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Agro Enterprise Project

Keells Agro Products Limited

Shiitake

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SUMMARY

Review of current production problems experienced at the Shiitake growing facility was conducted over a 2-week period during early November, 1993. Findings indicated that excessive and inconsistent heating of substrate during sterilization has resulted in poor mycelial colonization of substrate. In addition, use of non-precooked wheat for spawn production has resulted in inconsistent and non-uniform spawn.

The current rate of contamination (approximately 20-25%) of substrate has reduced yields and resulted in excessive waste of resources. Procedures required to address these problems have been outlined.

Finally, the company is encouraged to incorporate a clean room - dirty room concept for processing substrate at the earliest possible time. Current practices on the farm will not allow contamination rates to drop much below 5 to 10% without implementation of clean room technology. Continuation of such a high contamination rate may, in the future, place the company in a tenuous situation.

I INTRODUCTION

Shiitake production and consumption world-wide is increasing at about 15-20% per year. From an agricultural perspective, this growth rate is seldom duplicated. Keells Agro Products Limited has produced Shiitake since 1987 and is well positioned in the international market place.

Relative to other countries, Sri Lanka enjoys an inexpensive labor force. This provides an advantage over most other countries where labor is substantially more expensive. In developed countries, labor accounts for about 50% of production costs.

According to management, energy accounts for approximately 35 - 40% of costs associated with producing Shiitake at Keells Agro Products Limited. With a relatively high cost of energy, it was felt that emphasis should be placed on developing strains of Shiitake that require higher optimum production temperatures. It was suggested that development of such strains, through breeding and selection, would substantially reduce production costs. In addition, management desires to improve overall productivity by improving substrates and incorporating improved cropping procedures.

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My task was to review the current situation at the Shiitake production facility and provide information that may help in achieving the goals outlined above. This report is a summary of the findings over a 2-week visit to the Shiitake production facility November 2 - 15, 1993. The report has been organized into cropping phases and includes both research needs and outlook sections.

II SPAWN PRODUCTION

Wheat, soaked overnight in cold water, is the main ingredient used to make spawn. Approximately 500 g of soaked wheat is mixed with $CaCO_3$ and $CaSO_4$ and filled into polypropylene bags. The mixture is autoclaved for about 7 hours at 121 C, cooled, and inoculated with a macerated culture of Lentinus edodes. After about 10 days, the spawn is shaken to more evenly distribute the growing mycellium. Upon maturity the spawn is aseptically filled into sterile polypropylene bottles.

The procedures, as outlined above, have not resulted in the production of the highest quality spawn possible. Moisture contents usually average about 30 to 35% while the optimum spawn moisture content is in the range of 47 to 48%. Lower moisture contents result in uneven mycelial colonization of the grain. As a result, the spawn does not "fuzz out" as rapidly when inoculated into sterilized substrate.

An additional problem currently exists with the availability of a consistent variety of wheat. Both hard and soft wheats are used in the spawn making process. Use of both types of wheat makes it difficult to produce a consistent product.

Considering the above issues, it would be desirable to use a consistent variety of wheat and implement a pre-cooking process to increase the moisture content of the spawn. In addition, the use of solid inoculum instead of liquid inoculum would reduce the levels of contamination currently experienced in the spawn plant. Pre-cooked spawn successfully was produced in the laboratory with the desired consistency. For larger scale production, it will be necessary to install steam kettles in the filling area to precook the spawn grain.

In order to reduce the spawn contamination levels in the spawn lab, it is suggested that the polypropylene bottles, originally used for spawn production, again be used. Laboratory personnel are aware of the procedures necessary to implement the above procedures.

Finally, it would be desirous to have cold storage for both the mature spawn and for the wheat grain. Cold storage for spawn is necessary to maintain the material in optimum condition while cold storage for the wheat would allow a continuous supply of the same type (either soft or hard) with control of wevil infestations.

III SUBSTRATE PREPARATION

A mixture of rubber sawdust, millet, rice bran and a special additive are moistened (60% water) and filled into polypropylene bags. The bags are closed with polypropylene caps containing a foam rubber plug.

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To examine variability of bag fill weights, two samples of 20 bags each were collected and individual bags were weighed. Means, standard deviations (SD) and coefficients of variation were calculated for each sample (Table 1). Mean weights of the two samples were 964 g and 989 g. This is below the target range of 1 kg. Addition of more substrate, however, causes difficulties in the inoculation process due to inadequate head space between the substrate and bag collar. Thus, fill weights are near the practical maximum.

Coefficients of variation in bag weights were at or below 5%. For most biological systems, a 5% CV is an acceptable range. Therefore, variation in bag weights are near the expected values.

The current substrate formulation contains about 11.5% (dry weight) of millet. Current levels used in the USA average about twice as much (20 - 25%). Therefore, experiments are suggested to optimize millet levels with regard to the current strain of Shiitake used at the farm.

IV SUBSTRATE STERILIZATION

Autoclave containers (2) loaded with 180 Kg substrate each are placed by forklift into each of eight autoclaves. The substrate is subjected to 140 C for a desired time of 1 hr. Various problems have existed with the current mode of autoclave operation.

Blackening of the substrate and subsequent inhibition of mushroom mycelial growth is observed during the incubation period. Thus, Ceylon Institute of Scientific and Industrial Research (CISIR) was commissioned to conduct a study of temperature profiles of substrate subjected to autoclave temperatures.

Their study recommend the replacement of pressure gauges fitted to the autoclave. They also suggested the installation of an automatic system to regulate temperature of the autoclaves. This has not yet been initiated. Some improvements have been made, but autoclaves still are not functioning at the desired level of performance. Considerable variation exists in the time required to heat up to 140 C. Means, SDs, and CVs, for heat-up times are presented in Table 2. Heat-up times varied from a mean of 89.6 minutes to 106.1 minutes (Table 2). Standard deviation ranged from 20.7 (autoclave No.6) minutes to 11.6 (autoclave No.1) minutes. Coefficients of variation were all above 5% ranging from 11.1% (autoclave No.8) to 20.7% (autoclave No.6). These data point out the considerable variation that exists in the heat-up mode. A more controlled process should be developed.

Data similar to heat-up times were noted for sterilization times. An analysis of variation of sterilization times is presented in Table 3. Mean sterilization times ranged from a low of 56.6 minutes to a high of 83 minutes. Autoclave No. 3. had a SD of over 38 minutes with a CV of 46% (Table 3). None of the CVs were below 15%. These data point out the huge variation in sterilization times experienced from batch to batch and autoclave to autoclave. Such variation must be brought under control if a consistent, high yielding substrate is to be produced.

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The above problems can be addressed by the installation of automatic control systems or by increased supervision of the autoclaves by the operators. It is nearly impossible for a single person to operate the 8 or 9 autoclaves in an acceptable manner. Additional personnel will be necessary if automatic controllers are not installed.

The most serious problem with substrate sterilization at the farm, however, is the excessive temperatures used to heat treat the substrate. Our experience in the USA is that exposure of the substrate to temperatures above 126 C results in the generation of toxic products in the substrate. These products inhibit mycelial growth and thereby reduce productivity of the strain. It is suggested that autoclave temperatures be lowered to below 126 C. Maintenance of temperature of 123 C in the autoclaves for 2 hrs has proven sufficient to kill spore forming bacteria. Sterilization can be confirmed by a laboratory test within 48 hrs. Continuation of substrate heat treatment at autoclave temperatures of 140 C will continue to depress mushroom yields.

V INOCULATION

After the autoclaving process is complete, the cooled, bagged substrate is removed from autoclave skids and placed in plastic transport containers. Transfer of the bags from autoclave skids to transport containers occurs in the bagging area. This area is biologically dirty and exposure of autoclaved bags to this area soon results in contamination of collar surfaces. Contaminated collar surfaces increase the chances of contaminants gaining entry to sterile substrate during the inoculation process.

Contamination currently is a serious problem on the farm. Data collected on November 4, 1993 from three days of production reveal that approximately 25.7% of bags are contaminated (Table 4). Contamination percentages ranged from 33% (October 25 inoculation) to 15% (October 26 inoculation). Such high rates of contamination could place the company in a tenuous situation.

A closer look at contamination routes reveals that approximately 42.5% of bags contaminated eminate from holes punched in the bags by physical means (Table 5). Other routes of contamination account for about 57.5% of the total contamination. Physical damage of the bags can be reduced by tucking in bag corners at bag fill and eliminating exposed wires that gouge holes in the bags. Procedures currently are underway to correct the physical damage to the bags.

It is unclear as to the contribution of each aspect of the other means of contamination (Table 5). Such factors as dirty collars and caps, improper inoculation procedure, faulty cap plugs and use of contaminated spawn all contribute to the production of contaminated logs. Some factors contributing to contamination will be impossible to entirely eliminate due to design of the autoclave rooms. The current materials flow does not take advantage of a dirty room - clean room process. Autoclaves are not double-doored so that "dirty" material enters at one end of the autoclave and sterilized material exits into a clean room from the opposite end. For long term viability of the farm it will be necessary to implement the clean room - dirty room concept. Plans should be made to convert the current system to this technology at the earliest time.

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VI INCUBATION

Vegetative mycelial growth takes place in spawn run rooms controlled at the appropriate temperature. Inoculated substrate, contained in bags, is placed on wire racks during spawn run. Optimum spawn run should be completed in seven (7) weeks. This has not been the case, however, and spawn run often requires nine weeks or more to complete. Even after 9 weeks, mycelial growth may not reach the bottom of the bagged substrate. This slow spawn run is due to the overheating of the substrate during autoclaving as outlined under the section "substrate sterilization".

Exposed wires on shelves often are a source of physical damage to bagged substrate. Workers stacking substrate on shelves often gouge bags on the jagged edges of the wire shelves. To eliminate this problem it is suggested to "fold under" the exposed ends of the wires so that bags cannot be snagged. Personnel are aware of the procedure to "fold under" the edges of the wire racks.

VII CROPPING

Problems experienced in cropping, i.e., reduced yields are a result of earlier problems outlined in previous sections. Once improvements are made in earlier processes, yields will increase 20 to 25%. Purchase of a CO_2 meter is needed to monitor and control fresh air intake based on CO_2 levels in the production rooms. Monitoring of CO_2 levels may help to reduce stipe length and improve overall biological efficiency. In many markets, a larger mushroom is desired. Research has shown that greater volumes of substrate per bag i.e. 2-2.5 Kg result in increased mushroom size. Experimentation should be performed with substrate bag weights greater than 1 Kg to improve mushroom size.

VIII ENERGY CONSUMPTION

Air conditioning currently accounts for the bulk of electricity consumption on the farm. Ways should be found to reduce cooling costs. One obvious way is to control fresh air intake based on CO_2 levels in the room. Currently, CO_2 is not monitored and therefore fresh air is introduced by "feel" of the air. This is imprecise and should be based on CO_2 levels.

Finally, lighting needs in both growing rooms and incubation rooms should be reassessed. In the USA, growers typically use 3 to 4 hrs lighting per day in both the incubation rooms and production rooms. Lights are placed vertically along rack shelves to provide adequate light to bottom shelves. Current light placement at the farm is not ideal as most lights are located overhead so that adequate light does not reach bottom shelves. Relocation of lights to vertical positions along shelf posts would allow adequate light with much shorter duration. Thus, lighting costs could be reduced by 50%. Considering the 1,488-40 watt bulbs in operation on the farm 12 hrs/day, the savings would be significant. Reduced lighting would also reduce cooling demands due to less heat generated by light bulbs.

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IX RESEARCH

1. Establishment of a research laboratory and research production facility as proposed by management at the farm should continue to receive high priority. Operation of such a facility will pay substantial dividends in the future. A list of equipment and supplies needed for the research laboratory is presented in Table 6.

2. Project research oriented toward increasing productivity should receive high priority. The development of more productive substrate through improved nutrient supplementation would yield results within 9 to 12 months. In addition to, and coupled with, substrate development should be the screening of other commercially available germplasm. Such strains are readily available from the Pennsylvania State University Mushroom Culture Collection and from commercial sources.

3. Newly emerging cropping procedures, developed in the USA should receive attention in the research facility. Through-spawning, bag removal at 3 weeks and curing should be tried and developed to improve productivity and reduce energy costs (cooling of production rooms to 10 C).

4. Alternative wood species should be tried to improve productivity. More dense wood sources such as Jack fruit sawdust should be examined singly and in combination with rubber sawdust. Combinations may improve spawn run and subsequent productivity.

5. Breeding and selection of new lines should receive a lower priority than the above projects. Until more productive substrate is developed, selection of new hybrids would be an inefficient exercise. In addition, breeding and selection of lines for higher production temperatures would yield minimum gains relative to the research efforts required to run such a breeding and selection program.

X OUTLOOK

Given the labor cost situation in Sri Lanka, the company is in a good position to capitalize on the growing world-wide demand for Shiitake. If the company commits to the utilization of the latest technology then shiitake production can be much more profitable.

The company should consider sending their representatives to international conferences and workshops so that they are aware of the latest technological advances. In addition, the company should continue to have production procedures reviewed by a consultant 2 to 3 times per year. Such reviews should result in substantial savings and productivity increases.

Finally, it is suggested that the company establish a formal relationship (a sustaining membership with the library) at the University of Kelaniya. Such a relationship will allow use of reference materials, software, etc as needed.

In conclusion, I have been favorably impressed with the kind and generous personnel at Keells Agro Products, Ltd. This is a group composed of highly dedicated and professional individuals.

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Table 1.

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Mean, Standard Deviation (SD) and Coefficient of Variation (CV) for bagged substrate weights at time of fill.

Sample No.*	Mean (g)	SD (g)	CV (%)
1	964	42.5	4.4
2	989	53.9	5.5

* Each sample is average of 20 bags selected at random on Nov. 5, 1993.

Table 2.

Mean, Standard Deviation (SD) and Coefficient of Variation (CV) for times taken for autoclaves to reach 140 C on November 3, 1993.

Autoclave No.	Mean time (min)	SD (min)	CV (%)
1	89.6	11.6	12.9
2**	-	-	-
3	92.1	12.7	13.8
4	89.7	13.3	14.9
5	106.1	17.0	16.0
6	96.3	20.7	21.5
7	95.7	11.1	11.6
8	102.6	14.2	14.8
9	96.1	18.3	19.0

* Mean based on seven (7) autoclave batches/day.
** Autoclave used for spawn production only.

Table 3.

Mean, Standard Deviation (SD) and Coefficient of Variation (CV) for Autoclave times (after temperature reached 140 C) for 8 Autoclaves on November 3, 1993.

Autoclave No.	Mean time (min.)*	SD (min)	CV (%)
1	63.3	9.5	15.1
2**	-	-	-
3	83.0	38.2	46.0
4	64.6	11.0	17.1
5	56.6	27.7	48.9
6	58.0	18.9	32.6
7	74.0	18.6	25.1
8	60.2	18.4	30.6
9	58.0	14.6	25.1

* Mean based on seven (7) autoclave batches/day.
** Autoclave used for spawn production only.

Table 4.

Batch date, number of logs non-contaminated, and contaminated logs from those selected at random in incubation room on November 4, 1993.

Batch date	Number non-contaminated	Number contaminated	Total	% Contaminated
10/24/93	15	5	20	25.0
10/25/93	20	10	30	33.0
10/26/93	17	3	20	15.0
Total	52	18	70	25.7

Table 5.

Batch date, number of logs contaminated via hole punched in bag or via other means.

	Batch date**			
Contamination route	10/24	10/25	Total	%
Via hole	11	6	17	42.5
Via other means*	19	4	23	57.5
Total	30	10	40	100.0

* May include dirty collars, improper inoculation procedure, faulty cap plugs, and use of contaminated spawn.
** Samples collected on November 4, 1993.

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Table 6. List of equipment and supplies for research lab.

Item No	Catalogue No.	Item	Page No.*
1.	-	Refrigerator	-
2.	-	Computer	-
3.	-	Printer	-
4.	-	File Cabinet	-
5.	-	Book Shelves	-
6.	-	Mycology reference books	-
7.	-	CO_2 meter	-
8.	-	Transfer hood	-
9.	MJ630-10	Steromicroscope	281
	MJ632-15	20x Eyepiece	
10.	AU115-02	Top loading autoclave	15
11.	-	Microwave oven	-
12.	BC280-55	Balance (PJ6000)	29
13.	-	Beakers (250ml, 600ml,1000ml)	73
14.	-	Sink	-
15.	BU170-12	Bunsen Burner (microburner)	101
16.	CY600-26	Plastic Cylinders (100ml,500ml)	158
	CY600-34		
17.	-	Scalpels & Blades(Blades No.11)	173
18.	DS710	Forceps	175
19.	FK series	Flasks (100ml, 500ml, 1000ml)	213
20.	-	Funnel (plastic)	219
21.	OV142-20	Oven (bench top)	302
22.	-	Petri Dishes (plastic)	307
23.	PJ825-10	pH meter	326
24.	PP280-13	Pipettes (disposable 1ml)	333
25.	SE165-25	Parafilm	431
26.	SH305-15	Spatula (stainless steel)	440
27.	SM502-22	Test tube racks (4)	451
28.	TE250-32	Test tubes (100)	474
29.	SS705	Magnetic stirer/hot plate	459
30.	ST630-25	Stirer follower	461
31.	TG500-35	Thermometers (2)	477
32.	TG730-15	Thermometers (6)	479
33.	TH700-12	Temperature strips	485
34.	TL615-30	Timer (1-hr)	488
35.	TM900-40	Wall Clock	491
36.	SA710-12	Heat resistant gloves	502
37.	TT710-30	Platform Tray Trolley	507
38.	WB700-30	Wash bottles (2)	522

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