect infestations in Benin City, where the composition of insect taxa associated with an edible mushroom *Pleurotus tuberregium* was examined for informed management decisions using standard method. Fifteen samples of insects were collected during the duration of sprouting to harvest of the mushroom. Twelve insect taxa were observed on *P. tuberregium* after being identified to their respective orders and families using an insect identification key. Following three non-parametric estimators (first and second order Jackknife, and Chao estimates), between 76% and 93% of the 'true taxa richness' of insects associated with the mushroom were estimated to have been encountered. Of these, *Drosophila* species (Diptera: Drosophilidae) were the most abundant insects followed by the Staphylinids (Coleoptera: Staphylinidae). Larvae of *Drosophila* were found feeding within the tissues of infested fruiting bodies of *P. tuberregium*. Some of the insects found on the mushroom can threaten its commercial farming and growers' return on investment. The need to prioritise consumer-friendly pest management options as opposed to chemicals was discussed.

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Mushrooms are macrofungi that belong to the family Basidiomycota, which are either epigeous or hypogeous with fleshy spore-bearing fruiting body, equipped with stem (or stipe) and cap (or pileus) (Chang and Miles 2004). Without leaves, chlorophyll, roots or seeds, they flourish in damp and shady environment. Worldwide, commercial production of mushroom comes with numerous economic, nutritional and pharmaceutical benefits (Aina *et al.* 2012; Bellettini *et al.* 2018). Low calories, high proteins and other essential minerals available in mushroom may positively contribute to global health index given the number of people using it.

Mushrooms are a vital source of vitamins and minerals and a typical example of such valuable mushroom is the oyster mushroom, like *Pleurotus tuberregium* (Fr.) Singer. In Nigeria, mushrooms such as *P. tuberregium,* serve as good substitutes for meat protein in several suburbs (Ene-Obong and Camovale, 1992; Gbolagade *et al*., 2006). Several species of mushrooms have been reported as local delicacies across different tribes in Nigeria (Akpaja *et. al*., 2003, 2005; Gbolagade *et al*., 2006) and as tools for bioremediation (Kubatova *et al.* 2001).

To transform the potentials of edible mushrooms into wealth, large-scale production of unblemished products is needed. However, different factors, e.g., abiotic factors and biotic factors –insect infestation (Bellettini *et al.* 2018 and references cited therein)– may impinge on such production as insects have been anecdotally reported to plague *P. tuberregium* in different parts of Benin City in Nigeria. Besides insect infestation, secondary infections from insect vectors may arise (Bellettini *et al.* 2018). Given the value of mushrooms (e.g., Gbolagade *et al*., 2006; Akpaja *et. al*., 2003, 2005) and their susceptibility to insect infestations, which growers are striving to avoid, we surveyed the composition of insect taxa associated with *P. tuberregium* to document the array of insect taxa on it, and to discuss the probable management options.

MATERIALS AND METHODS

The study was conducted in 2018 at the botanical garden of Faculty of Life Sciences at University of Benin situated at N 6° 23' 51.0"; E 5° 36' 57" with an attitude of 107 m asl. Locally sourced sclerotia of *P. tuberregium* were cut into ten roughly uniform sizes

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of about 100 g, which were subsequently cultivated in perforated plastic pots containing heat-sterilised and water saturated soil. Prior to cultivation, dry masses of sclerotia of *P. tuberregium* were soaked in water for six hours for rehydration. Ten randomly placed soil-filled pots were apportioned for both control (i.e., pots filled soil without mushrooms) and mushroom-cultivated units.

Insect were sampled from sprouted mushroom using aspirators and pooters, and collected insects were introduced into a killing jar charged with ethyl acetate. Thereafter, the collections were mounted in an entomological box in Insect Ecology and Diversity Unit, where they were sorted into morphotaxa. After insect collection, mushrooms were further assessed for any insects within its parts using a dissecting blade along with the aspirators. Taxa composition of insects associated with the fruiting bodies of *P. tuberregium* was tabulated and counts were recorded. Statistical analyses were run using PAST™ for constructing the dendrogram of similarity in composition among samples and taxa rarefaction curves. The dendrogram was constructed with classical multivariate clustering using the Unweighted Pair-Group Method with Arithmetic Mean (UPGMA) algorithm coupled with a Bray-Curtis similarity index. Other diversity indices were computed also computed.

RESULTS AND DISCUSSION

The composition of insect families found in and on *Pleurotus tuberregium* were fruit flies (Drosophilidae), frit flies (Chloropidae), rove beetles (Staphilinidae) and springtails (Isotomidae), albeit they differ disproportionately in abundance (Table 1). Over fifteen sampling occasions, the majority of insects encountered on *P. tuberrgium* were those of adults and larvae of *Drosophila melanogaster* with the highest total count being 281 (i.e., the sum of 68 adult and 213 larval) individuals representing fifty two percent of the total number (536) of insects collected (Table 1). That was closely followed by members of the family Staphilinidae (Coleoptera) with 230 (42.9% of the total) individuals. The least abundant insects were two singletons and their true taxonomic statuses remain to be established. Understanding the composition of the insects associated with, and those culpable in inflicting damage on, *Pleurotus tuberregium* is vital to scale up mushroom production for local and international markets in Nigeria. Our findings here do not support any advocacy for an open-field cultivation of *P. tuberregium* (and perhaps of other economic species of mushrooms) as twelve insects, which belong to five orders (namely: Diptera, Collembola, Coleoptera, Hymenoptera, Hemiptera; and two undetermined) and five families (namely: Chloropidae, Isotomidae, Staphylinidae, Platygastridae, Drosophilidae; and two undetermined) were encountered on it. Unlike the observations in the units where mushroom were cultivated, no insect was encountered on the control units; thus suggesting that the insects found on the mushrooms were only attracted to them.

Table 1. Taxa composition associated with *Pleurotus tuberregium* that was set up in a secondary forest of the University of Benin botanical garden, Benin City, Nigeria.

		Sample (counts)															
S/No.	Order (Family)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Total
1	Diptera (Chloropidae)	5	0	0	1	0	0	0	0	0	0	0	0	0	0	0	6
2	Collembola (Isotomidae)	3	0	0	0	0	0	0	0	0	0	2	1	0	1	0	7
3	Coleoptera (Staphylinidae)	1	3	0	3	1	4	0	0	0	0	0	0	0	0	0	12
4	Coleoptera (Staphylinidae)	46	0	0	60	12	1	0	0	5	7	55	10	5	6	4	211
5	Coleoptera (Staphylinidae)	5	0	2	0	0	0	0	0	0	0	0	0	0	0	0	7
6	Hymenoptera[u]	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
7	Hymenoptera (Platygastridae)	1	1	0	0	0	0	0	0	0	0	1	1	0	0	0	4
8	*Unidentified nymph*	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
9	Diptera (Drosophilidae)*	0	115	0	0	0	0	0	0	3	64	6	2	7	11	5	213
10	Diptera (Drosophilidae)**	0	2	0	0	1	16	3	6	4	0	11	7	8	4	6	68
11	*Unidentified*	0	0	1	1	1	0	0	0	0	1	0	0	0	0	0	4
12	Hemiptera[u]	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
	Total Individuals	64	121	3	66	15	21	3	6	12	72	75	21	20	22	15	536
	Total species	8	4	2	5	4	3	1	1	3	3	5	5	3	4	3	12

*Note: [u] undetermined family; *larvae; **adults*

Sample-based species rarefaction curve for the insects associated with mushroom approached its asymptote at the fourteenth sample out of the fifteen sampling events (Figure 1). Over 90% of the insects associated with *P. tuberregium* were encountered after thirteen sampling events as indicated by the asymptote of the curve. Individual-based sample showed that the rarefaction curve was far from reaching its asymptote (Figure 2). The result suggests that additional individuals, but not necessarily new taxa, would have been collected if the sampling period was extended as the rarefaction curve of individuals did not reach its asymptote at the end of the growth phase of the mushrooms. Most of the insects encountered were dipterans, which have been implicated for inflicting damage on mushrooms elsewhere either directly as primary consumers or indirectly as mechanical vectors of mites, mold, bacteria, virus and nematodes (Rinker *et al.* 2017; Bellettini *et al.* 2018).

Insects encountered were not equally abundant as shown by evenness index (0.3252) from a total of 536 individuals belonging to eleven orders. On average, the number of species collected per sample was low (3.6 ± 0.12). Global beta diversity was low adjudging from Whittaker's index of 2.33, which suggests that all the samples were not homogeneous, but moderately heterogeneous. The true taxa richness of insects associated with the mushroom as computed using first and second order Jackknifes and Chao2 non-parametric estimators suggested that the extent of insect taxa collected during sampling were 81% 76% and 93%, respectively. On the average 83% of the true species associated with *P. tuberregium* were encountered according the three estimators. The similarity of taxa composition at each sampling event as schematically represented (Figure 3) shows that the incidence of observed insects varies largely across samples.

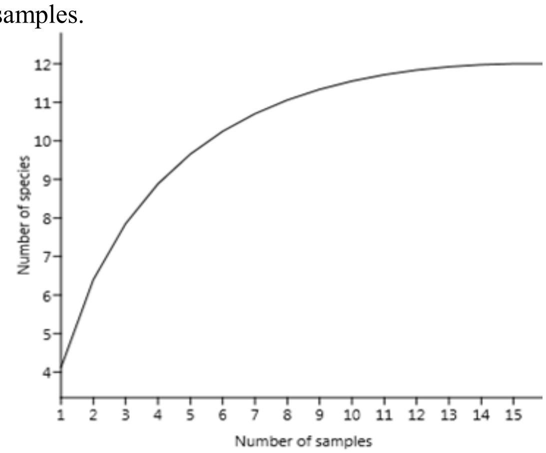

Fig 1 Sample-based species rarefaction curve for insects associated with mushrooms in university of Benin Botanical Gardens, Benin City, Nigeria.

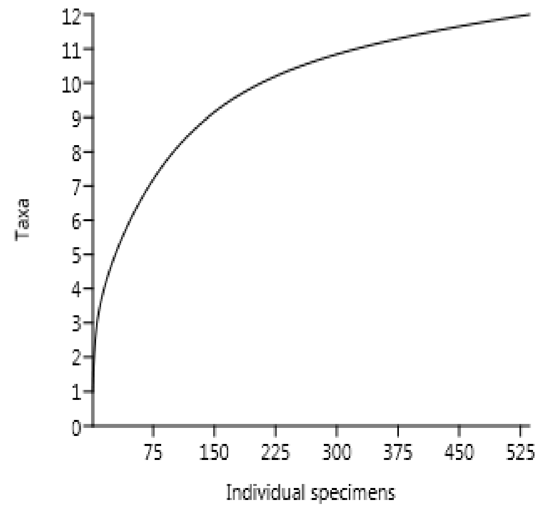

Fig 2. Individual-based species rarefaction curve for insects associated with mushrooms at the University of Benin Botanical Gardens, Benin City, Nigeria

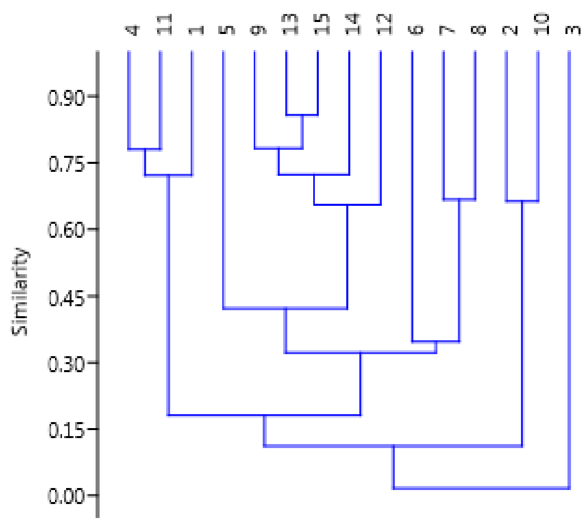

Fig 3. Dendrogram of similarity of taxa composition among sampling events

Generally, eight families of Diptera namely: Calliphoridae, Cecidomyiidae, Culicidae, Drosophilidae, Muscidae, Mycetophilidae, Phoridae and Sciaridae have been reported as notorious pest of mushroom as they feed on fruiting bodies and vector pathogens (Bellettini *et al.* 2018). Here, only two families (that is, Chloropidae and Drosophilidae) were encountered. Although Drosophilids have been earlier reported on mushrooms (Bellettini *et al.* 2018), reports on Chloropidae remains to be seen. Of these two families, records have it that the drosophilid flies (e.g., *D. funebris* and *Mycodrosophila* species) are notorious generalists that are attracted to fermenting organic matters. Their larvae feed on the internal stipe and pileus tissues of affected mushrooms thereby compromising their structure and nutritional quality

(Bellettini *et al.* 2018). Other examples of mycetophagous dipterans are *Lycoriella mali* Fitch (Sciaridae) and the fungus gnat *Rhymosia domestica* (Mycetophilidae); the latter is a vector of the gill-knot inhabiting nematodes (Bellettini *et al.* 2018). Such dipterous larvae as seen in this survey fed on the internal structure of *P. tuberregium* and that led to tissue rot. Other than flies, rove beetles (Staphilinidae) were equally abundant as the dipterous flies. Although report has shown the occurrence of mycetophilic beetles, they are minor pests. The orders Hymenoptera and Hemiptera were rare and occurred as doubleton and singleton respectively.

In managing insect infestation on mushrooms, proper hygiene, biorational (biological control inclusive) and chemical approaches have been advocated (Bellettini *et al.* 2018). Chemical application remains largely intolerable on mushrooms as their growth/pre-harvest duration is short (< 10 days). The short duration of growth *in situ* and its short pre-consumption shelf life may allow 'potent' residuals of such chemicals easy access to human food.

Nonetheless, according to Belletini *et al.*'s review (2018) a nematicide Thionazin can be used against mushroom nematodes without traces of residuals when applied at the rate of 80 ppm; such recommendation remains to be seen for insecticides. Thus, any attempt to use synthetic chemicals to control potentially problematic insects on edible mushrooms that are meant for human consumption should be discouraged. The use of botanicals, however, which have biocidal and/or repellent actions, and are non- or less- toxic to humans should be prioritised. Nonetheless, it remains unclear if botanicals would prevent the generalist drosophilid flies, which remains a threat to mushroom production in open-field systems. Beyond biopesticides, physical methods such as the use of nettings with mesh sizes smaller than the body sizes of the culpable insects may suffice towards attaining food safety in mushroom farming as opposed to mushrooms laden with harmful chemicals. In addition to physical methods, the use of baited traps could suffice to mitigate insect pest problems.

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