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Studies on *Volvaria diplasia* Berk & Br., The Paddy Straw Mushroom

by

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Introduction: Sporophores of some species of the genus *Volvaria*, occurring in nature in the tropical regions, are known to be edible. At least three of its species, *V. volvacea*, (Bull.) Quelet, *V. esculenta* Massee and *V. diplasia* Berk & Br., are reported to be artificially cultivated in some countries of Asia (2, 3, 4, 7, 9, 11, 14). All the three species grow well on rice straw and are popularly known as 'Paddy straw mushroom'. Another closely related species, *V. bresadolae* Trotter, has been reported to grow on decaying pine apple fruits in Palau Island (U. S. A. Trust), Pacific Ocean (6).

It is believed that *V. volvacea* has been under commercial cultivation prior to 1932 in China (2). The natural occurrence of this species in Malaya and the possibilities of its commercial cultivation was reported by Sands in 1935 (9). In 1936 Benemerito (3) described in detail the method of cultivation of *V. esculenta* in the Philippine Islands and Su that of *V. diplasia* in Burma (11). Thomas *et al* (14) were the first to grow *V. diplasia* in Coimbatore, India, and to describe in detail the method of its cultivation. Subsequently Bertus (4) reported successful cultivation of this species in Ceylon and Asthana on the possibilities of growing it in the Central Provinces, India, (1). Cultivation of *V. volvacea* has also been reported from Madagascar and Java by Bouriquet (5) and Reitsma and Hadiwidjaja (7) respectively.

Investigations into the possibilities of improving the yield of *V. diplasia* in straw beds were made by Su (12) and Seth (10). There was no difference in the yields obtained by either inoculating each layer or alternative layers of the bed, whereas increased yields could be obtained by using fresh spawn instead of four-month-old spawn. Increased yields could also be obtained by applying two gallons of water to the bed both in the morning and in the evening and also by adding ground seeds of pigeon pea (*Cajanus cajan* (L.) Millsp.) or chick pea (*Cicer arietinum* L.) to the spawn. Asthana (1) in 1947 also found that when powdered *dhal* (seeds of pigeon pea or chick pea) was not added to the medium the sporulation (sporophore formation) was scanty.

Investigations into some of the factors controlling the development of sporophores of *V. diplasia* in straw beds and the possibilities of increasing the yield of the mushroom were made and also some of the cultural and physiological properties of the fungus in various media studied and the results are reported in this paper.

Materials and methods: A fresh isolate of the fungus obtained from an unopened sporophore (button) and subsequently brought into pure culture by single hyphal tip method was used in all these studies. The method of preparation of spawn (inoculum) and cultivation of the mushroom were essentially the same as recommended by Thomas *et al* (14) and deviations, if any, have been indicated in the text. The beds were laid in shaded areas of the greenhouse well protected from wind and rain.

The media used for the cultural and physiological studies of the organism were prepared according to Riker and Riker (8). For plate culture studies 15 ml portions of melted agar medium were poured in 10 cm petri dishes and inoculated in the centre with 4 mm discs of the fungus taken from a week-old culture. The radius and nature of growth of the fungus was recorded after 5-10 days. For liquid culture studies 100 ml portions of the media in 250 ml Erlenmeyer flasks were inoculated with 4 mm culture discs as in plate cultures and allowed to grow for various lengths of time. The dry weight of the mycelium was obtained in the usual manner. Both the plate and liquid culture studies were made in quadruplicate and and the average of the results are presented.

Experimental Resuls: *Study of the factors involved in mushroom culture:* In general it is believed that April to June is the best period for the cultivation of this mushroom. In order to obtain some information on the factors involved in this phenomenon two beds were laid every month during 1947 in the recommended manner and the yields of mushroom recorded. The maximum and minimum atmosheric temperature and relative humidity during the period of each culture was also recorded and the results are presented in Fig. 1. The yield of the mushroom was found to be greatly influenced by the minimum temperature and relative humidity rather than the maximum temperature and humidity. The total number of mushrooms obtained from each bed is directly proportional to the total weight of the mushroom, indicating thereby the non-variability of the average size of the mushrooms throughout the year.

It was advocated that the beds should be watered every day either in the morning or in the evening or both times (10, 12). With a view to find out the influence of alternate wetting and drying on the yield, four beds were laid in identical manner and watered at different intervals, the total quanity of water applied to each bed being constant. The experiment was repeated three times during April, May-June and August, 1947 and the average yield recorded (Table I).

TABLE I

Effect of alternate wetting and drying on the yield of mushrooms

Treatments	No. of mushrooms per bed	Total Wt/bed (Oz.)	Average Wt. (Oz.)
Watering on the first and on the eighth day	168	119	0·71
Watering once in three days	162	99	0·61
Watering once in two days	140	39	0·64
Watering daily	126	82	0 65
No watering	0	..	..

Watering the beds at longer interval seems to favour sporophore formation. There was no significant difference in the average weight of the mushrooms in different treatments.

According to Su and Seth (13) the average size of a mushroom bed should be approximately $3\frac{1}{2}' \times 3\frac{1}{2}' \times 2'$ and according to Thomas *et al* (14) approximately $3\frac{1}{2}$ to $4'$ cube. Nearly 75 to 100 lb. of rice straw is required to heap such a bed. The possibilities of growing the musbroom in smaller-sized beds was investigated by the following methods:

1. Beds of $10'' \times 8'' \times 6''$ were laid on wooden planks in the manner recommended for tbe normal beds and the edges covered with a thin paste of equal parts of cowdung and clay to provide sufficient compactness inside the bed. The surface coating was kept moist by sprinkling water periodically.

2. Beds of $10'' \times 8'' \times 6''$ laid as above without the surface coating.

3. Beds of $10'' \times 10'' \times 9''$ laid inside open glass jars of $18'' \times 10'' \times 10''$ so as to cover only a portion of the area. The beds were kept sufficiently moist and the excess water accumulating at the base was syphoned out periodically.

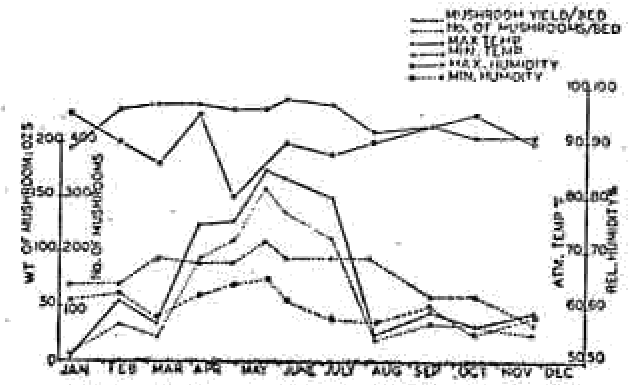

FIG. 1

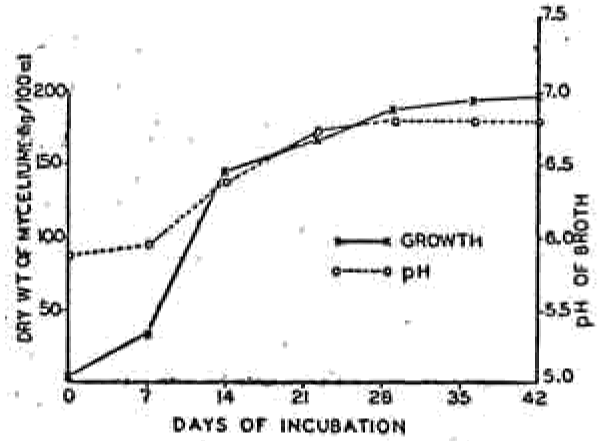

FIG. 2

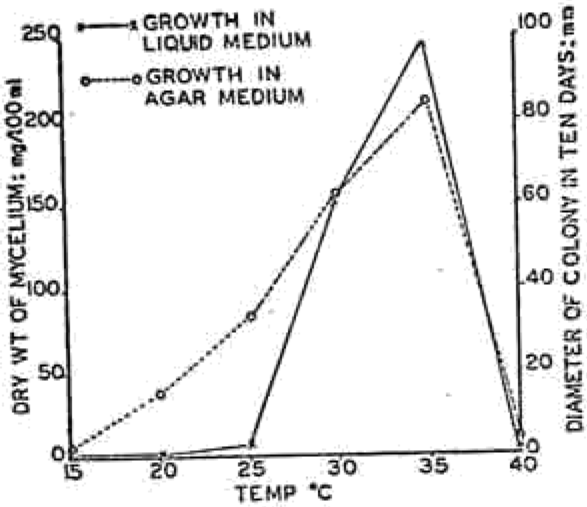

FIG. 3

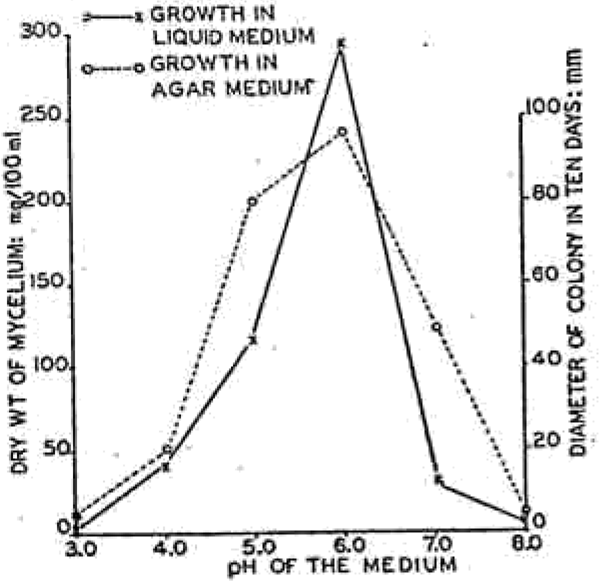

FIG. 4

FIG. 1: Yield of the mushroom during different seasons of 1947 as influenced by atmospheric temperature and relative humidity.
FIG. 2: Rate of growth of *V. diplasia* in nutrient broth.
FIG. 3: Influence of pH on the growth of *V. diplasia* in nutrient medium.
FIG. 4: Effect of temperature on the growth of *V. diplasia* in nutrient medium.

In the first case a few buttons appeared on the surface of the coating after 20 days but none of them developed into normal mushrooms, whereas in the second case a few normal-sized mushrooms developed from below the wooden plank on which the bed was laid but not on the straw layers. The fungal mycelium developed rapidly in the beds laid in glass jars and within 12 days there was normal development of mushrooms (Fig. 7).

Cultural and physiological properties of V. diplasia: At present very little is known about the cultural characteristics and nutritional requirements of *V. diplasia* and other species of the genus. The following studies were made to find out a suitable medium and optimum conditions required for growing the fungus in the laboratory and also to understand its capability to utilize different carbon and nitrogen sources.

Growth on different media: The cultural characters of *V. diplasia* were studied by growing on different complex organic and synthetic media at pH 6.0 to 6.4. On inculation the plates were incubated at room temperature (28 – 32°C) for 7 days and the results recorded (Table II).

TABLE II

Cultural characteristics of V. diplasia on different media

Medium	Diameter of colony (mm)	Nature of growth
Nutrient agar	49	Fairly good growth with colourless submerged hyphae. Greyish - brown pigment produced.
Potato dextrose agar	57	Soft woolly mycelium, mostly submerged and colourless.
French bean agar	53	Growth as above.
Richards' agar	38	Poor growth with thin hyaline submerged, mycelium.
Brown's agar	78	Very thin growth made of mostly submergod quickly spreading hyaline mycelium.
Oat agar	49	Very thick, whitish, cottony aerial mycelium and colourless thick submerged hyphae. No soluble pigment produced.
Carrot agar	50	Thin colourless mycelium, mostly aerial with few submerged hyphal strands.
Corn meal agar	76	Very good white aerial mycelium and light yellow submerged hyphae. No soluble pigment produced.

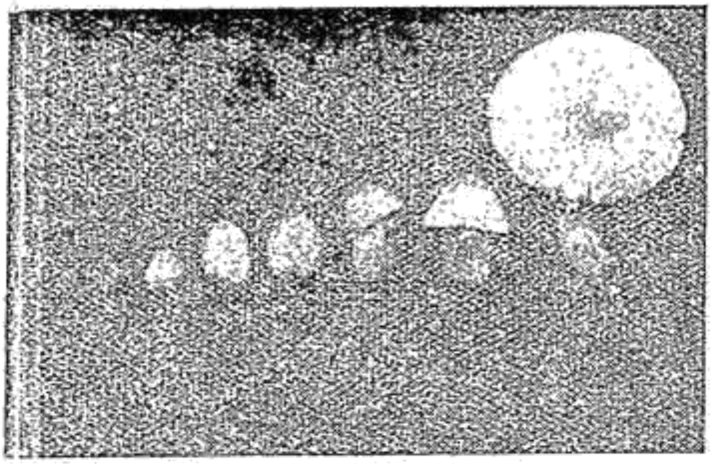

Fig. 5

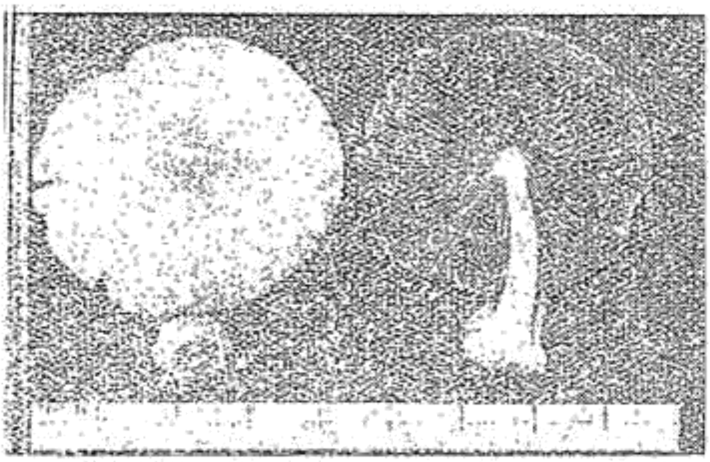

Fig. 6

Fig. 7

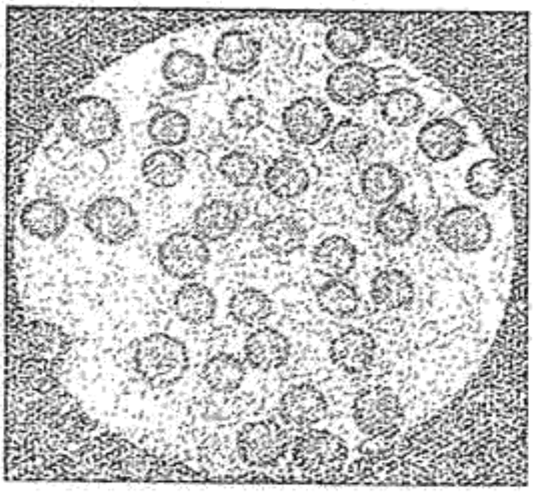

Fig. 8

Fig. 9

Fig. 5: Different stages in the development of sporophores of *V. diplasia*.
Fig. 6: Fully developed sporophores.
Fig. 7: Development of sporophores in miniature beds inside glass jar.
Fig. 8: Photomicrographs of chlamydospores of *V. diplasia*, (335X).
Fig. 9:- Growth and development of chlamydospores (dark growth along the edge and in the center of the medium) of *V. diplasia* in peptone broth.

Corn meal agar and oat agar were found to be highly satisfactory for growing the fungus and fairly good growth was also obtained in nutrient agar medium. There was abundant formation of dark brown chlamydospores after ten days in oat agar medium (Fig. 8), whereas no chlamydospores could be observed on other media even after thirty days' incubation.

Optimum pH and temperature for the growth of the fungus: The rate of growth of the fungus as well as the effect of pH of the medium and incubation temperature on the growth were studied in liquid and agar media. For this purpose nutrient broth and nutrient agar media were selected, despite the fact that corn meal and oat agar gave better growth of the fungus, because the latter media were found inconvenient for liquid culture studies. The growth and pH of the medium levelled off after 28 days (Fig. 2). The H-ion concentration around 6·0 and an incubation temperature around 35°C seem to be most suitable for the growth of the fungus (Figs. 3 and 4).

Utilization of carbon and nitrogen sources by V. diplasia: A basal medium containing 10 g of KNO_3, 5 g of KH_2PO_4, 2.5 gm of $MgSO_4.7H_2O$ and 25mg each of $FeSO_4.7H_2O$, $MnSO_4.H_2O$ and $ZnSO_47H_2O$ per liter (pH adjusted to 6·0) was prepared and 2% w/v of different carbon sources added. The growth of the fungus in each case was recorded as dry weight of mycelium in liquid media or as diameter of the colony on agar media (Table III).

TABLE III

Utilisation of carbon sources by V. DIPLASIA

Carbon source	Nature of growth*	Diameter of colony in agar medium after 7 days (mm)	Dry wt. of mycelium in liquid culture after 22 days (mg/100 ml)
Sucrose**	+++	72	103·5
Glucose	+	52	9·6
Maltose	+	37	5·1
Lactose	+	60	8·7
Cellulose	±	21	2·3
Starch	++++	74	252·1
No carbohydrate	0	62	..

* 0 to ++++ : increasing amounts of growth.

** : cane sugar.

Starch was found to be by far the best source of carbon for the growth of the organism, while cane sugar ranked next.

In a similar experiment using the same basal medium with 20 gm of sucrose substituted for 10 gm of KNO_3, the capability of the fungus to utilize different nitrogen sources was studied (Table IV).

TABLE IV

Utilization of different nitrogen sources by V. DIPLASIA

Nitrogen source	Nature of growth*	Diameter of colony in agar medium after 7 days (mm)	Dry wt. of mycelium in liquid culture after 22 days (mg/100 ml)
KNO_3	+++	48	67·9
KNO_2	0	0	1·8
$(NH_4)_2SO_4$	++	62	51·4
Asparagine	++	53	45·5
Urea	+	88	28·7
Peptone	++++	66	623·4

* 0 to ++++ : increasing amounts of growth.

Best growth was obtained with peptone while the organism could also utilize to some extent KNO_3, $(NH_4)_2SO_4$ and asparagine as nitrogen sources. There was abundant chlamydospore formation along the edges and surface of the medium containing peptone (Fig. 9.)

Discussion and Conclusions: Even though *V. diplasia* formed sporophores in straw beds throughout the year, comparatively high yields were obtained only during April-July. These results are in agreement with the previous reports (1, 13). Whenever the atmospheric temperature fell below 68-69°F, together with a similar drop in the relative humidity below 65%, there considerable reduction in the yield. These findings are supported by the fact that there was very little growth of the fungus in artificial media below 20°C (68°F) (Fig. 4). Besides other cultural factors have been found to greatly influence the sporophore formation; the yield was higher when the beds were watered at longer intervals; there was normal development of mushrooms in miniature beds laid in open jars. Watering at longer intervals results in alternate wetting and drying of the bed and probably creates favourable conditions for the reproductive phase of of the fungus. These results indicate that under certain conditions it is possible to induce sporophore formation and eventually increase the yield. Further detailed studies, however, are necessary to draw any final conclusions.

The capability of the fungus to utilize starch but not cellulose indicates that some complex metabolic processes are involved in the utilization of rice straw as a carbon source by *V. diplasia*. It is possible that the associative microflora of the straw bed influence the utilization of the nutritive material by the fungus and these micro-organisms in turn might be influenced by the climatic factors which are prevalent during certain seasons of the year.

The three species of *Volvaria* Viz., *V. volvacea*, *V. diplasia* and *V. esculenta*, are differentiated chiefly on some morphological bases but their habitat seem to be identical and very little is known about their physiological properties. It is believed that a comparative study of their morphological, cultural and physiological properties would result in better knowledge of these useful and economically important species. It would also be advisable to explore the possibilities of introducing the species into other countries, improving their yield by strain selection, induced mutation etc., and growing them on other agricultural waste products.

Summary: A study of the factors influencing the sporophore formation of *V. diplasia* in rice straw beds was made. There was some direct correlation between the minimum atmospheric temperature and relative humidity and the yield of the mushroom per bed. Under certain conditions it was found possible to induce sporophore formation in smaller-sized beds and also to increase the yield per bed by altering the spray schedule.

Among the complex organic and synthetic media tested for growing the fungus corn meal and oat agar were found to be most satisfactory, nutrient agar ranking next. The fungus was found to grow well in nutrient medium with a pH reaction of 6.0 around 35°C and to readily utilize starchas carbon source but not cellulose. Peptone was the best nitrogen source for the growth of the fungus even though it could utilize KNO_3, $(NH_4)_2SO_4$ and asparagine to some extent. There was abundant chlamydospore formation by the fungus in oat agar and peptone broth.

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