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MAXIMAL UTILIZATION OF RICE STRAW FOR FOOD AND FEEDS
BY MUSHROOM CULTIVATION
(Grant No. AID 492-5542-G-SS-6053-00)

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SUBMITTED BY:

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Film

A C K N O W L E D G M E N T

. . . . . to the officers of the USAID office of Rural and Agricultural Development (ORAD), Manila, for their patience and understanding in the projects' budget management

. . . . . to the officers of the USAID Program on Science and Technology Cooperation (AID-SCI) for making the financial assistance for the project possible

. . . . . to the officers of the University of the Philippines at Los Banos for the physical and moral support for the project

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1. TITLE OF PROPOSAL:

"Maximal Utilization of Rice Straw for Food and Feeds by Mushroom Cultivation"

2. RESEARCHERS:

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3. ORGANIZATION WHERE RESEARCH WAS CONDUCTED:

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Institute of Animal Science
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INSTITUTIONS VISITED IN RELATION TO RESEARCH:

American Type Culture Collection
Rockville, Maryland, USA

University of Viterbo (Tuscia)
Viterbo, ITALY

University of Puerto Rico
Mayaguez, Puerto Rico

4. DURATION AND TOTAL COST OF PROJECT:

Duration: 3 years
Project Started: July 1, 1986
Project Ended: December 30, 1989
Total Cost: US $107,732.00

5. OVER-ALL OBJECTIVE:

To maximize the use of rice straw by producing mushrooms and using the spent compost for animal feed. Rice straw is the main substrate for straw mushroom (Volvariella) cultivation in Southeast Asia and other rice-producing areas of Asia. The spent mushroom compost may be a good source of food for animals as it is as fertilizer to plants. If lignin which Volvariella only partly degrades, is a problem, the compost may be used to further grow Pleurotus, another edible mushroom known to produce lignin-degrading enzymes. By further growing this mushroom, more food is therefore produced from the straw; at the same time, the feed value of the straw is upgraded by lowering the lignin and increasing protein content, due to reported high level of nitrogen accumulation in the Pleurotus spent straw. If these hypotheses work, the above project will help solve the feed and food problem in most of the developing countries of Asia where rice straw abounds.

6. INNOVATIVE ASPECTS:

The usual practice in upgrading the feed value of rice straw besides physical and chemical methods is through microbial conversion, i.e., growing lignolytic fungi such as Chaetomium and Trichoderma on straw. Although the idea of using lignolytic edible mushrooms such as Pleurotus to improve the rice straw is not completely new, this will be the first time that such attempt will be made on Volvariella spent compost. Volvariella being the third important cultivated mushroom in the world is most popular in Southeast Asia. World production of this mushroom in 1981 is 58,000 tonnes mostly from Mainland China and Taiwna. With 20% yield biological efficiency, approximately 200,000 tonnes of fresh rice straw were used in mushroom cultivation during that year. Assuming only 20% of this straw remained as residual compost, then 50,000 tonnes of the compost would have been available as mushroom cultivation wastes or "spent composts". In most cases, this mushroom is grown using simple village level technologies. If their spent compost will be found appropriate as animal feed, these same rural folks can now keep livestock, thus alleviate spent compost disposal problem, at the same time increasing their source of food and livelihood.

Considering that Volvariella is harvested on the 3rd to 4th week after bedding the straw, it may be possible that after the first week harvest during which 50% already of the expected yield is obtained, the straw can still be recycled and used to grow another type of mushroom, the Pleurotus.

7. BACKGROUND:

Microbial biomass as a source of nutrients is receiving worldwide attention. Rolz's recent lengthy review (1983) shows so much activity in a field where biotechnology can play a unique and important role, i.e., the procurement of adequate supplies of food and feed utilizing local raw materials and technology. He considers it a first priority item for any country in the world.

Considerable attention has been directed towards the use of straws as feed because of the increasing demand for high quality meat for human food consumption. While it contains cellulose as an excellent source of energy for ruminants, the presence of lignin hampers digestibility thus reducing the nutritional quality of the straw for animal consumption. Numerous methods therefore for improving the nutrition as well as digestibility of the straw have been investigated. The most common is microbial conversion through the use of lignolytic organisms. Although it has been regarded with a high potential among unconventional sources of feed, its worldwide commercial production is still low (Rolz and Humphrey, 1982).

Previous studies have shown that Basidiomycetes fungi are good producers of lignin-decomposing enzymes (Kim, C.S., 1981), and that *P. ostreatus*, a cultivated edible Basidiomycete, not only renders the spent straw highly digestible due to its laccase-rich mycelium (Sermanni and Luna, 1981) but it also provides the compost with more nutrients especially proteins after harvesting (Zadrazil, 1974). In the tropics, the most popular mushroom being cultivated on rice straw

in most rural areas is Volvariella (Chang, 1980). The spent composts are either burned after harvesting or some are used as mulch. As far as the proponent knows, nobody has attempted to use these spent rice straw compost as feed for animals. This may be due to lack of information on its resulting digestibility and exact constituents.

In the Philippines as well as in the other hot-humid tropical countries, rice straw is fed directly to ruminants especially during periods of feed scarcity. Farmers supplement the feeding with some leguminous forages and/or grasses because rice straw has poor feeding value. In some instances, easily available and cheap concentrate feedstuff such as rice bran or corn bran is used as a supplement. Rice straw is also enriched by ensiling it with small amounts of urea. And to improve its palatability, molasses, if available and cheap, is used.

The proposed project may be the first attempt to analyze the chemical composition of the straw mushroom spent compost for possibility of using it as feeds for ruminants after harvesting the mushroom. If it is still not as digestible and as nutritious as a standard animal feed, further treatments will be devised such as further using it as substrate for another mushroom (Pleurotus) or other organisms (Chaetomium or Candida) to produce a high protein, digestible biomass.

If these will work, not only would more food be produced from rice straw but feed production can also be developed without the use of sophisticated expensive equipments to provide feeds for livestocks

in the rural areas. Most farmers can not keep livestocks due to excessive cost of feeds. In the Philippines alone, the cost of animal meat has gone up considerably due to limited supply of feeds. The project is expected to guide farmers on how theuse of rice straw can be fully maximized. The economy of Southeast Asian countries is still basically agricultural and consequently there is abundance of farm wastes in the form of straw. Recycling these wastes to human food then animal feed is probably the best way possible to utilize these wastes.

8. PROJECT PRESENTATION:

For purposes of clarity of presentation and to emphasize the systematic approach used in pursuing the objectives of the project, the various studies in the project are presented in 5 parts as follows:

STUDY I	-	Screening for Best Isolates and Technology for Rural Cultivation of *Volvariella* and *Pleurotus*
STUDY II	-	Biochemical and Environmental Changes Occurring in the Substrates During Mushroom Cultivation
STUDY III	-	Maximal Utilization of Rice Straw Compost for *Volvariella* and *Pleurotus* Cultivation
STUDY IV	-	Microbial Pre-treatment of Rice Straw for Optimal Mycelial Growth and Degradation for Better Compost
STUDY V	-	Animal Feeding Trials

STUDY 1

I. Screening for Best Isolates and Technology for Rural Cultivation of *Volvariella* and *Pleurotus*

A. INTRODUCTION

The cultivated mushrooms, *Volvariella volvacea* and *Pleurotus sajor-caju*, are represented by several strains that differ among others, in their rate of mycelial growth on a given substrate, the manner and quantity of fruiting, the appearance and size of their fruits, the resistance to pests, and their adaptation to specific environmental conditions. Thus, the first phase of one's cultivation activity should be devoted to screening for the best or good isolates that would adopt to the local conditions and the substrates that will be used in growing the mushroom.

Similarly there are several technologies available for growing the mushrooms. While *Pleurotus* can be grown in ways similar to other wood-inhibiting mushrooms like *Lentinula edodes* and *Auricularia* spp., the method for *Volvariella* is quite different just as *Agaricus* mushrooms are also grown on ways specific to them. All these cultivated mushrooms differ not only on their choice of substrates but also on their temperature requirements. In general, their relative humidity, light and aeration requirement are quite similar.

In like manner, strains within one genus may also behave differently on the way they are grown, or rather on the type of technology followed. For, within one type of mushroom, there is also a choice of technology, not only in terms of substrates. For example,

Volvariella may be grown indoor on composted pasteurized substrates or outdoor in non-composted non-pasteurized substrates, prepared, formed or molded in several ways to achieve the best condition necessary for fast mycelial growth that can overgrow other organisms naturally present in the raw substrates.

The specific objectives of this study were 1) to determine the best isolates of Volvariella and Pleurotus on hand. This is based only on the rate and the thickness of the mycelial growth on agar (for Volvariella) and on sorghum grains (for Pleurotus) 2) to determine the technologies that will be used for growing the mushrooms (these technologies will be consistently followed throughout the project to be able to produce a consistent quality of compost that will be used for animal feeding trails).

B. METHODOLOGY

Ten isolates of Volvariella volvacea and six of Pleurotus sajor-caju were used in this study. These were taken from the Culture Collection of Edible Mushrooms (FCUP 103) at the Department of Plant Pathology, UPLB. For subsequent tests (spawn substrates and actual growing) the best isolates resulting from the earlier tests, one of Volvarlella and one of Pleurotus, were used.

1. Mycelial growth on agar and grains:

Mycelial growth on PDA was used to determine best isolate (showing fastest and densest growth) of Volvariella. Slants of potato dextrose agar were prepared in test tubes and inoculated at the tip of the slant, with the same amount of mycelial inoculum of the different isolates of Volvariella. Growth was rated according to the linear growth rate of the mycelium or the ability of the mycelium to grow from the tip point of inoculation to the bottom of the slant surface.

Growth on sterilized grains was used as basis for best isolate of Pleurotus. Sorghum grains, traditionally used in Southeast Asia as substrate for preparing mother spawn, were boiled for at least 15 minutes, then drained and dried to almost 15% moisture content. They were equally distributed in catsup bottles and plugged with foam or cotton. After sterilization at pressure cooker for one hour, each bottle is inoculated with a mycelial plug of Pleurotus and incubated at room temperature. Rate and density of resulting mycelial growth was again used as basis for good growth of the strain.

2. Mycelial growth on different spawn substrates:

A variety of agricultural wastes was used to test mycelial quantity and quality of the chosen strain. From these, the substrate for preparing the spawn or planting material will be chosen. These were: water hyacinth, ipil (Leucaena glauca) leaves, cotton waste, rice hull, rice straw, sawdust, and coir dust (from coconut husk). These were

chopped fine when necessary then moistened to more or less 70% MC then packed in petri dishes before sterilization for one hour. A mycelial plug of the chosen Volvariella strain was then inoculated on the center surface of each of the substrates. Mycelial growth was observed after 7 days incubation.

3. Effect of supplementation on spawn and fruiting bags substrates:

Normally, easily accessible sawdust,rice straw or other substrates is supplemented with more nutritions and more expensive ones such as oatmeal, rice bran, corn meal substrates or ipil leaves. Tests were made therefore to determine the best supplement for Pleurotus and Volvariella. Ten percent of the supplement was added to the main substrate (chopped water hyacinth) before bottling, sterilization, then inoculated with mycelial plug of Pleurotus or Volvariella.

In another experiment, varied combination and amount of supplement to rice straw as main substrate was prepared in heat resistant plastic bags. These treatments were: 1) rice straw (control) 2) rice straw and sawdust 1:1 3) rice straw and corn meal (20%) 4) rice straw and corn meal (10%) and rice bran (10%) 5) rice straw and rice bran (20%) 6) rice straw and rice bran (10%) 7) rice straw and rice bran (20%) and sugar (1%) 8) composted (6 days) rice straw and rice bran (10%).

As usual, rate of growth and density of the resulting mycelial growth was basis for determining the best substrate supplementation/

combination to be used for preparing spawn of Volvariella and fruiting bags of Pleurotus.

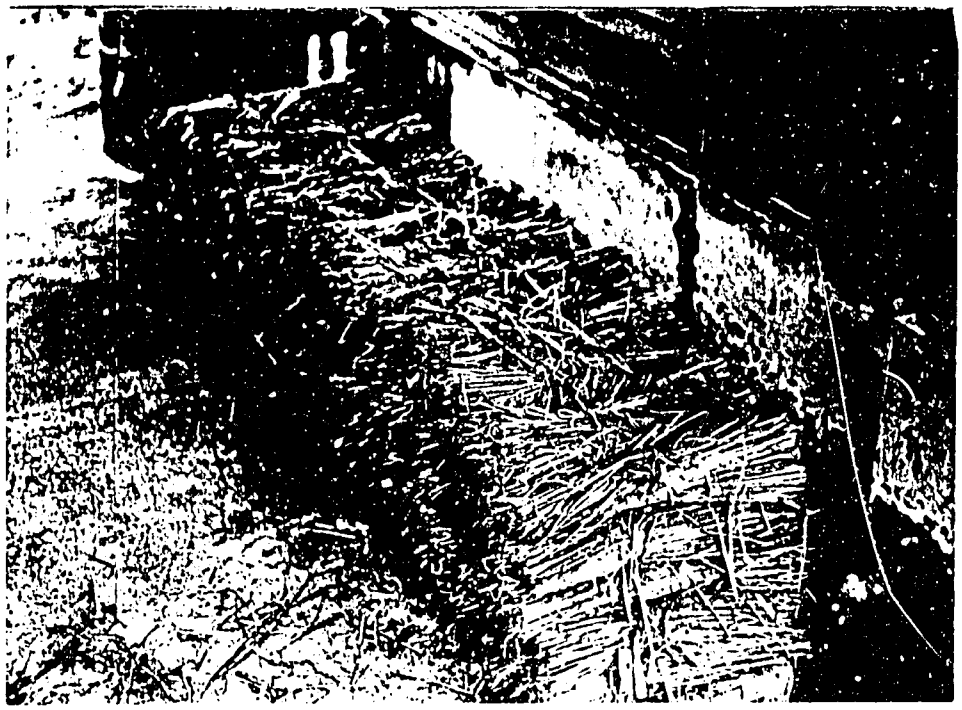
4. Evaluation of best technology for growing Volvariella:

Three different types of beds were used to grow the mushroom: (Fig. 1) 1) 2 m long high bed (3 tiers) 2) molded 1 m small beds, and 3) portable boxes. Rice straw was used as the main substrate. For the long beds and the portable boxes, the dried straw were bundled and cut into 18 inches long before soaking overnight. For each layer of bundles, bits of spawn were placed all along the sides of the layer of bundles. At least 4 to 5 layers make up a good height (at least 12-16 inches high) of a mushroom bed. To conserve space, the beds were made on a 3 tiered wood foundation where three mushroom beds can be prepared. The rice straw used for the molded 1 m beds were chopped then soaked before used to pack into the molds. Spawn was broadcast as the substrates are packed. The molds were then carefully lifted and removed and the resulting beds covered with plastic until fruiting.

C. RESULTS

1. Mycelial growth on agar and grain:

Table 1 and Figure 2 shows that Volvariella strain 560 and 600 were able to fill up the agar surface within 3 days compared with the other strains which took up to 8 days to reach the bottom of the slant.



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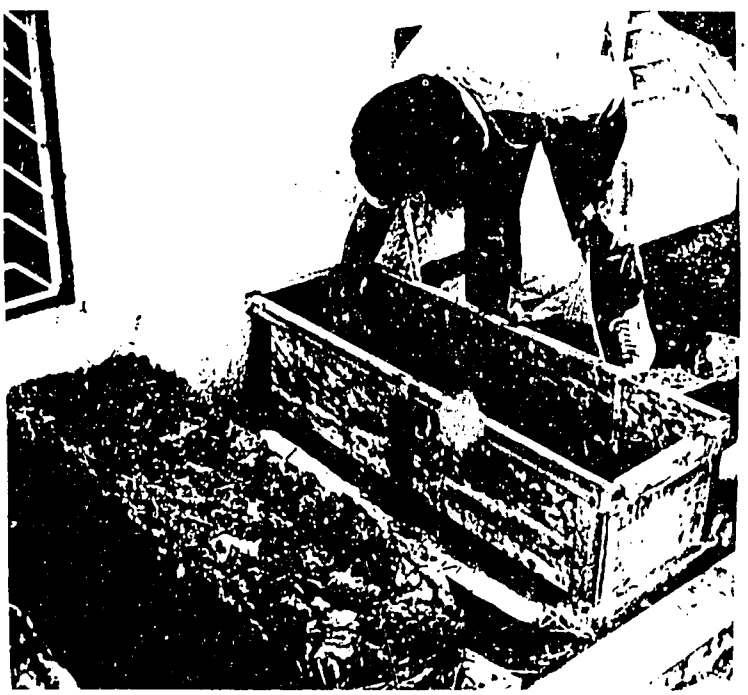

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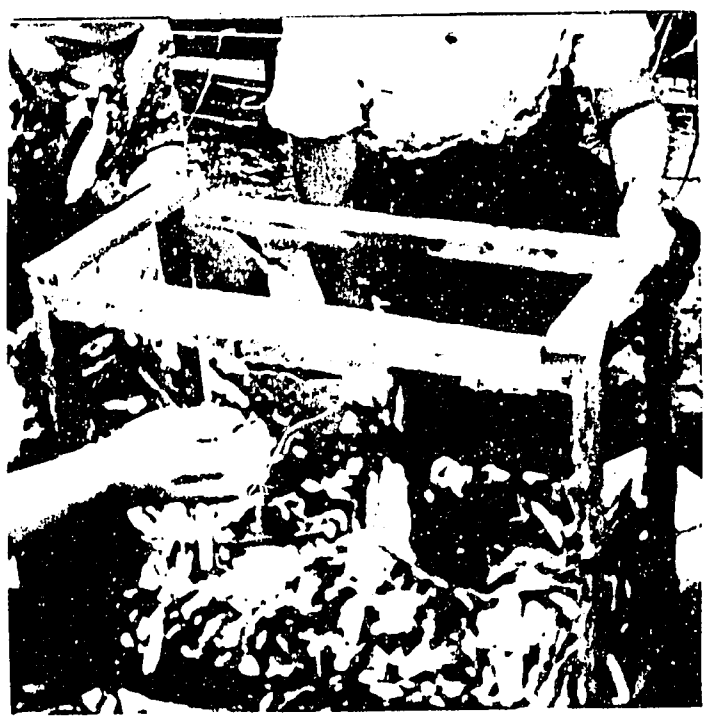

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Figure 1. Different types of bedding for Volvariella

A) 2 m high bed B) molded 1 m bed C) box - frame

Table 1. Strain Evaluation of *Volvariella* and *Pleurotus*

Volvariella	No. of days to reach bottom	Chlamydospore formation
560	3	+ + +
600	3	+
536	4	+ +
567	4	+
579	4	+
559	6	+ + +
535	6	+ + +
611	6	+
599	8	+ +
620	8	+

Pleurotus	Primordia formation in agar
660 (Hongkong)	+
657 (TLRC)	+ +
592 (Thailand)	+
503 (India)	+ +
572 (Bhutan)	+
662 (Antipolo)	+

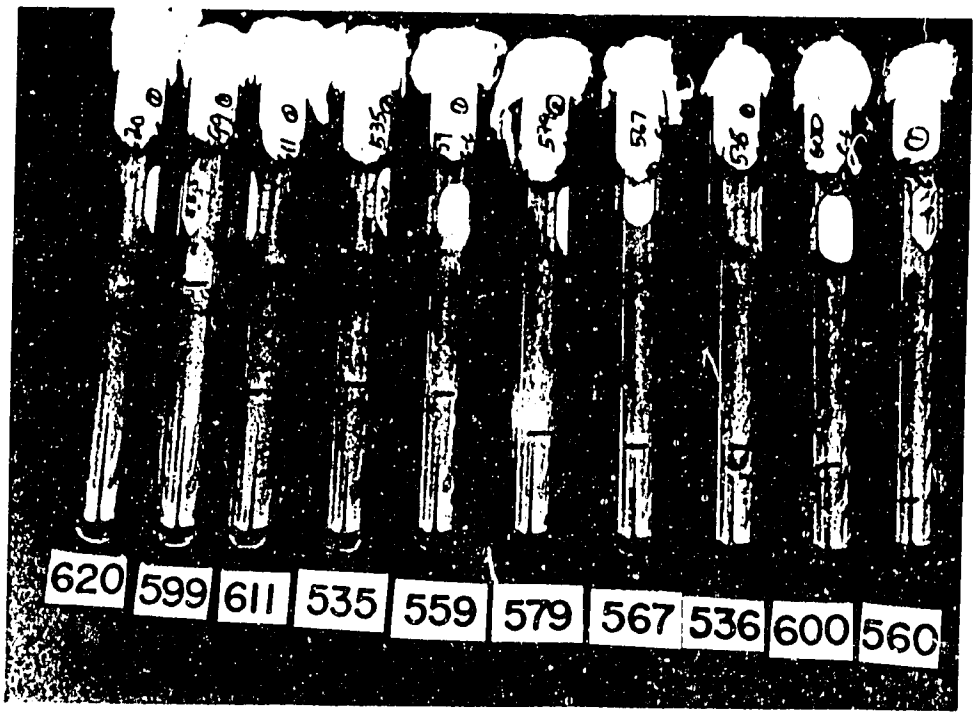


Figure 2. Strains of Volvariella and Pleurotus growing on agar and sorghum grains, respectively.

Between the two fast-grower strains, strain 600 was chosen since it produced more chlamydospores than strain 600. Production of chlamydospores is a good criteria for spawn production since these spores also serve as inoculum (besdies the mycelium) to the substrate when preparing the mushroom beds. Presence of chlamydospores can also be an indication that the spawn is of the right age,

Figure 2 also shows that Pleurotus strain 503 is the fastest grower on sorghum thus this strain is chosen to be used for all subsequent experiments.

2. Mycelial growth on different spawn substrates:

Figure 3 shows that Volvariella strain 600 grow on all the substrates tested but with varying thickness. Sawdust and coir dust showed the thinnest mycelium while water hyacinth, cotton waste, rice hull and rice straw showed all same thickness and rate of growth and appeared to be good substrates for Volvariella even without supplementation. Ipil leaves showed thick growth but rate of growth was very slow.

3. Effect of supplementation on mycelial growth of the mushroom:

Figure 4 shows that all the supplements used resulted equally to dense mycelium. Corn meal however, while resulting to the most dense mycelium, appeared to slow down mycelial growth due to over thickness of the resulting mycelium.

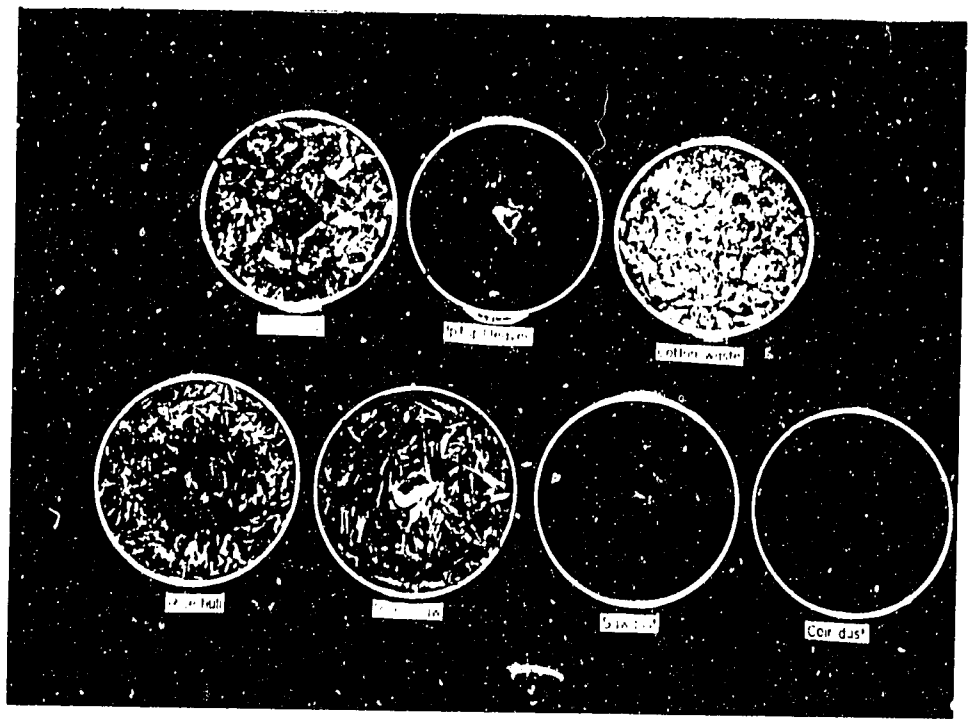


Figure 3. Mycelial growth of Volvariella volvacea 600 on different agricultural wastes

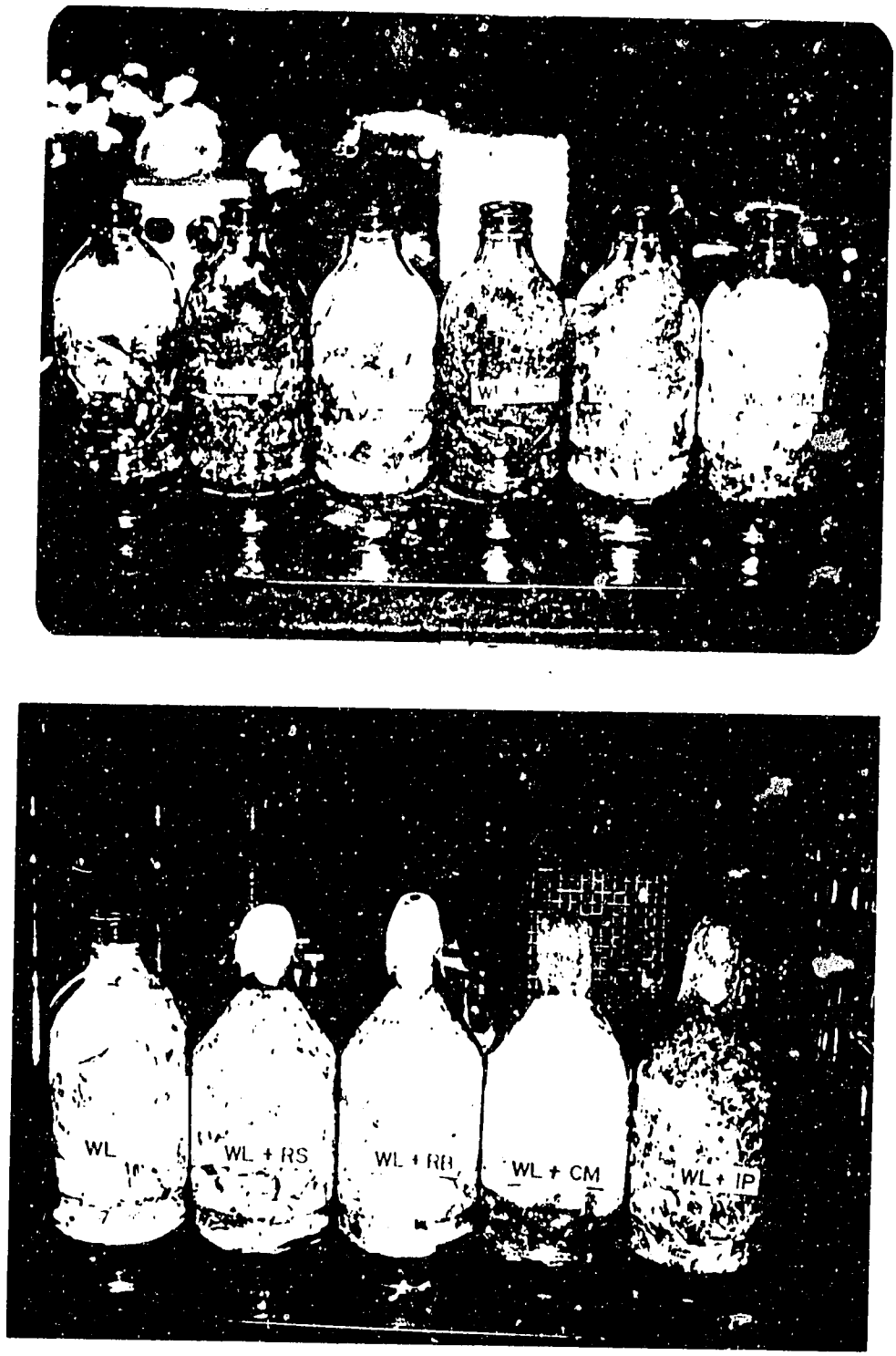


Figure 4. Tests to show effect of supplementing water lily substrate on mycelial growth of Volvariella (a) and Pleurotus (b)

Figure 5 and Table 2 show that supplementation increased density or thickness of the mycelium. Since the rate did not differ significantly, one can have a choice of the different substrate combination for preparing fruiting bags of Pleurotus.

4. Evaluation of best technology for Volvariella cultivation:

Table 3 shows that the long bed technique gave the best yield ratio. Earliest initiation was observed both at the long and short bed. It is possible that the delay of fruiting at the boxes may be due to the lack of heat usually generated by the bulk of the substrate. This heat (at least up to 38 C) hastens both composting of the substrate as well as growth of the spawn onto the substrate. The good yield produced by the long beds may also be due to the ability of the substrates to produce a second flush after a break of at least 7 days. The short beds and boxes did not form additional fruits after the initial growth.

Figure 5. Effect of varied supplementation on mycelial growth of Pleurotus on sawdust. Please see text on the composition of each bag.

Table 2. Evaluation of different substrate/supplementation combination on the mycelial growth of *Pleurotus*

Treatment	No. of days to fill the bag	Mycelial thickness	Days to form primordia
1. Rice straw +	25	+	35
2. Rice straw + sawdust (1:1)	25	+	35
3. Rice straw + corn meal (20%)	32	+ + + +	39
4. Rice straw + corn meal (10%) + rice bran (10%)	30	+ + + +	38
5. Rice straw + rice bran (20%)	28	+ + +	35
6. Rice straw + rice bran (10%)	28	+	40
7. Rice straw + rice bran (20%) + sugar (1%)	30	+ + + +	38
8. Composted rice straw + rice bran (10%)	28	+ + +	35

Table 3. Effect of different techniques on the yield of *Volvariella* on rice straw and banana leaves

Treatments	Quantity of substrate (k)	Days to fruiting	Ave. yield (k)	Biological efficiency
2 m long high bed	25 k	10	3 - 4	12 - 15%
1 m long molded bed	10 k	12	1	10%
box frame	10 k	14	1	10%

STUDY II

II. Biochemical and Environmental Changes Occurring in the Substrates During Mushroom Cultivation

A. INTRODUCTION

During the growth of the mushroom on its substrate, biochemical changes occur both on the mushroom mycelium and on the substrate itself. In a way, the mushroom grow on the substrate causing changes on its physical, chemical and biological characteristics. These changes may be both beneficial as well as deterin to the growing mycelium. The mushroom derives most of its required nutrients from the breakdown products of the substrate during the gorwth of the mushroom. In the case of Volvariella which use non-pasteurized substrates, the natural occurring organisms in the straw are activated during growth of the mushroom. This contributes to some inconsistent data or results taken.

It is the aim of this study to investigate the changes taking place during the various stages of Volvariella volvacea and Pleurotus cultivation. This will then provide clue on the best stage to harvest the spent substrate to be used for growing another mushroom and/or use the substrate for animal feed.

B. METHODOLOGY

Bundles of rice straw alternating with bundles of banana leaves were used in preparing 2 m long beds for Volvariella. Samples of the substrates were taken every seven days beginning bedding and spawning

time for a period of one month of growing Volvariella. Each sample consisted of a mixture of five random subsamples 5 g each taken at about 3-5 cm from the surface of the mushroom bed.

Temperature and pH were recorded at the same hour during the day. The samples were mixed with water and stirred after which pH of the resulting decoction measured with a pH meter.

For moisture content determination, a 50 g sample was dried for two days at 95% and the percentage of moisture calculated.

For evaluation of nutritive quality of the resulting compost, chemical analysis of compost taken from beds made up of pure rice straw, pure banana leaves and rice straw/banana leaves combination, were taken every seven days (for Volvariella) and every 2 weeks beginning primordial formation for Pleurotus.

The three types of composts used were 1) rice straw and banana leaves (RS + BL - V) spawned with Volvariella and harvested (samples taken) 30 days after, 2) rice straw and rice bran (RS + RB - P) spawned with Pleurotus and harvested 30 days after fruiting (60 days after spawning), 3) rice straw and rice bran (RS + RB - V - P) spawned with Volvariella, harvested after 15 days, added with rice bran, sterilized, inoculated with Pleurotus, harvested 30 days after fruiting. Proximate and detergent fiber analysis were made on these three composts and compared with raw rice straw.

C. RESULTS

Table I shows the changes in pH, temperature and mixture content of the beds during the growth of Volvariella on beds. The pH appeared to be constant between 8.3 to 8.8. The temperature rose to 37.4 C seven days after bedding probably due to the activities of the naturally occurring thermophilic organisms. Moisture content decreased until the end of the bed life altho there has been no need to water the beds as long as they are covered with plastic except during harvesting time.

Table II and III shows the changes in the lignin, cellulose and hemicellulose content of the substrates during Volvariella and Pleurotus growth. For Volvariella, lignin increased until the fruit harvest (15 days) but decreased after another 15 days. The same trend was observed with hemicellulsoe and cellulose. For Pleurotus there was consistent decrease in lignin, cellulose and hemicellulose during its growth until the resulting compost is again used for growing another cycle of Pleurotus.

Proximate and detergent fiber analysis of rice straw composts (Table IV) shows decrease in all fractions analyzed compared with raw rice straw except on crude protein which showed consistent increase in all the types of spent compost use. The table shows that the lignin increased after 30 days of Volvariella growth, but decreased after further growth of another mushroom, Pleurotus sajor-caju.

Table 1. Changes in the composting substrates (rice straw + banana leaves) during Volvariella growth.

Stages	Days after spawning	pH	Temp. (C)	Moisture content
At bedding	0	8.70	29.2	86.3
Before primordia	7	8.46	37.4	70.0
After first harvest	15	8.80	34.6	69.2
After full harvest	28	8.30	33.0	50.8

Table II. Changes in lignin, cellulose, and hemicellulose at different stages during *Volvariella* growth both on unpasteurized beds and on sterilized bags. *

Stages	Days	Lignin	Cellulose	Hemi-Cellulose
Raw rice straw	0	13.27	21.95	6.41
Before primordia	7	14.10	28.88	12.27
After first harvest	15	16.24	17.25	11.64
After full harvest	28	12.09	26.62	4.52

* Bed is made up of equal number of bundles of rice straw and banana leaves

Table III. Comparative changes in the two composting substrates during *Pleurotus* growth. **

Stages	Weeks	Lignin		Cellulose		Hemi-	
		Vspent	RS + RB	Vspent	RS + RB	Vspent	RS + RB
Raw rice straw + rice bran	0	15.96	9.49	26.67	33.98	13.13	16.38
Before primordia	4	15.49	18.43	28.12	28.79	9.65	11.51
After first harvest	6	12.92	11.04	10.37	24.77	7.50	12.44
After full harvest	8	12.04	10.57	18.84	21.63	9.32	12.45
After another P. harvest	14	10.31	7.71		52.56		10.99

** One group (V spent) used the 2 week old *Volvariella* spent compost in preparing Pleurotus bags while the other group (RS + RB) used raw rice straw and rice bran to prepare the Pleurotus bags.

Table IV. Proximate and detergent fiber analysis of rice straw used in broiler feeding trials.

	DM (%)	Crude Fiber (%)	Crude Protein (%)	NDF (%)	Hemi cellulose (%)	Cellu-lose (%)	Lignin (%)
A. Rice straw	92.34	5.64	27.78	71.11	20.25	30.19	13.66
B. RS + BL - V							
Pure (0 Cellulase)	91.88	6.10	24.15	66.50	9.85	26.42	14.80
1:1 Cellulase/RS	91.90	6.56	24.38	64.59	10.53	24.55	14.10
C. RS + RB - P							
Pure	91.85	6.11	21.52	66.67	4.95	21.52	10.29
1:1 Cellulase/RS	91.97	5.29	25.83	70.48	3.33	23.75	10.52
D. RS + BL - V - P							
Pure	91.09	6.47	24.05	62.42	4.19	21.19	12.20
1:1 Cellulase/RS	89.95	6.20	21.04	63.75	3.25	20.48	12.87

Volvariella volvacea have been reported as low lignin decomposer (Chang-Ho & Yee, 1977) but their particularly high cellulosic ability (Wang, 1981) must have released the lignin bonded by cellulose early during the growth of the mycelium. This also must explain the increase in lignin content up to fruiting. Normally, rice straw is pre-treated with cellulase when used as animal feed but the presence of lignin which the mushroom hardly degrades still created problems in improving digestibility of the straw.

Simultaneous application of cellulase and lignin-decomposing enzymes have been suggested as a better approach (Kim, 1981) but the use of lignin-degrading mushrooms have been proven as best approach and would seem to be the most economical among the numerous methods used to improve feeding value or satisfy the nutritional needs of ruminants. In this case Pleurotus appears to be a good lignin degrader of the Volvariella spent compost.

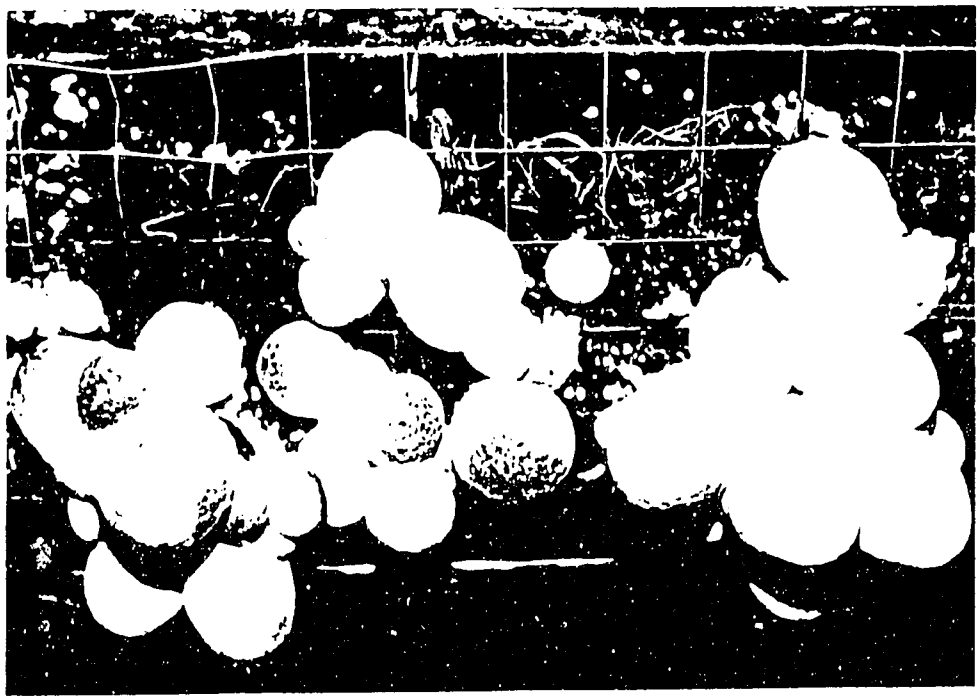
While protein of the resulting compost has slightly increased after Volvariella growth and fruiting, it was further increased by its subsequent use for further cultivation of Pleurotus. This continuous recycling of the rice straw resulted to further decrease in crude fiber and apparent digestibility for animal feed.

STUDY III. Maximal Utilization of Rice Straw Compost for *Volvariella* and *Pleurotus* Cultivation

A. INTRODUCTION

Rice straw is usually the main substrate component for mushroom growing in the tropics. Having rice as the primary crop in the region, Southeast Asian countries are blessed with abundant straw that can be used for the cultivation of the mushrooms. More seventy-three million tons of straw are produced in the region annually (Chang, 1980). In most places however where utilization of this agricultural waste product has not been popularized, farmers merely burn these straw in preparation for the next planting season.

Volvariella volvacea (Figure 1) is the most popular in the region. Grown mostly on rice straw, this mushroom is popularly called the straw mushroom. The outdoor technology of growing the mushroom on rice straw beds provides a biological efficiency of only 10 to 20% depending on many factors, mostly cultural mangement. This technology is highly adaptable as family or small scale projects in areas where rice straw is abundant and labor is cheap. For big scale commercial production, the technology of growing the rice straw is partially composted for at least 6 to 12 days depending on the bulk of the compost, then steamed inside the mushroom house before spawning. This method, being more controlled, gives higher and more stable yield than the outdoor beds.



a

b

Figure 1 a) Button stage of Volvariella volvacea, ready for harvesting

b) Harvesting of the Mushrooms

Pleurotus mushroom (Figure 2) is another species of edible fungi that can be grown in the tropics using rice straw as the main substrate. While some growers recommend a short composting period of 6 days, chopped dried raw straw may also be used and can the same or better yield than pre-composted substrates.

In most cases, the spent substrates or compost are merely disposed and burned after full harvest. These spent compost are still highly nutritious because of the breakdown of the rice straw and because the mycelium of the mushroom stays with the spent straw. For this reason, this spent compost can be recommended as plant fertilizer or animal feed. Zadrazil (1976) showed that the content of individual elements in Pleurotus substrates increases with the duration of fungus growth. While nitrogen, potassium and phosphorus slightly decreased with the harvesting of the fruiting bodies, calcium and magnesium showed a relative increase, thereby making the spent compost ideal as humus manure or for animal feed. Jana and Purkayastha (1983) has also shown increased in nitrogen in rice straw during growth of Pleurotus sajor-caju. In the present investigation, attempts were made to recycle the spent rice straw residues both from Volvariella and Pleurotus farms into either producing another crop of the same mushroom using different technology or producing another mushroom, thus fully maximizing the straw for the production of edible mushrooms before using the spent compost for feeds.

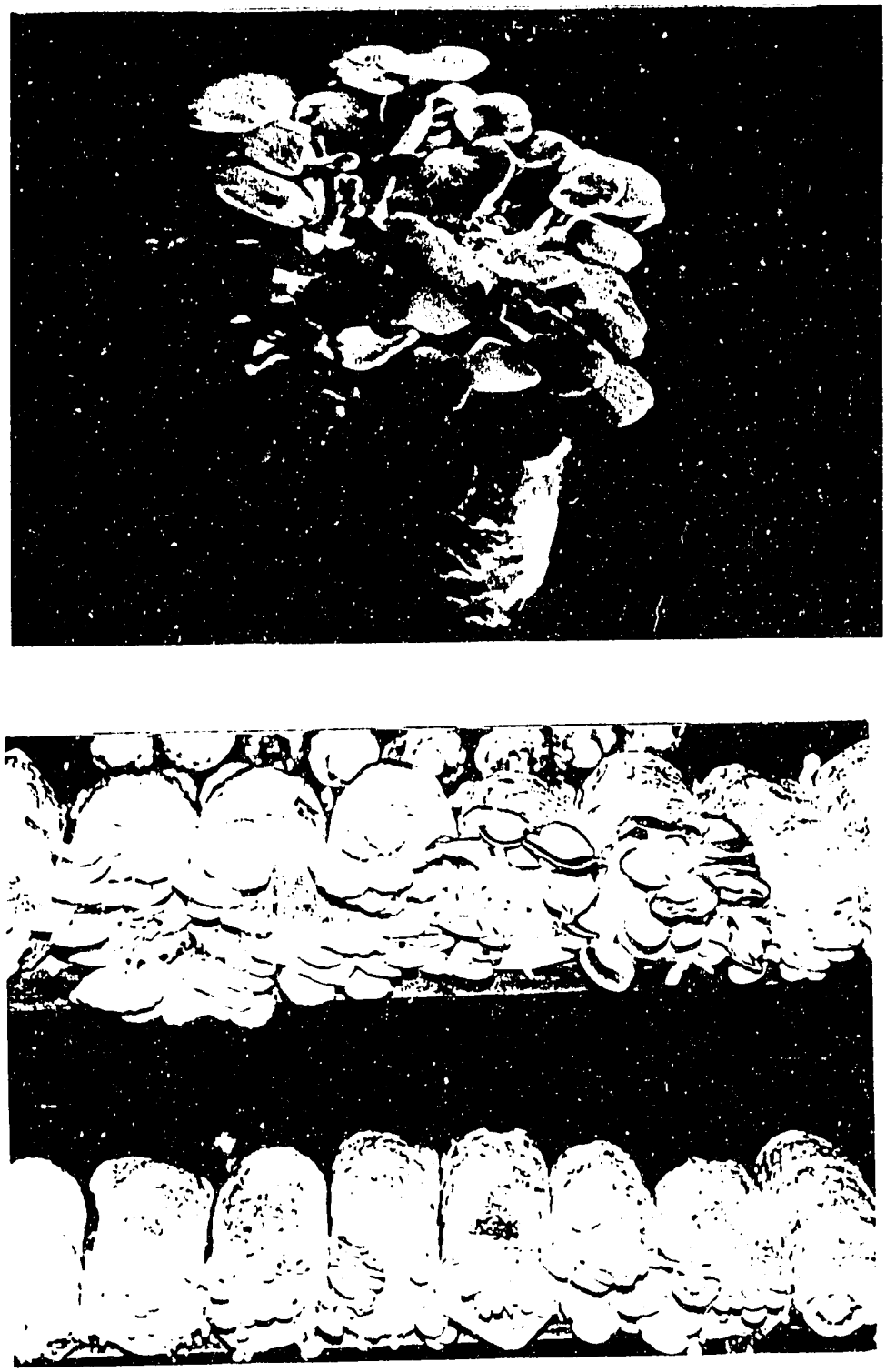


Figure 2. *Pleurotus sajor-caju* growing on rice straw substrates in plastic bags

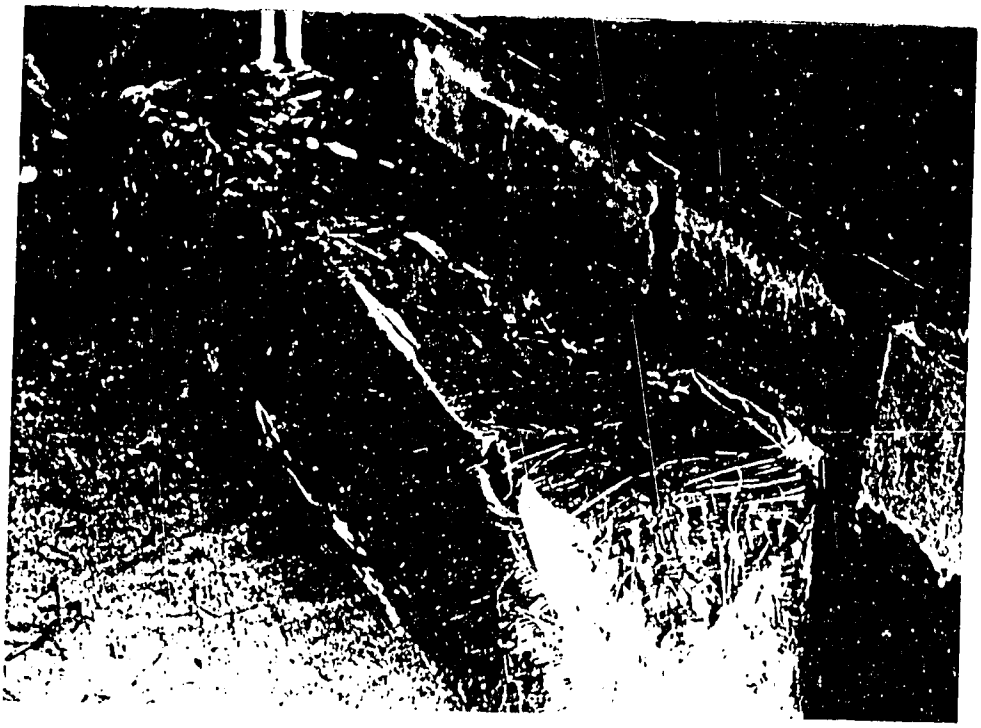
B. METHODOLOGY

The long bed (2 m) outdoor technology of growing Volvariella was used in this study (Figure 3). To maximize space, the beds were prepared in 3-tiered wood foundations. To prepare the beds, bundles of rice straw on one layer were alternated with another layer of bundles of banana leaves. Each bed composed of 3 layers of rice straw, alternated with 2 layers of banana leaves.

Exactly two weeks after preparing the beds (after the first harvest), the partially composted substrates were chopped and added with 20% rice bran (Figure 4). The resulting mixture is then packed in heat-resistant bags, sterilized then inoculated with Pleurotus. The same 2 week outdoor compost was also used to prepare the substrates for indoor cultivation of the same mushroom (Figure 5). Artificially composted rice straw and banana leaves (6 days composting) was used as control on the test. Mushroom yield of the beds made up of spent compost and artificial compost were compared.

C. RESULTS

Two weeks after preparing the rice straw and banana leaves bed and spawning with Volvariella, the substrate have already produced at least 75% of the expected yield of the mushroom. At this stage, Volvariella production can be terminated and the composted substrate was used to prepare fruiting bags for Pleurotus production or as Volvariella



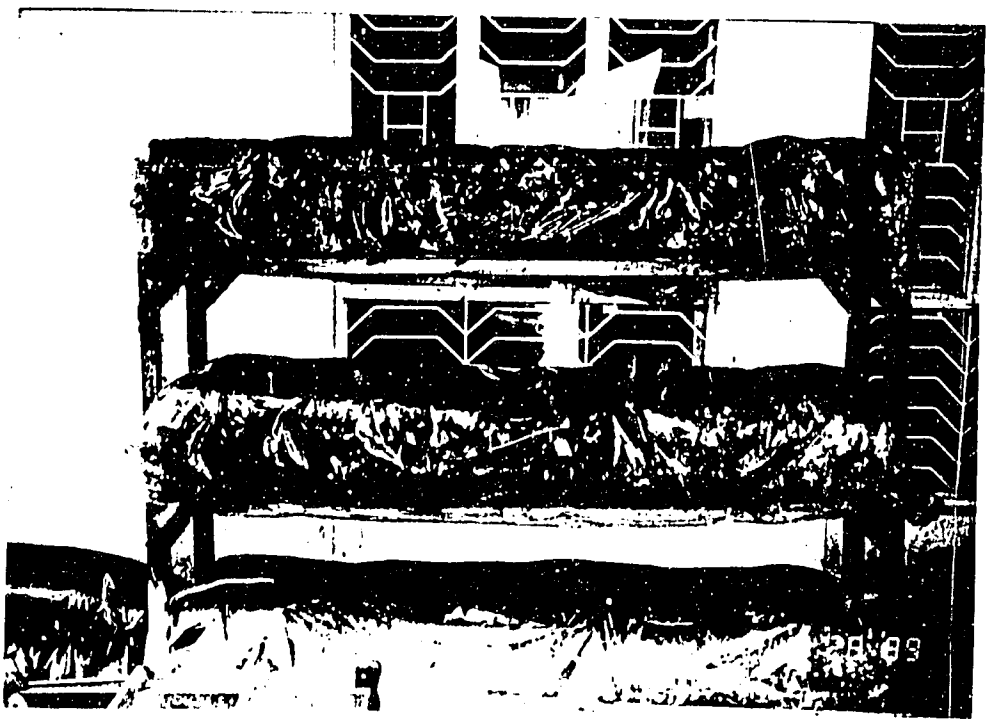


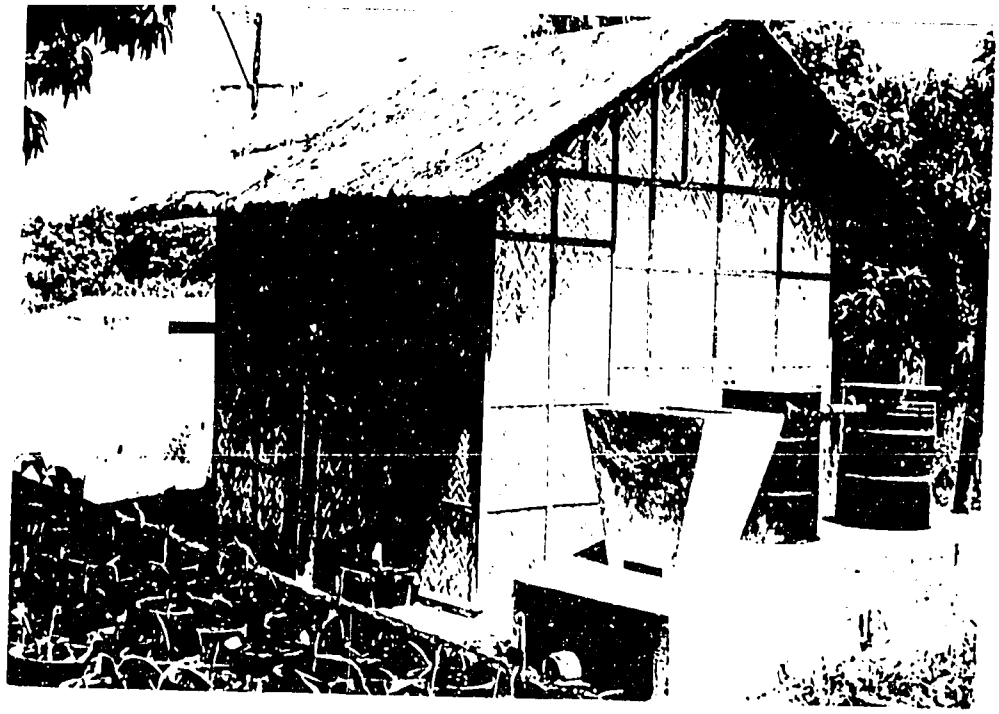
Figure 3. Three-tiered "bunk beds" of Volvariella volvacea

a

b

Figure 4. a) Volvariella spent compost, added with rice bran, then bagged

b) for Pleurotus growing



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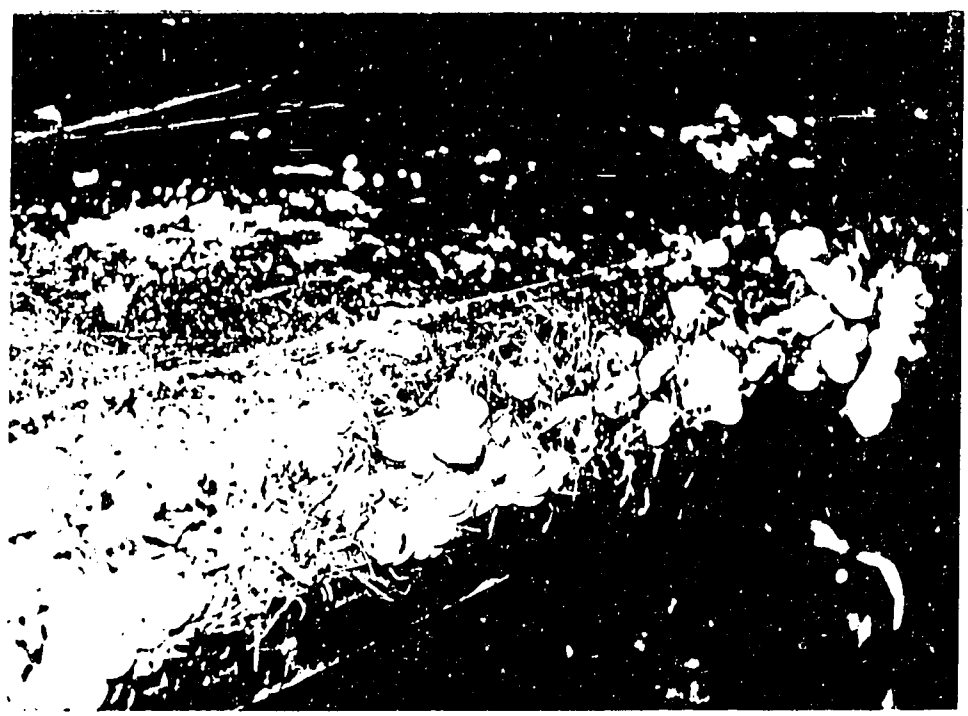


b

Figure 5. Indoor cultivation using Volvariella spent compost

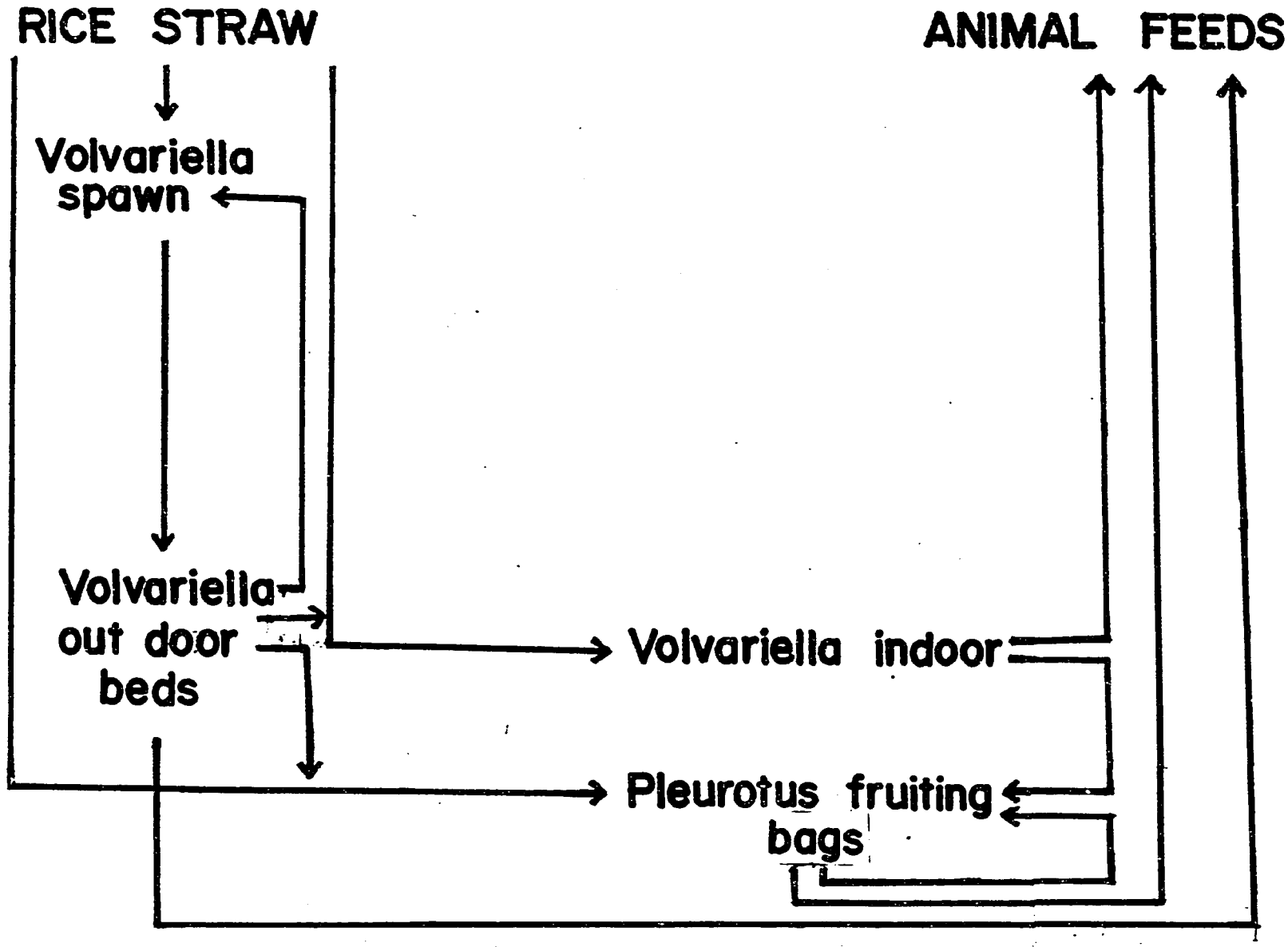
a) outside view of the house and steamer

b) indoor beds

spawn substrate or for preparing beds for Volvariella indoor cultivation. Table I shows a slight decrease in yield of Volvariella using the spent compost compared with the artificially composted rice straw substrates. This may be due to lesser nutrients present on the spent compost than the artificially composted substrates which was composted for six days with urea and lime. Yield of Pleurotus on bags containing spent compost is comparable with bags containing raw rice straw and rice bran, indicating that use of rice straw can be maximized by growing Volvariella fruit, then using the spent compost for growing Pleurotus. As shown in the previous study (Study II), the amount of nitrogen present increased with the growth of Pleurotus on spent compost of Volvariella thus improving the quality of the compost for animal feed.

Figure 6 shows a diagram showing how utilization of rice straw can be maximized and quality of spent compost improved by growing several mushrooms consecutively before using the spent compost for animal feed.

Figure 6. A diagram showing how utilization of rice straw can be maximized.



STUDY IV

IV. Microbial Pre-treatment of Rice Straw for Optimal Mycelial Growth and Degradation for better Compost

A. INTRODUCTION

While rice straw contains enough cellulose to make it an excellent source of energy for ruminants, the presence of lignin that surrounds the cellulose renders the cellulose indigestible. Microbial pre-treatment has been especially with many wood-inhibiting basidiomycetes which has been reported to increase in-vitro digestibility from 40 to 80%, according to species. Reports on the ability of these fungi to degrade lignin and render rice straw more digestible are numerous (Zadrazil, 1979; Plat et al, 1981; Kaneshiro, 1977; Latham, 1979; Streeter et al, 1981, 1982) had shown how bacteria, Erwinia, Carotovora, can supplement Pleurotus ostreatus in improving wheat straws nutritive value for ruminant animals. Traditionally, Tremella, and edible jelly fungus, is grown in China in mixed culture, with the mycelium of Hypoxylon, believed to serve as a biological factor or "friend of the mycelium", helping Tremella in the digestion of the wood, providing residual nutrition.

The objective of this study is to screen for organisms that can facilitate growth of Volvariella on rice straw. These organisms will be isolated from the straw beds of Volvariella. Several known lignin degrading fungi will also be tested for their ability to grow on rice straw and for their ability to

B. METHODOLOGY

a) Isolating and screening Actinomycetes

Several isolates of Actinomycetes were collected from rice straw beds of Volvariella. To isolate actinomycetes, samples were taken from mushroom bed and from the vicinity of fruiting bodies of V. volvacea, The sample was macerated and diluted to appropriate dilutions (10^{-6} to 10^{-9}). One ml portion of these dilutions was used to isolate bacteria and actinomycetes by dilution plate technique and 0.1 ml was applied to the other agar surface by pipette, then spread across the surface with sterile glass rod to isolate selected colonies. The plates were incubated at room temperature (28 - 30 C) for three days, afterwhich the colonies of actinomycetes were counted.

The isolated Actinomycetes were all grown in dual cultures with the mushroom mycelium. In a separate experiment, the actinomycetes were first grown on potato dextrose broth for 34 hr and the resulting culture filtrate was added to new PDB at different percentages. A mycelial plug of Volvariella was then allowed to grow on these mixtures with plain PDB serving as control. After 5 days of shaking, dry weights of resulting mycelia were taken.

b) Isolating and screening bacteria

Samples of rice straw were taken from Volvariella beds and used to isolate bacteria by the same dilution plate technique described used for actinomycetes. Each of the isolated organism was streaked on one side of the agar plate and a mycelial plug of *Volvariella volvacea* placed on the other side. The plates were incubated at room temperature to see if *Volvariella volvacea* could overgrow the opposing bacterium. In another set-up chopped rice straw substrate was osaked in water for two hours and packed in petri plates. The dishes were autoclaved at 121 C for 30 minutes. Inoculation of bacteria was done by suspending the 12 hour culture of each bacteria in 10 ml of sterile distilled water and poured immediately into the petri dishes containing sterile rice straw. Then one disc of four day culture of *V. volvacea* was seeded in the center of the petri dish. Mycelium growth was measured daily for 6 days, using the linear method.

c) Lignin-degrading fungi

Thirteen known lignin-degrading fungi were used in this study. These were:

Trametes versicolor (ATCC 16298)
Phanerochaete chrysosporium (ATCC 24725)
Pleurotus ostreatus (ATCC 58053)
Humicola grisea (ATCC 16298)

Lentinus edodes (ATCC 48855)
Agaricus bitorquis (ATCC 32675)
Pycnoporus cinnabarinus (ATCC 48748)
Merulius tremellosus (ATCC 48745)
Chaetomium globosum (FCUP)
Pleurotus sajor-caju (FCUP 503)
Volvariella volvacea (FCUP 509)
Auricularia polytricha (FCUP)
Ganoderma lucidum (FCUP)

These strains were first grown both on rice straw and sawdust and their specific rate of degradation on these substrates determined the resulting substrates ("spent") were then dried in an oven then moistened, rebottled, sterilized and inoculated with "Volvariella".

C. RESULTS

a) Test on Actinomycetes

Results show that while most of them didnot have any inhibiting effect on the mushroom mycelium, one isolate showed a marked stimulatory effect. Repeated test showed that the mushroom mycelium were able to grow over the actrinomycete colony and became profusedly branched to form a thick portion of the colony (Figure 1).

Agar discs containing the mushroom mycelium and agar discs cut from the mycelium overlapping the actinomycetes were transferred to new PDAplates and incubated at room temperature. Figure 2 shows that the actinomycete stimulated the growth of the mushroom mycelium. After 4 days of incubation, the diameter of the actinomycete containing disc was 86.0 mm compared with the 64.0 mm da. produced by the mycelium in the absence of the actinomycetes.

Table 1 and Figure 3 shows that mycelial growth slightly increased with 20% culture filtrate then decreased with higher concentration.

Table 1. Mycelial growth of *Volvariella volvacea* in media containing culture filtrate of an Actinomycete isolate 1/

Media	Dry mycelium weight (mg)
Potato dextrose broth	8.4 a
PDB + culture filtrate 20%	8.7 a
PDB + culture filtrate 40%	4.5 b
PDB + culture filtrate 60%	2.9 c
PDB + culture filtrate 80%	1.4 cd
Culture filtrate 100%	1.2 dc

1/ Average of 3 replicates. Values with the same letter are not significantly different at 5% level using DMRT.

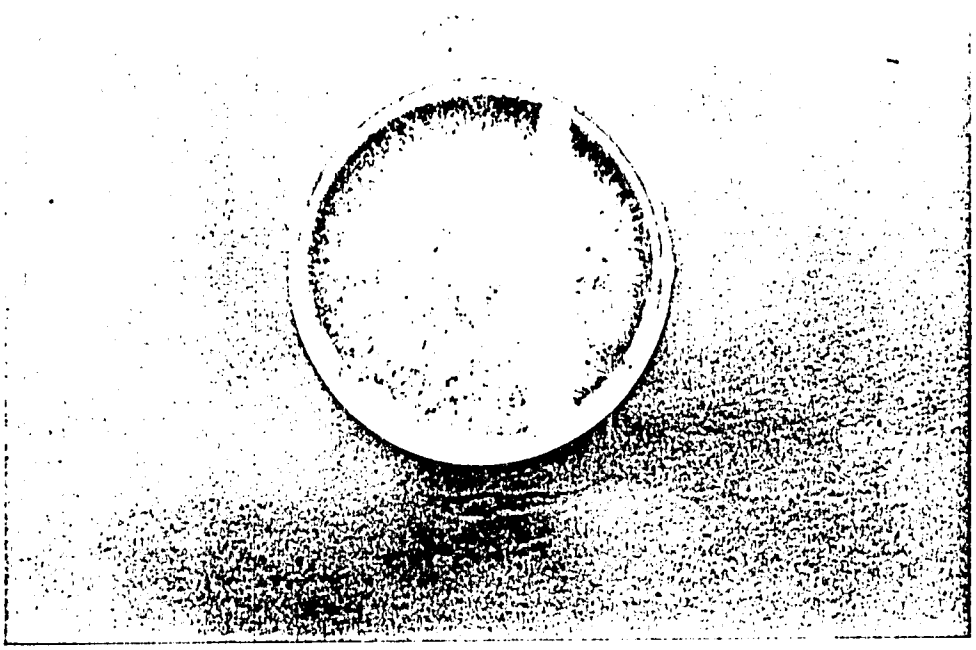


Figure 1. Growth of *Volvariella volvacea* and a thermoactinomycetes on agar showing profused mycelial growth on the streak line of actinomycete colony.

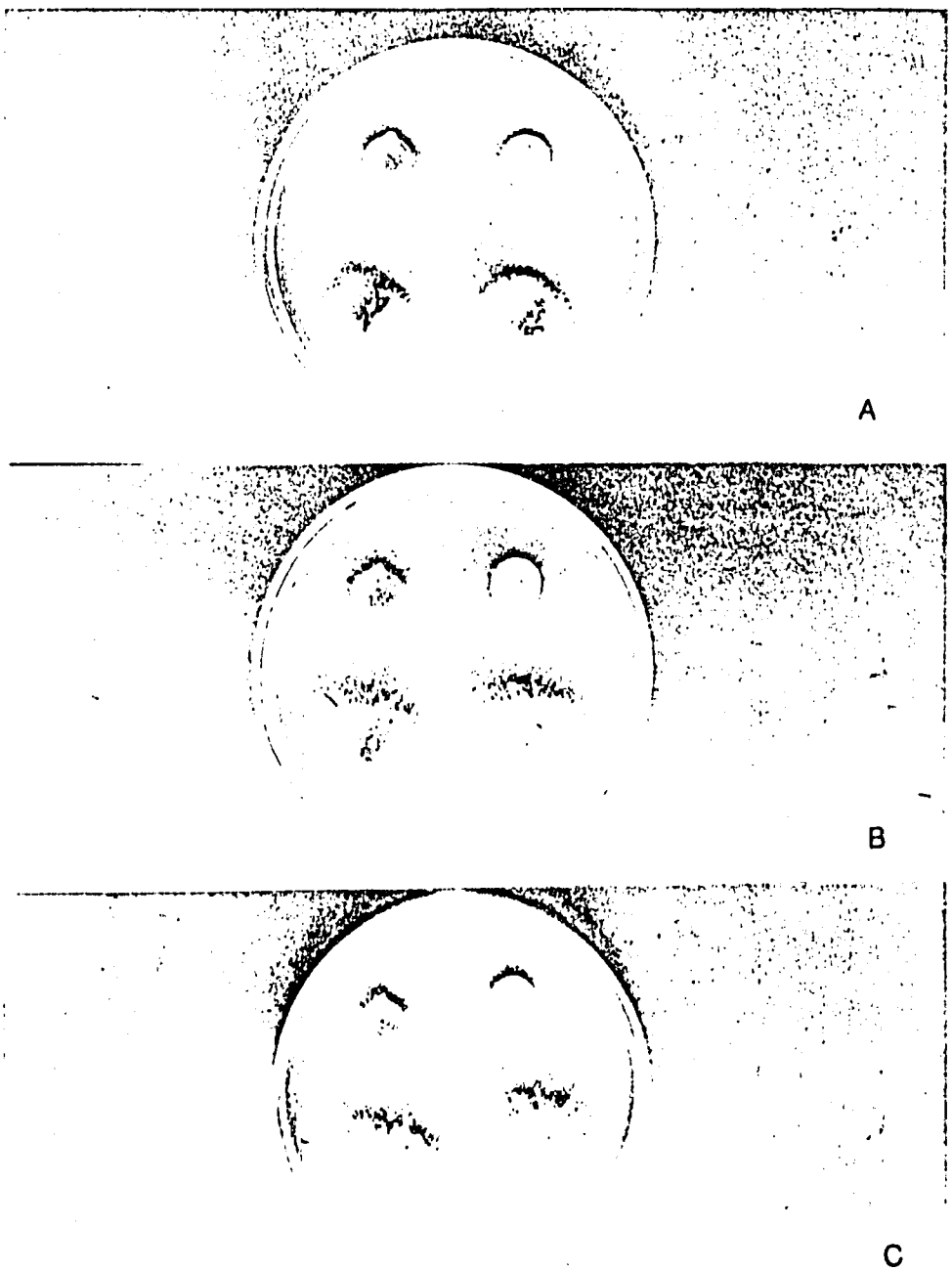


Figure 2. Agar cultures showing Volvariella mycelial enhancement by the actinomycetes

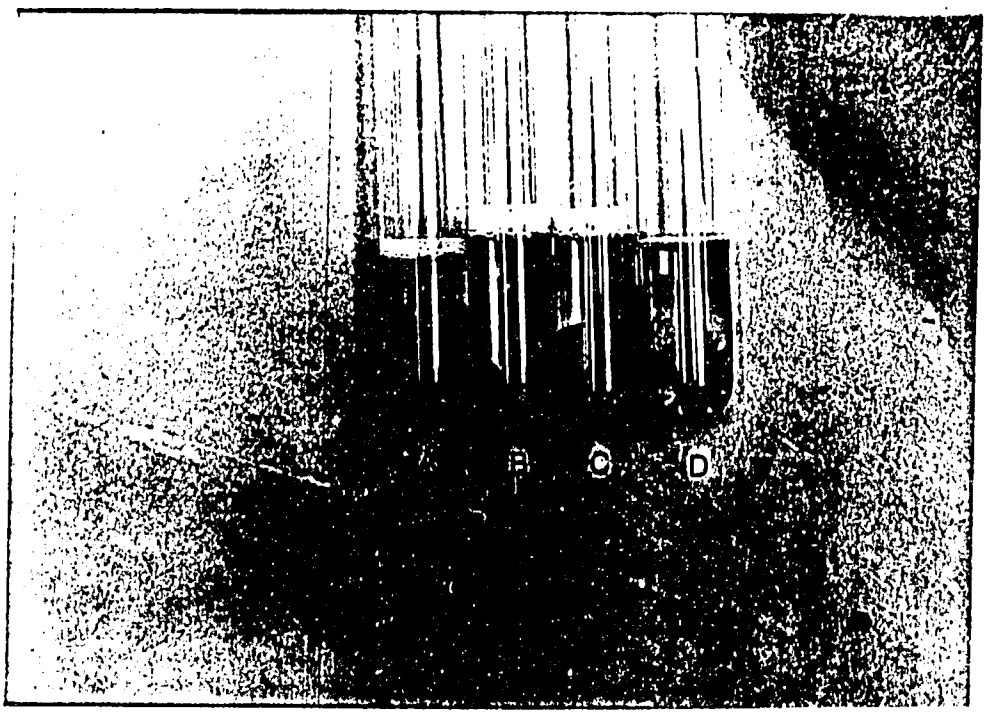


Figure 3. Liquid broth containing actinomycetes (C and D) causing enhanced mycelial growth of *Volvariella volvacea*

The stimulating effect of the actinomycete might have been due to some metabolities produced and secreted into the medium.

b) Tests on bacteria

Fourteen isolates of bacteria were collected from different parts of the Volvariella mushroom beds. These isolates were grouped into (Figure 4) isolates that completely inhibited mycelial growth of Volvariella; isolates that has no effect on the mycelial growth (their colonies were overgrown by the mycelium but there was no enhancement of growth) and isolates overgrown by the mycelium and growth was apparently enhanced. The last group was further tested for their ability to enhance Volvariella mycelial growth by growing them together in test tubes containing rice straw substrates (Figure 5). Table 3 showed that one isolate among the group (#009) apparently increased rate of growth of Volvariella as well as facilitated production of chlamydospores compared with other isolates including the control. Practical use of this bacterial isolate as a microbial catalyst for Volvariella production and lignin degradation should need further investigation.

c) Lignin degrading fungi

Out of the 13 strains tested, three were found to have no effect on the mushroom mycelial growth: These are: Chaetomium globosum,

Table 2. The length of Volvariella volvacea mycelia in test tube containing rice straw and bacterial suspension (Isolate 009).

TREATMENT	LENGTH OF MYCELIA (mm)	
	after 7 days	8 days
Inoculated (Isolate 009)	96.3	126.3
Uninoculated (Control)	65.7	91.3

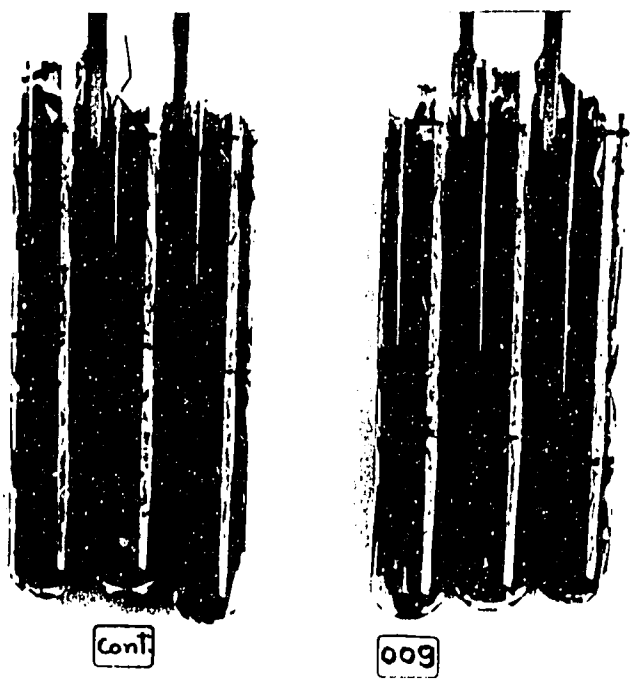


Figure 5. Rice straw added with actinomycetes decoction (009) inoculated with Volvariella showing increased rate compared with the control (no actinomycetes)

Table 3. Mycelial growth of *Volvariella volvacea* in pteri dishes containing rice straw and bacterial suspension.

BACTERIA (Isolates)	COLONY DIAMETER (mm) AFTER 6 days	MYCELIAL DENSITY (rate after 10 days)
001	37.7	+
002	50.3	+
003	0.0	0
004	35.7	+
005	0.0	0
006	57.3	+
007	52.0	+ +
008	67.3	+
009	90	+ + + +
010	73.3	+ + +
011	0.0	0
012	90	+ + +
013	36.0	+ +
014	33.6	+ +
Uninoculated (no bacteria)	90	+

0 = no growth

+ = very slight + + = slight + + + = moderate

+ + + + = abundant + + + + + = very abundant

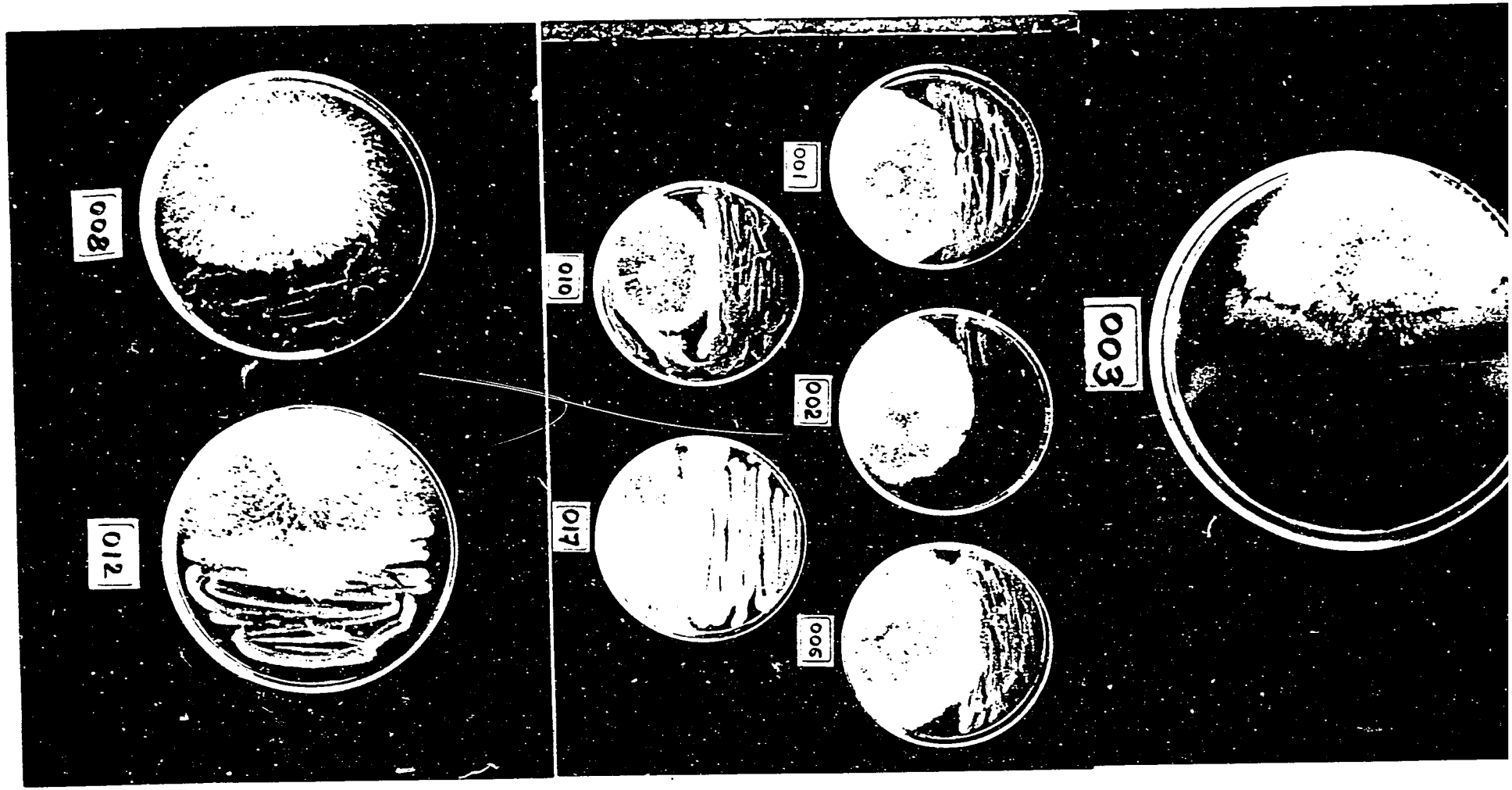


Figure 4. Three grouos of bacteria isolated from Volvariella mushroom bed.

a) inhibiting Volvariella mycelia; b) not inhibiting; c) not inhibiting and enhancing mycelial growth

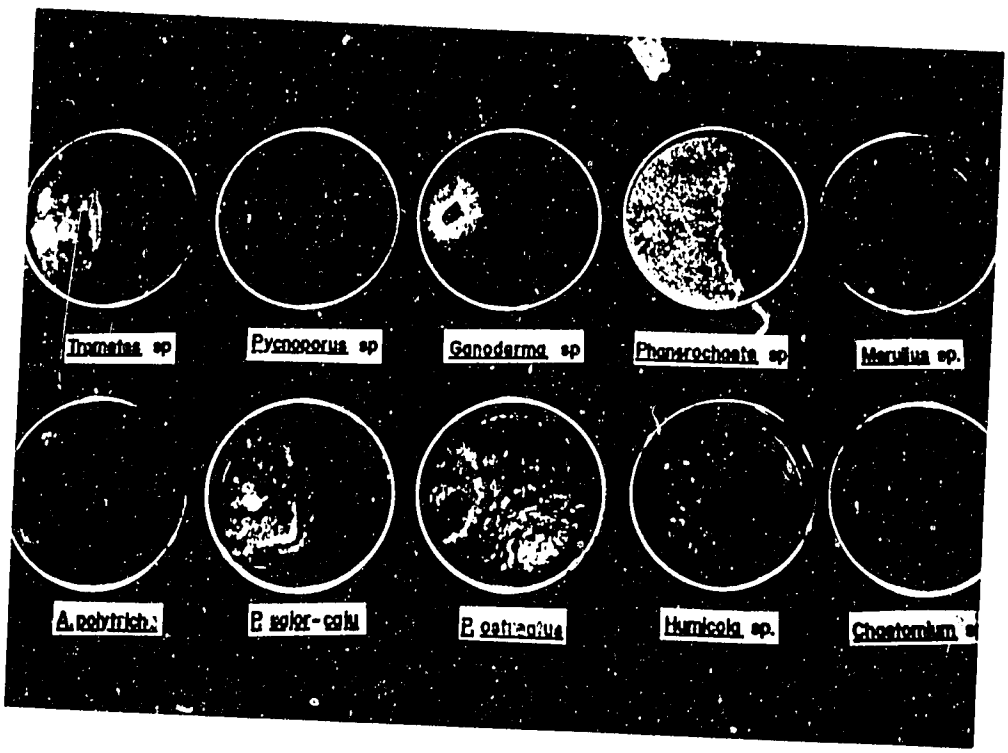


Figure 6. Dual cultures of bacteria and some known lignin degraders

Pycnoporus cinnabarinus and Humicola grisea. The rest seemed to inhibit Volvariella (Figure 6) hence cannot be used for the subsequent dual culture test.

Table 4 and 5 show that Trametes and Agaricus are the best degraders on rice straw while Phanerochaete, Pycnoporus and Agaricus are on sawdust. Table 6 and Figure 6 shows that Trametes and Humicola. altho didnot increase rate of growth of Volvariella, all caused increased in density which means increased mycelial growth probably due to better degraded substrate than the raw (unspent) rice straw used as control.

Volvariella hardly grows on sawdust, but when the sawdust was pre-grown with some drgraders, rate of mycelia of Volvariella increased on the spent sawdust substrate (Table 6 and Figure 8). The density of the mycelia also increased on the spent sawdust compared with that growing on raw sterilized sawdust. This suggests possible role of the lignin-segrading fungi on the mycelial growth of Volvariella.

Table 4. Mycelial density, days to filling, and rate of degradation of different fungal degraders after full growth on rice straw and rice bran.

FUNGUS	MYCELIAL DENSITY	DAYS TO FILLING * *	RATE OF DEGRADATION * * *
Trametes versicolor	+ +	45	47 a
A. bitorquis	+ + +	28	41 a
Phanerochaete chrysosporium	+ + +	32	35 ab
A. polytricha	+ + +	31	31 ab
P. sajor-caju	+ + +	19	25 bc
P. ostreatus	+ + +	28	24 bc
Humicola grisea (ATCC 16298)	+	60	22 bc
Volvariella volvacea	+	14	14 c
Ganoderma lucidum	-	-	-
L. edodes (ATCC 48855)	-	-	-
Chaetomium globosum	-	-	-
Merulius tremellosus	-	-	-
Pycnoporus cinnabarinus (ATCC 48748)	-	-	-

* Mycelial density

\- = no growth

\+ = scanty mycelia

\+ + = moderate growth

\+ + + = thick and abundant mycelia

* * = days to filling - number of days from inoculation of mycelial plug to filling of bottle

* * * Rate of degradation expressed as % weight loss of rice straw and rice bran according to the formula:

$$\% \text{ weight loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

$$= \frac{100 \text{ g} - \text{FW}}{100 \text{ g}} \times 100$$

All means having common letters are not significantly different from each other at 5% level using S - test.

Table 5. Mycelial density, days to filling, and rate of degradation of different fungal degraders after full growth on sawdust and rice bran.

FUNGUS	MYCELIAL DENSITY *	DAYS TO FILLING * *	RATE OF DEGRADATION * * *
Phanerochaete	+ + +	20	49 a
Pycnoporus	+ +	23	46 ab
A. bitorquis	+ + +	16	43 ab
Trametes	+ +	21	38 ab
P. ostreatus	+	30	36 bc
Merulius	+	11	35 bcd
A. polytricha	+ +	43	28 cde
Ganoderma	+	61	25 de
P. sajor-caju	+	40	24 e
Volvariella	+	28	6 f
Chaetomium	-	-	-
Humicola	-	-	-
L. edodes	-	-	-

* Mycelial density

- = no growth
+ = scanty mycelia
+ + = moderate growth
+ + + = thick and abundant mycelia

* * = days to filling - number of days from inoculation of mycelial plug to filling of bottle

* * * = rate of degradation expressed as % weight loss of sawdust and rice bran according to the formula:

$$\% \text{ weight loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

$$= \frac{250 \text{ g} - \text{FW}}{250 \text{ g}} \times 100$$

All means having common letters are not significantly different from each other at 5% level using S-test

Table 6. Growth of *Volvariella volvacea* on rice straw and sawdust spent substrates of selected fungal degraders

SPENT SUBSTRATE	RICE STRAW		SAWDUST	
	MYCELIAL DENSITY *	DAYS TO FILLING **	MD	DF
P. ostreatus	+ +	17	+ +	
A. polytricha	+ +	16	+ +	
Phanerochaete	+ +	18	+ +	
P. sajor-caju	+ +	16	+ +	
A. bitorquis	+ + +	8	+ +	
Humicola	+ + +	7	+ +	
Trametes	+ + +	8	+ +	
Control (unspent)	+	7	+	

* Mycelial density

\+ = scanty mycelia

\+ + = moderate growth

\+ + + = thick and abundant mycelia

* * = days to filling - number of days from inoculation of mycelial plug to filling of bottle

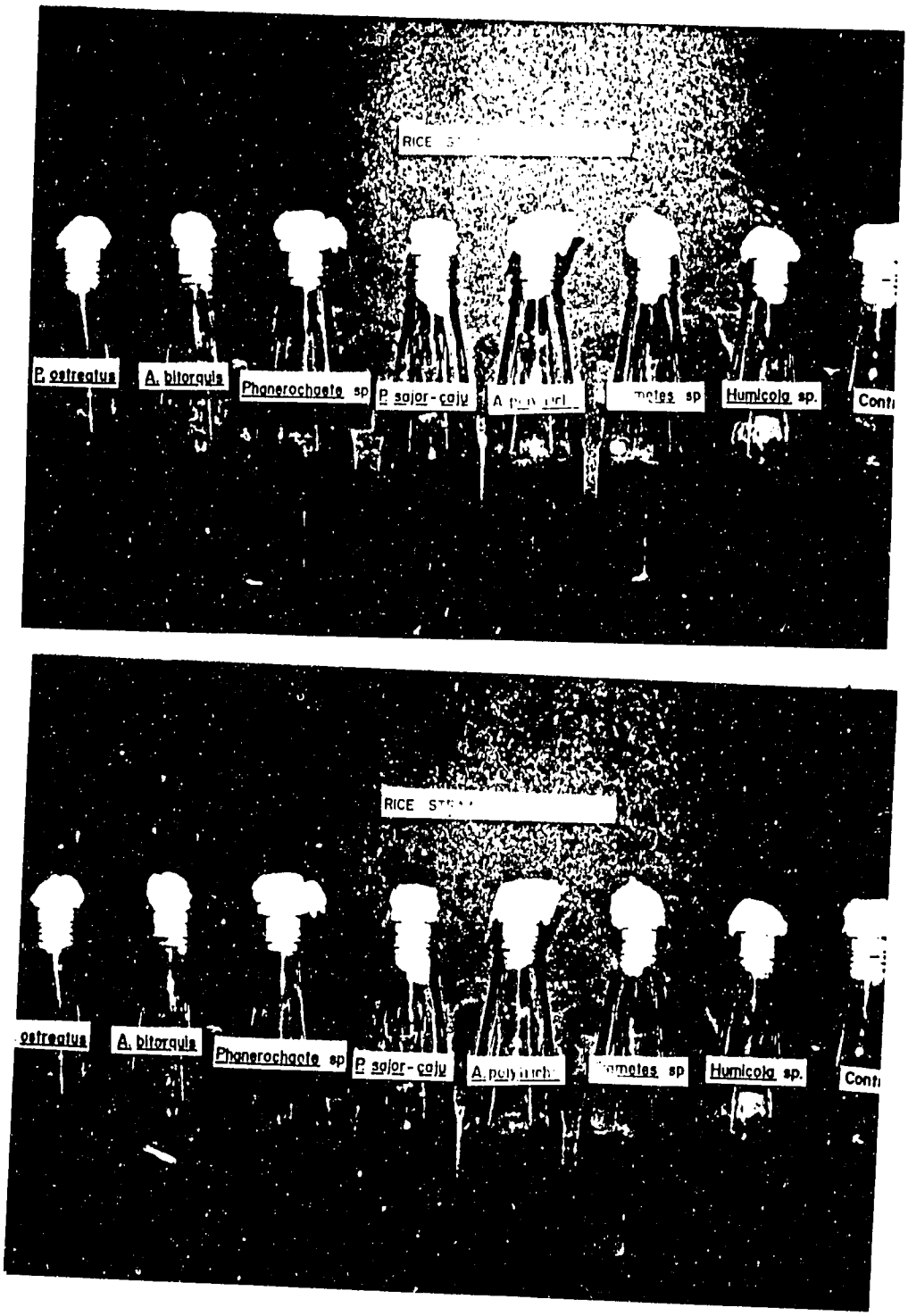


Figure 6. Rice straw and sawdust spent compost of several lignin degraders inoculated with Volvariella showing that of Trametes best enhancing mycelial growth of Volvariella

STUDY V

V. Animal Feeding Trials

A. INTRODUCTION

Rice straw is usually the main substrate component for mushroom growing in the tropics. Having rice as the primary crop in the region, Southeast Asian countries are blessed with abundant straw that can be used for the cultivation of the mushrooms. About seventy-three million tons of straw are produced in the region annually (Chang, 1980). In most places however where utilization of this agricultural waste product has not been popularized, farmers merely burn these straw in preparation for the next planting season.

In the village system of feeding ruminants, crop residues are important part of their diet. However, due to their low protein content and low bio-degradability, these crop residues are not efficiently utilized by the animal. The presence of polyphenolic compound lignin in the cell wall adversely affects digestion and consequently, celluloase rich but highly lignified materials such as cereal straws are of low feeding value as ruminant feedstuff. Their digestibility can, however be improved by physical treatment which increases the surface area available for microbial attack, by chemical treatment such as by using alkali aimed at dissociating lignin from the cell wall and by biological treatment such as by fungal growth which aims to produce microbial protein (Kristensen, 1978) and improve degestibility because of its abiiity to degrade lignin.

In rice-growing countries, rice straw is used as main substrate for growing mushrooms. As such, a lot of spent substrates are available after harvesting these mushrooms. These are merely disposed or burned after full harvest. In other cases, these spent compost are used as soil conditioners providing the soil with additional nutrients available in the compost.

Literature are numerous concerning the nutrients available in the compost after harvesting the mushrooms and their possible role as animal feeds. This is due to the breakdown of the lignin content of the substrate by the mushroom organism as well as the additional nutrients provided by the mycelium of the mushroom left on the spent substrate. Most of these reports have used straw other than rice straw, and have also used different compost formulation thus the performance of these spent compost as animal feeds vary accordingly.

In the present study, attempts were made to use the spent compost from Volvariella and Pleurotus farms as supplements to chicken and swine feeds as well as direct feed to sheep.

The animal feeding tests involved feeding trials of broilers and layers using three types of spent compost; i.e. Volvariella-spent, Pleurotus spent and Volvariella-Pleurotus spent compost. As previously explained in Phase IV, the Volvariella-spent compost is the product of growing Volvariella mushrooms on beds made up of rice straw and banana leaves. Harvesting of the mushroom starts at the 12th day after bedding and spawning and continues up to 30 days after which the substrate is considered spent. The Pleurotus-spent compost is the spent

substrate after harvesting Pleurotus for one month after the start of fruiting. The substrate used was rice straw and rice bran which was packed in heat resistant plastic bags, sterilized and inoculated. One month after inoculation, the bags were opened and allowed to produce fruiting bodies. After one month on intermittent fruiting, the substrate was considered spent, dried and used for animal feeding trials. The Volvariella-Pleurotus spent compost was prepared by growing Volvariella first on rice straw and banana leaves bed. After the first harvest or 15 days after spawning, the composted substrates were cut into at least 6 cm long, added with 20% rice bran, bagged, sterilized then inoculated with Pleurotus. The resulting spent compost after harvesting the mushroom is thus called Volvariella-Pleurotus spent compost.

B. METHODOLOGY

a) Broiler and layer feeding:

Each rice straw product was inocrporated in broiler and layer diets at 7.5%. This level was chosen since at 10% cost of ration (feed) will be much more expensive than the control ration. All diets were formulated to be isonitrogenous and isocaloric and to meet recommended nutrient requirements.

Eighty (80) day old Arbor Acre broiler chicks were all given the control ration for 3 days and then shifted to diets with rice straw

spent compost on the fourth day after randomization to eight dietary treatments. Ten birds per treatment were individually caged and given the diets for 32 days.

Criteria used were initial and weekly weights, weekly and total feed consumed, mortality (if any) and feed efficiency.

In case of layer feeding, same treatments were followed but using 42 ready-to-lay Hyline pullets. These were individually caged and given 100-110 grams feed formulation oer day for 14 weeks. Egg production was recorded daily.

RESULTS:

As shown in Table 1, diets with spent mushroom compost were comparable with the control diet in al criteria. Rice straw in which mushrooms are grown are more palatable than ordinary or plain rice straw. Significantly more feed was eaten and birds grew faster when rice straw spent compost was used, compared with plain or pure rice straw. Adjustment of weight gains to an equal protein indicating that the major effect was on feed consumption.

Statistically significant differences were obtained among the three types of rice straw spent compost products. More of diets containing rice straw + rice bran - Pleurotus was consumed followed by rice straw + banana leaves - Volvariella - Pleurotus. Least palatable was the rice straw + banana leaves - Volvariella. Again, observed weight gains suggested feed consumption as the major factor influencing growth of birds. Differences in feed consumption led to differences

Table 1. Summary of broiler performance: isolated effects of factors

FACTORS	Feed Intake (KG)	Gain in Weight (g) observed	Gain in Weight (g) adjusted 1/	Feed Efficiency	Feed adj. gain
Among levels of rice straw:					
0%	2.0152 (10)[a] 2/	835.6[a]	796.3[a]	2.526[a]	2.537[a]
7.5%	1.9473(70)[a]	803.6[a]	809.3[a]	2.475[a]	2.418[a]
Between RS (pure) and RS-mushroom:					
Rice straw	1.6903 (10)[b]	686.9[b]	831.4[a]	2.533[a]	2.041[b]
Rice straw-mushroom	1.9901 (60)[a]	823.1[a]	805.6[a]	2.466[a]	2.481[a]
Among RS with mushroom:					
RS + BL - V	1.8626 (20)[b]	759.7[b]	808.1[a]	2.533[a]	2.313[b]
RS + RB - P	2.0729 (20)[a]	869.9[a]	812.3[a]	2.394[a]	2.563[a]
RS + BL - V - P	2.0348 (20)[ab]	839.7[ab]	796.5[a]	2.470[a]	2.567[a]
Among levels of cellulase 3/					
0 Cellulase	1.9246 (40)[a]	791.9[a]	808.1[a]	2.485[a]	2.394[a]
1:1 4/	1.9774 (30)[a]	819.2[a]	810.9[a]	2.463[a]	2.450[a]
c.v. (%)	14.14	20 46	6.31	11.11	15.46

in feed intake and, therefore, to differences in protein intake (which is responsible for growth/tissue synthesis). When adjusted to a common/equal protein intake, all three products would have shown similar gains, as shown in Table 1.

Table 2 showed the summary of the layer performance. It showed siginificant differences in feed consumed (PL.01) and very little or no significant differences in percent hen-day egg production, egg weight, and feed efficiency expressed as feed per dozen eggs or feed per kilogram eggs produced.

Although not indicated in the table, medium egg production occurred at the 6th to 9th week after start of feeding the treatment diets to the 21-week old pullets, with 80.58%, 79.90%, 82.62% and 79.56%, resp-ctively. There was a perceptible drop in egg production from the 11th week to the 14th week.

Use of 7.5% "spent" rice straw reduced yellow corn usage in the diets by 18.1%, or from 50% in the control diet to 40.95% in diets containing spent rice straw-mushroom by-products. Economy of substitution will depend on the relative prices of the different feed ingredients at any time and on their availability in the market, as well as cost of hauling, drying and grinding of the spent straws for mixing into layer diets.

Table 2. Summary of layer performance (14 weeks feeding), mean of 6 reps/treatment 1/

TREATMENT	Feed Consumed (total, kg)	Total Egg Prod'n (% hen-day)	Egg Wts. (g/egg)	Feed Efficiency/ dozen eggs	Feed Efficiency/ kg eggs
T1 - Control (0 RSM)	56.5^{c}	72.11^{a}	51.4^{ab}	1.615^{a}	2.689^{a}
T2 - RS + BL = V (0 Cellulase)	51.3^{e}	67.86^{a}	51.9^{ab}	1.600^{a}	2.442^{a}
T3 - RS + BL = V (1:1 Cellulase)	54.7^{d}	77.38^{a}	53.2^{a}	1.458^{a}	2.384^{a}
T4 - RS + RB = P (0 Cellulase)	55.2^{d}	73.47^{a}	53.4^{a}	1.538^{a}	2.465^{a}
T5 - RS + RB = P (1:1 Cellulase)	58.2^{a}	75.51^{a}	52.0^{ab}	1.612^{a}	2.645^{a}
T6 - RS + BL - V - P (0 Cellulase)	55.3^{d}	69.90^{a}	50.1^{b}	1.685^{a}	2.813^{a}
T7 - RS + BL - V - P (1:1 Cellulase)	57.2^{b}	70.07^{a}	50.3^{b}	1.705^{a}	2.876^{a}
c.v. (%)	16.18	14.92	15.54	16.34	15.08

1/ Means with common superscripts are not significantly different ($P < .05$)

b) Swine experiment

Two types of RS-Mushroom by-products were tested in this experiment, i.e. 1) rice straw + rice bran planted to Pleurotus and 2) a combination of rice straw and banana leaves planted first to Volvariella and then to Pleurotus. The products were dried, ground, analyzed for chemical constituents and incorporated at 7.5% each in grower diets for feeding from 30 to 60 (±1) kg liveweight and in finisher diets from 60 kg up for a total of 70 days feeding period.

Three littermates from each of three sows were distributed in a randomized complete block design in the experimental site at the Institute of Animal Science. Initial weights were taken. Feeding was ad libitum or as much as each animal can consume per day.

Total feed per pig was determined weekly. Liveweights were measured biweekly. One piglet in treatment 3 fell sick early in the experiment and, although it recovered, was not able to catch up with the rest, and data for this pig was not included in the statistical analysis.

RESULTS:

Statistical analysis of all data did not show any significant difference in performance among pigs fed commercial type rations (corn-based) and treatments containing "spent" rice straw by-products of mushroom cultivation, even without cellulase treatment. Except for a slight decrease in feed intake, which was not significant, comparable

Table 5. Summary of pig performance (mean of 3 reps/treatment, 70 days feeding)

	No. of piglets	Initial wt (kg)	Final wt (kg)	Feed consumed total (kg)	AD gain (kg)	Feed Efficiency
T1 (control)	3	34.50[a]	78.75	192.8	0.632[a]	4.371[a]
T2 (RS + RB - P)	3	37.17[a]	81.33	174.0	0.631[a]	3.997[a]
T3 1/ (RS + BL - V - P)	3	33.25[a] 1/	76.0	177.2	0.611[a]	4.153[a]
C.V. (%)		14.88			12.13	14.25

1/ Data for one pig in T3 who became ill was not included in the statistical analysis

growth rate and feed efficiency were shown by pigs given diets with "spent" straw. These suggest that the spent straw can be a source of energy for pigs, up to 7.5% of the diet, without deterioration in performance. Economic viability will depend on prices of conventional feeds, however, and on cost of processing (hauling, drying and grinding) of spent straw as feed ingredient.

c) Sheep (ruminant) feeding

Twelve male sheep were randomly distributed to two dietary treatments; 6 head per treatment. For the control diet (Treatment 1) pure rice straw (PRS) and concentrate were given and spent mushroom substrate (SMRS) plus concentrate were offered to the other treatment (Treatment 2).

Initially, SMRS and PRS were fed *ad libitum* and concentrate was given at 1% of body weight (BW) in the early phase of the feeding trial. More than two weeks after, stargrass was incorporated in the ration at 1% BW, SMRS and PRS reduced to 1% BW and concentrate also at 1% BW for the next phase of the study. Consequently, SMRS and PRS were further reduced to .5% BW, stargrass at 1% BW and concentrate at 1.5% BW (Phase 3). For the next phase until the end of the feeding trial, SMRS and PRS were again increased to 1% BW, stargrass .5% BW and concentrate remained the same at 1.5% BW on dry matter basis.

The animals were dewormed before the start of the actual feeding trial. They were individually penned and cleaning of peus were done daily for proper hygiene and sanitation.

The feeding periof lasted for 146 days.

RESULTS:

In the early days of the feeding period ad libitum feeding of SMRS and PRS was done and for concentrate, 1% on dry matter basis was offered for the two treatments. After about two weeks, two animals in treatment 1 and three animals in treatment 2 died of rumen atony. Post-mortem examination showed that the rumen was found to exhibit doughy mass. The small and large intestines contained watery materials. No observable changes where noted in the riticulum, omasum and abomasum. The rumen malfunction could be attributed to a very low fiber intake because of the absence of fresh roughage in the diets of the animals. Hence, stargrass was incorporated in the ration to prevent rumen atony.

In one animal on SMRS diet for instance, an abrupt decrease in weight was observed after two weeks of feeding (6.8 kg decrease in weight) and the rest of the animals on the same diet also exhibited decrease in weight. The animals on PRS diet had positive gain in weight during the same period. The trend continued on an up and down trend for SMRS while the group on PRS steadily increased in weights. After the end of the feeding trial, SMRS diet had a negative result whereas PRS had an average daily gain (ADG) of 11.99 gm (see Table 2).

The actual total dry matter intake per day of the two treatments was lower than the daily requirement.

The results in this study appeared to be negative for SMRS. Despite the inclusion of stargrass and concentrate at 50% fo total ration, the animals fed SMRS diet had an average net loss in weight of 2.6 kg. The negative result in this study can be attributed to low intake and low digestibility of the spent substrate.

Table 2. Mean value of sheep fed the experimental diets.

	Treatment 1	Treatment2
No. of animals[a]	4	3
Ave. initial weight	17.65	21.20
Ave. final weight, kg	19.40	18.60
Ave. daily gain (loss) g	11.99	17.8
Ave. daily DM intake, g		
Mushroom spent substrate	-	152.53
Rice straw	177.67	-
Stargrass	98.27	94.83
Concentrate[c]	244.64	231.64
Ttoal	520.58	479.00
Feed efficiency g feed[d] / g gain	68.04	negative

[a]Two animals in treatment 1 and three animals in treatment 2 died of rumenatony

[b]Treatment 2 has negative result

[c]Composed of 60% rice bran, 30 copra meal, 5% salt and 5% bone meal

[d]Treatment 2 has negative result

While most of the literatures regarding the use of most mushrooms showed an improvement in the feeding value of the residual substrate due to lignin degradation and improvement in the chemical composition (Zadrazil, 1979; Hayes & Lim, 1970, 1979; Langar et al, 1980) chemical analysis of Volvariella-spent compost in the Study III, showed no improvement in the chemical composition of the spent substrate. Altho, there was lower cellulose and hemicellulose content, other cell wall constituents (acid detergent fiber, neutral detergent fiber, lignin and silica) were high. This agrees with previous reports that Volvariella is low lignin decomposer (Chang-Ho & Yee, 1977).

Unfortunately, due to lack of materials, spent compost of Pleurotus mushroom, reportedly good lignin degrader, was not used in this study.

9. SUMMARY AND CONCLUSION:

a) The first study had shown that different isolates and/or strains of the mushrooms differ in their rate of mycelial growth, the manner and quantity of fruiting, appearance and size of fruits, resistance to pests and their adaptation to specific environmental conditions. Thus before one should start growing mushrooms, the best isolate should be chosen or screened in order to obtain best yield of the mushroom.

Out of several isolates and strains kept at the Culture Collection of Edible Fungi (FCUP) of the Department of Plant Pathology, Volvariella isolate 600 and Pleurotus isolate 503 were chosen as the test materials for all subsequent stduies.

The method of growing Volvariella on tiered 2-m long beds and the plastic bag technique of growing Pleurotus were adopted based on preliminary exercises where these technologies were found to be highly adapted to local conditions.

b) The next study (II) shows that growth of Volvariella had caused increased in lignin, cellulose and hemicellulose until the end of its cycle (4th week) at which time only when decrease on these components were noticeable. At this stage however, the compost beds being formed outdoor appear to be fully colonized by some other organisms or contaminants that may cause adverse changes in the composition of the spent substrate. It is therefore made that

the substrates be used after only the first harvest (2 weeks) at which time, 3/4 of the expected yield has been obtained. At this time however, lignin is still high, even higher than raw straw. It is therefore desirable that this V-spent substrate is further used to grow Pleurotus before the final spent compost is used to supplement feeds for chicken and swine.

Raw substrates (RS + RB) used for growing Pleurotus showed consistent decreasing amount of lignin, cellulose and hemicellulose as well as resulting increase in protein after full harvest of the mushroom.

c) Tests made in Study III have shown how utilization of rice straw can be maximized and quality of spent compost improved. A diagram shows that rice straw can be used to prepare Volvariella spawn (planting material). It can also be used to prepare Volvariella outdoor beds. After 2 weeks and first harvest of the mushroom, the rice straw compost substrate is used either to prepare Pleurotus fruiting bags or prepare Volvariella beds for indoor cultivation, after which the spent compost can be used as animal feeds.

d) A strain of actinomycetes and a strain of bacteria were isolated from Volvariella beds and shown to enhance mycelial growth of the mushroom in vitro.

Among the lignin degrading fungi tested, Trametes was shown to enhance mycelial growth of Volvariella, apparently by degrading lignin of the substrate to give way to the growing mycelium of Volvariella.

e) Feeding trials with broilers and layers showed that rice straw in which mushrooms were grown are more palatable than plain or raw rice straw. Significantly more feed was eaten and birds grew faster when rice straw spent compost was incorporated at 7%. With layers, results showed siginificant difference in feed consumed and very little or no significant differences in % hen-day egg production, egg weight and feed efficiency expressed as feed per dozen eggs or feed per kilogram eggs produced. Use of 7.5% spent rice straw reduced yellow corn usage in the diets by 18.1%. Results with feeding trials on swine didnot show any significant difference in performance among pigs fed with commercial type rations (corn-based) and treatments containing "spent" mushroom compost. Except for a slight decrease in feed intake, which was not significant, comparable growth rate and feed efficiency were shown by pigs given diets with "spent" straw. These suggest that the spent straw can be a source of energy for pigs, up to 7.5% of the diet, without deterioration

in performance. Economic viability will depend on prices of conventional feeds, however, and on cost of processing of spent straw as feed ingredient.

Results of sheep feeding with Volvariella spent compost appeared to be negative. Animals fed with the spent compost had an average net loss in weight of 2.6 kg. The negative result can be attributed to low intake and low digestibility of the spent substrate. This is however expected since the analysis of V-spent compost (Study III) shows still high amount of lignin and low protein. Unfortunately, due to lack of materials, spent compost grown with Pleurotus whose analysis showed improved chemical composition was not used in this study.

10. LIST OF PUBLICATIONS AND PRESENTATIONS FROM THE PROJECT:

a) "Mushroom Spent Compost as Animal Feed"

A Diamond Jubilee Second Professorial Chair Lecture delivered by the author, February 2, 1989, UPLB.

b) "Mushroom Spent Compost for Animal Feed and Fertilizer"

A lecture delivered by the author to the staff of the Mushroom Section, University of Puerto Rico, Mayaguez, Puerto Rico, October, 1988.

c) "Continuous Recycling of Rice Straw in Mushroom Cultivation"

Paper presented at the GIAM VII Hongkong, August, 1988 and published at "Recent Advances in Biotechnology and Applied Biology" Chang et al (eds) pp. 595-602.

d) "Microbial Substrate Pre-treatment for Optimal Mycelial Growth of *Volvariella volvacea*"

Abstract submitted to the International Symposium on Mushroom Biotechnology China, November 6-10, 1989.

e) "Maximal Bioconversion of Rice Straw into Edible Mushrooms"

Abstract for Poster Session on XIIIth International Congress on the Science and Cultivation of Edible Fungi, Braunschweig, September 20-26, 1987.

f) "Maximal Utilization of Rice Straw Through Mushroom Cultivation"

Paper presented at the meeting of PSTC grantees on Biomass Production and Conversion. University of Faisalabad, Pakistan December 13-15, 1989.

g) "Screening for Maximal Mycelial Production of some Tropical Mushrooms for Increased Substrate Bioconversion"

Manuscript prepared for publication; research done at University of Viterbo, July 1-30, 1988.

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