

## Review

# The genus *Calvatia* ('Gasteromycetes', Lycoperdaceae): A review of its ethnomycology and biotechnological potential

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Accepted 8 May, 2009

Several members of the fungal puffball genus *Calvatia* Fr. have found widespread use amongst various cultures world-wide, especially as sources of food and/or traditional medicine. Hitherto the biotechnological potential of only a handful of *Calvatia* species, namely *C. cyathiformis*, *C. craniiformis*, *C. excipuliformis*, *C. gigantea* and *C. utriformis* has been investigated. However, despite promising results, information regarding the biotechnological potential of the rest of the genus, in particular the African species, is still completely lacking. In the hope that it might stimulate interest and further research on this topic, the current paper provides a brief overview of the literature pertaining to the importance of *Calvatia* to man in terms of its pathogenicity, its ecology and role as bioindicator, its food and nutritional value and also its potential as biotechnological tool in the pharmaceutical and other industries.

**Key words:** Biotechnology, *Calvatia*, ethnomycology, gasteromycetes, *Handkea*, *Langermannia*, Lycoperdaceae, pathogenicity.

## INTRODUCTION

*Calvatia* Fr. (Basidiomycetes, Lycoperdaceae) is a cosmopolitan gasteromycetous genus of about 35-45 species of mostly medium- to large-sized epigeous puffballs. The genus is characterized by stalked or sessile, globose, subglobose, turbinate, pyriform or agaricoid fruit-bodies that dehisce by irregular fragmentation of the peridium (spore-sac wall) and not by an apical pore as, for example, in *Lycoperdon* Pers.

The genera *Langermannia* Rostk and *Handkea* Kreisel have often been treated as distinct from *Calvatia*, but the current authors do not ascribe to that concept and for the purpose of this review, *Langermannia* and *Handkea* are included in the genus *Calvatia* sensu lato (the '*Calvatia*-complex'). Recent taxonomic work on the *Calvatia*-

complex (Coetzee, 2006) has resulted in a considerably improved understanding of the infrageneric classification and nomenclature of the group.

In the hope that it may stimulate more research with regard to the beneficial and possible detrimental properties of this genus, this paper discusses the documented traditional and more modern uses of *Calvatia* species worldwide, as well as the results of biotechnological investigations involving the genus. As far as could be ascertained, the discovery of *C. utriformis* (Bull.: Pers.) Jaap fruit-bodies in pre-Hadriatic deposits ( $\pm$  A.D. 85-125) at the Roman fort of Vindolanda in Northern England (Watling and Seaward, 1976) represents the earliest physical evidence concerning the use of *Calvatia* by man. According to those authors the fruit-bodies could not have arrived at the site fortuitously and must have been collected deliberately, probably for medicinal (perhaps haemostatic) use and/or as tinder.

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## ECOLOGY AND ROLE AS BIOINDICATOR

All *Calvatia* species are terrestrial saprophytes and are such responsible for the breakdown and recycling of organic matter in nature. No parasitic taxa are known. Although Kreisel (1962) regarded mycorrhizal associations involving species of *Calvatia* as unlikely, Trappe (1962) listed *C. utriformis* [as *C. bovista* (Pers.) Kambly & Lee (sic)] as an ectotrophic mycorrhizal symbiont of *Pinus sylvestris* L. and *C. excipuliformis* (Scop. : Pers.) Perdeck [as *C. saccata* Vahl (sic)] as a similar symbiont of both *Picea abies* (L.) H.Karst. and *Pinus strobus* L.

An endotrophic mycorrhizal relationship between *Liquidambar styraciflua* L. and *C. craniiformis* (Schwein.) De Toni under controlled conditions has also been reported by Filer and Toole (1966). Riffle (1968) included *C. subcretacea* Zeller (= *Calvatia arctica* Ferd. & Winge) in a list of mycorrhizal- or suspected mycorrhizal fungi, but was unable to establish such an association between *C. craniiformis* and *Pinus ponderosa* Douglas ex P.Lawson & C.Lawson (Riffle, 1973). Rimóczi (1987) also did not rule out facultative mycorrhizal relationships in the case of *C. gigantea* (Batsch : Pers.) Lloyd, but that was purely speculative.

Species such as *C. gigantea*, *C. cyathiformis* (Bosc) Morgan, *C. fragilis* (Vittad.) Morgan [= *C. cyathiformis* f. *fragilis* (Vittad.) A.H.Sm.] and *C. polygonia* Lloyd have been reported to form fairy rings (Shantz and Piemeisel, 1917; Dickenson, 1979), the latter three with a stimulatory effect on the autotrophic vegetation inside the ring (Shantz and Piemeisel, 1917). Kamo et al. (2006), on the other hand, observed that azo- and azoxyformamides isolated from *C. craniiformis* (and *Lycoperdon hyemale* Bull.) caused considerable inhibition of radicle growth in lettuce seedlings.

Apart from some pedological data in Demoulin (1968) concerning *C. excipuliformis* and *C. utriformis*, detailed ecological studies pertaining to specific members of the *Calvatia*-complex are virtually non-existent. Rimóczi (1987), however, provided valuable information regarding *C. gigantea*, which seems to be a strongly nitrophilous, acido-subacidophilous species (pH 5.7-5.9) avoiding basic soils. It is mesophilous with regard to temperature as well as soil humidity and requires complete shade during the early developmental stages. Later it remains shade-loving, although it can tolerate intense light. It is also more common in disturbed areas, especially cultivated lands, than in natural habitats. Dörfelt et al. (1988) confirmed the nitrophilous character of this fungus, suggesting also that, in central Europe at least, it probably is a species of nutrient-rich old-forest, with secondary distribution into non-forest habitats. Over-exploitation of this popular edible species has resulted in its protection by law in Poland (Lawrynowicz, 1988).

In contrast to the mesophilous *C. gigantea*, most other members of the *Calvatia*-complex are known from arid to semi-arid habitats, including the high arctic desert

(Lange, 1990). A number of species are, however, also known from tropical forest (Dissing and Lange, 1962; Demoulin and Dring, 1975). Particularly noteworthy is Lange's (1990) statement that the arctic species, which can stretch their development over more than one season by overwintering under the snow, are, in contrast to *C. gigantea*, more or less confined to basic soils.

It is well known that fruiting bodies of higher fungi are efficient bioaccumulators of heavy metals and radionuclides from the ecosystem (Sesli and Tüzen, 1999; Kalač and Svoboda, 2000; Kalač, 2001; Skwarzec and Jakusik, 2003; Turkecul et al., 2004; Cocchi et al., 2006) and as such, also potential bioindicators of environmental pollution with heavy metals (Kalač and Svoboda, 2000). After analysing a considerable number of wild-harvested mushrooms, Seeger (1976) and Aichberger (1977) both concluded that the Lycoperdaceae were, in general, more active accumulators of mercury than most of the other taxa investigated.

Seeger (1976), for instance, reported a mercury content of 4.57 mg/kg dry mass for *C. excipuliformis* (as *C. saccata*) and in only twenty other species, out of a total of 236 investigated, were higher mercury levels measured. Falandyasz et al. (2003) found the same species to be the greatest mercury accumulator out of fourteen fungi investigated, measuring similar levels as those reported by Seeger (1976), with mercury levels in the carpophores close to 900 times higher than in the substrate. Corroboration for those findings also comes from Pokorny et al. (2004), who reported a very similar mercury content for *C. utriformis* and whose findings also indicate a higher accumulation of toxic heavy metals by this puffball than most of the other fungi assayed.

In the same study, the levels for arsenic, lead and cadmium were 10.4, 6.63 and 8.70 mg/kg dry mass respectively. Aichberger (1977), however, found that *C. utriformis* (as *C. bovista*) had the lowest mercury content (0.018 mg/kg dry mass) of 24 species analysed. This low figure is out of line with the general trend in the Lycoperdaceae, however, and needs to be verified. According to Vetter (1990 and 2004) species of *Calvatia*, especially *C. utriformis* also seem to have the ability to accumulate arsenic and informative data on the arsenic content of *C. excipuliformis*, *C. utriformis* and *C. gigantea* (as *Langermannia gigantea*) from various localities in Hungary are provided in those papers.

The same author also reported a lithium level of 0.171 mg/kg dry mass for *C. excipuliformis* (Vetter, 2005) while Cocchi et al. (2006) measured a lead concentration in *C. utriformis* almost ten times as high as the average for the 58 edible mushroom species assayed. Skwarzec and Jakusik (2003) found *C. excipuliformis* to be the most active bioaccumulator of  $^{210}\text{Po}$  of all the fungi investigated from the Elblag area in Poland. More information on the heavy-metal content of *C. utriformis* and *C. excipuliformis* is contained in Cocchi et al. (2007) and Sesli et al. (2008) respectively.

## FOOD VALUE

### Culinary use

All *Calvatia* species are edible (Morris, 1987), but only in the immature state before the commencement of spore maturation and while the gleba is still firm and white (Gray, 1973; Rinaldi and Tyndalo, 1974; Grigson, 1978; Purkayastha and Chandra, 1985; Læssøe and Spooner, 1994). *C. gigantea* and *C. utriformis* are rated particularly high with respect to their organoleptic properties (Rinaldi and Tyndalo, 1974; Grigson, 1978; Christensen, 1981; Purkayastha and Chandra, 1985) and Legg (1987) placed *C. gigantea* (as *Langermannia gigantea*) in joint seventh position (with *Cantharellus cibarius* Fr.).

In a preliminary survey of the best edible fungi of Britain. *C. excipuliformis*, *C. craniiformis* and *C. cyathiformis* have also been reported as good edible species (Mendoza, 1938; Gray, 1973; Rinaldi and Tyndalo, 1974; Christensen, 1981; Purkayastha and Chandra, 1985), while *C. sculpta* (Harkn.) Lloyd has been listed as a traditional food of the Central Miwok Indians of North America (Burk, 1983).

Apart from reports of *C. cyathiformis* being eaten in Nigeria (Oso, 1975, 1977; Aletor, 1995), no further confirmation regarding the culinary use of *Calvatia* in Africa could be found. The information on the use of various *Calvatia* and *Langermannia* species as food items in sub-Saharan Africa as contained in Rammeloo and Walley (1993) should be treated with circumspect since some of the entries in that work have been based on incorrect identifications in the original publications cited. Despite the fact that the Chewa of Malawi use a wide variety of fungi as food (Williamson, 1975; Morris, 1984 and 1994) they classify puffballs as 'chirombo' (a useless thing), and the two *Calvatia* species recorded for that country, namely *C. gardneri* (Berk.) Lloyd [= *C. pyriformis* (Lev.) Kreisel] and the excellent *C. utriformis*, are apparently both spurned as inedible (Morris, 1990).

Calonge et al. (1997) could not find any evidence of the traditional use of any gasteromycete as food in Tanzania either. Puffballs are also conspicuously absent from a list of food plants for Zimbabwe (Tredgold et al., 1986), as well as the lists of edible mushrooms of the Upper-Shaba region of Zaire (Parent and Thoen, 1977) and Zambia (Pegler and Pearce, 1980).

The organoleptic properties and pharmaceutical potential of *Calvatia* spp., as well as the possible health risk associated with the consumption of these heavy-metal-accumulators from the wild, make the pursuit of cultivating various species desirable. This has stimulated a number of research projects, such as those reported on by Rimóczi (1987) and Alexander and Lippert (1989). To date, however, attempts at inducing carpophore formation in controlled environments seem to have met with little success.

### Nutritional and chemical analysis

Apart from the relatively detailed compositional informa-

tion provided for *C. gigantea* in Vetter (1994a, b and c) and Agrahar-Murugkar and Subbulakshmi (2005) and for *C. cyathiformis* in Adriano and Cruz (1933) and Crisan and Sands (1978), the latter repeated also in Purkayastha and Chandra (1985), and as determined independently by Aletor (1995), relatively little is known regarding the nutritional value of the *Calvatia*-complex in general. According to Aletor (1995) a washed, air-dried, wild-harvested *C. cyathiformis* sample from tropical Nigeria contained 13.2% crude protein and had a gross energy value of 3.07 kcal/g.

That compares well with the 13.69% protein content reported for *C. gigantea* grown on brewery spent grain press liquor (Shannon and Stevenson, 1975a), but differs substantially from the 27.3% reported for *C. gigantea* by Agrahar-Murugkar and Subbulakshmi (2005), the 46% crude protein cited for *C. cyathiformis* [as *Lycoperdon lilacinum* (Mont. & Berk.) Speg.] in Crisan and Sands (1978) and Purkayastha and Chandra (1985), and even more so from the 52% and >65% (expressed in terms of dry mass) reported by Bauer Petrovska (2001) for *C. utriformis* [as *C. caelata* (Bull.) Morgan] and Adriano and Cruz (1933) for *C. cyathiformis* respectively.

In *C. gigantea*, at least, the protein content appears to be substrate-dependent and when grown on trub press liquor, Shannon and Stevenson (1975a) reported a protein content of 17.5%. The latter authors' protein values, as well as that of Adriano and Cruz (1933), Bauer Petrovska (2001) and Agrahar-Murugkar and Subbulakshmi (2005), were all calculated as Kjeldahl N  $\times$  6.25, but, as has been explained elsewhere (Crisan and Sands, 1978; Wehmeyer et al., 1981), a conversion factor of 4.38, which compensates for the non-protein nitrogen in the fungus's chitinous cell walls, is widely accepted to provide a closer approximation of the crude protein content of mushrooms. The protein values reported by the above mentioned authors might, therefore, in all probability, be somewhat inflated.

Vetter (1994a, b and c) compared the potassium, copper, manganese, zinc and phosphorus content of *C. gigantea* (as *L. gigantea*) with a large number of other edible macrofungi. The values for *C. gigantea* as determined by Agrahar-Murugkar and Subbulakshmi (2005) differ considerably from those reported by Vetter, however, while Alonso et al. (2003) also reported much higher copper and zinc levels for *C. utriformis* from Spain. Vetter (2003) and Agrahar-Murugkar and Subbulakshmi (2005) reported on the sodium content of *C. excipuliformis* and *C. gigantea* respectively and more data on trace element levels is provided by Sesli and Tüzen (1999) and Turkecul et al. (2004) for *C. utriformis*, by Borovička and Řanda (2007) and Sesli et al. (2008) for *C. excipuliformis* and by Borovička and Řanda (2007) for *C. gigantea* (as *L. gigantea*). Borovička and Řanda (2007), however, questioned the high iron levels reported by Sesli and Tüzen (1999) and Turkecul et al. (2004).

As seems to be the norm for the Fungi in general, linoleic acid was found to be the major component of the

lipids of both *C. gigantea* and *C. utriformis*, with smaller but significant amounts of palmitic and oleic acid also present (Sumner, 1973). Unlike the other fungi examined, however, the short-chain fatty acids octanoic acid, decanoic acid and in particular lauric acid also formed a significant component of the lipid content of both *Calvatia* species. More information on the fatty acid composition of various members of the Lycoperdaceae, including *C. utriformis*, is provided by Nedelcheva et al. (2007). In an analysis of the fatty acid composition of mushroom glycerophospholipids, only trace amounts of phosphatidylcholine and phosphatidylethanolamine were found in *C. gigantea*, while phosphatidylinositol and phosphatidylserine were barely detectable (Proštenik et al., 1983). The glycoinositolphosphosphingolipids (basidiolipids) of *C. excipuliformis* have been isolated and reported on by Jennemann et al. (2001).

According to Dijkstra (1976) 1-octen-3-ol, an alcohol with a typical mushroom-like odour and which is known to be an important contributor to the flavour of *Agaricus bisporus* (Lange) Imbach, has been found to be the dominant volatile also in *C. gigantea*, accounting for 89% of the total volatile components detected. Glutamic acid and 5'-GMP, two other known flavour components of mushrooms, were also detected in concentrations high enough to significantly contribute to the flavour of *C. gigantea*.

Overton's (1994) analysis of the volatile and semi-volatile compounds of *C. gigantea* (only referred to as the 'giant puffball *Calvatia*' in his paper) did not support Dijkstra's findings on 1-octen-3-ol, however. Overton reported a very high methoxybenzene concentration but no 1-octen-3-ol, which is somewhat suspect, especially in view of the fact that he also did not detect any 1-octen-3-ol in *A. bisporus*.

## BIOTECHNOLOGICAL POTENTIAL

### Pharmaceutical investigations

*Calvatia* species have found widespread use in the folk medicines of various cultures, especially as a haemostatic (styptic) and wound dressing [on which topic Baker (1989) provides an informative overview] as well as for a variety of other ailments such as leucorrhoea, pneumaturia, inflammation, diarrhoea in calves, etc. (Swanton, 1917; Patouillard, 1924; Rolfe and Rolfe, 1925; Watling and Steward, 1976; Oso, 1977; Dickenson and Lucas, 1979; Burk, 1983; Liu, 1984; Morris, 1987; Rai et al., 1993; Guzmán, 1994; Kawahara et al., 1994; Læssøe and Spooner, 1994).

A summary of these uses is contained in Coetzee (2006). It is noteworthy; however, that Watt and Breyer-Brandwijk (1962), as well as two recent works on southern African traditional medicines (Hutchings et al., 1996; Van Wyk et al., 1997), list no traditional use for this group of fungi in the region. According to Pearce (1981) macrofungi are also not used in Zambian traditional medicine.

*C. gigantea*, *C. utriformis*, *C. craniiformis* and *C. cyathiformis* are all known to produce tumour inhibitors (Lucas et al., 1959), and the mucoprotein calvacin, extracted and purified from *C. gigantea*, has been shown to possess anti-tumour activity against 13 out of 24 different mouse, rat and hamster tumours (Roland et al., 1960; Beneke, 1963). Continued use of the drug induced an apparent allergic reaction in dogs and monkeys, however (Beneke, 1963).

According to Sevilla-Santos and Bernardo (1966) extracts of *C. cyathiformis* [as *C. lilacina* (Mont. & Berk.) Henn.] carpophores from the Philippines also displayed strong anti-tumour activity. Kim et al. (1992) observed that protein-bound polysaccharides extracted from cultured *C. craniiformis* mycelium suppressed the growth of sarcoma 180 in mice by up to 74.1%. The anti-tumour activity of at least one of the extracted fractions, referred to as calvatan, was believed to be as a result of immunopotentiality rather than cytotoxicity.

Takaishi et al. (1997) reported anticarcinogenic activity against K562 leukaemia cells by two substances isolated from *C. craniiformis*, identified as craniformin and the phenolic tautomer of rubroflavin respectively. The latter substance was originally isolated from *C. rugosa* (Berk. & M.A.Curtis) D.A.Reid [as *C. rubroflava* (Cragin) Morgan] by Gill and Steglich (1987) in their study of the pigments of *C. craniiformis* and *C. rugosa*. More recently, Lam et al. (2001) reported high antiproliferative activity toward breast cancer cells by an ubiquitin-like peptide isolated from *C. utriformis* (as *C. caelata*) fruit-bodies. The same substance also displayed antimitogenic activity toward splenocytes.

The discovery of the oncostatic properties of *Calvatia* species directly inspired an investigation into their possible antiviral activity (Cochran, 1978). In this regard, *C. gigantea* provided partial, but significant, protection in mice against infection by the poliomyelitis virus (Cochran and Lucas, 1959), while strains of the same species also significantly inhibited five out of thirteen types of ECHO enteroviruses in monkey kidney cell cultures (Goulet et al., 1960). *C. gigantea* also displayed significant activity against the A<sub>2</sub>/Japan 305 virus in in vitro studies with calf kidney cells and against the A/PR8 influenza virus in in vivo studies with mice (Cochran et al., 1967), as well as against the A/PR8/34 influenza virus in eggs and intraperitoneally infected mice (Gainer et al., 1967).

Extracts from *C. utriformis* (as *C. caelata*), on the other hand, were inactive against both poliomyelitis and influenza viruses (Cochran and Lucas, 1959; Cochran et al., 1967). Calvacin (cf. previous paragraph) displayed no activity against the poliomyelitis virus and the antiviral and antitumour activity of *C. gigantea* thus seems to be caused by different active substances (Cochran, 1978).

Gasco et al. (1974) and Umezawa et al. (1975) more or less simultaneously, but independently, isolated and identified another compound with possible therapeutic potential from *C. cyathiformis* (as *C. lilacina*) and *C. craniiformis* respectively. This substance, p-carboxypheny-

lazoxyanide [= 2-(4-carboxyphenyl)diazene-carbonitrile 2-oxide], commonly called calvatic acid, has subsequently also been found in *C. gigantea* and *C. utriformis* (Okuda and Fujiwara, 1982).

According to Gasco et al. (1974) and Viterbo et al. (1975) calvatic acid displays antibacterial as well as anti-fungal activity, but Umezawa et al. (1975) were unable to support those claims, detecting no activity against most yeast and other fungi. More recently, however, Sorba et al. (2001) reported strong antibiotic activity by calvatic acid and some of its analogues and derivatives against *Helicobacter pylori*, the bacterium implicated in a number of gastric pathologies such as peptic ulcers and gastric cancers.

Calvatic acid has also been demonstrated to have a definite antitumour effect, significantly inhibiting the growth of Yoshida sarcoma in cell culture, as well as increasing the survival time of mice with Leukaemia 1210 (Umezawa et al., 1975). Subsequent investigations have therefore also focussed on the antitumour properties of calvatic acid, which, according to Antonini et al. (1997), may represent a model for the synthesis of more specific glutathione transferase-P1-1 inhibitors with possible therapeutic relevance.

Ng et al. (2003) isolated a novel ribosome-inactivating protein with translation-inhibiting and antimitogenic activities from *C. utriformis* (as *C. caelata*). This protein, named calcaelin, reduced the viability of breast cancer cells but displayed no antifungal or antibacterial activity. More recently, Dulger (2005) reported on antibacterial, but poor antimycotic activity of a 60% methanolic extract of *C. utriformis*. Suay et al. (2000), however, were unable to detect any microbial activity by methanol extracts of both *C. cyathiformis* and *C. utriformis* against six bacteria and three fungi. Such conflicting reports warrant further investigation.

The use of *Calvatia* species in Chinese and Japanese traditional medicine has served as an additional motivation for continued pharmaceutical investigation of these fungi and has led to the isolation of ergosterol from *C. excipuliformis* (as *C. saccata*) (Kwon et al., 1980), a polyoxygenated ergosterol derivative, various other steroids as well as a calvatic acid derivative from *C. nipponica* Kawam. [as *Lasiosphaera nipponica* (Kawam.) Kobayasi] (Takaishi et al., 1992) and the novel steroids calvasterone, cyathisterone, cyathisterol, calvasterol A and B from *C. cyathiformis* (Kawahara et al., 1993, 1994 and 1995).

Deng et al. (2007) also makes further reference to a number of steroids isolated from *C. argentea* (Berk.) Kreisel (as *Lasiosphaera fenzi* Reichardt), *C. craniformis* and *C. gigantea*. The pharmaceutical relevance of these substances, most of which were reviewed also by Kovganko (1999), has yet to be established, however.

### Other industrial investigations

Shannon and Stevenson (1975a, b) reported very posi-

tively on the potential of *C. gigantea* as a producer of microbial protein from brewery wastes and as an agent for the reduction of the chemical and biochemical oxygen demand of such wastes. The same fungus has subsequently also been found to be a producer of various enzymes of possible biotechnological significance. Kekos and Macris (1983) reported *C. gigantea* as a prolific producer of  $\alpha$ -amylase in starch-containing liquid growth media.

The amylase yield has been described as '... among, if not the highest reported in the literature' (Kekos et al., 1987). Significant also is the fact that the *C. gigantea*-amylase is tannin-resistant (Kekos and Macris, 1983, 1987a; Kominos et al., 1988), tannins being well known as inhibitors of enzyme activity. *C. gigantea* has also been found to be able to utilise toxic phenolic and polyphenolic compounds, particularly condensed tannins, as sole carbon sources (Galiotou-Panayotou and Macris, 1986) due to its ability to degrade catechin, the building units of condensed tannins.

A novel catechin-degrading enzyme has subsequently been purified from this fungus (Galiotou-Panayotou et al., 1988). *C. gigantea* thus seems to hold potential for the production of biomass and amylase from carbon sources such as acorns containing high levels of toxic tannic compounds that would otherwise restrict the biotechnological upgrading of such substances (Kekos and Macris, 1987a, b).

The cost of chitinase production has been an impediment to the economic feasibility of the bioconversion of chitinous wastes to single cell protein (Zikakis and Castle, 1988). Since the autodigestion occurring in the gleba of ripening *Calvatia* fruit-bodies is suggestive of the genus being a potential source of both the chitinolytic enzymes chitinase and chitobiase, it is not surprising that investigations into cheaper chitinase sources also involved members of this genus.

In this regard, Tracey (1955) reported 'powerful' chitinolytic activity in crude extracts from ripening, but not yet dry, *C. gigantea* (as *Lycoperdon giganteum* Batsch) carpophores. The extracts, which normally effected complete hydrolysis of chitin samples within ten days, contained both chitinase and chitobiase. Zikakis and Castle (1988) also reported strong chitinase activity for extracts of *C. cyathiformis*. The extracts, which did not lose its activity after freezing, could be used in chitin hydrolysis studies without the need for further purification and *C. cyathiformis* has been described as an effective and most convenient source of chitinolytic activity (Zikakis and Castle, 1988).

Fungi are generally regarded as excellent lipase sources and in this regard Christakopoulos et al. (1992) determined that the lipase yield from *C. gigantea* compares well with other lipase-hyperproducers. The fact that this is an edible fungus could make it a valuable source of lipase for use in the food industry. Recently Jaya Prakash Goud et al. (2009) also reported on moderate lipase and carboxyl esterase activity by *C. sculpta* (Harkn.) Lloyd from southern India.

## OTHER USES

Smoke from smouldering fruit-bodies of *C. gigantea* is known to have been used by beekeepers to drive bees from their hives (Swanton, 1917) or merely to calm the bees in order to gain access to hives (Rolfe and Rolfe, 1925; Cook, 1970; Dickenson and Lucas, 1979). More recently, Wood (1983) reported on the similar use of *C. argentea* [as *Langermannia wahlbergii* (Fr.) Dring] by Tanzanian beekeepers, ascribing the anaesthetic effect of the smoke partly to the presence of hydrogen sulphide and speculating that hydrogen cyanide and other unidentified substances might also be involved.

The use of smoke from burning fruit-bodies of *C. argentea* (as *Lasiosphaera fenzi*) for hut-fumigation in Kenya has been reported by Duke (1926). Due to the apparent 'injurious effects of the fumes on the eyes', huts need to be vacated for some time after fumigation, however. [See also under *Langermannia wahlbergii* in Walley and Rammeloo (1994).]

*C. gigantea* has found use as tinder (Dickenson and Lucas, 1979) and the addition of saltpetre to dried *C. utiformis* fruit-bodies provided an alternative source of amadou, used in older times as tinder or haemostatic (Læssøe and Spooner, 1994). The use in ancient times of *C. utiformis* as insulation material to plug up holes in draughty dwellings, as mentioned by Burk (1983), is, however, unlikely (Watling and Seaward, 1976).

## PATHOGENICITY

According to Læssøe and Spooner (1994) it has been reported in the literature that certain *Calvatia* species may cause 'violent gastrointestinal upsets'. Substantiation is lacking, but such cases might have resulted from the consumption of specimens of which the gleba had already ripened.

The inhalation of massive quantities of puffball spores (*Lycoperdon* spp.) has been reported to cause an allergic respiratory condition known as lycoperdonosis (Strand et al., 1967; Henriksen, 1976) and it is reasonable to expect that, in the unlikely event of the inhalation of very large quantities of *Calvatia* spores, similar symptoms might develop in sensitive individuals. According to Simon-Nobbe et al. (2008), *Calvatia* is one of the most prominent genera of the Basidiomycota responsible for the induction of fungal type 1 allergy. Basidiospores of *C. cyathiformis* have been identified in aerobiological studies and spore extracts of this species have been demonstrated to cause significant skin and radioallergosorbent test (RAST) reactivity in sensitive patients (Levetin et al., 1992).

According to Horner et al. (1995) isoelectric focussing analysis of *C. cyathiformis* revealed 21 allergens, while O'Neil et al. (1990) reported cross-reactivity between allergens from *C. cyathiformis*, *Alternaria alternata* and *Fusarium solani*. Levetin et al. (1992) regard *C. rugosa*

(as *C. rubroflava*) and *C. craniiformis* also as potentially important aeroallergens. It is quite probable, therefore, that those species and most likely other *Calvatia* species as well, may, like many other fungi, contribute to causing allergic conditions such as asthma and rhinitis. Horner et al. (1995) and Simon-Nobbe et al. (2008) may be consulted for reviews on fungal allergens.

## Conclusion

A recent taxonomic study of the genus *Calvatia* in southern Africa (Coetzee, 2006), but conducted in world context, revealed the existence of eleven species in the region (four new to science) whose biotechnological potential have not yet been investigated at all. The same situation applies to various other species reported from the continent. Much still needs to be learned regarding the fungal biodiversity of Africa and it is reasonable to anticipate that further studies will uncover the existence of even more *Calvatia* species from this continent.

In view of this exciting prospect and also the biotechnological potential already reported from the limited investigations on only a handful of species, as has been touched upon in this paper, continued taxonomic work on this genus in Africa is imperative. Such taxonomic studies will lay the foundation and pave the way for meaningful continued biotechnological investigations aimed at determining and unlocking the potential benefits of these organisms to humankind.

## REFERENCES

- Adriano FT, Cruz RA (1933). The chemical composition of Philippine mushrooms. Philipp. J. Agric. 4: 1-11.
- Agrahar-Murugkar D, Subbulakshmi G (2005). Nutritional value of edible wild mushrooms collected from the Khasi hills of Meghalaya. Food Chem. 89: 599-603.
- Aichberger K (1977). Untersuchungen über den Quecksilbergehalt österreichischer Speisepilze und seine Beziehungen zum Rohproteingehalt der Pilze. Z. Lebensm. Unters. Forsch. 163: 35-38.
- Aletor VA (1995). Compositional studies on edible tropical species of mushrooms. Food Chem. 54: 265-268.
- Alexander JP, Lippert BE (1989). The effects of phytohormones on the mycelial growth of *Calvatia gigantea* and related species. Mushroom Sci. 12(2): 401-410.
- Alonso J, García MA, Pérez-López M, Melgar MJ (2003). The concentrations and bioconcentration factors of copper and zinc in edible mushrooms. Arch. Environ. Contam. Toxicol. 44: 180-188.
- Antonini G, Pitari G, Caccuri AM, Ricci G, Boschi D, Fruttero R, Gasco A, Ascenzi P (1997). Inhibition of human placenta glutathione transferase P1-1 by the antibiotic calvatic acid and its diazocyanide analogue. Evidence for multiple catalytic intermediates. Eur. J. Biochem. 245: 663-667.
- Baker T (1989). Fungal stypitics. Mycologist, 3: 19-20.
- Bauer Petrovska B (2001). Protein fraction in edible Macedonian mushrooms. Eur. Food Res. Technol. 212: 469-472.
- Beneke ES (1963). *Calvatia*, Calvacin and cancer. Mycologia, 55: 257-270.
- Burk WR (1983). Puffball usages among North American Indians. J. Ethnobiol. 3: 55-62.
- Borovička J, Řanda Z (2007). Distribution of iron, cobalt, zinc and selenium in macrofungi. Mycol. Prog. 6: 249-259.

- Calonge FD, Härkönen M, Saarmimäki T, Mwasumbi L (1997). Tanzanian mushrooms and their uses 5. Some notes on the Gasteromycetes. *Karstenia*, 37: 3-10.
- Christakopoulos P, Tzia C, Kekos D, Macris BJ (1992). Production and characterization of extracellular lipase from *Calvatia gigantea*. *Appl. Microbiol. Biotechnol.* 38: 194-197.
- Christensen CM (1981). Edible mushrooms, edn 2. University of Minnesota Press, Minneapolis.
- Cocchi L, Vescovi L, Petrini LE, Petrini O (2006). Heavy metals in edible mushrooms in Italy. *Food Chem.* 98: 277-284.
- Cochran KW (1978). Medical effects. In Chang ST, Hayes WA, The biology and cultivation of edible mushrooms, pp. 169-187. Academic Press, New York.
- Cochran KW, Lucas EH (1959). Chemoprophylaxis of poliomyelitis in mice through the administration of plant extracts. In Welch H, Marti-Ibañez F, *Antibiot. Annu. 1958-1959*: pp. 104-109. Medical Encyclopedia Inc, New York.
- Cochran KW, Nishikawa T, Beneke ES (1967). Botanical sources of influenza inhibitors. *Antimicrob. Agents Chemother.* 1966: 515-520.
- Coetzee JC (2006). Contributions towards a new classification of *Calvatia* Fr. (Lycoperdaceae) in southern Africa. PhD dissertation, University of Pretoria, Pretoria, South Africa.
- Cook VA (1970). Puff ball control of bees. *N. Z. J. Agric.* 120(5): 72-75.
- Crisan EV, Sands A (1978). Nutritional value. In Chang ST, Hayes WA, The biology and cultivation of edible mushrooms, 137-168. Academic Press, New York.
- Demoulin V (1968). Gastéromycètes de Belgique: Sclerodermatales, Tulostomatales, Lycoperdales. *Bull. Jard. Bot. Nat. Belg.* 38: 1-101.
- Demoulin V, Dring DM (1975). Gasteromycetes of Kivu (Zaire), Rwanda and Burundi. *Bull. Jard. Bot. Nat. Belg.* 45: 339-372.
- Deng ZP, Sun LR, Ji M, Yuan YM (2007). Steroids from *Bovistella radicata* (Mont.). *Pat. Biochem. Syst. Ecol.* 35: 700-703.
- Dickenson CH (1979). Fairy rings in Norfolk. *Bull. Br. Mycol. Soc.* 13: 91-94.
- Dickenson C, Lucas J (1979). The encyclopedia of mushrooms. GP Putnam's Sons, New York.
- Dijkstra FY (1976). Studies on mushroom flavours. 3. Some flavour compounds in fresh, canned and dried edible mushrooms. *Z. Lebensm. Unters. Forsch.* 160: 401-405.
- Dissing H, Lange M (1962). Gasteromycetes of Congo. *Bull. Jard. Bot. État. Brux.* 32: 325-416.
- Dörfelt H, Kreisel H, Benkert D (1988). Karten zur Pflanzen-verbreitung in der DDR. 7 Serie. Ausgewählte Makromyzenen (11). *Hercynia n.F.* 25: 84-106.
- Duke MM (1926). Fungi from Kenya colony. *Bull. Misc. Inf. (Kew)* 1926: 305-320.
- Dulger B (2005). Antimicrobial activity of ten Lycoperdaceae. *Fitoterapia*, 76: 352-354.
- Falandysz J, Gucia M, Brzostowski A, Kawano M, Bielawski L, Frankowska A, Wyrzykowska B (2003). Content and bioconcentration of mercury in mushrooms from northern Poland. *Food Addit. Contam.* 20: 247-253.
- Filer TH, Toole ER (1966). Sweetgum mycorrhizae and some associated fungi. *For. Sci.* 12: 432-437.
- Gainer JH, Cochran KW, Lucas EH (1967). Anti-influenzal activity of plant extracts. *Pharmacologist*, 9: p. 193.
- Galiotou-Panayotou M, Macris BJ (1986). Degradation of condensed tannins by *Calvatia gigantea*. *Appl. Microbiol. Biotechnol.* 23: 502-506.
- Galiotou-Panayotou M, Rodis P, Macris BJ, Stathakos D (1988). Purification of a novel enzyme involved in catechin degradation by *Calvatia gigantea*. *Appl. Microbiol. Biotechnol.* 28: 543-545.
- Gasco A, Serafino A, Mortarini V, Menziani E, Bianco MA, Seruti Scurti J (1974). An antibacterial and antifungal compound from *Calvatia lilacina*. *Tetrahedron Lett.* (38): 3431-3432.
- Gill M, Steglich W (1987). Pigments of Fungi (Macromycetes). *Prog. Chem. Org. Nat. Prod.* 51: 1-317.
- Goulet NR, Cochran KW, Brown GC (1960). Differential and specific inhibition of ECHO viruses by plant extracts. *Proc. Soc. Exp. Biol. Med.* 103: 96-100.
- Gray WD (1973). The use of fungi as food and in food processing. Part II. CRC Press, Cleveland.
- Grigson J (1978). The mushroom feast. Penguin, Middlesex.
- Guzmán G (1994). Los hongos en la medicina tradicional de Mesoamérica y de México. *Rev. Iberoam. Micol.* 11: 81-85.
- Henriksen NT (1976). Lycoperdonosis. *Acta Paediatr. Scand.* 65: 643-645.
- Horner WE, Helbling A, Salvaggio JE, Lehrer SB (1995). Fungal allergens. *Clin. Microbiol. Rev.* 8(2): 161-179.
- Hutchings A, Scott AH, Lewis G, Cunningham AB (1996). Zulu medicinal plants. University of Natal Press, Pietermaritzburg.
- Jaya Prakash Goud M, Suryam A, Lakshminpathi V, Singara Charya MA (2009). Extracellular hydrolytic enzyme profiles of certain South Indian basidiomycetes. *Afr. J. Biotechnol.* 8: 354-360.
- Jennemann R, Geyer R, Sandhoff R, Gschwind RM, Lavery SB, Gröne HJ, Wiegandt H (2001). Glycoinositolphosphosphingolipids (basidiolipids) of higher mushrooms. *Eur. J. Biochem.* 268: 1190-1205.
- Kalač P (2001). A review of edible mushroom radioactivity. *Food Chem.* 75: 29-35.
- Kalač P, Svoboda L (2000). A review of trace element concentrations in edible mushrooms. *Food Chem.* 69: 273-281.
- Kamo T, Kashiwabara M, Tanaka K, Ando S, Shibata H, Hirota M (2006). Plant growth inhibitory activity of azo and azoxyformamides from *Calvatia craniiformis* and *Lycoperdon hiemale*. *Nat. Prod. Res.* 20: 507-510.
- Kawahara N, Sekita S, Satake M (1993). A novel dimeric steroid, calvasterone from the fungus *Calvatia cyathiformis*. *Chem. Pharm. Bull.* 41: 1318-1320.
- Kawahara N, Sekita S, Satake M (1994). Steroids from *Calvatia cyathiformis*. *Phytochem.* 37: 213-215.
- Kawahara N, Sekita S, Satake M (1995). Two steroids from *Calvatia cyathiformis*. *Phytochemistry* 38: 947-950.
- Kekos D, Galiotou-Panayotou M, Macris BJ (1987). Some nutritional factors affecting  $\alpha$ -amylase production by *Calvatia gigantea*. *Appl. Microbiol. Biotechnol.* 26: 527-530.
- Kekos D, Macris BJ (1983). Production and characterization of amylase from *Calvatia gigantea*. *Appl. Environ. Microbiol.* 45: 935-941.
- Kekos D, Macris BJ (1987a). Effect of tannins on growth and amylase production by *Calvatia gigantea*. *Enzyme Microb. Technol.* 9: 94-96.
- Kekos D, Macris BJ (1987b). Kinetic studies of amylase and biomass production by *Calvatia gigantea*. *Biotechnol. Bioeng.* 30: 681-684.
- Kim BK, Kwun JY, Park YI, Bok JW, Choi EC (1992). Antitumor components of the cultured mycelia of *Calvatia craniiformis* (sic). *J. Korean Cancer Res. Assoc.* 24: 1-18.
- Kominos J, Kekos D, Macris BJ, Galiotou-Panayotou M (1988). Tannin-resistant  $\alpha$ -amylase from *Calvatia gigantea*. *Biotechnol. Bioeng.* 32: 939-941.
- Kovganko NV (1999). Ecdysteroids and related compounds in fungi. *Chem. Nat. Comp. Engl. Transl.* 35: 597-611.
- Kreisel H (1962). Die Lycoperdaceae der Deutschen Demokratischen Republik. *Feddes Repert.* 64: 89-201.
- Kwon TJ, Park DW, Lee CO, Kang CY, Kim BK (1980). Studies on the constituents of higher fungi of Korea (XXI). A sterol from *Calvatia saccatum* (Vahl) Fr. *Korean. J. Mycol.* 8: 25-28.
- Læssøe T, Spooner B (1994). The uses of 'Gasteromycetes'. *Mycologist*, 8: 154-159.
- Lam YW, Ng TB, Wang HX (2001). Antiproliferative and antimutagenic activities in a peptide from puffball mushroom *Calvatia caelata*. *Biochem. Biophys. Res. Commun.* 289: 744-749.
- Lange M (1990). Arctic Gasteromycetes II. *Calvatia* in Greenland, Svalbard and Iceland. *Nord. J. Bot.* 9: 525-546.
- Lawrynowicz M (1988). Threatened macrofungi and their conservation in Poland. *Mycologist*, 2: p. 113.
- Legg A (1987). The top twenty edible fungi? *Mycologist*, 1: 109-125.
- Levetin E, Horner WE, Lehrer SB (1992). Morphology and allergenic properties of basidiospores of four *Calvatia* species. *Mycologia*, 84: 759-767.
- Liu B (1984). The Gasteromycetes of China. *Beih. Nova Hedwigia* 76: 231-235.
- Lucas EH, Byerrum RU, Clarke DA, Reilly HC, Stevens JA, Stock CC (1959). Production of oncostatic principles in vivo and in vitro by species of the genus *Calvatia*. In Welch H, Marti-Ibañez F, *Antibiot. Annu. 1958-1959*: pp. 493-496. Medical Encyclopedia Inc., New

- York.
- Mendoza JM (1938). Philippine mushrooms. Philipp. J. Sci. 65: 1-128 & 79 plates.
- Morris B (1984). Macrofungi of Malawi: some ethnobotanical notes. Bull. Br. Mycol. Soc. 18: 48-57.
- Morris B (1987). Common mushrooms of Malawi. Fungiflora A/S, Oslo.
- Morris B (1990). An annotated check-list of the macrofungi of Malawi. Kirkia, 13: 323-364.
- Morris B (1994). Bowa: ethnomycological notes on the macrofungi of Malawi. In Seyani JH, Chikuni AC, Proceedings of the XIIIth plenary meeting of AETFAT, Zomba, Malawi, 2-11 April 1991, Volume 1: 635-637. National Herbarium and Botanic Gardens of Malawi, Zomba.
- Nedelcheva D, Antonova D, Tsvetkova S, Marekov I, Momchilova S, Nikolova-Damyanova B, Gyosheva M (2007). TLC and GC-MS probes into the fatty acid composition of some Lycoperdaceae mushrooms. J. Liq. Chromatogr. Relat. Technol. 30: 2717-2727.
- Ng TB, Lam YW, Wang H (2003). Calcaelin, a new protein with translation-inhibiting, antiproliferative and antimitogenic activities from the mosaic puffball mushroom *Calvatia caelata*. Planta Med. 69: 212-217.
- Okuda T, Fujiwara A (1982). Calvatic acid production by the Lycoperdaceae 2. Distribution among the Gasteromycetes. Trans. Mycol. Soc. Jpn. 23: 235-239.
- O'Neil CE, Horner WE, Reed MA, Lopez M, Lehrer SB (1990). Evaluation of Basidiomycete and Deuteromycete (Fungi Imperfecti) extracts for shared allergenic determinants. Clin. Exp. Allergy. 20: 533-538.
- Oso BA (1975). Mushrooms and the Yoruba people of Nigeria. Mycologia, 67: 311-319.
- Oso BA (1977). Mushrooms in Yoruba mythology and medicinal practices. Econ. Bot. 31: 367-371.
- Overton SV (1994). Determination of volatile organic compounds in mushrooms. Mass Spec. Source, 18(2): 4-7.
- Parent G, Thoen D (1977). Food value of edible mushrooms from Upper-Shaba region. Econ. Bot. 31: 436-445.
- Patouillard N (1924). Note sur une variété de *Lanopila bicolor* (Lev.). Bull. Trimest. Soc. Mycol. Fr. 40: 227-228.
- Pegler DN, Pearce GD (1980). The edible mushrooms of Zambia. Kew Bull. 35: 475-491.
- Pearce GD (1981). Zambian mushrooms customs and folklore. Bull. Br. Mycol. Soc. 15: 139-142.
- Pokorny B, Al Sayegh-Petrovšek S, Ribarič-Lasnik C, Vrtačnik J, Doganoc DZ, Adamič M (2004). Fungi ingestion as an important factor influencing heavy metal intake in roe deer: evidence from faeces. Sci. Total Environ. 324: 223-234.
- Proštenik M, Častek A, Čosović Č, Gospočić L, Jandrić Z, Kljaić K, Ondrušek V (1983). The patterns of the constituent fatty acids of glycerophospholipids in mushrooms: Lipids of higher fungi. Exp. Mycol. 7: 74-81.
- Purkayastha RP, Chandra A (1985). Manual of Indian edible mushrooms. (Int. Biosci. Monogr. 16) Today & Tomorrow's Printers and Publishers, New Delhi.
- Rai BK, Ayachi SS, Rai A (1993). A note on ethno-mycology-medicines from Central India. Mycologist, 4: 192-193.
- Rammello J, Walley R (1993). The edible fungi of Africa south of the Sahara: a literature survey. Scr. Bot. Belg. 5: 1-62.
- Riffle JW (1968). Effect of an *Aphelenchoides* species on the growth of mycorrhizal fungi. Nematologica 14: p. 14.
- Riffle JW (1973). Pure culture synthesis of ectomycorrhizae on *Pinus ponderosa* with species of *Amanita*, *Suillus* and *Lactarius*. For. Sci. 19: 242-250.
- Rimóczi I (1987). Ecology, cenology, and distribution of the giant puffball (*Langermannia gigantea* [Batsch ex Pers.] Rostk.) in Hungary. Acta Bot. Hung. 33: 279-294.
- Rinaldi A, Tyndalo V (1974). Mushrooms and other fungi, an illustrated guide. Hamlyn, London.
- Roland JF, Chmielewicz ZF, Weiner BA, Gross AM, Boening OP, Luck JV, Bardos TJ, Reilly HC, Sugiura K, Stock CC, Lucas EH, Byerrum RU, Stevens JA (1960). Calvacin: a new antitumor agent. Science, 132: p. 1897.
- Rolfe RT, Rolfe FW (1925). The romance of the fungus world. Chapman & Hall, London.
- Seeger R (1976). Quecksilbergehalt der Pilze. Z. Lebensm. Unters. Forsch. 160: 303-312.
- Sesli E, Tüzen M (1999). Levels of trace elements in the fruiting bodies of macrofungi growing in the east Black Sea region of Turkey. Food Chem. 65: 435-460.
- Sesli E, Tüzen M, Soylok M (2008). Evaluation of trace metal contents of some wild edible mushrooms from Black sea region, Turkey. J. Hazard. Mater. 160: 462-467.
- Sevilla-Santos P, Bernardo ZP (1966). Tumor inhibitors in Philippine basidiomycetes. Philipp. J. Sci. 95: 189-196.
- Shannon LJ, Stevenson KE (1975a). Growth of fungi and BOD reduction in selected brewery wastes. J. Food Sci. 40: 826-829.
- Shannon LJ, Stevenson KE (1975b). Growth of *Calvatia gigantea* and *Candida steatolytica* in brewery wastes for microbial protein production and BOD reduction. J. Food Sci. 40: 830-832.
- Shantz HL, Piemeisel RL (1917). Fungus fairy rings in eastern Colorado and their effect on vegetation. J. Agric. Res. Washington DC. 11: 191-245.
- Simon-Nobbe B, Denk U, Pöll V, Rid R, Breitenbach M (2008). The spectrum of fungal allergy. Int. Arch. Allergy Immunol. 145: 58-86.
- Skwarzec B, Jakusik A (2003). <sup>210</sup>Po bioaccumulation by mushrooms from Poland. J. Environ. Monit. 5: 791-794.
- Sorba G, Bertinaria M, Di Stilo A, Gasco A, Scaltrito MM, Brenciaglia MI, Dubini F (2001). Anti-*Helicobacter pylori* agents endowed with H<sub>2</sub>-antagonist properties. Bioorg. Med. Chem. Lett. 11: 403-406.
- Strand RD, Neuhauser EBD, Sornberger CF (1967). Lycoperdonosis. N. Engl. J. Med. 277: 89-91.
- Suay I, Arenal F, Asensio FJ, Basilio A, Cabello MA, Díez MT, García JB, González Del Val A, Gorrochategui J, Hernández P, Peláez F, Vicente MF (2000). Screening of basidiomycetes for antimicrobial activities. Antonie van Leeuwenhoek 78: 129-139.
- Sumner JL (1973). The fatty acid composition of Basidiomycetes. N. Z. J. Bot. 11: 435-442.
- Swanton EW (1917). Economic and folk lore notes. Trans. Br. Mycol. Soc. 5: 408-409.
- Takaishi Y, Adachi R, Murakami Y, Ohashi T, Nakano K, Tomimatsu T (1992). A polyoxygenated steroid from *Lasioisphaera nipponica*. Phytochem. 31: 243-246.
- Takaishi Y, Murakami Y, Uda M, Ohashi T, Hamamura N, Kido M, Kadota S (1997). Hydroxyphenylazoforamamide derivatives from *Calvatia craniformis* (sic). Phytochem. 45: 997-1001.
- Tracey MV (1955). Chitin. In K Peach & MV Tracey, Modern methods of plant analysis, Vol. II: 264-274. Springer-Verlag, Berlin.
- Trappe JM (1962). Fungus associates of ectotrophic mycorrhizae. Bot. Rev. 28: 538-606.
- Tredgold MH, Biegel HM, Mavi S, Ashton H (1986). Food plants of Zimbabwe with old and new ways of preparation. Mambo Press, Gwero.
- Turkekel I, Elmastas M, Tüzen M (2004). Determination of iron, copper, manganese, zinc, lead, and cadmium in mushroom samples from Tokat, Turkey. Food Chem. 84: 389-392.
- Umezawa H, Takeuchi T, Iinuma H, Ito M, Ishizuka M, Kurakata Y, Umeda Y, Nakanishi Y, Nakamura T, Obayashi A, Tanebe O (1975). A new antibiotic, calvatic acid. J. Antibiot. 28: 87-90.
- Van Wyk B-E, Van Oudtshoorn B, Gericke N (1997). Medicinal plants of South Africa. Briza, Pretoria.
- Vetter J (1990). Mineral element content of edible and poisonous macrofungi. Acta Aliment. 19: 27-40.
- Vetter J (1994a). Kalium-Gehalt von essbaren Wildpilzen. Z. Lebensm. Unters. Forsch. 198: 33-35.
- Vetter J (1994b). Die Kupfer, Mangan and Zink-Gehalte einiger essbarer Grosspilzarten. Z. Lebensm. Unters. Forsch. 198: 469-472.
- Vetter J (1994c). Phosphorus content of edible wild mushrooms of Hungary. Acta Aliment. 23: 331-336.
- Vetter J (2003). Data on sodium content of common edible mushrooms. Food Chem. 81: 589-593.
- Vetter J (2004). Arsenic content of some edible mushroom species. Eur. Food Res. Technol. 219: 71-74.
- Vetter J (2005). Lithium content of some common edible wild-growing mushrooms. Food Chem. 90: 31-37.
- Viterbo D, Gasco A, Serafino A, Mortarini V (1975). *p*-Carboxypheny-

- lazoxy-cyanide-dimethyl sulphoxide: an anti-bacterial and antifungal compound from *Calvatia lilacina*. *Acta Crystallogr. Sect. B* 31: 2151-2153.
- Walley R, Rammeloo J (1994). The poisonous and useful fungi of Africa south of the Sahara: a literature survey. *Scr. Bot. Belg.* 10: 51-56.
- Watling R, Seaward MRD (1976). Some observations on puff-balls from British archaeological sites. *J. Archaeol. Sci.* 3: 165-172.
- Watt JM, Breyer-Brandwijk MG (1962). *Medicinal and poisonous plants of southern and eastern Africa*, edn 2. E & S Livingstone Ltd, Edinburgh.
- Wehmeyer AS, Coetzee JC, Eicker A (1981). Nutrient content of *Macrolepiota zeyheri* and *Agaricus brunnescens*. *S.A. J. Sci.* 77: 426-427.
- Williamson J (1975). *Useful plants of Malawi*. University of Malawi, Zomba.
- Wood WF (1983). Anaesthesia of honeybees by smoke from the pyrolysis of puffballs and keratin. *J. Apic. Res.* 22: 107-110.
- Zikakis JP, Castel JE (1988). Chitinase-chitobiase from soybean seeds and puffballs. In Wood WA, Kellogg ST, Biomass. Part B. Lignin, pectin and chitin. *Methods Enzymol.* 161: 490-497. Academic Press, San Diego.