



Morchella



Tuber spp.



Cordyceps sinensis



Ganoderma lucidum

Mycorrhizal, Entomopathic and Novel Mushrooms

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OUR CONCERN

- ❏ Mycorrhizal mushrooms- *Tuber, Morchella*
- ❏ Entomopathic mushrooms- *Cordyceps sinensis*
- ❏ Novel Mushrooms- *Ganoderma lucidum*

1. MYCORRHIZAL MUSHROOMS

- ❏ Form symbiotic associations with their host plants (Smith and Read, 1997).
- ❏ Includes about 200 common **edible ecto-mycorrhizal mushrooms (EEMs)**.
- ❏ A few species have well established worldwide-markets in excess of US\$2 billion.
- ❏ Edible ecto-mycorrhizal mushrooms (**EEMs**) are livelihood for many people who collect them from their natural habitats or cultivate them.

Some most valuable mycorrhizal mushrooms

Mushroom spp.	Estimated world production (t)	Approx. wholesale price (US\$/kg)	Approx. retail market (US\$)
<i>Tuber melanosporum</i>	150	250-1200	>150 million
<i>Tuber magnatum</i>	50-200	1,000-13,000	>150 million
<i>Tricholoma matsutake</i>	2,000	40-500	500 million
<i>Boletus edulis</i>	20,000-1,00,000	13-198	>250 million
<i>Cantharellus cibarius</i>	2,00,000	8-19	1.62 billion

TUBER

- 🏠 About 180 species of *Tuber* were reported around the world (Bonito et al., 2010), of which 13 have commercial interest.
- 🏠 *T. melanosporum*- first cultivated in France 200 yrs ago (Olivier et. al., 1996).
- 🏠 Now spread in France, Italy, Spain, USA, New Zealand, China and Australia.
- 🏠 Production valued to 18 million euro in Italy (Pettenella et. al., 2004), 20 million in France, 7.5 million in Spain and 4 million euro in Australia (Reyna & Barreda, 2014).
- 🏠 Total economic impact from all resources of *T. melanosporum* produce was 70 million euros per annum in France (Escafre & Roussel, 2006) and 100 million euros in Italy (Gregori, 2013).

***Tuber melanosporum* production in France, Italy and Spain**

	France	Italy	Spain
Mean Production (2003-04 to 2012-13; t per year)	31.3	11.0	15.9
% truffles produced in plantations vs harvested in wild	90:10	50:50	60:40
Plantation surface (ha)	24,000	7,500	10,000
Rate of plantation (ha/year)	800	400	1000
No. of growers/harvesters	20,000	1,80,000	10,000
Price of seedlings (euros)	5-19	8-14	4-8
No. of truffle fairs and retail markets	129	68	15

Commercial exploitation of *T. melanosporum* in various countries

Feature	USA	New Zealand	Australia	Chile	South Africa	Argentina
Year of first plantation	1979	1987	1993	2003	2008	2010
Production (kg/year)	40	<50	4500	7	0	0
Plantation surface (ha)	120	100	700	200	30	40
Plantation increased (ha/year)	20	Very low	30	35	10	20
Price of seedlings (euro)	11-19	28-33	15-46	10-15	11	12

Morchella spp.

Different *Morchella* species



M. esculenta



M. deliciosa



M. hybrida (*M. semilibera*)



M. angusticeps (*M. elata*)



M. conica



M. crassipes

Production and trade of *Morchella* species

- ❖ Total world production-150 tons (dry wt) which amounts to 150 million tons fresh morels (**Negi, 2006**).
- ❖ India & Pakistan shared 50 tons each dry wt. (**Negi, 2006**).
- ❖ All the produce come from natural harvesting, however, some **Patents** for cultivation are as follows-
 - a) Neogen Corporation, Publication No. WO 1986006247A1, Nov. 06, 1986.
 - b) Neogen Corporation, Publication No. US 4757640A, July 19, 1988.
 - c) Neogen Corporation, Publication No. US 4866878A, Sept. 19, 1989.

2. ENTOMOPATHIC MUSHROOM-*Cordyceps sinensis*



Cordyceps sinensis
(fruiting body)

Hepialus armoricanus
(Larvae)

Distribution- *C. sinensis*

- The *C. sinensis* species are distributed in China, India, Nepal, Bhutan, Japan, Germany, U.S.A, Canada, Denmark and Italy.
- Native occurrence of the fungus is confined to the high Himalayan mountains in Tibet-China, India, Nepal and Bhutan.
- In India, the fungus is reported from Uttarakhand, Himachal, Arunachal and J & K at an altitude ranging from 3000m to 5000m.
- In China, Tibet and Qinghai regions are producing 80% of the Chinese production (100-120 tones/annum). However, another 20% comes from Sichuan, Yunan and Gansu provinces.
- In Nepal, it is reported from Northern Districts namely Dolpa, Darchula, Jumla, Bajura, Kalikot, Muga, Humla, Rukum, Jajarkot, Bajhang & Mustang.

Annual production (Qtl) of *C. sinensis*

Location	Production Min – Max
1) China	743-1400
Tibet	350-500
Qinghai	200-500
Sichuan	150-300
Yunnan	3.0-10
Gansu	40-90
2) India	16-24
Uttarakhand	12.5 – 18
HP, Sikkim and Arunanchal Pradesh	3.5 – 6.0

Location	Production Min – Max
3) Nepal	10-30
Dolpa	5.0-12
Jumla, Humla, Kalikot	5.0-18
4) Bhutan	1.0-10

Life Cycle

Host- *Hepialus armoricanus*

- The insect host *Hepialus armoricanus* belongs to order Lepidoptera and family Hepialidae.
- Insect hosts go through – egg, larvae, pupa and adult stages.
- It takes about 3 yr to complete the whole life history and the stage of larvae lasts about 2 yr.
- Larvae of the host insects live underground, feeding on roots of alpine plants. it builds tunnel and move up and down in the tunnel.
- The Larvae pupate in the tunnels close to the ground after winter and then pupae emerge into adults. The adults looks to spouses , mate , and reproduce.



Eggs



Larvae



Pupa



Moth

Contd....

Life Cycle

Fungus – *C. sinensis*

- Life cycle of the host larvae is to change if encountered with the *C. sinensis*.
- The fungus lives on the ground in the form of ascospores which germinates & form spiny germ tube that invaded the body of larvae & form mycelium in its body.
- Mycelium feed on the body of the larvae until the larvae life runs out, leaving the exo-skeleton intact full of mycelium.
- The fungal stroma comes out in the next spring.
- There is a fertile perithecium which can eject ascospores, at the upper of the stroma.
- If the spores encounter the larvae, a new life cycle of the fungus begins again.



Perithecium



Ascospores



Infected larvae



Stroma emergence

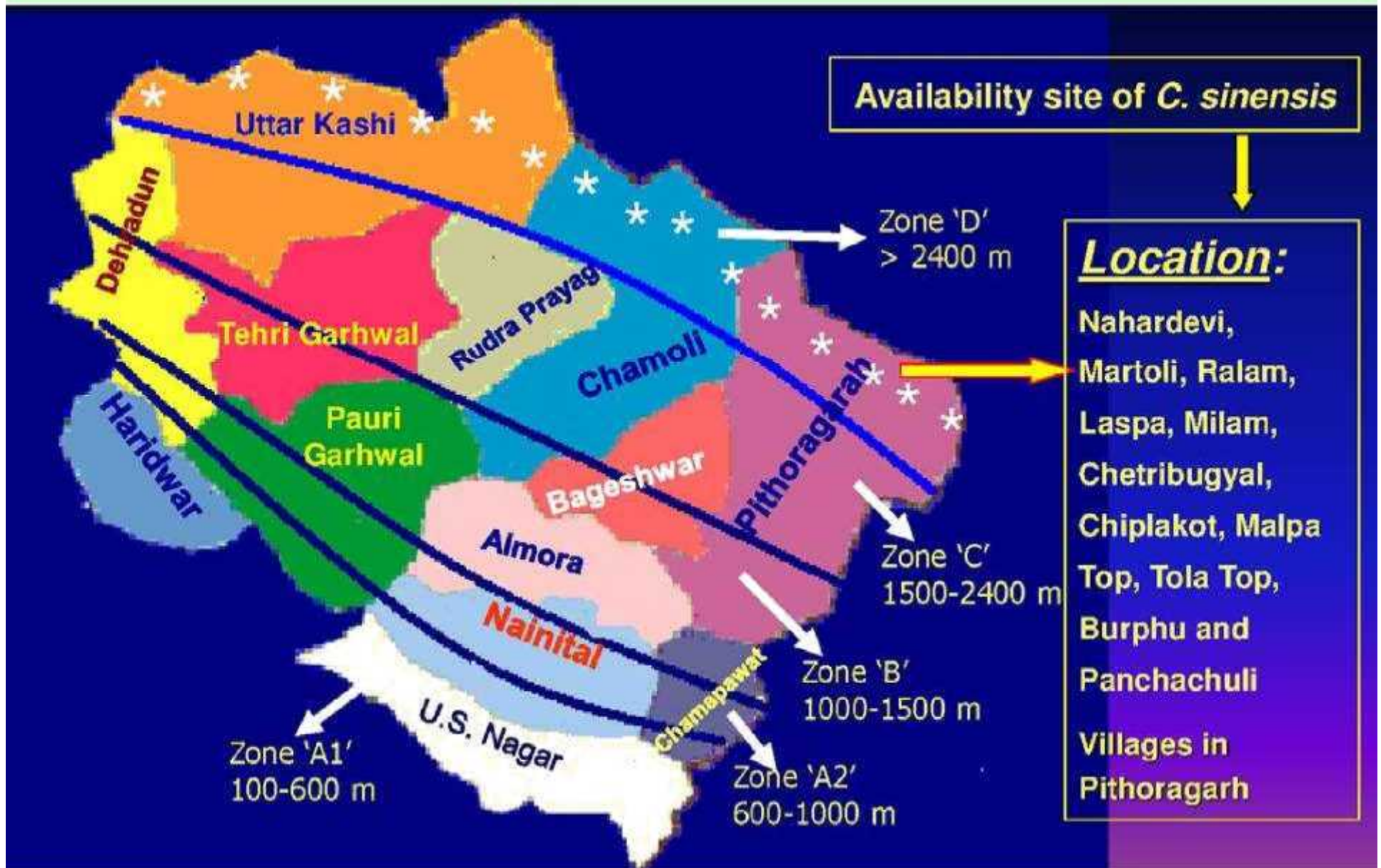


C. sinensis



Occurrence in Uttrakhand, India

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COLLECTION SITES-UTTARAKHAND,INDIA



Medicinal Mushroom-*Cordyceps sinensis*.....cont.....

C. sinensis sun drying by a tribe woman/local gatherer



Status & Trade – Pithoragarh, UK, India

- 🏠 Most appropriate time for collection is April-June.
- 🏠 *C. sinensis* emerged as an important cash crop.
- 🏠 A family of 4 collects 200-500g during season.
- 🏠 Its distribution in Uttarakhand, India is limited to temperate forests areas where average annual precipitation varies 350- 400mm.

C. sinensis trade in local market of Munsiyari and Dharchula

Year	Av. price (Rs./kg)		Approx. Quantity (Kg)	
	Gatherers	Dealers/Agents	Munsiyari	Dharchula
2004	60,000	90,000	1000	500
2005	70,000	100,000	1200	600
2006	120,000	125,000	900	400
2007	200,000	300,000	800	600
2008	250,000	400,000	760	500
Total	700,000	1015,000	4,660	2600

- Cost of the fungus in local market varied Rs. 60,000 – 90,000 in 2004 to Rs. 250,000 - 400,000 in 2008 while in 2014 it went up to Rs. 8,00,000-12,00,000.
- The estimated volume of trade in Uttarakhand varied between 1260-1800kg during 2004 to 2008.

Trade system in China

- There are gatherers market in the capital of *Cordyceps* producing provinces.
- Of the total Chinese production more than 60% comes from Tibet.
- There are 12 Pharmacological/Companies in Xining, Quinghai, using more than 100MT *Cordyceps* /annum.
- From gatherers market the fungus purchased by the Pharmacological Industry and showrooms for export.
- There is a taxation system on purchase of *Cordyceps* from the local market of gatherers and show rooms.

GATHERER'S TRADE MARKET IN XINING CHINA



Chinese Products of *Cordyceps* in Market



MORPHOLOGY & PHYSIOLOGY



- Fruiting bodies of *C. sinensis* varies from 4 to 7 cm in size.
- The fruiting bodies are mostly erect, stalked, slightly swollen at tip; emerged single, double or triple from the heads of larvae.
- Stalks are alike grass straw, slightly thickened at the base and tapered towards the end.
- Caterpillar cadaver had worm-like head, body and eight pairs of legs with numerous thin and fine transverse wrinkles.

Cultural characteristics

SDYA (Sabouraud's Dextrose Yeast Agar)

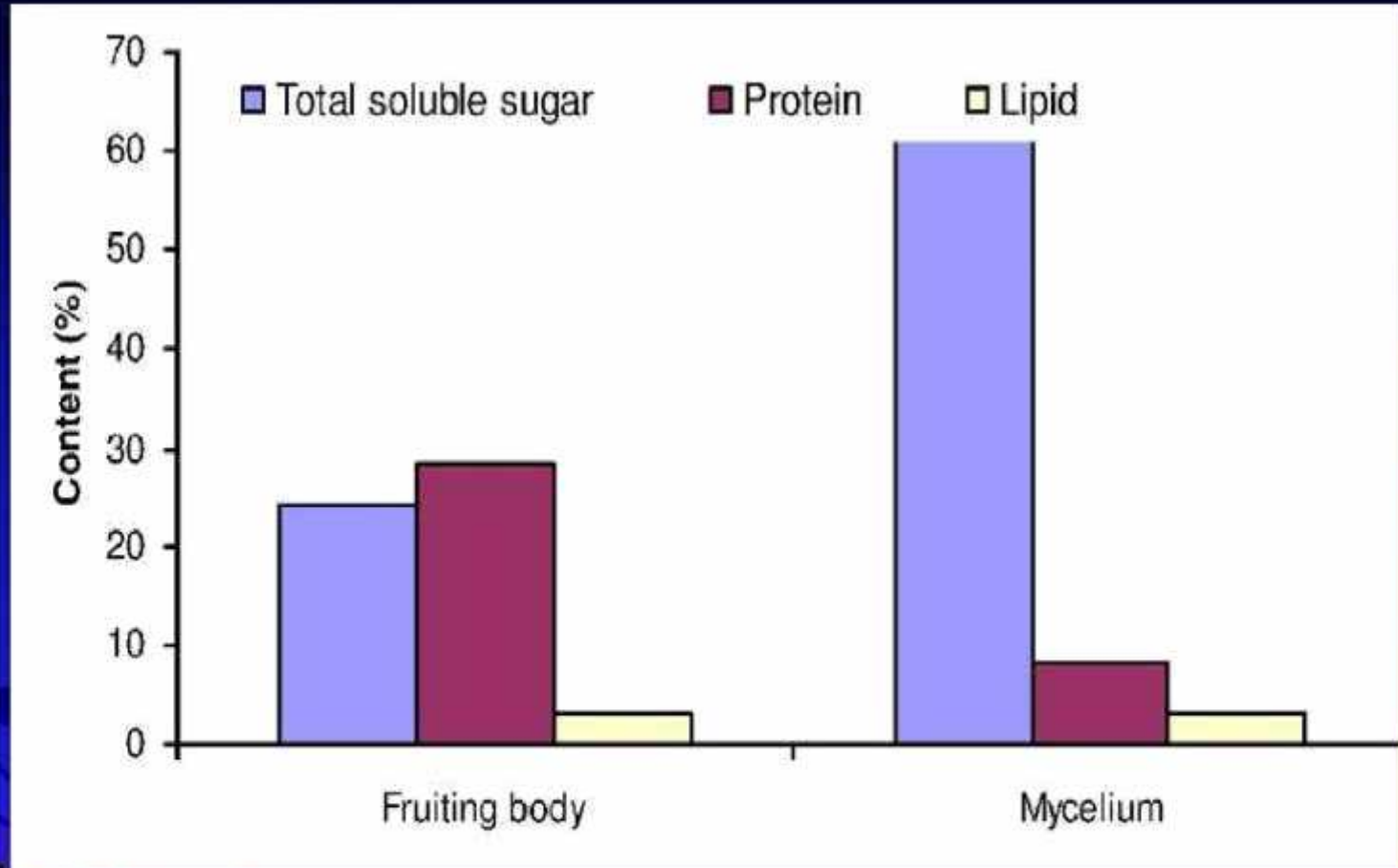


- Fresh fruiting bodies of *C. sinensis* and larva cadaver are used for isolation.
- Colony initially white changing to pinkish red or orange and from reverse creamish to purplish red to dark tan.
- Medium SDYA, pH-6 and temp. 15⁰C have been reported to be most suitable for growth.

Effect of the C and N sources on the mycelial growth of *C. sinensis*

C-sources	Dry wt. (g/l)	N-sources	Dry wt. (g/l)
Mono-saccharides		Inorganic	
Glucose	3.74	Potassium nitrate	3.33
Fructose	3.18	Sodium nitrate	2.75
Disaccharides		Ammonium nitrate	2.52
Sucrose	5.15	Ammonium chloride	2.30
Maltose	4.72	Ammonium sulphate	2.16
Lactose	4.25	Urea	1.67
Check (no carbon)	2.83	Organic	
--	--	Beef extract	5.24
--	--	Check (no nitrogen)	1.85
CD at 5%	0.26	CD at 5%	0.31

Total soluble sugars, proteins and lipids in fruiting bodies and mycelium of *C. sinensis*

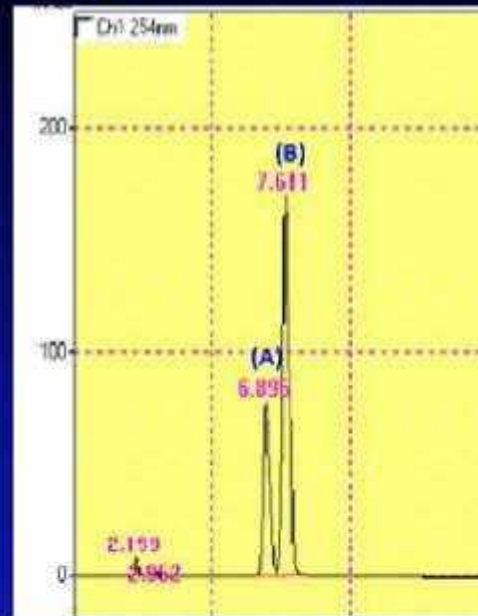


- The TSS was found much higher in mycelia in SDY broth (63.1%) as compared to fruiting bodies (24.2%).
- Protein content was higher in fruiting bodies (28.6%) as compared to mycelia (8.2%).

Medicinal Importance

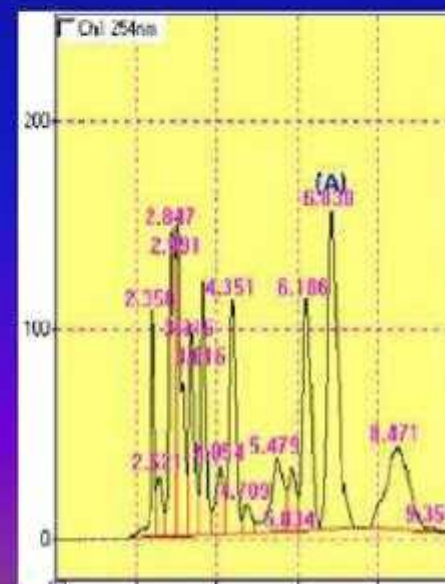
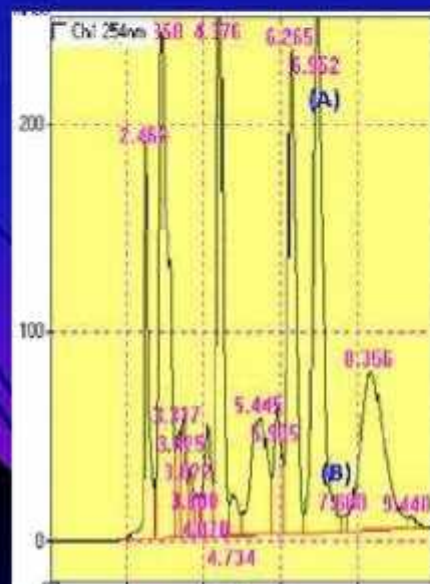
- Activate immune response.
- Controls the blood sugar level.
- Treatment of hepatitis B.
- Improve respiratory functions.
- Maintains level of cholesterol & improves functioning of heart.
- Improves the functioning of kidney and adrenal gland.
- Reduces of the tumor size.
- Protection against free radical damage.
- Reduces of fatigue.
- Combats sexual dysfunction.

Detection of adenosine, cordycepin and ergosterol from fruiting bodies and mycelia of *C. sinensis* by HPLC analysis

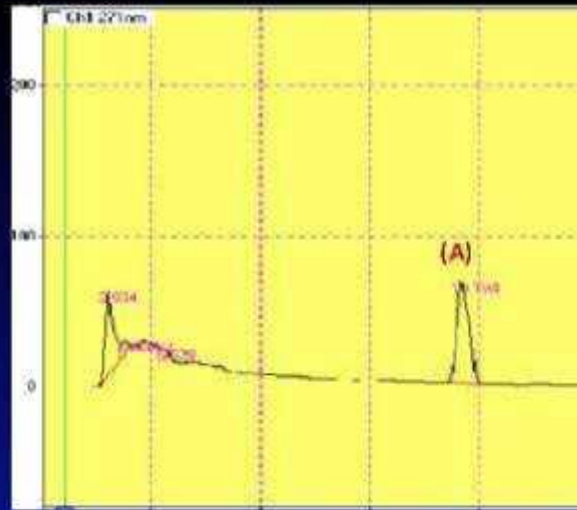


Chromatogram of standard adenosine (A) and Cordycepin (B) at 100ppm. Retention time were 6.895 and 7.611 min

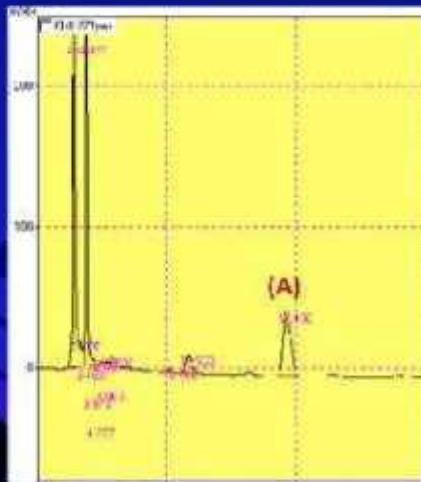
Chromatogram of purified extract of fruiting bodies of *C. sinensis* showing adenosine (A) and Cordycepin (B)



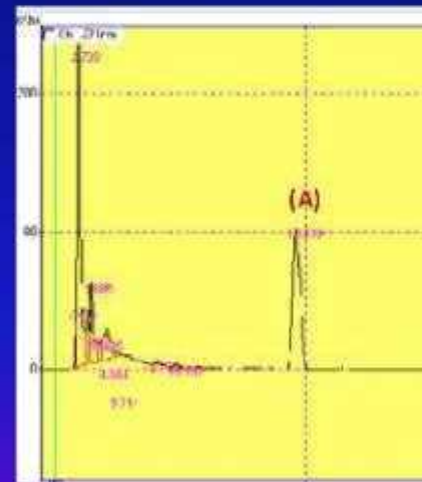
Chromatogram of mycelium of *C. sinensis* showing adenosine (A)



Chromatogram of standard- ergosterol (A) at 100ppm. Retention time was 19.194



Chromatogram of purified extract of fruiting bodies of *C. sinensis* showing ergosterol (A)



Chromatogram of mycelium of *C. sinensis* showing ergosterol (A)

3. NOVEL MUSHROOMS

Edible and medicinal mushrooms can produce a variety of biologically active compounds and can be, therefore, described as a novel class of nutraceuticals which are widely used as dietary supplements.

Jiang and Sliva, 2010

Important mushrooms:

- *Ganoderma lucidum*
- *Agaricus blazei*
- *Coriolus versicolor*
- *Grifola frondosa*
- *Lentinula edodes*

G. lucidum



1- Medicinal significance

- 🏠 Immune system - cancer, influenza
- 🏠 Respiratory system - bronchitis, asthma
- 🏠 Endocrine system - menopause, menstruation
- 🏠 Nervous system - muscular dystrophy
- 🏠 Circulatory system - coronary heart disease
- 🏠 Excretory system - nephritis
- 🏠 Reproductive system - erectile dysfunction

2- Properties of an adaptogen

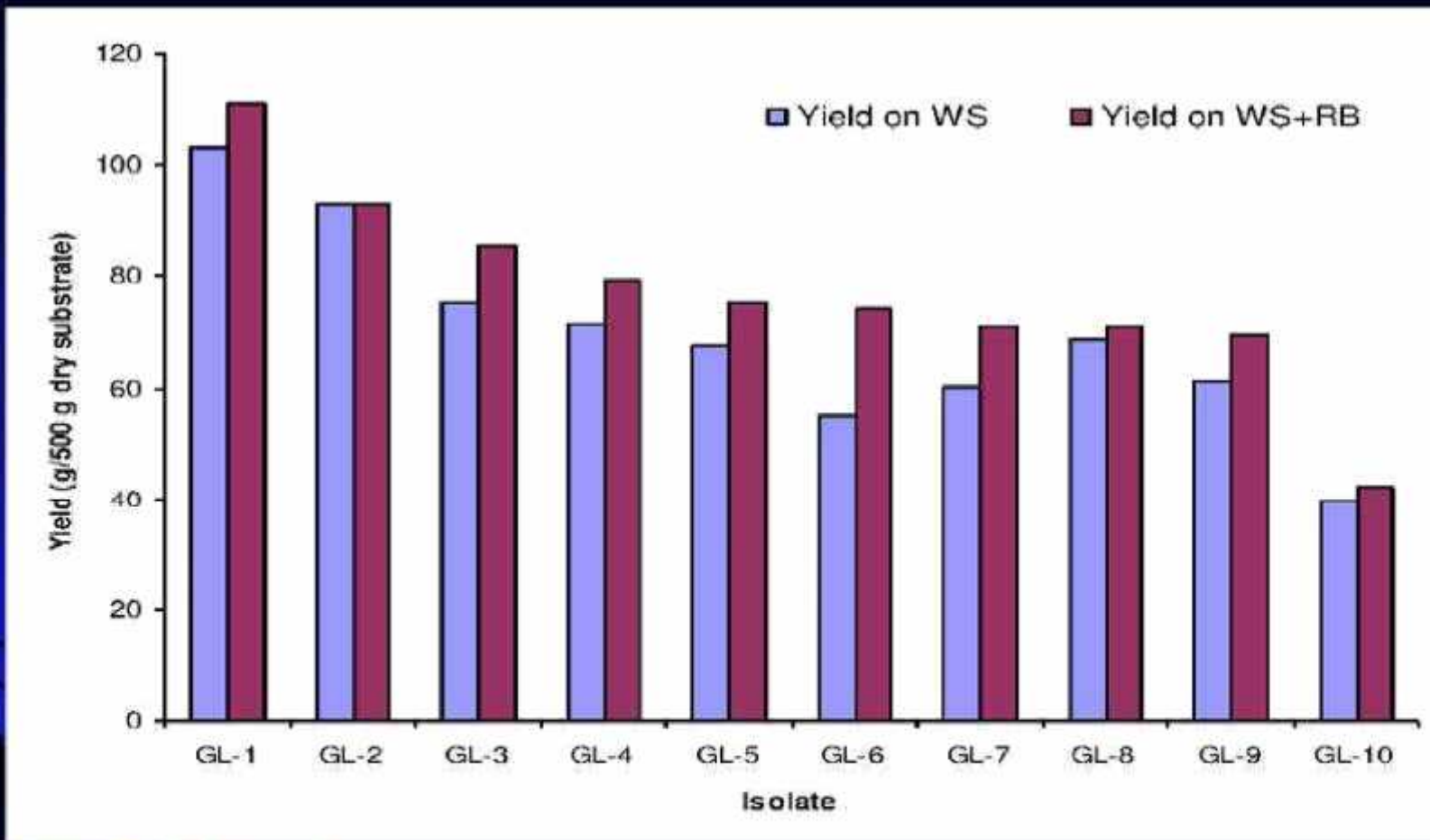
3- Effective for “terminal illness”

PRODUCTION STATUS



- Global 6000 tons in 2005 (Chang, 2005).
- China – 3,800 tons.
- Global turnover > US \$ 300 million (Organo Gold review, 2012).
- India – yet to commercialize.

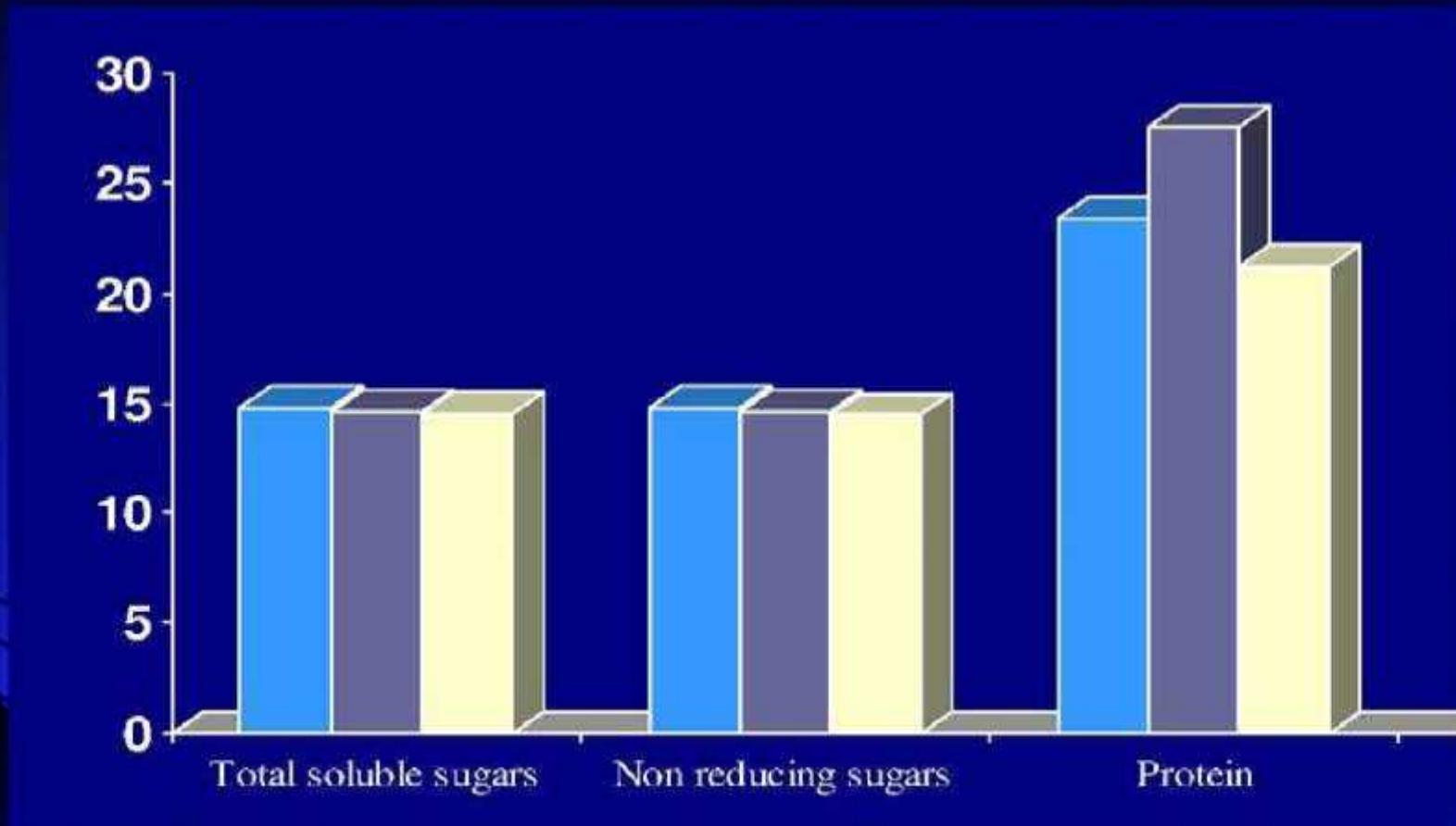
Effect of supplementation of RB @ 10% on yield of *G. lucidum*



- Out of different substrate tested, wheat straw out yielded all other substrate.
- Supplementation of rice bran @ 10% resulted in enhanced yield, in general.

Mishra & Singh, 2008

Total soluble sugars, non reducing-sugars and protein contents (% dry weight) in *G. lucidum* isolates



- TSS and NRS varied from 14.58–14.88 and 14.55-14.80%.
- Protein content varied 21.0- 27.0%.

Detection of terpenoids in *G. lucidum* isolates

Isolate	Ganoderic acid A	Ganoderic acid C ₂	Ganoderic acid H
GL-1W	++	++	++
GL-2W	++	++	++
GL-3W	++	++	++
GL-1 C	++	++	+
GL-2 C	+	+	+
GL-3 C	++	++	+

HPLC profile of Ganoderic acid A

- ❏ The HPLC profile of the fruiting bodies of *G. lucidum* showed the presence of ganoderic acid-A.
- ❏ The ganoderic acid A is a complicated mixture and usually have α,β - unsaturated carbonyl groups.
- ❏ Other organic acids may also be extracted and purified together with Ganoderic acid.

Amount of glucosaminoglycan in *G. lucidum*

Isolate	Place of Collection	Amount of glucosaminoglycan (%)
GL-1W	Ranikhet	2.166
GL-2W	Almora	2.248
GL-3W	Lansdowne	2.298
GL-1C	Cultivated	1.142
GL-2C	Cultivated	1.212
GL-3C	Cultivated	1.348

- Wild *G. lucidum* had more amount of glucosaminoglycan as compared to cultivated isolates.
- Wild isolate GL-3 had the highest amount of glucosaminoglycan (2.298%).
- The cultivated isolate GL-3 had the highest content of glucosaminoglycan (1.348%).

Singh *et. al.*, 2008

Conclusion

- 🏠 Cultivation of Edible Ecto-mycorrhizal Mushrooms has made considerable progress over last century.
- 🏠 More focus was given on truffles while other mycorrhizal mushrooms yet to get proper attention.
- 🏠 Molecular sequencing of *T. melanosporum* genome done so far will lead as a valuable resource for future studies on biology and ecology of the fungus.
- 🏠 Metagenomics and comparative genomics of all other mycorrhizal mushrooms will stimulate new frontiers of variety.
- 🏠 There should be more focused research projects on the commercial cultivation of mycorrhizal mushrooms.

Contd.....

Conclusioncont.....

- Pharmacological products of medicinal mushrooms proved that it inhibit tumor cells, regulates blood pressure, strengthen body's immunity, improves kidney and liver function and enhances stamina.
- The price increase of *C. sinensis* in the past decade brought along a bright new lift to farmers and gatherers at the high altitudes of Himalaya, particularly in China, India, Nepal and Bhutan.
- The combination of modern chemistry with bio-based materials, like bio-metabolites, offers the scope for revolutionizing mushroom based pharmaceutical industries.

Thanks

for the patience hearing

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