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Wonder drug - an insect and fungus relationship (*Hepialus - Cordyceps*) through biotechnological approaches

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Abstract
Yartsa gumba or dbyar-rtswa-dgun-bu is Tibetan name of an entomo-fungal combination between *Hepialus armoricanus* (Lepidoptera; Hepialidae) larva and its parasitizing fungus *Cordyceps sinensis* (Berk), which is traditionally used in Tibetan and Chinese System of Medicine (TCM). This medicinal entomo-fungal product is known in Chinese as Dong Chong Xia Cao (winter worm and summer plant or grass in summer and worm in winter), Yarchagumba means herbs of life in Nepal and Tochukaso in Japan. It is also known as Caterpillar mushroom and Caterpillar fungus, and in India it is commonly known as Keera Ghas. This entomo-fungal combination is known to be used for many centuries as tonic, medicine and aphrodisiac and in religious ceremonies in China, Indonesia and Upper Himalayas. Yarsa gumba is also known as the "Himalayan Viagra" or "Himalayan Gold" for its high medicinal and commercial value. It is mainly used as a treatment for impotency in many countries. Numerous scientific studies reveal that it has properties of antibiotic in it. *Cordycep sinensis* is used for lung and respiratory infection, pain, sciatica and backache. It also provides vitality and increases physical stamina of the body. Yarsa gumba is used by the Chinese to cure chronic hepatitis B and immune function disorder such as dysfunctioning of liver. Approximately 5 grams was stuffed into the stomach of a duck that was roasted until well cooked, then the *Cordyceps* was removed and the duck was slowly eaten, twice daily, over a period of 8-10 days.

Keywords: *Cordyceps*; Mass production; Yartsa gumba; Culture medium.

Introduction

Yartsa gumba or dbyar-rtswa-dgun-bu is Tibetan name of an entomo-fungal combination between *Hepialus armoricanus* Oberthur (Lepidoptera; Hepialidae) larva and its parasitizing fungus *Cordyceps sinensis* (Berk), which is traditionally used in Tibetan and Chinese System of Medicine (TCM). This medicinal entomo-fungal product is known in Chinese as Dong Chong Xia Cao (winter worm and summer plant or grass in summer and worm in winter), Yartsa Goenbub in Bhutan, Yarchagumba means herbs of life in Nepal and Tochukaso in Japan. It is also known as Caterpillar mushroom, Caterpillar fungus, vegetable wasp, plant worm, vegetable caterpillar (Cooke, 1892) and in India, it is commonly known as Keera Ghas. This entomo-fungal combination is known to be used for many centuries as tonic, medicine and aphrodisiac and in religious ceremonies in China, Indonesia and Upper Himalayas. The medicinal importance of this combination is due to a fungus viz., *C. sinensis* parasitizing the host caterpillars i.e. *H. armoricanus* (Arif and Kumar, 2003).

Materials and Methods

Live specimens of *Cordyceps* were carefully collected from their natural habitat in the high altitude region (Laspa area) at an altitude of 13000 feet during May and June 2004. The specimens were wrapped inside the moss plants and then packed in the ice cubes. The specimens were washed with tap water to remove the adhering dust particles on it. The stroma of the fresh specimen of *Cordyceps* were washed 2-3 times in double distilled water and dipped in 0.1% HgCl solution for one minute. Further the stroma was washed with sterile distilled water, surface dried by pressing between sterilized filter paper. In order to propagate the mycelium in vitro, tissues were taken from the different parts of the *Cordyceps* body like spores, stalk tissue and tissue from stroma region. These tissues were excised from the *Cordyceps* body with the help of a sterilized scalpel inside a laminar flow and cultured in to the various culture media. Eight different types of media were prepared to get the pure culture of the fungus. Culture media utilized for pure mycelium culture were Potato Dextrose Agar (PDA), Casein Hydrolysate Dextrose Agar (CHDA), Beef Extract Dextrose Agar (BEDA), Soya bean Seed Extract Dextrose Agar (SEDA), Rice Extract Dextrose Agar (REDA) and Black Soya Seed Extract Dextrose Agar (BSEDA). The chemical composition of each culture media is given in Table 11. pH of media varied from 4.5 to 6.5. The cultures were incubated at the various range of temperature (5 to 25^{0}C) inside the incubator.

Results

Observations were taken for the mycelium spread on the different media. Results are shown in Fig. 1. It was observed that the tissue taken from the stroma region of the *Cordyceps* is the most suitable inoculum to get the mycelia run in the culture media. However, spores and stalk tissue did not response at all. Out of the 8 culture media the mycelial growth was successful on 5 culture media viz., PDA, BEDA, CHDA, SEDA and REDA. During the experiment it was also observed that the optimum growth of the *Cordyceps* occurred under low temperature condition between 5 to 15^0 C and more acidic pH 5-5.5 (Fig. 4). However, sclerosis was observed in the mycelium obtained on all the types of the culture media. With the result, mycelia having numerous spores were observed under the compound microscope (Fig. 2 and 3).

Discussion

The availability of Yartsa gumba is scanty in nature and it involves a high labour cost to collect from its natural habitat as wild harvest. Under such circumstances, laboratory culture of this fungus is the only solution to fulfill the demand of such a high value medicinal and highly priced fungus. Hence, the standardization of laboratory culture technique of present investigation of the fungus needs to be the prime importance. Thus, laboratory production of the mycelium of *Cordyceps* will definitely prove a great success in preparation of various products from the dried mycelium which has numerous potential therapeutic applications.

In the early 1970's, Chinese government promoted cultivation of over 200 species of wild *Cordyceps*, looking for the best type, finally isolated and selected *C. sinensis,* after conducting many studies using scientific standards to verify the safety and better medicinal properties of *C. sinensis*. The product of Chinese strain is of artificial medium are commercially available in USA and Canada. The medicinal properties of fermented mycelium products have been examined in experimental and clinical trials which showed promising results. In Korea, an association of mushroom biologists and mushroom growers is providing knowledge to farmers on *Cordyceps* fruiting body inoculation on synthetic media (Zhu *et al*., 1990; Ikumoto, 1991; Manbe *et al*., 1996; Kiho, 1996; Yamaguchi *et al*., 2000; Lie *et al*., 2001 and Zhao *et al*., 2002).

In India, biotechnological study and extension service for its culture is badly needed .The present author suggest following steps for the benefit of the local people.

- Screening natural population of *Cordyceps* for its constituent.
- To develop a protocol for growing Indian strain in artificial medium.
- To compare the chemical composition of in vitro culture with naturally occurring *Cordyceps* for its quality.

Since wild *Cordyceps* is rare and difficult to harvest due to its growing in harsh environment, location and season specific efforts have been made to cultivate *Cordyceps* mycelia for commercial application. Commercial cultivation of *Cordyceps* began in the early 1980's making the herb readily available for clinical research. The active ingredients of CS-4 strain of *C. sinensis* are quite different (Zhao *et al.*, 2002; Zhou *et al.,* 1990 and Li *et al*., 2001).

Liquid culture of *C. sinensis* is a common practice in China. The fermentation in which the organism is introduced into a tank of sterilized liquid medium, which has been formulated to provide all the necessary nutritional components for rapid growth of the mycelium. After the growth in the liquid medium, the mycelium is harvested by straining out from the liquid broth and drying, after which it can be used for further processing. In this method the extra cellular compounds, which were exuded by the fungus during the growth period are discarded with the spent broth. Thus causes a major loss of bioactive compounds as many of the active ingredients are extra cellular in nature and are found only in small concentrations in the mycelium.

USA and Japan are practicing the solid-substrate cultivation method. In this system, the mycelium is grown in plastic bags or glass jars containing sterilized medium, which contain some type of cereal grains viz., rice, wheat or rye. After some period of growth, the mycelium is harvested along with the residual grains. This method is based on low capital investment cultivation technique and is easy for the growers. The down side of this method is that the grain content is usually greater than the mycelium content. However, in this method the extra cellular compounds are harvested along with the substrate and mycelium. The compound cordycepin is primarily extra cellular in nature. The tests have shown the presence of cordycepin in solid substrate grown *Cordyceps* and absent in liquid cultured *Cordyceps*. Recently (Zhou *et al*., 1990; Kiho, 1996; Yamaguchi *et al*., 2000; Lie *et al*., 2001 and Zhao *et al*., 2002) have obtained Hydroxy Ethyl Adenosine (HEA) from

a new hybrid *Cordyceps* after cross breeding by advanced techniques, the wild strain of *C. sinensis* occurring at an altitude of 21000 feet on the snow covered peaks of Himalayas for the quality of product and *C. sobolifera* occurring in the low bamboo forest of China. Continuous efforts have been made to increase concentration of HEA and cordycepin, the compound much in demand worldwide. The improved techniques available to the growers have resulted in an increase of 1500% higher HEA and cordycepin yield. Further, the products are pure and without contamination of soil particles.

Zhou *et al*. (1998) and Hobbs (1986) reported that genus *Cordyceps* produce some potent antibiotics. Further, these authors doubted whether the species of genus *Cordyceps* are single organism or they are symbiotic colonies of more than one organism. They doubt that today's *C. sinensis* will one day be known as a fungal/bacterial symbiosis. However, DNA sequencing is inconclusive in this regard as the DNA sequence tends to change with time, as if the fungus were incorporating some of the insect DNA into its own DNA code for the initiation of its fruiting body form, then loosing the insect DNA when it goes back into its mycelial form, microscopic examination of growing *C. sinensis* mycelium reveals some very interesting morphology including the concurrent anamorphous of filamentous mycelium and rapidly moving single celled yeast like morphological form. This has been observed in other *Cordyceps* spp. as well as in *C. sobolifera.*

Thus, the culture method itself has an effect on the quality of the resultant *Cordyceps* product. Besides methodology, the next factor in the production of particular secondary metabolized or target medicinal compounds is dependant on the nature and composition of the substrate itself. Further, a substrate that favours rapid and strong growth of the mycelium would be an ideal substrate for use (Zhang *et al.,* 1992). The culture medium for the development of *C. sinensis* is in progress (Fig. 1). The chemical analysis of initial products *in vitro* is in progress (Table 1). Further studies are in progress.

culture media.

Table 1. Chemical composition of different

Constituents	Media (g/ liter)							
	PDA	CHDA	BEDA	SEDA	REDA	MEDA	SBEDA	BSEDA
Peptone	10	-	10	-	-	-	-	-
Dextose	40	40	40	40	40	40	40	40
Casein Hydrolysates	-	10	-	-	-	-	-	-
Beef extract	-	-	3	-	-	-	-	-
Sodium Chloride	-	-	5	-	-	-	-	-
Mushroom powder	-	-	-	-	-	50	-	-
Soya bean powder	-	-	-	80	-	-	-	-
Rice powder	-	-	-	-	100	-	-	-
Black Soya powder	-	-	-	-	-	-	-	80
Soya bean powder	-	-	-	-	-	-	40	-
Agar powder	15	15	15	15	15	15	15	15

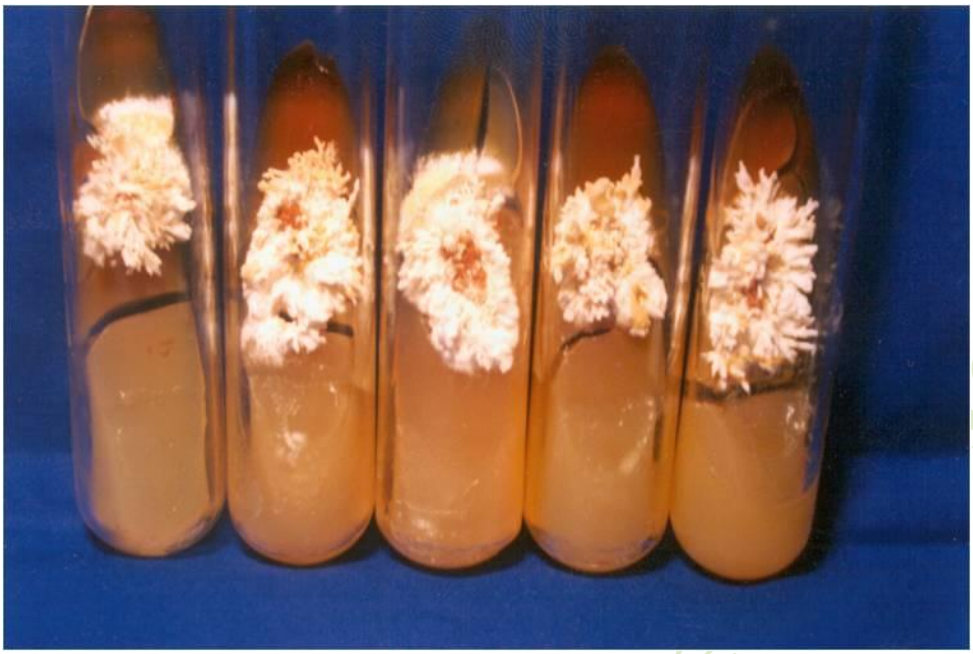

Fig 1. Successful *in vitro* culture of *Cordyceps sinensis* on artificial medium under laboratory conditions.

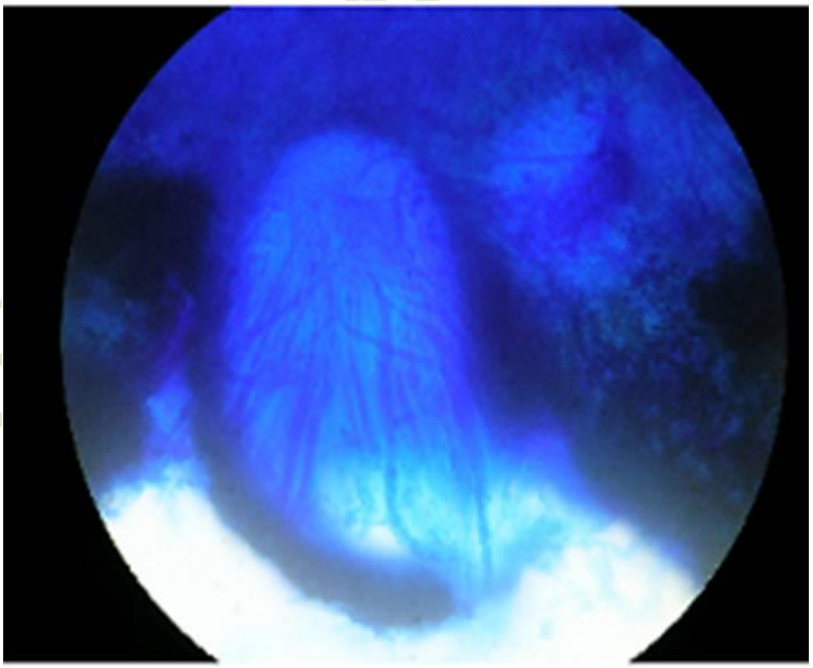

Fig 2. An ascus showing ascospore (T.S.).

Fig 3. T.S. of stroma showing peritheca attached.

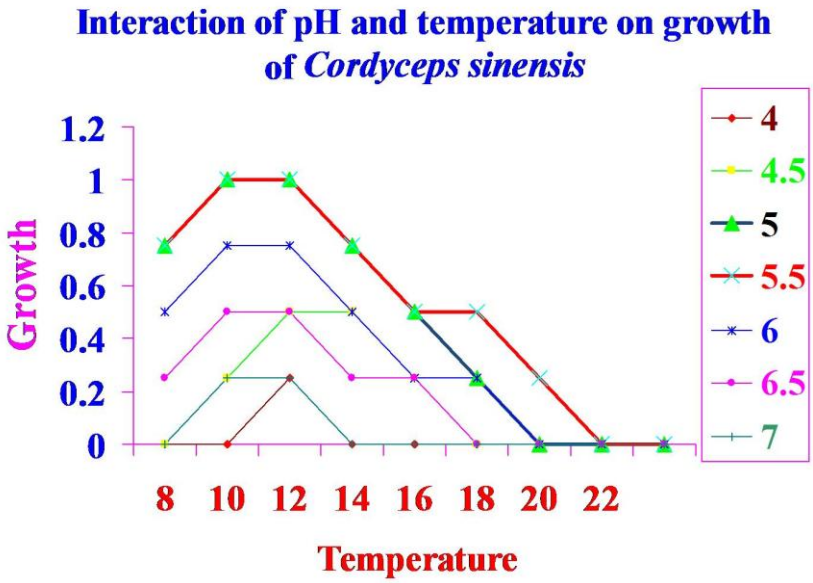

Fig 4. Interaction of pH and temperature on growth of *Cordyceps sinensis*.

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