Colegio de Postgraduados

Microhabitat of Totolcozcatl mushroom [*Entoloma abortivum* (Berk. & Curtis) Donk, Basidiomycotina] for restoration in the cloud forest

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ABSTRACT

Objective: to characterize the microhabitat and requirements of *Entoloma abortivum*, which is considered as a wild fungus (known as Totolcozcatl, in Nahuatl), and as a non-timber forest resource in the cloud forest.

Design/Methodology/Approach: *E. abortivum* is used for food (as a mushroom); it has ecological, cultural and economic importance for rural communities. Their non-regulated collection has led to depletion and over-exploitation in the mountanous cloud forest of Xaltepuxtla (Puebla) Mexico. Habitat characterization of the fungus, which it requires for development and reintroduction is presented. Sensors for temperature and relative humidity were placed in each site determined in the field visits, at the depth of emergence of the carpophores, in order to record these microclimate variables. These sensors were used before and during the whole period of development of the fungus in two seasons, 2016-2017 and 2017-2018; pH and substrate temperature sensors were also used.

Results: the surrounding plant composition, site-specific shade density and soil-substrate chemical characteristics were described. The emergence of carpophores occurred during the winter when the microenvironmental temperature was recorded between 15.8 and 17.5 °C, relative humidity between 64%-67%, shade density 93.6%. In the substrate, temperatures fluctuated between 8.5 and 14 °C in the same period; the pH of the substrate, between 5.0-5.5; Chemical characteristics and base-type of the bamboo stem, leaf litter; tillers from decaying grasses, and rotten pieces of jonote are other conditions for the development of this fungus.

Limitations/Implications of the study: female collectors identified and delimited the emergency sites of the fungus in the field visits. The capabilities of these female collectors are based on morphological traits, and they reach identification up to the level of species.

Findings/Conclusions: this is the first site-specific characterization of the native habitat of the Totolcozcatl mushroom (*Entoloma abortivum*).

Keywords: emergency period, substrate, microclimate.

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INTRODUCTION

The wild mushroom "Totolcozcatl" (*Entoloma abortivum*) is a species of great nutritional, ecological, cultural and economic importance for the rural communities of Xaltepuxtla (Puebla), Mexico. This use has led to resource depletion and overexploitation

in the cloud forest. It is known that this fungus only emerges in the winter season and its propagation by conventional means has not been successful. Since it is evident that it requires particular ecological conditions, such restrictions have made it impossible to adequately restore it (Mateo, 2018). The reproduction of wild fungi in natural conditions is determined by the biology of each taxon and some ecological characteristics such as number of trees in the site, height, normal diameter, canopy cover, and average age of the trees. It is important to have information, as precise as posible, on temperature, average monthly and annual precipitation, which correspond to the period considered for sampling (Arteaga y Moreno, 2006; Velasco *et al.*, 2010). In addition, Lorenzana (2008) recognized the importance of evaluating the characteristics of the soil microhabitat, such as pH, soil texture class, organic matter contents, nitrogen, phosphorus, potassium and cation exchange capacity. The assessment of the optimal ecological conditions for fungi development is decisive in the restoration process of edible mushrooms (Sözbir *et al.*, 2015; Villanueva-Jiménez *et al.*, 2006).

In addition to considering ecological factors, the technique for *E. abortivum* reproduction is another conditioning factor for restoration. Research by Mateo (2018) showed that *E. abortivum* can be isolated and reproduced by the context method, and that the malt-bran medium is the most viable for the induction process in terms of mycelial growth and hyphae production (Buendía-Espinoza *et al.*, 2020). This is particularly important because this fungus is prone to be parasitized (aborted); in this condition spore isolation is not possible. So, research is important because it provides information on a little-known species whose restoration is urgent in ecosystems where extinction appears as imminent. The objective of this research was to characterize the ecological conditions of the microhabitat where the Totolcozcatl fungus (*Entoloma abortivum*) develops, in order to select key sites for propagation in the cloud forest of Xaltepuxtla, Puebla.

MATERIALS AND METHODS

The research was carried out in the community of Xaltepuxtla, Puebla; at the Ocotitla farm with an area of 40 ha (Ruíz, 2016). On the property there are remnants of severely disturbed cloud forest, predominantly dedicated to the production of local ornamental plants, such as “chima” (*Chamaecyparis lawsoniana*), myrtle, rhododendron and *Cedrela* sp. The area is located between the extreme coordinates 20° 11’ 23.06” N, 97° 58’ 53.03” W; and 20° 10’ 57.124” N, 97° 57’ 30.836” W; at an altitude of 1280 m. The climate (A)Cb(fm)(e)gw” semi-warm humid with rainfall all year round, the driest month rainfall greater than 40 mm, with less than 18% winter rainfall related to total average precipitation. The average annual temperature ranges between 18 and 24 °C, extreme weather, the hottest month before June (Ganges-type temperature march), and intra-summer drought.

The type of vegetation in the remnants corresponds to cloud forest species, with abundance of *Liquidambar* sp., *Platanus* sp., *Pinus patula*, tree-ferns of the genus *Cyathea*, *Heliconia* sp., locally called papatla leaf or apatla which is endemic to the area. Due to anthropocentric activity in the middle herbaceous stratum there is abundance of

Pteridium aquilinum (ocopetate), *Rhododendron simsii*, (azalea) and 'chima', *Chamaecyparis lawsoniana*; an some Bambusae individuals. The herbaceous (middle) stratum is composed of multi-purpose, medicinal and ornamental shrubs. In the lower stratum, vegetables, ornamental plants, short-cycle crops such as chili, tomato, radishes, coriander, and small aromatic plants, where 'papalo' (*Porophyllum ruderale*) predominates (López, 2013).

Natural distribution areas of *Entoloma abortivum*

The participation of commoners (community members), both 'medieros' and owners, is key to the process of restoration of depleted plant species; as well as to forest conservation and its resources (Durston and Miranda, 2002). Given the importance of Totolcozcatl mushroom for the community, female collectors who can identify the fungus up to the species level, participated in field trips to identify the areas where it develops. Field visits for delimiting aproximate areas were made in June 2016, before the period in which the mushrooms begins to emerge. According to the collectors, this occurs at the end of November or beginning of December, and occasionally it extends until February. Four of the sites identified by the collectors were chosen because of greater fungal emergence (Table 1).

Microclimate conditions at the sites

Once the sites were georeferenced and delimited, a HOBO Pro v2l sensor was installed in each. This sensor was placed in the centre of the site at ground level, in order to record temperature, relative humidity; maximum, minimum, and average dew point, with a recording frequency every 15 minutes. This information began to be recorded before fungi emergency to identify precisely the microclimatic requirements of this fungus. In addition, an instrument for soil studies (model SK-300B) was placed to measure pH and temperature. Monitoring of maximum, minimum and average ambient temperature and relative humidity at all sites was performed at 15-day intervals with a hygrothermograph. The canopy coverage density was obtained with a concave density meter with direction to the four cardinal points. These records were averaged and expressed as a percentage of visible sky, *i.e.* canopy opening.

Site-specific soil collection and chemical analysis

Twenty days after the emergency period, samples were taken from the substrate-soil where the fungi developed. Blocks of approximately 15×25×2.5 cm were extracted, stored in plastic bags and transported in a cooler. They were then refrigerated for processing and analysis. In the laboratory each block was cut into four sections; in one

Table 1. Location of the sites where *Entoloma abortivum* emerges.

Coordinates	Site 1	Site 2	Site 3	Site 4
N	20° 11' 12.1"	20° 11' 15.8"	20° 11' 15.4"	20° 11' 04.8"
W	097° 57' 42.5"	097° 57' 51.5"	097° 57' 51.4"	097° 57' 47.8"
Altitude	1215 m	1252 m	1251 m	1229 m

of those the moisture content was determined; the rest of the fractions of each sample were also dried in the oven at the same temperature for grinding. The determinations included organic matter (Walkley and Black); inorganic N, available P (Olsen technique); exchangeable K, Ca and Mg (ammonium acetate at neutral pH); Fe, Mn Cu, Zn (extracted with DTPA); B (extracted with $CaCl_2$); and S (by turbidimetry) according to the methodologies indicated in the Mexican Standard NOM-021-RECNAT-2000 (SEMARNAT, 2002).

Once the emergency sites were identified, data were taken from the place of collection, date, common names of the nearest trees, photographs illustrating characteristics of the habitat of the species, color, size and shape. Once the emergence period of the Totolcozcatl fungus began, specimens in the reproductive phase were collected. The specimens placed in aluminum foil were transported in a cooler to the laboratory of the Institute of Ecology, for taxonomic and phenotypic characterization (Mateo, 2018).

RESULTS AND DISCUSSION

Two forms of occurrence of the fungus were identified at the sites, most of the fruiting bodies in the form of an "aborted" white carpophore that lacks the well-formation of lamellas, and the shape of a typical agaricoid fruiting body with a stipe, as indicated by Lindner *et al*. (2001).

Description of the Sites where *Entoloma abortivum* emerges

For sites Two and Three, the description is generalized because they have a similar composition and structure, due to the proximity between them. It is also necessary to specify that only in these two sites, the emergence of the fruiting bodies of the species of interest was recorded.

Site One. The upper layer in the vegetation structure is composed of sweetgum (*Liquidambar styraciflua*); the middle stratum is made up of specimens of tree ferns (Pterophyta). In the lower stratum there are ornamental plants of 'chima' (*Chamaecyparis lawsoniana*), and the shrubby fern (*Nephrolepis exaltata*). The substrate is a mixture of leaf litter of the plant species identified on site, with pieces of wood, both decomposing (Figure 1).

Figure 1. Vegetation structure on monitoring Site One.

Sites Two and Three. They were very close, only separated by a drainage stream where water derived from a spring runs. In both sites, the upper and middle stratum is bamboo vegetation (*Guadua* sp.), better known by the people as "Otate"; in site Two, there is also a specimen of sweetgum. The lower stratum is made up of a kind of tiller grass. The substrate where the fungi develop is predominantly bamboo leaf, with the presence of sweetgum litter, decomposing wood debris, a 'jonote' trunk (*Heliocarpus appendiculatus*), and rotten tillers of grass (Figure 2).

Site Four. The vegetation is diverse because it is a remnant of the forest that exists in the place, *i.e.* the vegetation that once predominated in the locality. The upper stratum presents specimens of sweetgum and poplar (*Platanus* sp.); the middle stratum is made up of tree ferns, also, citrus and vines species. In the herbaceous stratum we found fern (*Nephrolepis* sp.), selaginels, cycads, some palms and grasses (Figure 3). The substrate is decomposing leaf litter, combined with a large amount of dead plant material.

Figure 2. Conditions of Site Two and Site Three.

Figure 3. Site Four location.

The identification of the plant species that complement the microhabitat of the fungus is important because the leaf litter is what generates a substrate conducive to the reproduction of the fungus. Therefore, it is essential to know the plant composition that is present. As indicated by Velasco *et al.* (2010), the production of wild mushrooms under natural conditions is determined by the conformation of the tree dimension present in the stands where they develop. As well as by the edaphic and climatological elements of their habitats. The average diameter and average age of the trees are also important ecological features (Arteaga y Moreno, 2006).

What was observed also coincides with Villanueva *et al.* (2006) in proving that the lower density of trees and decrease in the humus layer impacts the development of macroscopic sporomes. Another parameter of importance in the characterization of the habitat was the average shade density, in site Two, this was 94.54%; and in site Three, 92.72%. With these results we can affirm that *E. abortivum* requires considerable tree cover for its production, which coincides with what was identified by Velasco *et al.* (2010) regarding that the total fungal production depends on the number of trees on site, and the canopy cover they provide to the site.

Substrate at the emergence sites

The substrate where the fungus emerged was basically composed of bamboo leaves and decaying wood; base of bamboo stalks, decaying tillers from grasses, and on a rotten section of jonote (*Heliocarpus appendiculatus*); all of them mixed with the soil. Díaz (2002) also described the species growing at the base of trees, among leaf litter or on decaying wood. As a sapoptroph species, *E. abortivum* obtains its nutrients from the decomposition of dead organic matter (Montañez, 2013). Similar observations were recorded by Watling (1974) and Huffman *et al.* (2008) in deciduous forests. The chemical analysis of the substrates where the fungus emerged is summarized in Table 2.

As it can be seen, a common denominator in both sites is that fungus requires high contents of organic matter in the soil, high level of K and S; and adequate of

Table 2. Chemical properties of the substrate-soil in the emergence sites of *Entoloma abortivum*.

	Site Two										
	MO	**Ca**	**Mg**	**K**	**S**	**P**	**Zn**	**Mn**	**Fe**	**B**	**Cu**
	%	**me 100 g^{-1}**			**mg kg^{-1}**						
$\bar{X}$	12.7	9.53	1.78	1.27	20.4	5.8	9.8	49.7	70.2	2.4	0.78
σ^{Z}	2.66	8.83	0.50	0.25	12.4	7.9	5.4	35.3	36.1	1.1	0.57
Level	high	medium		high	high	medium	adequate				marginal
	Site Three										
$\bar{X}$	9.0	6.72	1.48	1.27	12.5	3.3	2.3	6.3	119.1	2.1	0.35
σ^{Z}	1.41	8.73	0.29	0.41	7.3	2.3	0.42	2.0	16.6	0.63	0.09
Level	medium			high	high	low	adequate				marginal

ZN=10.

micronutrients Zn, Mn, Fe and B. According to Olfati and Peyvast (2008) high K content in the substrate (6200-7920 mg per 100 g DM) is essential for the production of fruiting bodies of *Pleurotus ostreatus* var. sajor caju. Detailed research in regard to the valuation of substrates in the production of *Pleutorus ostreatus* and *P. cystidious*, indicates that the content of Ca, Fe, Mn and Zn is strongly related to the biological efficiency of *Pleurotus ostreatus* and *Pleurotus cystidiosus* (Ha *et al.*, 2015). Substrate requirements for *E. abortivum* have not yet been reported. Due to mushroom cultivation is a multifactorial process (Krupodorova y Barshteyn, 2015), the data obtained in this research should be considered in the preparation of a culture medium for *E. abortivum*, since these requirements are unique for each species of fungus.

Emergence period and climate requirements of *Entoloma abortivum*

The averages of the temperature and micro-environmental relative humidity data recorded at the two sites, during the two monitoring periods on the emergence of the fungus, indicated that its requirements fluctuate between 15.8 °C to 17.5 °C temperature, and 64 to 67% relative humidity. In the case of the substrate, in order to emerge the species needs temperature conditions between 8.5 °C and 14 °C, and a neutral to slightly acidic pH (5.0 to 5.5). Figures 4 and 5 show the temperatures recorded in the substrate during the period of emergence of the fungus in the collection sites, in which the requirements for temperature drop, that the species demands to be able to emerge, can be observed.

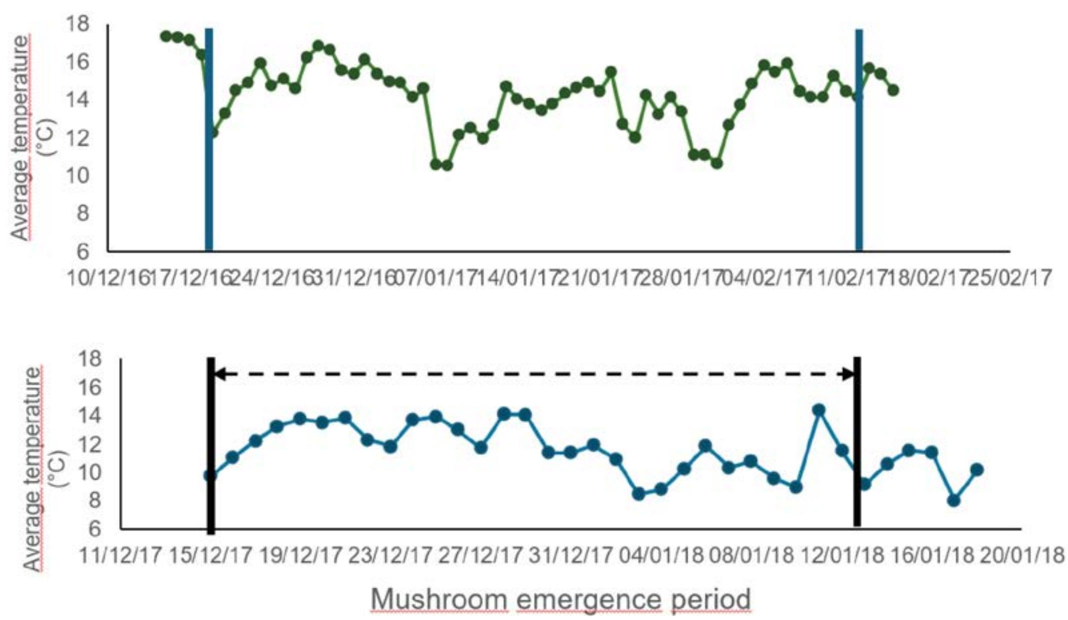

Figure 4. Microenvironmental temperature and emergence period of *Entoloma* at site two (years 2016-2017 and 2017-2018).

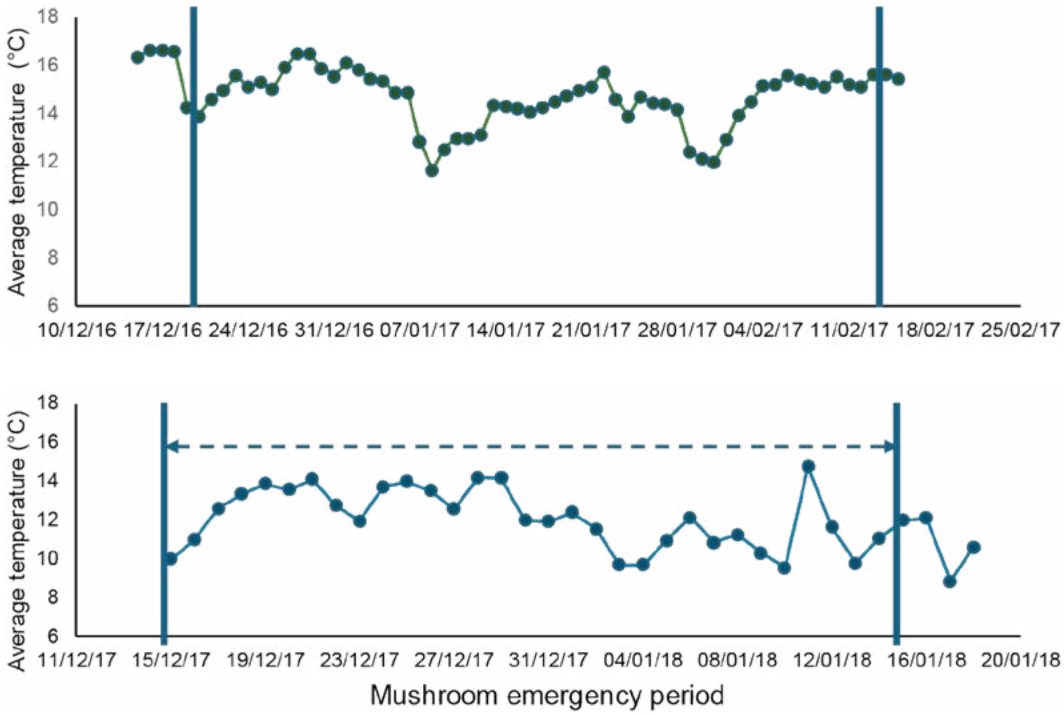

Figure 5. Micro-environmental temperature and emergence period of *Entoloma abortivum* at site Three (2016-2017 and 2017-2018).

CONCLUSIONS

The reintroduction of the wild fungus *Entoloma abortivum* known as Totolcozcatl, in the mountain cloud forest in the community of Xaltepuxtla, (Puebla) Mexico, requires the location of microhabitats with a shade percentage of almost 100%, and high relative humidity (64 to 67%). Other requirements, linked to the emergence period of this fungus (December-February), are low microenvironmental temperatures (15.8-17.5 °C) and substrate temperatures (8.5-14 °C). Materials derived from the base of the bamboo stem, as well as its leaf litter, as well as tillers from decaying grasses and rotten pieces of jonote (*Heliocarpus appendiculatus*), which provide slight acidity (pH 5.0-5.5) and adequate nutritional conditions are other restrictions for this fungus development.

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