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FURTHER INVESTIGATIONS ON PSYCHOACTIVE MUSHROOMS OF THE GENERA *PSILOCYBE, GYMNOPILUS* AND *CONOCYBE*

Abstract - JOCHEN GARTZ - Further investigations on psychoactive mushrooms of the genera *Psilocybe, Gymnopilus* and *Conocybe*.

The analysis of three bluing species of the genera *Psilocybe, Gymnopilus* and *Conocybe* from Germany revealed psilocybin and baeocystin and in some cases psilocin as well. The content of the alkaloids in cultured basidiocarps of *Psilocybe semilanceata* and *Gymnopilus purpuratus* was in the same order of magnitude as that found in naturally grown mushrooms. For the first time, the psilocybin containing non-bluing mycelium of *Conocybe cyanopus* could be cultivated.

Key words: Psychoactive mushrooms, *Conocybe, Gymnopilus, Psilocybe*.

Riassunto - JOCHEN GARTZ - Nuove ricerche su funghi psicoattivi dei generi *Psilocybe, Gymnopilus* e *Conocybe*.

L'analisi di tre specie bluificanti dei generi *Psilocybe, Gymnopilus* e *Conocybe* provenienti dalla Germania ha rivelato la presenza di psilocibina, baeocistina e, in qualche caso, psilocina. Il contenuto di alcaloidi nei basidiocarpi di *Psilocybe semilanceata* e *Gymnopilus purpuratus* coltivati era dello stesso ordine di grandezza rispetto a funghi selvatici. Viene inoltre comunicata, per la prima volta, la possibilità di coltivazione del micelio, non bluificante ma contenente psilocibina, di *Conocybe cyanopus*.

Parole chiave: Funghi psicoattivi, *Conocybe, Gymnopilus, Psilocybe*.

INTRODUCTION

The use of hallucinogenic fungi containing the indole alkaloids psilocybin, psilocin and baeocystin has, during the past three decades, spread from Mexico to the western world (BEUG & BIGWOOD, 1982; BIGWOOD & BEUG, 1982; CHRISTIANSEN *et al.*, 1981; FRANCIS & MURRAY, 1983; WEIL, 1977).

The most common European fungus of this kind is *Psilocybe semilanceata* (Fries) Kummer (Christiansen *et al.*, 1981; Ohenoja *et al.*, 1987; Stijve & Kuyper, 1985).

Psilocybin levels of these mushrooms are uncommonly high (up to 2,0%) in European samples (Christiansen *et al.*, 1981) and in basidiocarps from North America (Beug & Bigwood, 1982).

Little is known about the cultivation of this species. Repke *et al.* (1977) analysed mushrooms which were cultivated on sterilized rotted branches of *Quercus* sp. Basidiocarps were produced by cultures after 84 weeks.

Recently, quantitative detection of psilocybin and its derivatives has been described in other European species by Gartz and Müller (1989). In this study we have also cultivated *Psilocybe bohemica* Sebek from Czechoslovakia on rice grain. In continuation of studies in which we reported the analysis of other psilocybin containing species like *Gymnopilus purpuratus* (Cooke & Massee) Singer (Gartz, 1988a) and *Psilocybe cubensis* (Earle) Singer (Gartz, 1989b), in this paper the analysis and cultivation of various species of Germany are described.

Experimental

Basidiocarps of *Psilocybe semilanceata* from a single location (Dübener Heide G. D. R., Sept. 21, 1987) were dried at room temperature. Possibly present residual water was removed from the mushrooms by freeze-drying. Mycelium obtained from the spores of one mushroom (Gartz & Müller, 1989) was kept as a stock culture on 6% malt agar. Cotton-plugged 500 ml Erlenmeyer flasks were filled with horse manure compost (Stamets & Chilton, 1983), sterilized by autoclaving, cooled, innoculated from stock cultures and incubated at 21 °C.

Fruiting of the mycelia occured without casing 12 till 16 weeks after inoculation. The strain of *Gymnopilus purpuratus* cultivated in this study originated from a spore print taken in the northern G.D.R. in Sept. 1987.

Mycelium of this species was grown on 4% malt agar. A 2:1 rice grain saw dust mixture with twice the amount of water was used to obtain fruiting without casing. The first basidiocarps were produced by cultures of *Gymnopilus purpuratus* in 8 weeks.

The cultures continued to produce mushrooms in five flushes as *Psilocybe semilanceata*. The two species required diffuse day light for pinhead initiation. Each flush was harvested as soon as the basidiocarps were mature. The mushrooms were immediately freeze-dried, sealed in plastic, and stored at -10 °C until analysis. Each flush was analysed to determinate the average alkaloid level in the mushrooms.

Basidiocarps of *Conocybe cyanopus* (Atk.). Kühner were harvested from a

location near Potsdam (G.D.R., July 2, 1989). A very slowly growing mycelium on malt agar was obtained from spores.

No fruiting on rice grain or compost could be observed. The mycelium was also harvested from the agar after 12 weeks cultivation. Then it was freeze-dried, extracted and analysed as the mushrooms.

The extraction procedure of the mushrooms and the quantitative analysis of psilocybin, baeocystin and psilocin by means of HPLC were described in previous papers (Gartz, 1985, 1989a, b; Semerdžieva *et al.*, 1986). Results obtained by HPLC were confirmed by TLC using various mobile phases (Gartz, 1985; Semerdžieva *et al.*, 1986).

Results

Psilocybe semilanceata occurs in all parts of Germany. Its main season seems to be September and October. Mushrooms are found scattered on various grassy areas in the country. They usually are frequent at moderately grazed, old pastures with a high ground water level.

There the mushrooms could be collected in large enough quantities for use (fig. 1). From 1986 to 1988 we found 2,800 mushrooms (140 g dry weight) at this single location in the Dübener Heide.

High concentrations of psilocybin were detected in all extracts of *Psilocybe semilanceata*. Basidiocarps with the highest psilocybin levels also contained the largest amounts of baeocystin (tab. 1).

The psilocybin content was in the same order of magnitude as that found in basidiocarps of *Psilocybe bohemica* (Gartz & Müller, 1989) and in *Psilocybe semilanceata* of the Pacific Northwest, U.S.A.

(Beug & Bigwood, 1982). Psilocin was not found in *Psilocybe semilanceata.* Beug & Bigwood (1982) also reported the absence of psilocin in collections of this species from North America. The highest concentations of psilocybin and baeocystin were found in the smallest basidiocarps (tab. 1).

It was found that the horse manure compost actually produced basidiocarps of *Psilocybe semilanceata* much earlier than the cultivations on another compost substratum (Repke *et al.*, 1977).

Laboratory grown *Psilocybe semilanceata* (fig. 2) failed to produce detectable amounts of psilocin. The levels of psilocybin and baeocystin varied from one flush to the next but generally were much the same as those in the naturally grown mushrooms of the present experiments (tab. 2) and in literature (Semerdžieva *et al.*, 1986).

Psilocybe semilanceata is a species that blues inconsistently. The high levels of psilocybin and baeocystin making it one of the most potent species as well

as one of the most constant in amount in comparison with other species (Gartz & Müller, 1989; Semerdžieva *et al.*, 1986). In a self experiment a small sample of baeocystin from *Psilocybe semilanceata* (Gartz, 1989b) (4 mg) has caused a gentle hallucinogenic experience.

Gymnopilus purpuratus is an agaric from the austral floral zone. But since 1983 this species has been observed on heaps of mixtures of pig dung and wood chips in the district Rostock, northern G.D.R. It seems that this species was introduced with grain from Argentina used for forage in pig-breeding during the last years. Recently, the detection of psilocybin, baeocystin and psilocin in extracts of *Gymnopilus purpuratus* has been described by Gartz (1989a).

Fruiting of this species in controlled culture has not been previously reported.

In cultivated *Gymnopilus purpuratus* psilocybin was found to be accompanied by only slight amounts of baeocystin and high levels of psilocin (tab. 3). The amounts of the indole derivatives varied from one flush to the next.

The content of alkaloids was in the same order of magnitude as that found in naturally grown basidiocarps (Gartz, 1989a). In literature several other species of *Gymnopilus* of North America are mentioned as to contain psilocybin (Hatfield *et al.*, 1978).

Most chemical studies of *Conocybe cyanopus* have involved samples of North America. But some doubt still exist about the taxonomic conformity of some species from locations of Europe and America (Gartz & Müller, 1989). Benedict *et al.* (1962) found psilocybin in *Conocybe cyanopus* from the U.S.A. Repke *et al.* (1977) reported on the presence of psilocybin as well as baeocystin in *Conocybe cyanopus* and *Conocybe smithii* Watling of Pacific Northwest, U.S.A. Beug & Bigwood (1982) found psilocybin (0,93% dry weight) in *Conocybe cyanopus* from the same area but did not find psilocin. Samples of this species from Norway contained 0,33-0,55% psilocybin and 0,004-0,007% psilocin (Christiansen *et al.*, 1984). The occurrence of similar amounts of these alkaloids in Finnish mushrooms of the same species has also been reported (Ohenoja *et al.*, 1987).

The collection of *Conocybe cyanopus* of Germany contained similar amounts of psilocybin and baeocystin as that found in basidiocarps of *Psilocybe semilanceata* (tab. 4) but also no psilocin.

A variation in the levels of the alkaloids from one sample to another was also found.

Conocybe cyanopus is the only Central and North-European species of the genus *Conocybe* exhibiting a distinguished bluing stipe. This characteristic distinguishes it easily from other species of the same genus. Psilocybin was also found to be contained in the cultivated non bluing mycelia of *Conocybe cyanopus* (0,25% dry weight).

No other alkaloids were detected in the mycelial extracts. The detection of psilocybin in mycelia of *Conocybe species* has not been previously reported.

In agreement with an investigation about *Psilocybe bohemica* (Gartz & Müller, 1989) the amount of psilocybin was lower in the mycelia than in the naturally grown basidiocarps.

Conclusions

Psilocybe semilanceata is a widespread species in Germany and could be collected in large enough quantities for use as well as the hallucinogenic fungus *Inocybe aeruginascens* Babos (Semerdžieva *et al.*, 1986). The levels of psilocybin, psilocin and baeocystin in the studied species were found to be variable even from a single location. No large variations between the content of indole derivatives in naturally grown and cultivated mushrooms of *Psilocybe semilanceata* and *Gymnopilus purpuratus* could be detected.

The bluing reaction is not a guide to psilocybin, psilocin or baeocystin levels.

In contrast to *Psilocybe semilanceata* the small and very uncommon fungus *Conocybe cyanopus* has no practical importance for use in Central-Europe.

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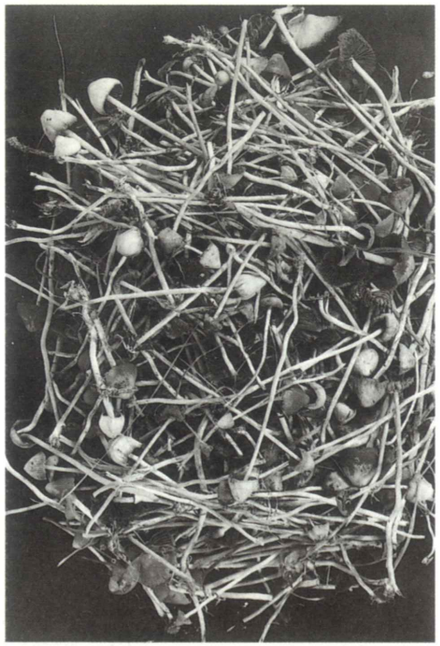

Fig. 1 - Naturally grown *Psilocybe semilanceata* (Fr.) Kumm. Photo: Gartz, Leipzig, Germany.

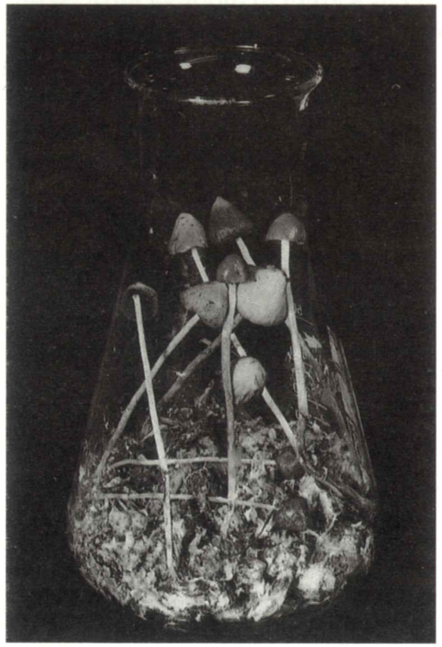

Fig. 2 - *Psilocybe semilanceata* (Fr.) Kumm. Fruit bodies from compost substrate. Photo: Thiel, Leipzig, Germany.

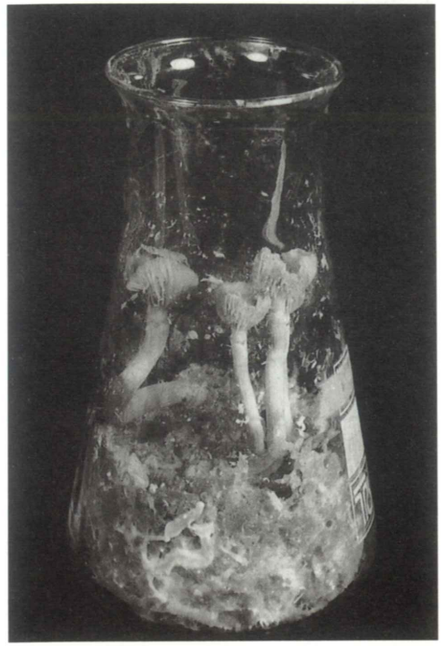

Fig. 3 - *Gymnopilus purpuratus* (Cooke & Massee) Singer. Fruit bodies on rice-grain-medium. Photo: Gartz, Leipzig, Germany.

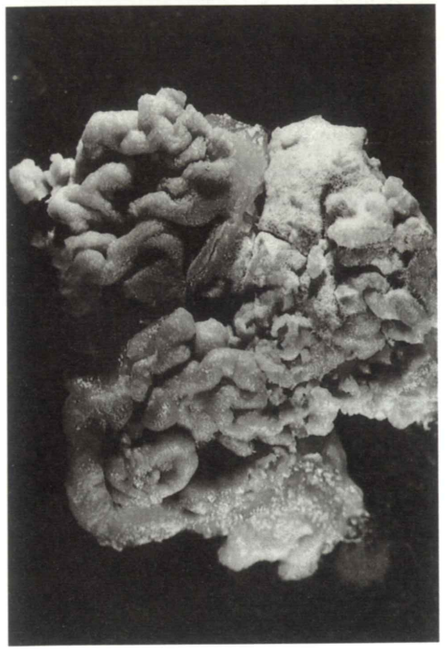

Fig. 4 - *Conocybe cyanopus* (Atk.) Kühner. Mycelia on 6% malt agar. Photo: Thiel, Leipzig, Germany.

PSILOCYBIN AND BAEOCYSTIN LEVELS IN NATURALLY GROWN FRUIT BODIES OF *PSILOCYBE SEMILANCEATA*

Table 1

SAMPLE	DRY WEIGHT OF THE MUSHROOM (MG)	PSILOCYBIN (% DRY WEIGHT)	BAEOCYSTIN (% DRY WEIGHT)
1	15	1,34	0,42
2	22	1,29	0,38
3	30	1,10	0,29
4	35	0,98	0,25
5	41	0,97	0,26
6	50	0,82	0,21
7	63	0,81	0,13
8	78	0,73	0,12

PSILOCYBIN AND BAEOCYSTIN CONTENT IN CULTIVATED FRUIT BODIES OF *PSILOCYBE SEMILANCEATA*

Table 2

FLUSH N.	PSILOCYBIN (% DRY WEIGHT)	BAEOCYSTIN (% DRY WEIGHT)
1	1,02	0,21
2	0,82	0,23
3	0,97	0,15
4	0,90	0,14
5	0,99	0,19

THE DRY WEIGHT VARIATION OF PSILOCYBIN, BAEOCYSTIN AND PSILOCIN LEVELS IN *GYMNOPILUS PURPURATUS* AS A FUNCTION OF FLUSH NUMBER

Table 3

FLUSH N.	PSILOCYBIN (%)	PSILOCIN (%)	BAEOCYSTIN (%)
1	0,29	0,21	0,05
2	0,15	0,16	0,04
3	0,21	0,18	0,03
4	0,23	0,20	0,04
5	0,18	0,15	0,01

AMOUNT OF INDOLE ALKALOIDS IN *CONOCYBE CYANOPUS*

Table 4

SAMPLE	DRY WEIGHT OF THE MUSHROOM (MG)	PSILOCYBIN (% DRY WEIGHT)	BAEOCYSTIN (% DRY WEIGHT)
1	5	0,84	0,15
2	6	0,78	0,12
3	7	1,01	0,20
4	10	0,91	0,16
5	12	0,89	0,14

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