From The Published Works of John W. Allen

The Ethnomycology, Biochemistry, and Cultivation of Psilocybe samuiensis Guzmán, Bandala and Allen, A New Psychoactive Fungus from Koh Samui, Thailand

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ABSTRACT

Several specimens of Psilocybe and Copelandia species in Koh Samui, Thailand were recently collected by JWA and native residents for herbarium deposit and scientific study. The following paper presents an ethnomycological and biochemical study of one of the species; Psilocybe samuiensis Guzmán, Bandala and Allen, a new

psychoactive gill fungus reported from Thailand.

Mycelium for the cultivation of Psilocybe samuiensis was obtained on 6% malt agar from the spores of a dried specimen. The growth of Psilocybe samuiensis was similar to that of Psilocybe tampanensis Guzmán and Pollock; but grew more rapidly than the mycelium of Psilocybe semilanceata (Fr.:Sacc.) Kumm. Laboratory analyses indicates that the alkaloid content in cultured fruit bodies of Psilocybe samuiensis is in the same order of magnitude as that found in naturally occurring mushrooms of this species.

HPLC analyses of both naturally occurring and in vitro cultivated fruit bodies of Psilocybe samuiensis revealed high concentrations of psilocybin and psilocin. Small amounts of baeocystin were also detected. Psilocybin levels varied from 0.23% up to 0.90%. The psilocybin content was highest in the caps.

Psilocybin was also found in the cultured non-bluing mycelia of Psilocybe samuiensis and varied from 0.24% to 0.32% dry weight. The relative alkaloidal content of psilocybin, psilocin, and baeocystin found in Psilocybe samuiensis was similar to that measured in other psychoactive fungi, but completely different from that found in Psilocybe semilanceata.

KEYWORDS: Psilocybe samuiensis, psilocybin, psilocin, cultivation, Koh Samui, Psilocybe sp., psychoactive fungi.

Introduction

Recent ethnomycological investigations on Koh Samui Island in Thailand (Allen, 1991; Allen and Merlin, 1992a, 1992b; Guzmán et al., 1993), confirm reports that some species of psychoactive fungi are ingested for recreational purposes by foreign tourists and some indigenous people (see Allen et al., 1992).

Koh Samui (280 km2 located at 10° N latitude, 100° E longitude), is a small tropical Island in the Gulf of Siam where psychoactive fungi are harvested by local farmers and their children. The psychoactive dung fungi (Psilocybe cubensis (Earle) Singer and/or Psilocybe subcubensis Guzmán) are known locally as hed keequai (literally, "mushrooms which appear after water buffalo defecates"). These mushrooms are sold by some farmers or their families directly to tourists and resort restaurants. At some resort restaurants the fungi are offered in a variety of meals (Allen and Merlin, 1992a).

Fungi specimens collected on Koh Samui Island (1991) for

herbarium deposit and scientific study include the following species: Psilocybe cubensis, Psilocybe subcubensis, Copelandia cyanescens (Berkeley et Broome) Singer, and a previously unreported bluing Psilocybe species, Psilocybe samuiensis Guzmán, Bandala and Allen, sp. nov. (fig. 1). **Chemical investigations of the psychoactive properties of some of these fungi include an unpublished study by Stijve (Nestec laboratories, Vevey, Switzerland) who analyzed Koh Samui collections of P. cubensis and P. samuiensis, as well as a Swiss collection of Psilocybe semilanceata (Fr.:Sacc.) Kumm. Tryptamine indoles were found in all three species. A second study by the authors was later conducted utilizing naturally occurring material and material grown only in vitro. Results of the HPLC and TLC analysis of both studies of P. samuiensis revealed high concentrations of tryptamine indole alkaloids. Furthermore, the senior author (JG) was successful in isolating a pure strain of P. samuiensis on malt agar and grass seed.**

Description of the cap, gills and stipe of Psilocybe samuiensis Guzmán, Bandala and Allen, sp., nov.

CAP:7-15 mm in diameter, subconvex to conic-convex, conic umbonate or campanulate-umbonate, frequently with a small papilla. Viscid with a separable pellicle, even and striate to sulcate at the margin. Hygrophanous. Chestnut or reddish-brown to straw-color, becoming pale straw-color or brownish clay when dry.
GILLS: Adnate to adnexed, clay color, becoming violaceous brown or chocolate brown-violet when dry, with white edges.
STIPE:40-65 x 1.52 mm, equal or slightly subbulbous. Hollow. White or whitish to pale straw color. Covered with white fibrils. Context concolorous with pileus, bluing with slightly farinaceous taste and odor.
HABITAT: Psilocybe samuiensis was first collected in soil of mixed sand and clay, among fan palms in rice paddies situated 2 km west of the village of Ban Hua Thanon, Koh Samui, Thailand. Unlike Copelandia, and some species of Psilocybe which are coprophilous, P. samuiensis does not fruit directly in manure but appears scattered or gregarious in the manured soil of rice paddies. This fungus fruits from early July through late August.

Methodology

While foraging for psychotropic fungi on Koh Samui

island in August of 1991, John W. Allen, accompanied by several Samui children, harvested P. samuiensis for herbarium deposit and scientific examination (See Guzmán et al., 1993). Psilocybe samuiensis first attracted the attention of the collector (JWA) because of its macroscopic similarity to Psilocybe semilanceata (the "liberty cap" mushroom). Both can be found in similar environments (i. e., pasture lands, rice paddies, etc.), occurring in the manured soil of ruminants. Psilocybe samuiensis was then photographed in situ, as were several carpophores of P. cubensis and/or P. subcubensis (which is macroscopically indistinguishable from P. subcubensis), and a few specimens of C. cyanescens which were also observed growing in the same rice paddy where P. samuiensis occurred.

Psychoactive fungi species described in this study were harvested from manured soil and/or the decomposed manure of the Asian water buffalo (Bubalus bubalis), and/or cattle (Bos indicus and/or Bos sundaicus). All of the above mentioned fungi were collected (August 2-8, 1991) in rice paddies at four different locations near the villages of Ban Saket, Ban Hua Thanon, Bo Phut and Ban Lipa Yai on Koh Samui Island.

Several collections of the above mentioned fungi (including P. samuiensis, labeled as collection F), were sun dried and forwarded to Dr. Gastón Guzmán of the Instituto

de Ecologia, in Xalapa, México for botanical identification. Specimens were also forwarded to Dr. T. Stijve of Nestec Ltd., Vevey, Switzerland for chemical analyses to determine the presence or absence of toxic and/or psychoactive alkaloids.

Experimental

Mycelium was obtained from the spores of a dried specimen of P. samuiensis by methods described by Stamets and Chilton (1983) and was stored as stock culture on 6% malt agar. Strains on agar of a related species Psilocybe tampanensis Guzmán and Pollock and P. semilanceata from Germany were also obtained. In a ratio of 1 to 6% on malt agar, the whitish mycelium of P. samuiensis grew at a faster pace than that of similar mycelium of P. semilanceata. The rapid growth of P. tampanensis was similar to the growth of P. samuiensis; however, the former species soon formed brownish sclerotia on the agar as it does with P. mexicana (Stamets and Chilton 1983). Even after a relatively long growth period (3 months), the mycelium of P. samuiensis formed only a few small brownish sclerotia.

Similar conditions were observed while cultivating the three species on Lolium seed / water (1:1.5). The same conditions were also observed in complete darkness; and P. tampanensis and P. samuiensis both grew with rapid speed. Observations on the rapid formations of sclerotia in P. tampanensis after a few weeks of cultivation has already been reported by Stamets and Chilton (1983). In contrast, P. samuiensis under cultivation only formed thick whitish mycelium (rhizomorphs, diameter 2 to 3 millimeters) throughout the media, and produced no sclerotia. Under the same conditions of cultivation, P. semilanceata grew slowly, producing only a fine and whitish mycelium with no formation of sclerotia or rhizomorphs.

Psilocybin was found to be present in the cultured, non-bluing mycelium of P. samuiensis grown on 6% malt agar. Amounts of psilocybin, ranging from 0.24 to 0.32% dry weight, were analyzed in 5 different batches of mycelium grown over a four week period. Analyses also revealed that these quantities of psilocybin were much lower than those detected in the naturally occurring fruit bodies obtained from the field. Interestingly, no other indole derivatives were detected in the extracts of the in vitro grown mycelium.

The alkaloidal levels obtained from the slightly bluing sclerotia of P. tampanensis were high. Additionally, the amount of psilocybin obtained from five different

cultivations grown on 6% malt agar and Lolium seed ranged from 0.34 to 0.68% by dry weight, and from 0.41 to 0.61% in three sections of sclerotia obtained from a single cultivation on Lolium seed. The sclerotia obtained from malt agar also contained 0.21 to 0.52% psilocin, but no baeocystin was detected. The sclerotia obtained from Lolium had a concentration of psilocin from 0.11 to 0.32%. Until now, it was not possible to produce complete fruit bodies of P. samuiensis on either malt agar or Lolium seed. Some small incomplete fruit bodies of P. samuiensis (up to 2 centimeters high) appeared, but failed to develop into normal sporulating mushrooms. These premature formations only occurred on agar with a low concentration of malt (0.5 to 1.5%). After stopping their natural growth, these incomplete fruit bodies began to exhibit a slight spontaneous bluing reaction.
< br> At this time it was not possible to be able to cultivate mushrooms on a Lolium seed / water mixture (Stamets and Chilton. 1983). Psilocybe samuiensis also grows well on some grains such as rye or rice. A mixture of rye/horse dung/water (2:1:2) did produce fruit bodies of P. samuiensis after 4 months cultivation, and 3 weeks after casing with peat/chalk (2:1) (Stamets and Chilton, 1983) (fig. 2). Two flushings producing eight mushrooms were observed; six of the mushrooms were analyzed (see fig. 2 and table 1).

Results

Two separate chemical studies were undertaken to determine the tryptamine alkaloid content of Psilocybe samuiensis. The first involved naturally occurring field specimens; the second analyzed material cultivated in the laboratory.

In the first study, 15 specimens of naturally occurring fruit bodies of P. samuiensis were analyzed by HPLC and TLC techniques (Gartz, 1987). High amounts of psilocybin were detected (0.23% - 0.90% dry weight); and a few specimens contained similar amounts of psilocin (0.05% - 0.81% dry weight). Baeocystin, a precursor to psilocybin, was also detected (0.01% - 0.5% dry weight) in all naturally occurring specimens of P. samuiensis but in much smaller concentrations than psilocybin. This is in sharp contrast to the high concentrations of baeocystin and very small amounts of psilocin (only in a few specimens) which were detected in naturally occurring field specimens of P. semilanceata from various origin (Gartz, 1993), and in vitro cultivated fruit bodies of P. semilanceata (Gartz, 1991a, 1991b).

In contrast to the cultivated P. cubensis (Gartz, 1987), where the accumulation of psilocin is often higher in the

stems than in the caps, analyses of P. samuiensis revealed that the caps contained more psilocybin than the stems. Identical concentrations of the alkaloids (psilocybin, psilocin, and baeocystin) were found in the cultivated fruit bodies of P. samuiensis and P. semilanceata grown in rye/ horse dung (Gartz, 1991a, 1991b). Stijve also found similar concentrations of psilocin and psilocybin in 5 naturally occurring fruit bodies of P. samuiensis (collection F, 8 August, 1991, psilocybin, 0.14%; psilocin, 50%; baeocystin, <0.01%).

Plate I (fig. 3) reveals the qualitative results of analysis of P. samuiensis (collection F, 8/8/91), along with the comparative study of 2 Thai collections of P. cubensis (collection C and G, August 3, 1991 and August 8, 1991), and analysis of a Swiss collection of P. semilanceata. Analyses were performed by Thin-Layer Chromatography (TLC) on cellulose 10 x 20 cm Nano plates. BAW=Butanol acetic acid-water 60:15:25 V/V. pDMCA reagent.

Plate II (fig. 4) reveals the qualitative results of analysis of P. samuiensis (collection F, August 8, 1991), a Swiss collection of P. semilanceata and a Thai collection of P. cubensis (collection G, August 8, 1991). Analyses were performed by Thin Layer Chromatography (TLC) on NANO-cellulose 10 x 10 cm., N-propanol-10% ammonia 5:2 V/V., PDMCA-reagent.

Discussion

Previous studies by Allen and Merlin (1992a, 1992b) and Guzmán et al. (1993) confirm reports that several species of psychoactive fungi in Thailand are used for non-traditional recreational purposes. The most commonly used species is P. cubensis (and/or P. subcubensis). Although psychoactive fungi are currently illegal in Thailand, such use is still common at many resorts on Koh Samui, Koh Pha-Ngan, and possibly other areas of this country. Allen and Merlin (1992) also reported that some adults as well as some children have eaten (or attempted to smoke) psychoactive fungi species for recreation. Furthermore, some tourists have apparently influenced a small segment of native inhabitants, who are enticed by their foreign companions into consuming such fungi. Foreign visitors may well have been responsible for introducing the use of psychoactive fungi to Koh Samui and other resort areas in South and Southeast Asia.

During the collection of field specimens, JWA questioned native children and a few adults whom he encountered in the rice paddies concerning their relationship with "hed keequai" and other fungi found in their environment. Some

children were aware of numerous varieties of edible fungi as well several poisonous and psychoactive fungi species occurring on Koh Samui. On one occasion, several children warned JWA not to eat Panaeolus antillarum (Fr.) Dennis, explaining that it was "antaray" (dangerous). A toxin is not known from this species at present. The authors were unable to confirm if the collection of psychoactive fungi by Samui farmers and their families had caused any serious poisonings due to the possible misidentification of species. However, the native farmers and their children are very knowledgeable regarding the natural flora of their environment. Regarding the new species, P. samuiensis is readily differentiated from other mind-altering mushrooms by a few adults and some native children who frequently collect psychoactive dung fungi. However, it is not known if this species is harvested for human consumption by foreign tourists or immigrants living on Koh Samui Island. It is possible that some European mycophagists may have collected and experimented with this species after noticing its macroscopic similarity to P. semilanceata. A few children and one adult apparently recognized fresh carpophores of P. samuiensis (harvested by JWA) as a unique type of fungus. At least some of the Samui cattle tenders and their children are aware that the psychoactive effects of P. samuiensis are similar to the mind-altering effects of the larger specimens of "hed keequai" (i.e., P. cubensis), which they often gathered. However, when compared to P.

cubensis, P. samuiensis is a small inconspicuous fungus, not more than 2-3 inches in height and can be easily overlooked by both tourist and native collectors seeking the larger specimens of P. cubensis.

After noticing that several carpophores of P. samuiensis exhibited a slight bluing reaction after handling, JWA bioassayed 25 fresh specimens (weighing approximately 6 grams wet weight); This resulted in an intensely visual experience similar to the action of equal amounts of P. semilanceata from Germany.

Prior to the botanical identification of P. samuiensis by Guzmán et al. (1993), a small collection of P. samuiensis was sent to Dr. T. Stijve of Nestec Ltd., Vevey, Switzerland for botanical identification. Unable to properly identify the fungi, Stijve (Pers. Comm., 1992a) forwarded several carpophores of P. samuiensis to Klaus Høiland of the Botanical Garden and Museum in Oslo, Norway. Høiland, in a personal communication to Stijve (11 June 1992), reported that he "examined the dried specimens according to Guzmán's taxonomic key of the genus Psilocybe," suggesting that the dried material "corresponded to Psilocybe mexicana Heim or a very closely related species." Furthermore, Høiland suggested that "since it [P. mexicana] is only known [of] from [the North] America[n] [continent], care should be undertaken to accept the species from Thailand. It may occur there naturally [Koh

Samui], or it may have been introduced by people from [North] American samples, or it is a close, but undescribed species (Stijve, Pers. Comm. to JWA, 12 June 1992)."

Psilocybe samuiensis is microscopically similar to P. mexicana, but the form and size of the spores, as well as the presence of pleurocystidia, its macroscopic features and the habitat, are very close and somewhat similar to P. semilanceata. Guzmán et al. (1993) placed this species in the section Mexicanae because of the big rhomboid or subrhomboid spores which separate this species from P. mexicana and other species in the Psilocybe section Mexicanae; it is the first species of that section to be found outside of the New World.

The chemical composition of P. samuiensis is also quite different than that of P. semilanceata which contains much more baeocystin than P. samuiensis (Gartz, 1991a, 1991b, 1993).

Both species are macroscopically distinguished and/or separated by the height or length of their respective stipes and the color of the fruit bodies. Psilocybe samuiensis attains a height of from 40-65 x 1.52 mm and P. semilanceata has a natural height of from 70-110 x 1.52 mm. During crossing experiments, complete reproductive barriers have been found between 4 mono karyons of P. semilanceata from Germany (2) and Austria (2), and in (3

strains of) P. samuiensis from Thailand. It is clear that both are autonomous species which do not form hybrid dikaryons.

Recent chemical analyses of both naturally occurring and cultivated specimens of P. samuiensis by the authors, as well as analysis of 5 naturally occurring fruit bodies by Stijve, indicate that it is a relatively potent psychoactive species containing high concentrations of both psilocybin and psilocin.

Herbarium deposits

Duplicate collections of fungi specimens referred to in this study (collected August 2-11, 1991) have been deposited at the Instituto de Ecologia in Xalapa, Veracruz, México (including P. samuiensis, holotype XAL, Allen F, 1991) and at the Pacificum Herbarium in the Bernice P. Bishop Museum in Honolulu, Hawaii (including P. samuiensis, isotypes in BISH and 0, Allen F, 626452, Allen F1, 626825). Additional specimens were sent to Dr. Rolf Singer, Field Museum of Natural History, Chicago, Illinois, and to Dr. Prakitsin Sihanonth, Chulalongkorn University, Bangkok, Thailand.

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TABLE 1. Indole derivatives in cultivated fruit bodies of Psilocybe samuiensis.

Fruit Body	Psilocybin	Psilocin	Baeocystin
1.	0.58----	0.34----	0.02
2.	0.43----	0.21----	0.03

3. 0.36---- 0.52---- 0.04
4. 0.47---- 0.31---- 0.04
5. 0.62---- 0.23---- 0.05
6. 0.73---- 0.25---- 0.03

PHOTOCAPTIONS

Figure 1. Fresh harvested specimens of Psilocybe samuiensis Guzmán, Bandala and Allen. Ban Hua Thanon, Koh Samui. Photograph by John W. Allen. Photograph is actual size.

Figure 2. Psilocybe samuiensis Guzmán, Bandala and Allen. Grown on rye/horse dung. Photograph by Jochen Gartz.

Figure 3. Plate I. TLC analytical results are 1=psilocin. 2=turquoise spot often encountered in extracts of hallucinogenic mushrooms. 3=psilocybin. 4=baeocystin. Photograph by T. Stijve.

Figure 4. Plate II. TLC analytical results are 1=psilocin (to front). 2=metabolite characteristic of Psilocybe cubensis; resembles tryptophan, but does not match in other systems. 3=turquoise spot. 4=psilocybin. 5=baeocystin. Photograph by T. Stijve.