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Some results of systematical and ecological research on Agaricales, IX.

By

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Abstract: On the basis of taxonomical examinations, 2 new taxa (Tricholoma nodulosporum sp.n., Agaricus campester var. xanthodermatoides var.n.), and a new combination (Hebeloma subcaespitosum var. psammocolum comb.n.) are described. The ecological researches included laboratory experiments with respect to salt tolerance and low temperature tolerance in Agaricus species; a further aim of the research was to establish whether there exist mycelium thalli without a formation of any fruit body.

1.

Tricholoma nodulosporum Babos & Bohus sp.n.
Figs. 1-2

Iorigida Sing. section, which was a monospecific one in European flora up till now, has been increased with one species. The characteristic features of this section are the mainly violaceous colour of the gills and the stipe as well as the special form of the spores.

The spores are as nodulose as in Inocybe praetervisa Quél., on the outline with 5-8 coarse obtuse warts. This is interesting because by SINGER's statement (1962): "The nodulose spores of many Inocybes are unique in the Agarics, and, for that matter in the Agaricales..."

Pileus 4-6,5 cm latus, carnosus, compactus, e convexo expansus, parum viscidus, pubescens-brevifibrillosus, luride albidus aut luride violaceus, brunnescens, parum argenteo nitidus, margine primitus involuta. Lamellae confertae aut moderate, violaceae vel lilacino-violaceae, tactu luride brunneae, emarginatae - dente decurrentes. Stipes solidus, 2,5-4 cm longus, 1-2 cm crassus, ventricosus aut basi parum bulbosus, metallice violaceus vel lilacino-violaceus, praecipue tactu brunnescens. Caro albida vel violacea, parum brunnescens. Odor nullus. Sapor non specialis. Sporae in cumulo albae. Sporae nodulosae, 7,8-9,5 x 6-8,5 µm. Basidia clavata, 30-35 x 6-8,5 µm.

Habitatio: ad folia coacervata in silva frondosa.

Typus: 73168 in Herbario Musei Hist.-nat.Hung., Budapest. Mts. Budai: Mt. Szarvashegy, in Fageto-Orneto hungaricum, 5 Aug. 1980, leg. Sarkadi - Babos- Bohus - Nehéz - Rimóczi.

Agaricus campester (L.) Fr. var. xanthodermatoides Bohus var.n.

This is a variety which reminds of Agaricus xanthodermus, since the cuticle of the pileus often radially cracked and the flesh in the base of the stipe is lemon-yellow or lemon-yellowish

in each individual. Since the weather was vapory considerable part of the fungi growing in mass reached a relatively large size so the similarity to Agaricus xanthodermus was even more conspicuous.

A typo differt: Caro in basi stipitis ± citrinolutea. Cuticula pilei saepe radialiter diffractus.

Typus: 67844, in Herbario Musei Hist.-nat.Hung., Budapest.

Sárszentágota, com. Tolna, in pascuo, 18 Sept. 1980, leg.: Bohus - Lendvay.

Pileus 6-12 cm; semiglobate then expanded; white, whitish or light ochre; first silky then breaking into scales, often radially cracked; at margin not rarely dentate from veil. Gills rather rarely rosy; edge fertile. Stem 4-6 x 0.8-1.2 cm; attenuated downwards; first with a rich floccose veil; white, at the base yellowing when touched. Ring at least small. Flesh whitish, in the base lemon-yellow or lemon-yellowish. Smell null. Spores ellipsoid, short ellipsoid; 7-7.5 x 4.7-5.2 µm. Gregarious in moderately saline meadows.

Hebeloma subcaespitosum Bon var. psammocolum (Bohus) Bohus comb.n.

Basonym: H. psammocolum Bohus: Hebeloma Studies, II. - Ann.Hist.-nat.Mus.Natl.Hung., 70 (1078), p. 103, Fig. 4.

In the course of an intensive research carried on recently by M. BABOS on the fungus flora of sandy areas in Hungary, rich H. versipelle ss. Konrad & Maublanc = H. subcaespitosum Bon material and several H. psammocolum Bohus specimens could be collected. While studying these, it turned out that the big sand tubercle - which is one of the characteristic features of H. psammocolum - can to a lesser or greater extent be seen also on certain H. subcaespitosum specimens. The other difference, the cylindrical marginal cells among contra lageniform cells, also disappeared. During microscopic examinations it turned out that both forms of marginal cells occur. Cylindrical marginal cells can be found on the gill edge of certain specimens in great number, they occur in other specimens only in small number.

A typo differt: Stipes basi cum bulbo ex arena non raro magno.

Cortinarius rigentoides Bohus nom.n.

Basonym: C. pseudorigens Bohus: Interessantere Cortinarius-Arten aus dem Karpaten-Becken, III. - Ann.Hist.-nat.Mus.Natl.Hung., 68 (1976), p. 54-56, Fig. 4.

It escaped the attention, that R. Henry used the name "pseudorigens" in 1969.

II.

The effect of sodium sulphate on Agaricus species living in saline soils

The easily soluble sodium salts (sodium cloride, sodium sulphate and sodium carbonate), which spoil the clayey structure in alkali soil, causing thereby that the soil becomes more or less barren, on the other hand they have a toxic effect on plants if they occur in greater con-

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Fig. 1. Tricholoma nodulosporum Babos & Bohus sp.n. - Fruit bodies (3/4 nat.size) Photo: Z. Sarkadi.

Fig. 2. Tricholoma nodulosporum Babos & Bohus sp.n. - Spores. Scanning electron micrograph by Mrs. I. Gondár.

Fig. 1.

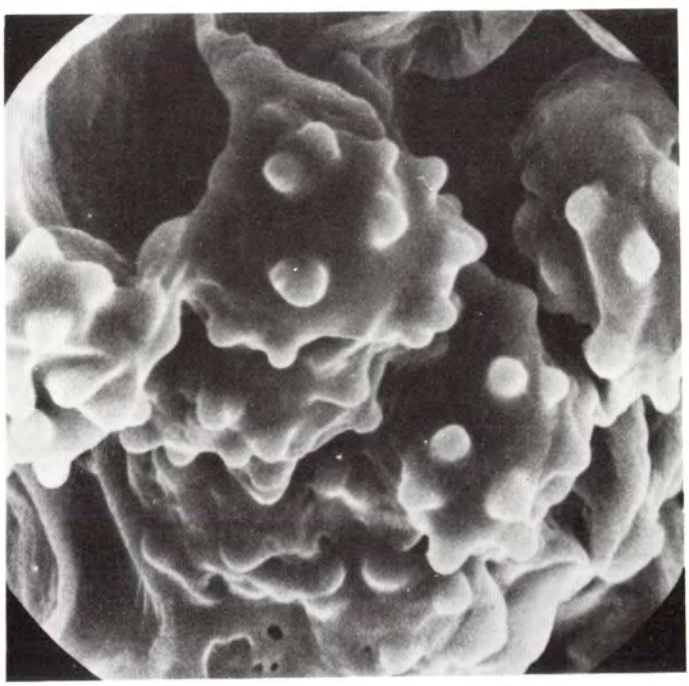

Fig. 2.

centration. Sodium carbonate causes the highest toxic effect, while sodium cloride and sodium sulphate are only moderately harmful. However, there are some plants, that can grow just in such areas of relatively high salt concentration. Of the fungi living on seashores, certain Agaricus species grow in mass in the saline soils in Hungary, and certain species do occur also in strongly fertilized pastures, grasslands as well.

During laboratory experiments the manifestation of the inhibiting toxic effect was examined by raising the sodium sulphate concentration in the case of these fungus species. The inhibition of the mycelium growth could be observed in the species drawn into the experiment (Agaricus macrosporoides, A. bernardii, A. fissuratus), and in Agaricus bisporus which was used as a control. In the case of Agaricus macrosporoides, which usually fructifies well under laboratory conditions, the inhibiting effect of sodium sulphate on the fructification process could be observed.

Methods

Cultivation: Culture vessels are 800 ml cylinder glasses covered with Petri dishes and paper wadding air-filter. The composition of the culture media: 80 g scob grist; 2.4 g $(NH_4)_2SO_4$; 4.8 g $CaCO_3$; 0.8 g commercial mineral premix; 220 ml hot water. Sterilization at 1 atm over-pressure, for 50-60 min. Inoculation material: spawn sprinkled over the culture media surface.

Spore germination: preparation of synthetical nutrient solution with two and a half times the nutrient material: 12.5 g d-glucose; 1.25 g White pepton; 0.25 g KCl; 0.25 g $MgSO_4$; 0.25 g $CaCl_2$; 0.25 g KH_2PO_4; 1.08 g Na_2HPO_4. 7 H_2O; 12.5 ml $FeCl_3$ solution of 0.1% concentration; 100 γ B_1 vitamin; 0.125 g malt extract; 2.5 g agar-agar completed with distilled water to 500 ml; 20-20 ml of synthetical nutrient were poured into 100 ml Erlenmeyer flasks; 30-30 ml buffer solution of required pH were poured into test tubes and all of the flasks and tubes were sterilized at 0.5 atm for 30 minutes. After sterilization, the buffer solutions were mixed with nutrient solution while they were hot and they were kept at 80°C for 20 min. Inoculation: pieces of malt extract agar containing the disperged spores were placed on the surface of the nutrient solution. Spore germination in the case of Agaricus macrosporoides was observed after 20 days at the earliest.

The number of repetitions was only 2, because in the course of numerous experiments carried out since 1970 it turned out that as regards the interlacing in Agaricus macrosporoides the error is of such a small extent that it is practically negligible, especially in case it is carried out in a way that the inoculation bud material is sprinkled on to the surface of the culture media and thus the interlacing starts simultaneously at the same time at several points. As for fructification, it always ensues in the case of this species under favourable conditions (BOHUS 1978).

Results

Agaricus macrosporoides

Repetition of the preceding experiment, with a 0.5% sodium sulphate: One of the two collateral cultures produced a crop of 50% of the dry matter weight of the media within 80 days, while in the other culture no fruit body had grown yet during this period, except on the 105th day a fruit body production was observed in a weight of 46%. Thus, 0.5% sodium sulphate in the culture medium inhibits or delays the fruit body formation to a certain extent. In the case of a concentration higher than this (1.0%) fruit body formation does not even occur and the growth rate of mycelium also slows down. At a sodium sulphate concentration of 1.5% already full inhibition was demonstrated.

Table 1. The effect of rising sodium sulphate concentration on the growth of the mycelia and the fructification

Percentage of sodium sulphate in the culture media	Extent of mycelium intrusion into the culture media on the 35th day	90 day's crop related to the dry matter weight of the culture media
-	4.5 cm	103%
0.5	3.5 cm	-
1.0	3.0 cm	-
1.5	-	-

Agaricus bernardii

In the case of this species no "in vitro" fruit body formation could be attained, therefore the effect of the treatment was only examined in the vegetative phase. It could be stated that 1% sodium sulphate in the culture media caused some inhibitory effect: the growth rate of mycelium was reduced by 1/3 (similarly as in Agaricus macrosporoides).

Agaricus fissuratus

In the case of this species, too, only the effect on the vegetative phase could be examined. It can be detected that 1% sodium sulphate moderately inhibits. The growth rate of mycelium was reduced to some one half of its usual rate.

Agaricus bisporus control

1% sodium sulphate in the culture medium causes full inhibition. On the 25th day after inoculation, the inoculation material did not even become pubescent, while in the culture without sodium sulphate, the interlacing was already in progress. This examination demonstrated the sensitivity of this species - not living on saline ground - to sodium sulphate, well.

On the basis of in vitro examinations it may be stated that temporary decrease of salt concentration during rainy weather may be an impulse among the factors which act upon fructification during the favourable rainy weather.

It is just possible that fungus species living on saline soils "are not fond of" salts; they only tolerate the higher salts concentration. In consequence of this ability they can live on moderately saline areas from which, however, the species more sensitive to higher salt concentration are ousted.

In the case of Agaricus bernardii, which is well known as living in saline soils, and which is very common in saline soils in Hungary, the location of the thalli could be well observed in the field. It is more frequent on those parts of Achilleo-Festucetum pseudovinae and Artemision-Festucetum pseudovinae associations where Festuca pseudovina is dominant. Its fairy rings of great size can be seen well - because of fading of Festuca pseudovina - even at a time when the thallus temporarily does not form fruit bodies. At the levels a few cm lower (see Fig. 3) no Agaricus bernardii fruit bodies were found in the Camphorosmetum annuae and Puccinellietum limosae associations. In the areas flooded with water for longer periods, on the saline plains of higher salt concentration, on the "blind saline soils", the fairy rings are interrupted.

Mycelium thalli without fruit body formation?

By reason of observations in the nature a question was stated, whether it is possible that in soils of certain pH-value the spores of some fungus species are still able to germinate and the formation of mycelium thalli takes place, but the fruit body formation in case of such soil chemical reaction does no longer ensue.

In order to examine this question experiments were carried on with Agaricus macrosporoides, because this species is able to fructify regularly under experimental conditions.

In the earlier experiments related to the above question, the following data had already become known (BOHUS, 1978).

1) Mycelium thallus formation could be observed at a pH between 3.7 and 7.0.

2) In vitro experiments, fruit bodies were observable even at pH 4.2, but this was the lowest value. Fruit bodies grew also in the compost of manure of horses, in culture premises (on the culture media of Agaricus bisporus). The pH value of the substratum was about 7.

In the present experiments it was examined what are the pH limits are between which spore germination still occurs. It was stated that the spores germinate between pH 4.0 and 7.0, but it is possible that a little below pH 4.0 there is still spore germination and the upper limit can be a little higher than pH 7.0. This can be inferred from the fact that the growth of thalli originated from spores germinating at pH 7.0 is more protected than the growth of the control: the radius of the thalli is 2-3 mm when in the case of the control that germinated at pH 5.2 it is ten times more than that.

Spores of Agaricus macrosporoides can germinate at about a value of pH 4.0, but mycelium growth can be observed at even pH 3.7. In the experiments presented, the pH of culture media was 4.2 and it proved to be suitable for fruit body formation. To answer the question whether it is possible to attain the fruit body formation in a culture media with a lower pH value than the former an opportunity was offered by the use of ammonium salts of strong acids as nitrogen source. If no $CaCO_3$ is used as a chemical binding the acid, the culture media turn sour strongly as a result of the using of ammonium ion, and this creates unfavourable conditions for a number of fungus species. This does not however happened in the case of Agaricus macrosporoides, which is able to grow even in a strongly acidic medium, and the interlacing of the culture medium does ensue. The observed pH values were as follows: on the 47th day of interlacing, 3.35; on the 61th day, 3.7. No fruit body was, however, formed in the thalli during the 90 days' - usual - observation period. Since it is a good characteristic of this species that preceding the fruit body formation, a great number of primordia appear in the thalli, it was remarkable in the present examination that below pH 4.0 values there was only one primordium which could be found in one the cultures. It is thus at such low pH values that the mycelium thalli of this species can live in the soil without a result that fruit body formation could take place. On the basis of the example it can be supposed that this is possible also with other species.

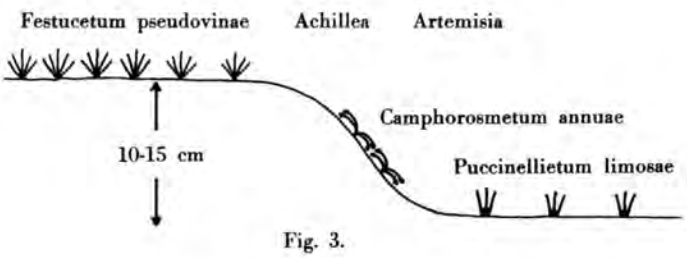

Fig. 3.

Fig. 3. Microrelief on a saline pasture on Hortobágy (Hung.)

The effect of low temperature on Agaricus macrosporoides

The examination of the effect of temperature is interesting from an ecological viewpoint in the case of soil-inhabiting and xylophagous fungi. Examples can be found in the researches presented earlier: Mycelium of Flammulina velutipes is still viable after 138 days at -21°C (PEHRSON cf. COCHRANE 1958) and fruit bodies of Schizophyllum commune withstand exposure to -15 to -40°C (BUXTON et al. cf. COCHRANE, 1958).

The experiments presented here were carried out with cultures of 320 g culture media in weight. The interlacing of the culture media at 25°C lasted 15-20 days. It was after this that freezing effect was introduced in several varieties. After freezing, incubation was continued again at 25°C, then cultivation followed at 17°C, when - if there was no freeze damage - fruit bodies were formed.

The four varieties of experiment were as follows:

Variety 1: Freezing effect held on one day, at -8°C. During cultivation, the fruit bodies were formed on the 57th day after inoculation, their weight was 69% of the dry matter weight of the culture medium. This can be taken as identical with the usual quantity observable under normal conditions after a growth of 50-60 days. Consequently, the above freezing effect was practically not harmful, or inhibiting.

Variety 2: Freezing effect continued for 1.5 days, at -24°C. There was no visible damage on the mycelia but the fruit body appeared 30-40 days later than usual. The weight of the body appeared 30-40 days later than usual. The weight of the fruit body was 66% of the dry matter weight of the culture medium, measured on the 90th day after inoculation. The influence of freezing is documentated by prolongation of the fruit body formation.

Variety 3: Freezing effect continued for one day, at -8°C, then for 2 days cultivation at about 23°C, then again for one day at -8°C. Frost damage could not practically occur because one fruit body started to form on the 40th day after inoculation - that is relatively early - and when it developed (on the 50th day) its weight was 56% of the dry matter weight.

Variety 4: Freezing effect lasted for 4 days, at -8°C. In this case the frost damage could already be observed, some part of thalli were damaged and the growth of the other parts were inhibited. Keeping the culture at 25°C, the continuation of the interlacing could not be noticed even after two weeks had passed. After this further interlacing started, presumably from those parts of the thalli that had not been damaged. A fruit body appeared on the 95th day and the observation was finished.

Discussion of some phenomena related to freezing effect

In the experiments carried out in consequence of relatively slow freezing (at -8°C 1.5-2 cm/h), and the bulk of media, the rate of freezing was different on the surface and inside the culture. In the case of 1st and 3rd experiments extracellular ice-crystals - withdrawing water from the cells - did not form even in the mycelia grown on the surface of the culture, cooling rapidly. It was documented by the practically normal fruit body formation.

In the case of 2nd experiment real frost damage could not also been observed but the fructification was extended in consequence of fairly short but low temperature (-24°C for 1.5 days). In the course of 4th experiment the duration (-8°C for 4 days) causes a stronger inhibition in growth.

The rate of thawing is another factor in the frost injury induced. In the case of experiments carried out, the thawing was relatively rapid, although it was different in rate on the surface and inside of the culture. Any kind of damage was observed.

The extent of frost-resistance depends on the type of the cells, and their physiological condition, too. Since, the cells of mycelia were young (about 20 days old) at the time of freezing, resting cells could not have formed. In the case of species examined, the cells of mycelia themselves were able to endure the frost effect even repeatedly.

A typical case of frost injury was shown by the fruit body, which was held at -8°C for one day and it perished. In consequence of higher water content ice crystals formed in the cells and the effect of damage was conspicuous.

Another observation is if the media is poor in nutrients (e.g. agar-agar with malt extract) the fructification goes on slowly and the primordia remain alive for weeks without any change in its condition, and it can be used for inoculation. On the other hand, in media rich in nutrient the fruit bodies grow up quickly and after a few days they are destroyed. The lower biotic potential or resistance capacity of the normal fruit body cells may be another factor which plays an important role in lower temperature tolerance.

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