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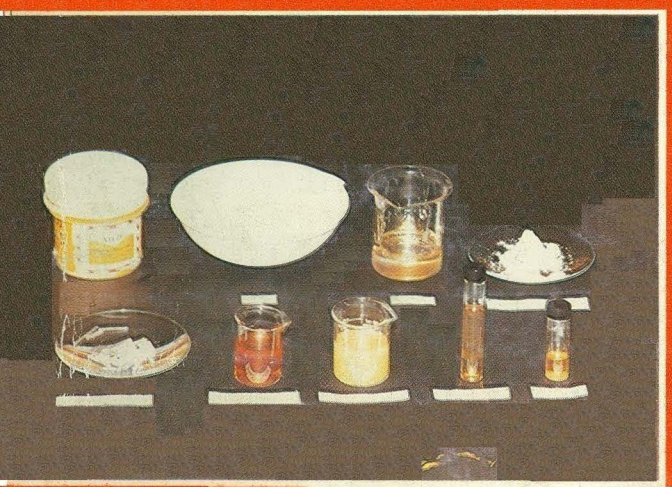

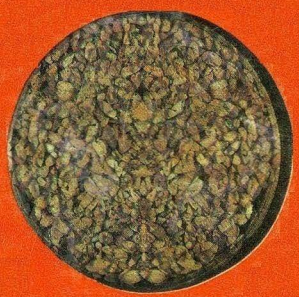

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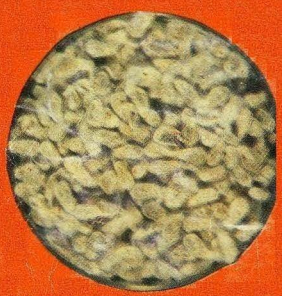

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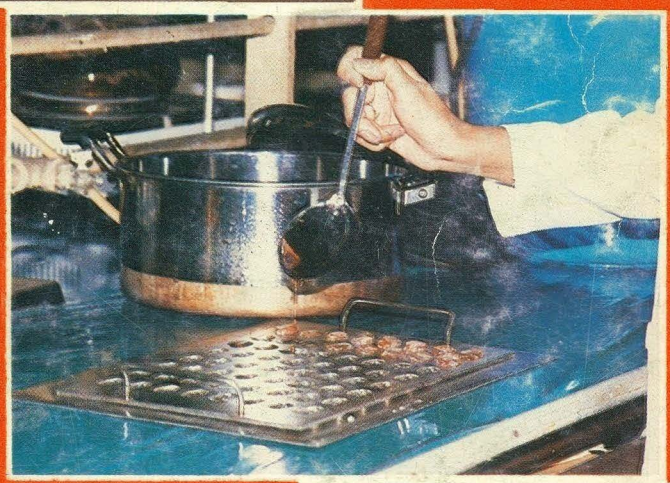

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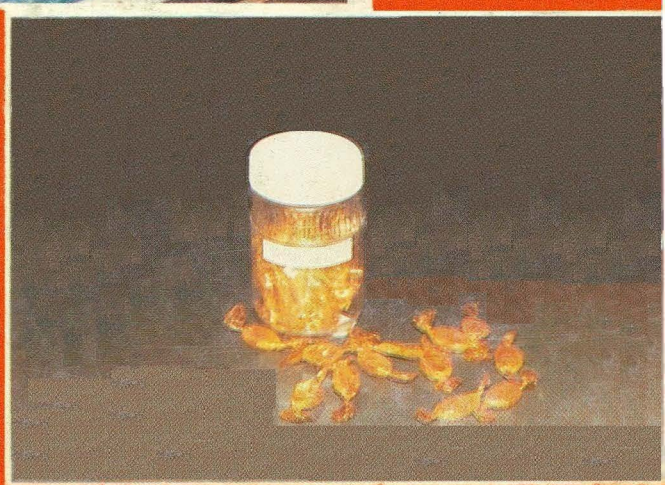

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Raising the Yield Potential of Rice

Benito S. Vergara

ABSTRACT

With the introduction of the modern high yielding rice varieties and appropriate cultural practices, yields have increased in the last two decades. Subsequent efforts to improve yielding ability by increasing photosynthetic rate increasing biomass production and increasing harvest index have not resulted in significant increase in yields. An approach being pursued to achieve this is the manipulation of the weight of single grains. Results of studies have shown the following.

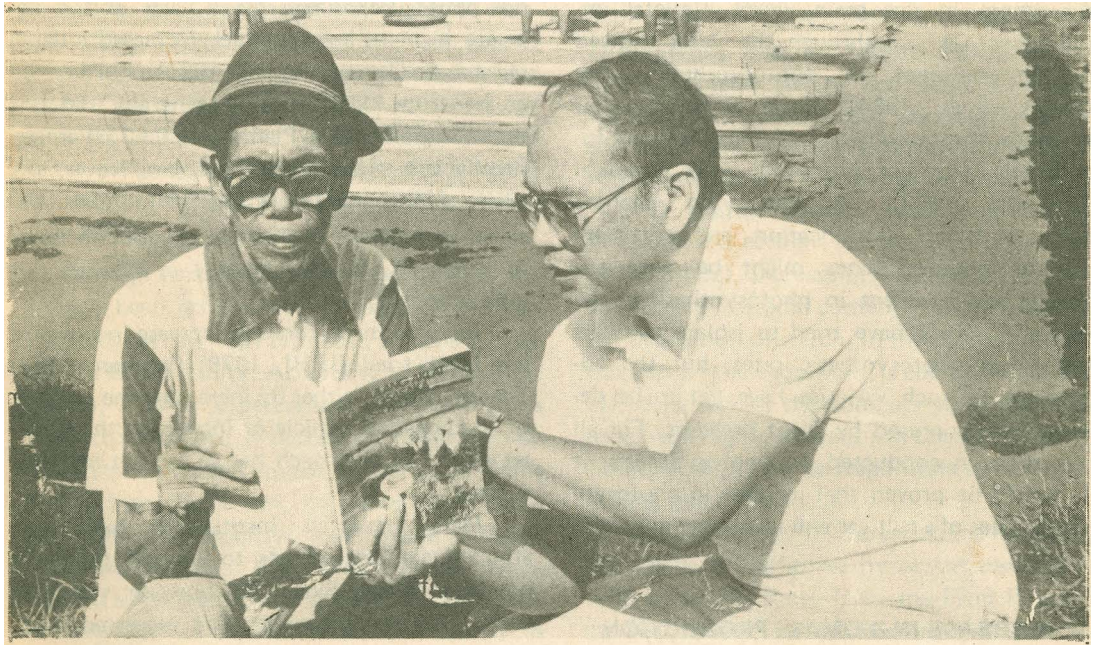
Increasing the number of high density (HD) grains can increase yield potential. HD grains result in better milling recovery and higher head rice recovery. Varietal differences in the number of HD grains per panicle exist.

With a panicle, certain spikelets invariably develop into HD grains. Most spikelets on the secondary branches have low grain weights. Leaves near the panicle are more important in grain filling. Removal of the 4th leaf from the top increased grain weight and number of HD grains

Lower temperature or higher photosynthetically active radiation after anthesis results in higher number of HD grains. Applied nitrogen fertilizer had no effect on the number of HD grains.

Limitations in grain filling may be the result of several factors. Although sufficient carbohydrate is available not all spikelets develop into HD grains. Factors limiting grain filling include structure of the pedicel, the spikelets and growth regulators.

In view of the above findings a new plant type is proposed to break the yield ceiling, Further studies are being conducted to identify the limitations of the current varieties in order to develop the new plant type being proposed



Dr. Benito S. Vergara discusses the Tagalog edition of his book, *A Farmer's Primer on Growing Rice* with a farmer in Calauan, Laguna.

The author is Plant Physiologist, International Rice Research Institute (IRRI, Los Baños, Laguna).

INTRODUCTION

Rice yields have greatly increased in the last two decades mainly through crop improvement and the accompanying cultural practices. With the development of IR8 and subsequent cultivars of similar type rice yields increased in the tropics, but now yields have apparently reached a plateau (Flinn et al., 1982). Subsequent efforts to improve yielding ability have not resulted in visible gains.

The present efforts to raise the yield potential focuses on increase in photosynthetic rates biomass production, and in harvest index (HI) (IRRI 1982).

INCREASE IN PHOTOSYNTHETIC RATE

Research on high photosynthetic rates in the last several years has not really benefited or increased grain yields in most crop plants. Identification of varietal differences in chlorophyll content (Sasahara et al , 1983, Yamakawa and Oshima 1977, Kariya and Tsunoda 1980) and photosynthetic rates (Murata, 1957; Murata and Iyama, 1963) has not led to improvement in rice grain yields. Varietal improvement in rice through the years showed no improvement in photosynthetic rates (Evans et al., 1984). There is no clear-cut evidence that a cultivar with high leaf photosynthetic rate has improved yield potential (Yoshida, 1972). Accompanying changes such as better translocation and partitioning of photosynthates might be necessary for an improvement in photosynthesis to be effective. Many have tried to isolate cultivars with high photosynthetic rates, but the advantages of such varieties have yet to be demonstrated or used by plant breeders. For all the research conducted on photosynthesis, it is yet to be proven that increase in photosynthetic rates of a cultivar will increase grain yield.

INCREASE IN BIOMASS PRODUCTION

Varietal differences in biomass production, more specifically in crop growth rates, have been studied but no improvement has been reported (Evans et al., 1984). The theoretical limit for biomass production has not been reached, but available data suggest that the present high production can be effectively increased only if the growth duration is increased and proper partitioning is obtained. Without proper partitioning, increase in biomass only leads to higher proportion of non-photosynthesizing plant parts or increase in plant height. Without a strong and thick culm, such increases in biomass would only result in lodging and mutual shading and eventual decrease in grain yield instead of the desired increase. This is the case in traditional varieties whose high biomass production in the early stages results in mutual shading so that the mean photosynthetic rate per unit leaf area and crop growth rate decrease.

INCREASE IN HARVEST INDEX

The HI has increased from less than 0.10 to 0.55 in the modern varieties (IRRI, 1978; Evans et al., 1984). This is one of the main features responsible for the yield increase. The increase in HI resulted in less straw or less non-photosynthesizing plant parts and a decrease in plant height, which increased lodging resistance (Tanaka et al., 1966). Further increase from 0.55 to 0.60 generally did not improve grain yields. Plants with 0.60 HI are generally very short with telescoping leaves, low tiller number, and low spikelet number. Because an increase in biomass production tends to lower HI, further increase in HI does not look promising.

Increase in HI through increase in sink size has been tried (IRRI, 1978; Rahman, 1984; Takeda, 1984) either by increasing the number of spikelets per panicle or increasing the spikelet size. This approach has so far not met any success.

Many research institutions have not stopped exerting efforts to increase the yield potential of rice. Since IR8, however, yield potential has not increased. The suggested pathways for increasing yield potentials do not look promising but we still need to look into them until we find other possible pathways.

YIELD COMPONENTS

Another way of looking at the possibility of increasing the yielding ability is to examine the yield components.

Grain yield is the product of the number of panicles per unit area x number of spikelets per panicle x percent fertility of the spikelets x weight of a single grain.

Normally and under tropical conditions, an increase in panicle number per unit area reduces the number of spikelets per panicle and vice versa (IRRI, 1968). Although agronomic practices can improve the number of spikelets per unit area the maximum possible has already been achieved and further increase is very difficult (Takeda 1984).

Increasing the number of spikelets per panicle often results in a large number of empty spikelets (Kumura and Takeda 1962; Matsushima 1957 Wada 1969, Venkateswarlu et al., 1981). This is apparently due to the reduced supply of carbohydrates in relation to the total demand of the spikelets. The optimum number has been reached for the present plant type.

Increasing spikelet size to increase yield potential has also been tried (IRRI, 1978; Rahman 1984, Takita 1986); but without success so far. Generally an increase in spikelet size resulted in a lower number of spikelets per panicle or square meter (IRRI 1978). There is also a tendency for large spikelets to have only partially filled grains (Takita, 1986; Xiong et al 1986).

A high percentage of spikelet fertility has already been achieved in the modern cultivars (Yoshida et al., 1972), most of which have around 85% fertility. According to Matsushima (1966), a fertility percentage of around 85 is the correct balance. A percentage lower than 85 indicates a possible source limitation and one higher than 85, a sink limitation. One could aim for 95% fertility that would increase yield by at most 10%. This increase would have to come from better pollination and better development of the spikelets. The former is greatly modified by environmental conditions such as wind, rain, and high and low temperatures. One has very little control of these environmental conditions.

Another alternative in increasing grain yield is to increase weight per grain within a variety. Very little work has been done along this line because workers have accepted the fact that grain weight is the most stable character of a variety (Matsushima 1970), and have variability within a variety is very small (Yoshida, 1981). A medium grain variety will always produce medium grains regardless of environment and cultural practices. Studies by Venkateswarlu and others (1986b) have shown, however, that weight per grain within a variety is highly variable (Figure 1). One could therefore increase grain yield by increasing the number of heavy or high density (HD) grains.

HIGHER PERCENTAGE OF HIGH DENSITY GRAINS

Within a panicle, some grains are heavier and also have higher density (Figure 2). Usually the 5th and 6th spikelets in a panicle branch have HD grains (Nagato and Chaudhry, 1969; Ahn, 1986). Thus, if we improve the density of the other filled spikelets, one can increase grain yields by as much as 30% in IR8 (Venkateswarlu et al., 1986b). HD grains have not only higher volume and weight (Venkateswarlu et al., 1986b) but also higher milling and head rice recovery (Venkateswarlu et al., 1985a), which is the final market yield of rice.

HD grains, however, have lower protein and crude fat content (Juliano and Ibabao, personal comm.). Increase in grain weight is due to an increase in starch content. Varieties differ in the deposition of starch. In the indicas, the central part of the endosperm is compact and hard; in the japonicas, the compact starch is on the peripheral region (Nagato and Chaudhry, 1969). This property may be responsible for the lower milling loss of japonicas.

The possibility of increasing the number of HD grains is confirmed by recent research results especially those from the Plant Physiology Department at the International Rice Research Institute.

Varieties differ in the number of HD grains per panicle; therefore, selections for varieties with HD grains can be made (Table 1). The HD

Table 1. Varietal differences in high density grains. IRRI, 1985 dry season.

Designation	High density grain index (%)
IR29725	63
IR42	57
IR28222	55
IR28178	50
IR29744	48
Peta	44
IR58	40
IR8	39
Binato	22

grain character is heritable and showed increases in form F_1 hybrids (Figure 3). Late maturing varieties have more uniformity in grain filling than early maturing varieties, and this uniformity resulted in a higher varieties, and in a higher percentage of head rice (Jongkaewattana and Geng, 1986). The occurence of HD grains had no correlation with 1,000-grain weight in the range of 20.0 to 28.0 g (Venkateswarlu et al, unpublished paper). This would mean that rice grains of varying sizes can be developed while maintaining a higher percentage of HD grains.

Contrary to expectations, increasing N application from 0 to 250 kg/ha did not decrease the number of HD grains (Figure 4). In IR28178, the number of percentage of HD grains actually increased with increase in nitrogen applied.

Wada (1969) reported that increased N fertilization increased spikelet number because of the increase in spikelets on the secondary branches. However, this increase resulted in a higher number of low density grains. However, varietal responses to N fertilization differ in terms of HD grains produced (Venkateswarlu et al., unpublished paper). The non-decrease or increase in HD grains with N fertilization may be the result of a varietal increase in spikelets on the secondary branches accompanied by a higher study as it is important in future selection of breeding lines.

Studies on environmental factors such as temperature showed that low temperature or a longer ripening period resulted in a higher number of HD grains (Figure 5). This indicates that production of HD grains is partly dependent on duration of the ripening period. In the tropics where temperatures are higher, production of HD grains would be hampered because the ripening period is shorter.

Higher photosynthetically active radiation (PAR) from anthesis to harvest greatly increased the number of HD grains (Figure 6). Low PAR can be a limiting factor in increasing HD grains during the rainy season. HD grains were not realized in all the filled spikelets irrespective of PAR level.

Kato (1986) reported that low PAR resulted in lower weight of all grains in a large-grain variety. In a small grain variety, the grains on the secondary branches and lower branches decreased in weight while the rest remained constant.

Within a panicle, certain spikelets invariably had HD grain (Figure 2). Spikelets on the secondary branches had low grain weights and removal of other spikelets did not inccrease the individual weights of spikelets on the secondary branches (Figure 7). HD filling of spikelets on the secondary branches is not completely related to the amount of available photosynthates.

The HD grains or vigorous spikelets generally flower earlier and fill up earlier (Choi, 1986).

LIMITATIONS ON GRAIN FILLING

The factors that affect or limit grain filling in obtaining HD grains need further studies.

Carbohydrate supply. The leaves are important in grain filling, depending upon their position on the tiller (Figure 8). The flag leaf and penultimate leaf supply most of the assimilates to the grains. Removal of the 4th leaf from the top increased grain weight and number of well-filled grains (Ahn, 1986). In the present plant type, carbohydrate is not a limiting factor in obtaining HD grains (Figure 8). Reduction of sink size by removing various spikelets did not increase the weight of the grains that are normally lightweight (Figure 7). This was also reported earlier with different varieties of various 1000-grain weights (IRRI, 1978). Kato (1986), however, reported varie-

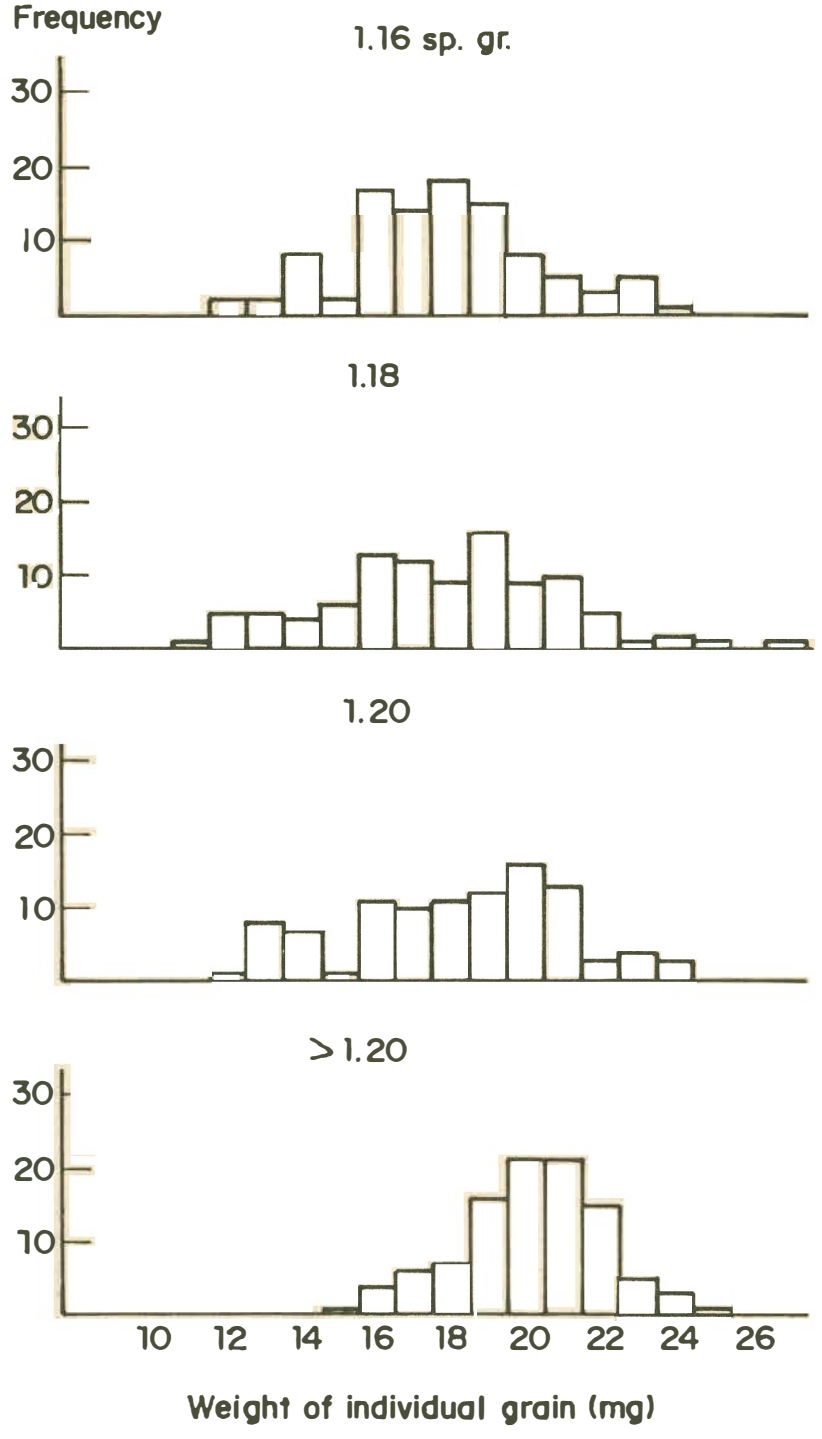


Figure 1. Frequency distribution of weight of individual grains of IR36, with 1.16 to $1.20_2{}^{sp}{}_1$ gr. when exposed to 780 μmo 1 m s.

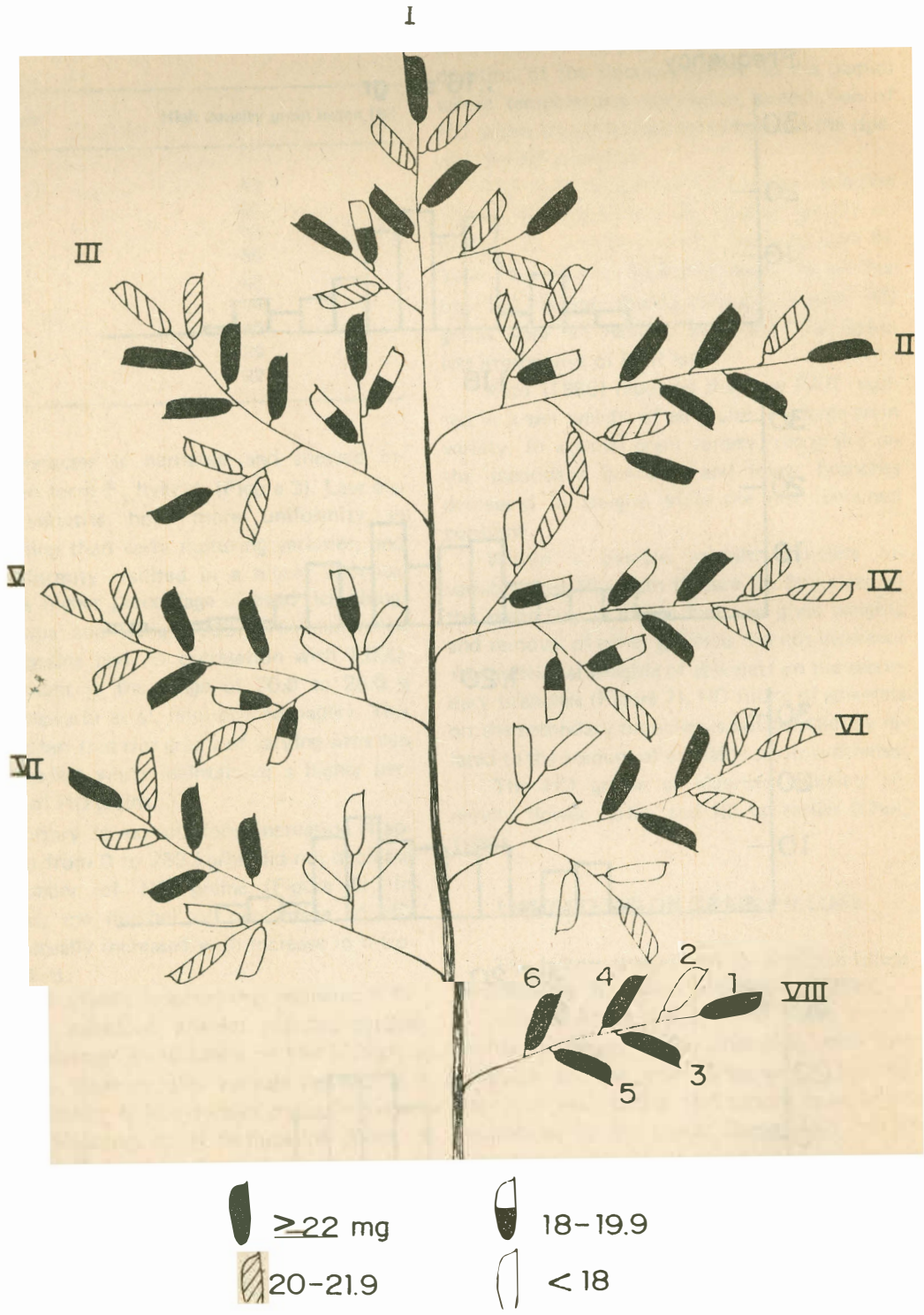


Figure 2. Location of grains of different weights in a panicle of IR58 (Ahn, 1986). Roman numerals indicate branch numbers, Arabic numerals indicate spikelet numbers.

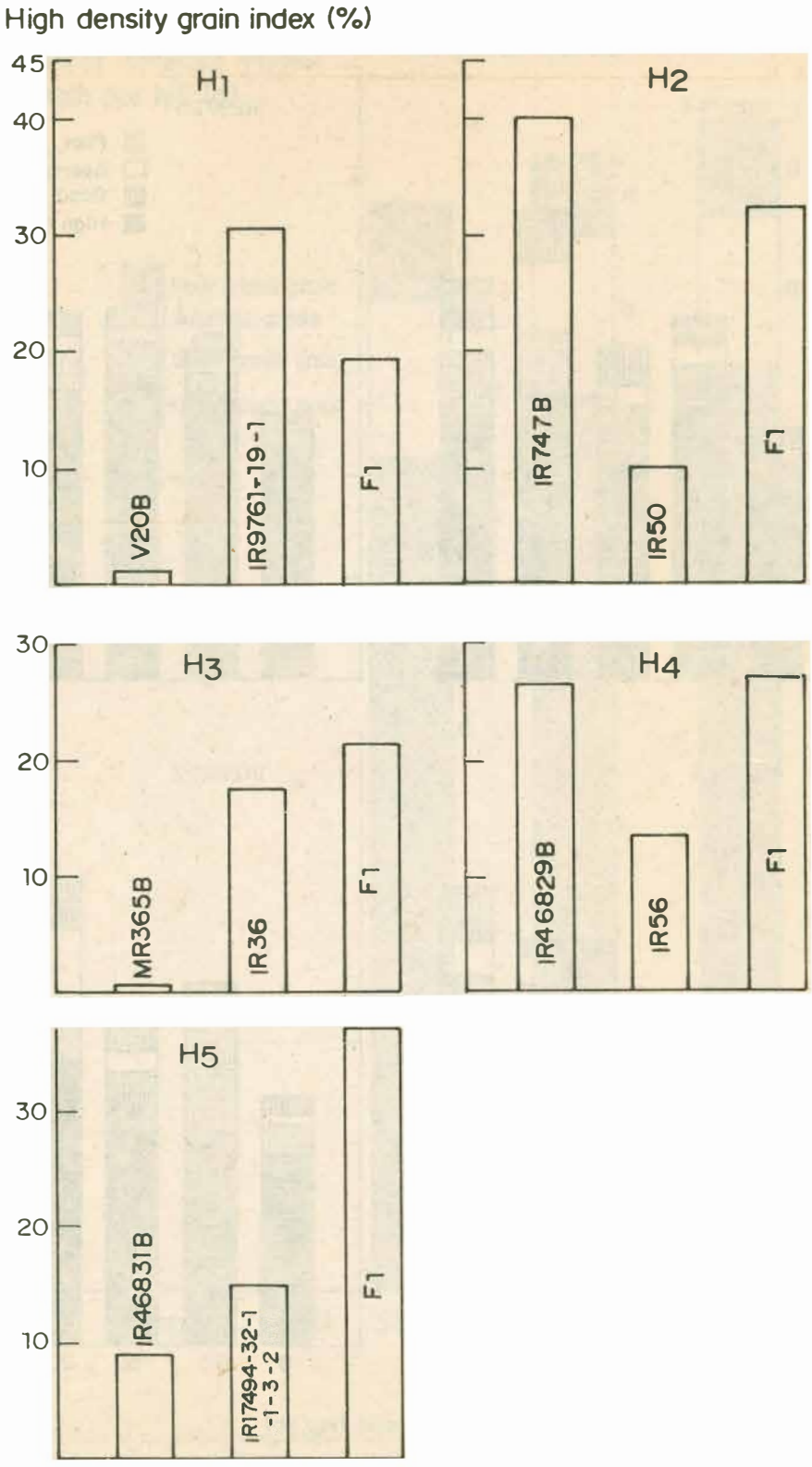


Figure 3. High density grain index in parents and hybrids of rice (Venkateswarlu, 1986b).

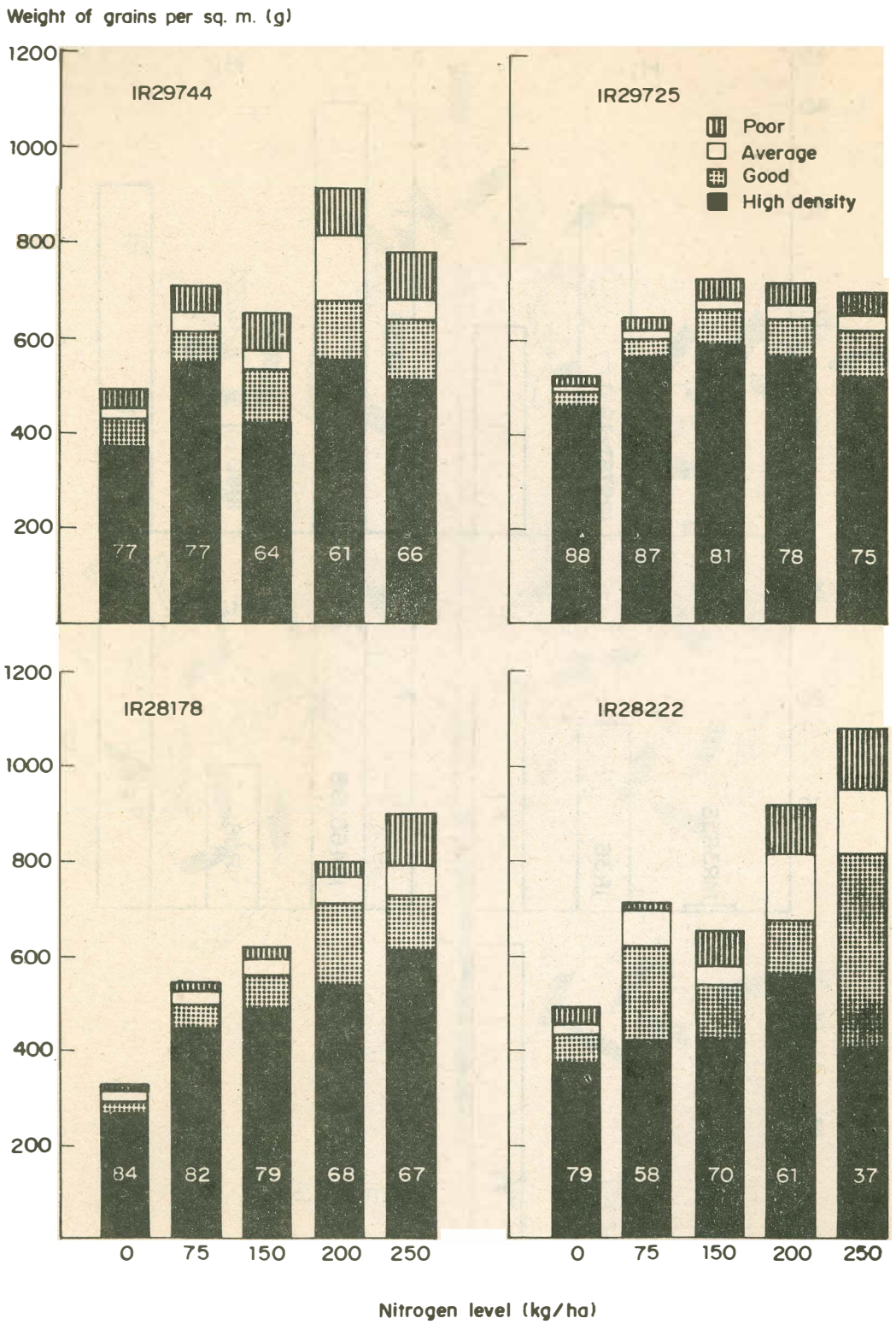


Figure 4. Number of grains of different grades at varied nitrogen levels (Venkateswarlu, 1986b).

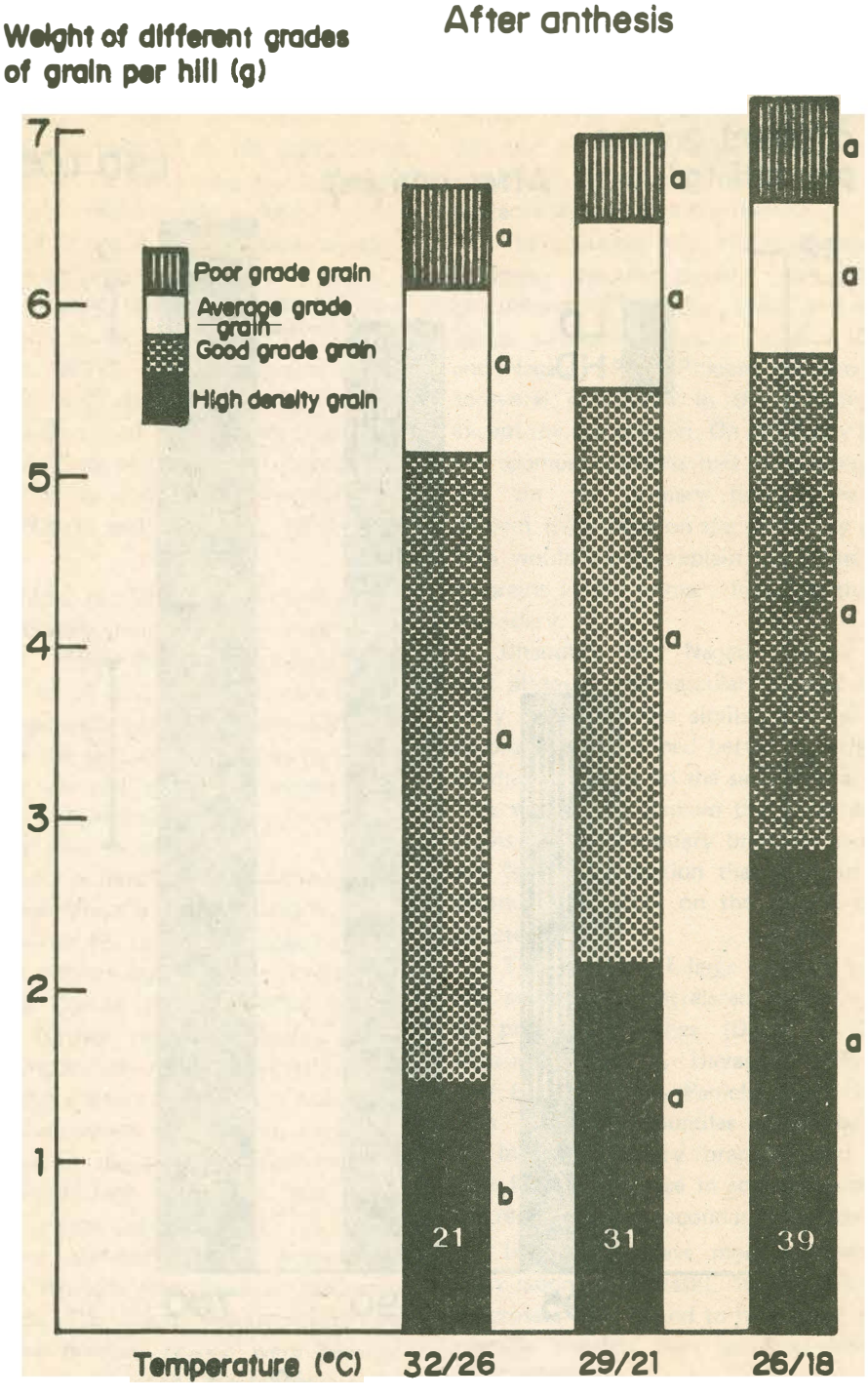


Figure 5. Influence of temperature regimes on the weight of different grades of grain in IR36 (Venkateswarlu et al., unpublished). The figures in dark shade are percent values. Bars of the same shade followed by the same letter are not significantly different at the 5% level.

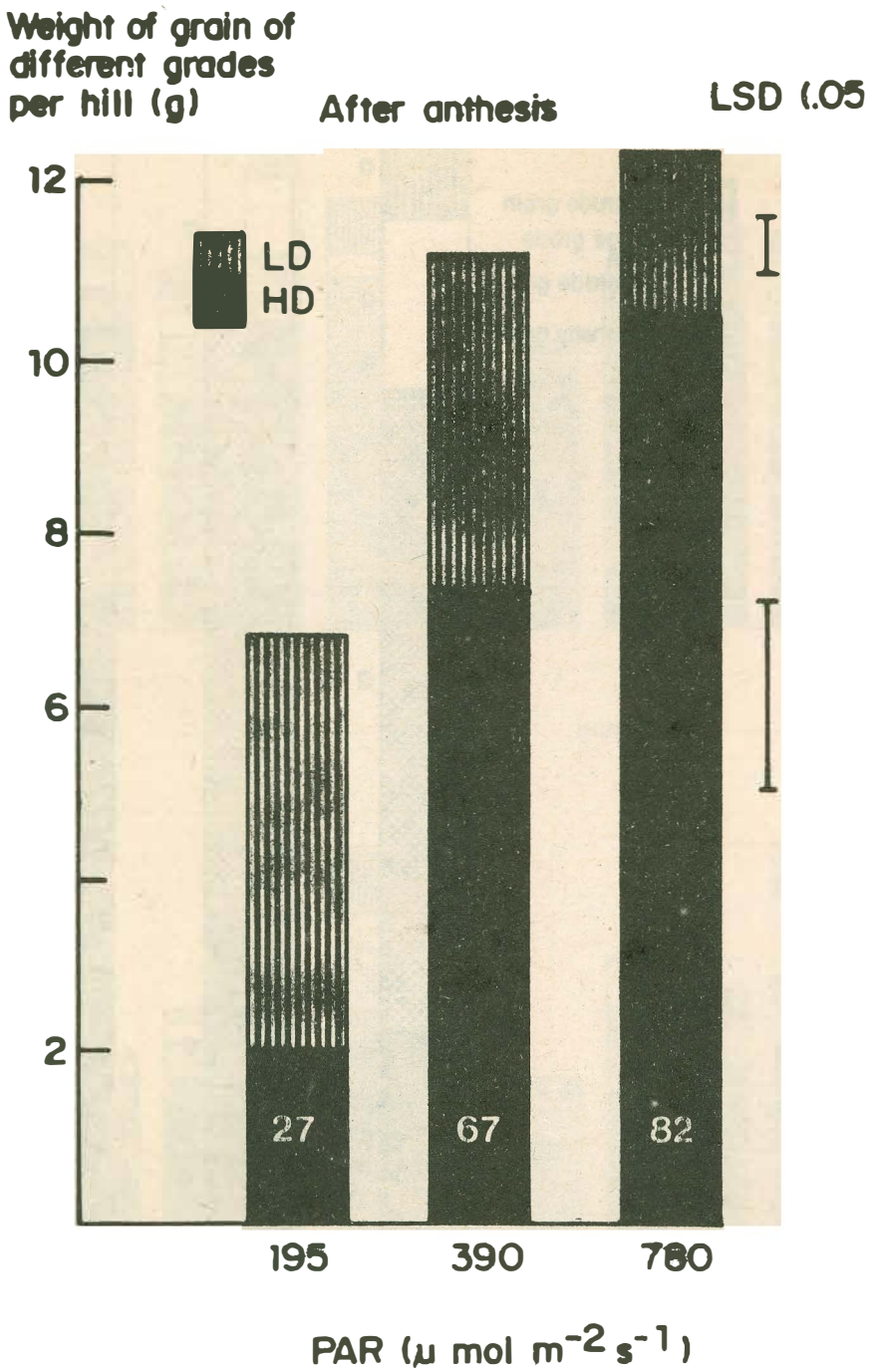


Figure 6. Weight of low density (LD) and high density (HD) grains of IR36 when exposed to different PARs after anthesis (Venkateswarlu et al., unpublished data).

tal differences: the large grain varieties showed a significant increase in their final grain weight while the small grain varieties did not have any increase.

The supply of sugar precursors did not limit starch accumulation in the grain (Singh and Juliano, 1977). Something else is limiting in the small and medium grain varieties; in some cases, it is as simple as having smaller or poorly developed spikelets to start with.

Rate of Filling. Spikelets are filled to capacity within 11 to 21 days (IRRI, 1978; Singh and Juliano, 1977). The large grains (40 g) mature in 16 to 21 days, small grains (18 g) in 11 to 12 days, and medium size grains (20-30 g per 1000 grains) in 11 to 21 days (IRRI, 1978). Indica varieties mature earlier than japonicas (Nagato and Chaudhry, 1969; Choi, 1986).

Grain filling rate and duration are positively correlated with grain size (Jones et al., 1979, Fujita et al., 1984). Grain filling duration was shorter (12 to 18 days in the primary branch than in the secondary branches (12 to 29 days). Spikelets on the secondary branches had lower grain filling rate and lower final weight (Ahn, 1986). This would indicate that rate of grain filling affects grain density.

Low 'sink-pulling' force. Although sufficient carbohydrate is available, many of the spikelets do not fill up to HD capacity. Whether or not growth regulators are involved, as suggested by Thorne (1974) on wheat and barley, needs further studies. Preliminary data show that spikelets resulting in HD grains have high IAA content and the peak occurs early in the development of the grains (Robles et al., unpublished data). Respiration measurements showed high rates for HD grains (Shanghai Teachers College, 1978).

Spikelets on the primary branch had greater sink strength than those on the secondary branch. The lower spikelets (5th and 6th) on the primary branch were heaviest. On the secondary branch, the topmost spikelet was always heavier (Ahn 1986). Even with all leaves removed at flowering, the same spikelets filled up first.

Structural limitations. In rice, the transport of assimilates from the vascular bundle to the endosperm is mediated by the pigment strand. At 12 days after anthesis, no structural evidence in the pigment strand was found to restrict the flow of assimilates to the endosperm (Oparka and Gates, 1981 and 1984). Whether or not the pigment strand becomes sealed off during grain filling would have importance in assimilate translocation.

The spikelets with HD grains have bigger pedicellar vascular bundles, specifically, larger phloem (Nishiyama, 1983) and more and better developed vascular bundles (Chaudhry and Nagato, 1970). Phloem size decreased by acropetal succession in the primary branch except the top spikelet. On secondary branches, the topmost spikelets had the thickest. Spikelets on the primary branches had thicker phloem than those on the secondary branches. This would partly explain the greater density of grains in the primary branches than in the secondary.

Chaudhry and Nagato (1970) reported that although the vascular bundles in all primary branches were similar, the 1st secondary branches developed better than the 2nd secondary branches on the same primary branch. This would also explain the lower density of grains on the secondary branches and the reason for the suggestion that cultivars with no secondary branches on the panicle should be selected.

The number of large vascular bundles in the peduncle is correlated with the number of primary branches (Dana et al., 1969; Matsushima, 1970, Hayashi, 1976; Joarder and Eunus, 1980). Panicles with large numbers of vascular bundles should be selected to increase primary branches and compensate for the decrease in spikelet number with the removal of the secondary branches.

Indica rices have more vascular bundles than japonica (Hayashi, 1976). Indica/japonica crosses were found to have more and larger vascular bundles than japonica varieties (Lee et al., 1985).

Thick culms have more vascular bundles. There is a high correlation between the diameter of the first node at the top of the culm and the length of the primary rachis branch and also the number of grains per panicle

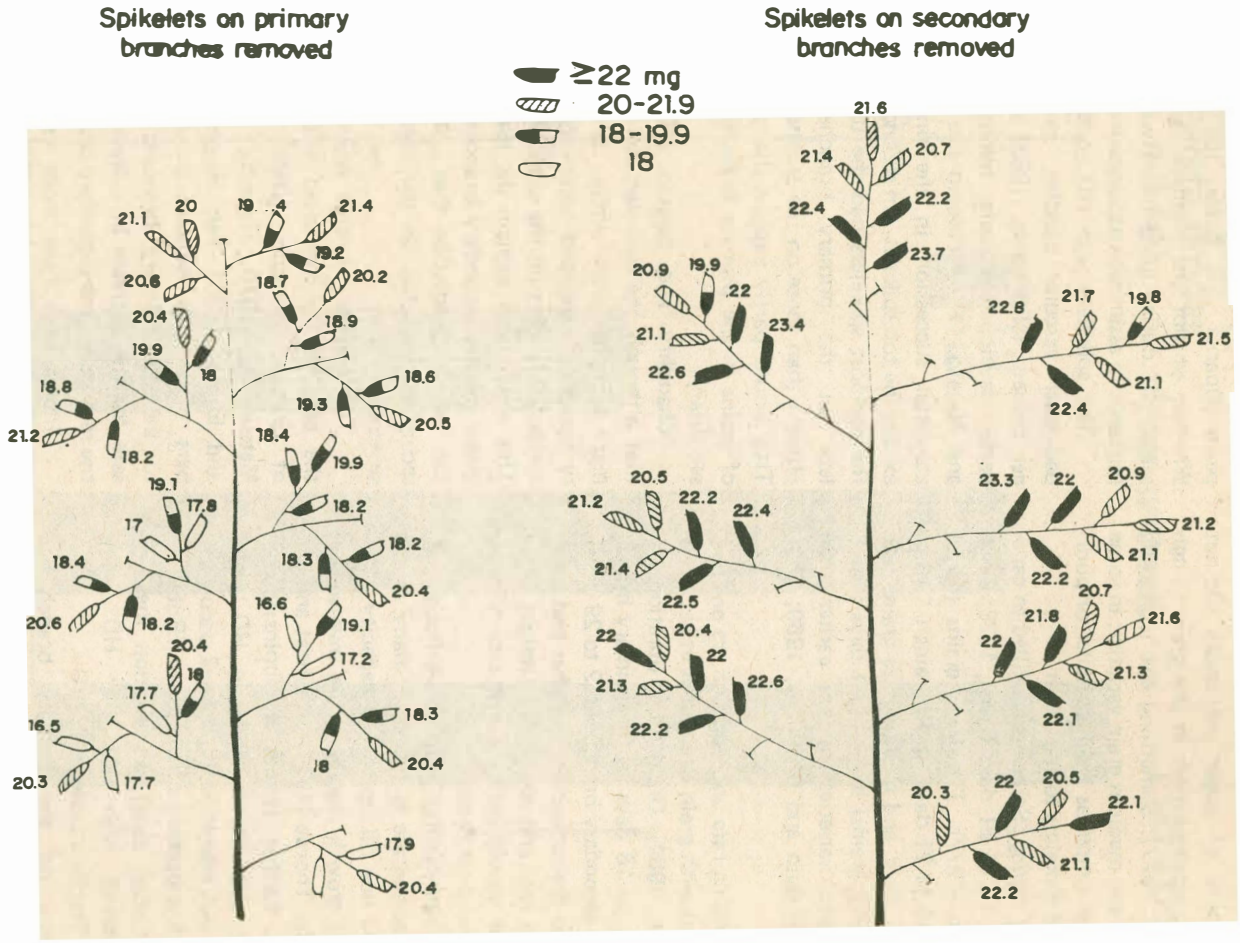
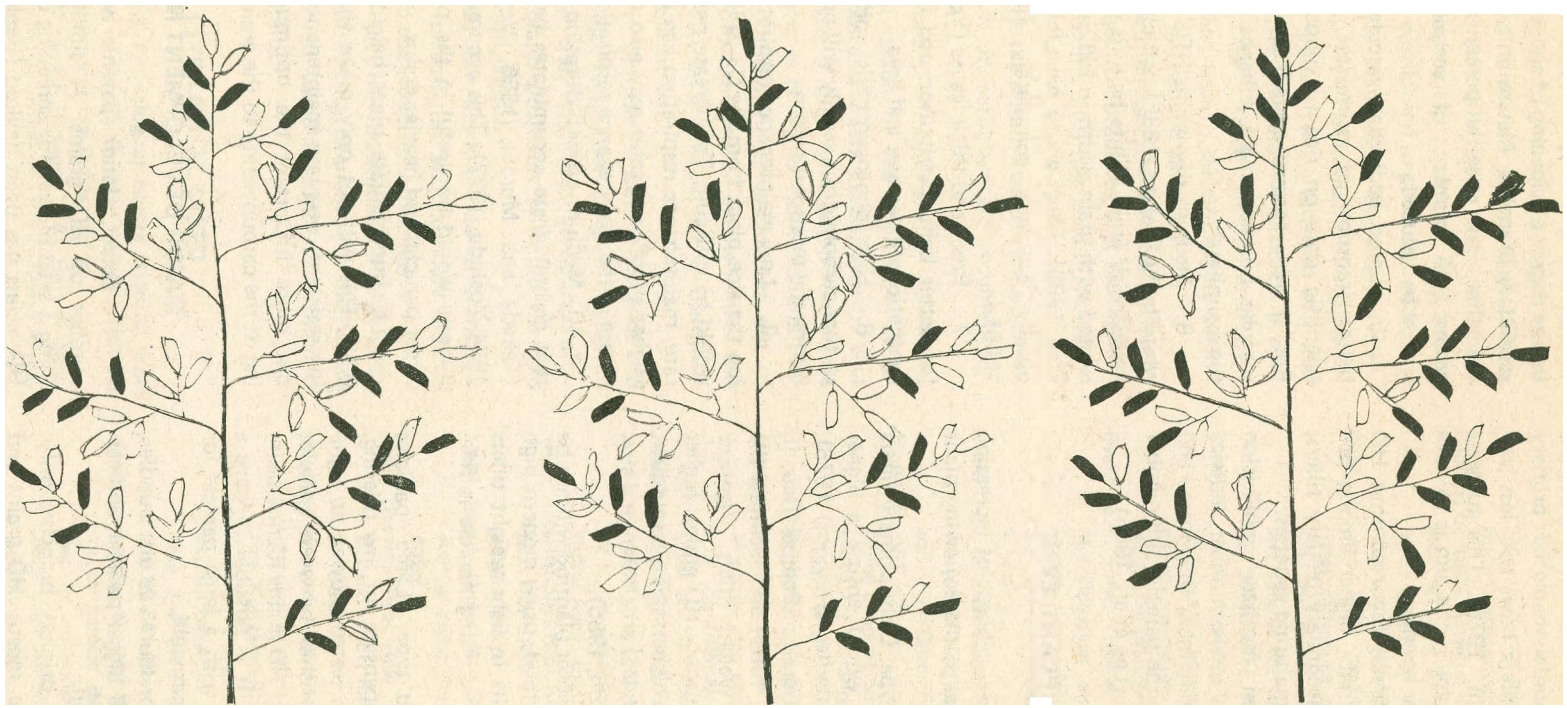


Figure 7. Effect of sink size and position on the location of IR58 grains of different grades (Ahn, 1986).



Flag leaf, 2nd leaf attached
Leaf area = 90 sq. cm.
Heavy grains = 34

All leaves intact
Leaf area = 183 sq. cm.
Heavy grains = 35

Flag leaf, 2nd & 3rd leaf attached
Leaf area = 142 sq. cm.
Heavy grains = 48

Figure.8. Effect of leaf removal at flowering on the number of high density grains produced in IR58 (Ahn, 1986).

(Hayashi, 1980). The secondary tillers have one less vascular bundle than the primary tillers. The tertiary tillers have two less vascular bundles (Hayashi, 1976). This suggests a low tillering plant type if the aim is to have high number of vascular bundles.

PadmajaRao (in press) reported that HD grain index was generally higher among primary tillers than in secondary/tertiary tillers, especially in the early maturing varieties.

Increased nitrogen fertilizer application resulted in an increase in the number and size of the vascular bundle, number of primary and secondary branches of the panicle, and number of spikelets per panicle (Lee et al., 1985).

SUGGESTED PLANT TYPE

In line with the new concept of increasing the number of HD grains, the following plant type is suggested:

1. Low tillering type. Only primary tillers should develop. This would ensure a higher number of vascular bundles (Hayashi, 1976), higher number of HD grains (PadmajaRao, in press; Choi and Kwon, 1985) and facilitate the production of heavy weight tillers. Vigorous or large tillers result in more HD grains; higher sink/source ratio; and higher spikelet number, percent filled spikelets, leaf area/tiller, and sink capacity (Choi and Kwon, 1985).

Low tillering by denser planting will not be practical since this method, using modern high tillering varieties, results in light weight tillers with thin culms. The resulting panicle is relatively small.

2. Panicle weight type. Large panicles will be needed to compensate for low tillering. Data from 86 varieties tested showed no significant negative relationship between spikelet number per panicle and HD grains (Samantasinhar and Sahu, 1986). It is possible to have a high HD grain index with a large panicle for stable and sustained grain yield.

3. Thick culm for more vascular bundles, less lodging, support of bigger panicle, and carbohydrate accumulation.

4. Panicles with primary branches only. Primary branches have mostly HD grains and fewer empty and halffilled spikelets. The percentage of ripened grains is governed mainly by the degree of ripening of the spikelets on the secondary branches. Matsushima (1976) suggested that, to raise the percentage of ripened grains, the number of secondary branches should be reduced.

5. Large pedicellar vascular bundle for better transport of assimilates. There are no scientific data on rice to support this aspect. But, if the transport system is limiting, larger vascular bundles might enhance movement of the assimilates.

6. Medium size grains (IR8 size) with less white belly (Takita, 1985), which is essentially low-density grain. White belly is positively correlated with grain width in indica cultivars (Takita, 1986). Large grains have low density and usually are not completely filled (Takita, 1986).

7. Erect and thick leaves (Yoshida, 1972) for better light distribution and higher photosynthetic rate per unit leaf area.

8. High photosynthesis under low PAR so that carbohydrate supply will not be limiting during the monsoon season.

9. Low maintenance respiration. Converting the rice plant from the C_3 to the C_4 system would be difficult. To increase net assimilation rate, maintenance respiration can be decreased. Higher shoot/root ratio may also result in a decrease in the maintenance respiration of roots.

10. Medium growth duration is needed so that carbohydrate accumulates before heading (Takeda and Murata, 1956, Vergara et al., 1964 Yoshida, 1972). This accumulated carbohydrate would be useful in the production of larger panicles and heavier grains.

11. Intermediate plant height with HI of 0.55. This will not only make the plant lodging resistant, decrease maintenance respiration but more important the optimum partitioning of the carbohydrate to the grains.

MAJOR DEVELOPMENT NEEDS

1. Select donor parents with a high number of HD grains. A simple procedure using a seed blower for screening cultivars with HD grains has been devised (Venkateswarlu et al., 1986a).

2. Select plants with a high number of vascular bundles or of primary branches in the panicle and testing for HD grains. Choi (1985) suggested that sink size/tiller is an effective indicator of high yield potential. This aspect should also be considered in plant selection.

3. Identify plants with low tillering ability. If such plants are not available, breeding for that character should be started. Use of tissue culture and other methods to produce a low-tillering plant type should be explored. Unless such a plant type is developed, its usefulness and potential cannot be tested.

4. A low-tillering type will need different cultural management practices that should also be studied. The use of a row seeder should be evaluated.

5. Study the role of cytokinin, gibberellin, and auxin on carbohydrate accumulation in the spikelets.

The movement of water and of assimilates in the dorsal region of the grain seem to be linked. Oparka and Gates (1984) suggest that studies be made to determine whether the rate at which water is removed from the grain influences the movement of assimilates out of the phloem. Silica deposition on the lemma and palea might play an important role in transpiration and translocation.

6. Study the role of slow senescence and low maintenance respiration on grain filling. Indications are that leaf area at 30 days after heading correlates positively with grain weight (Shin and Kwon, 1985).

7. Study the limiting rate of translocation to the endosperm and compare varietal differences in translocation efficiency.

8. Conduct genetic studies on inheritance of HD grains, tillering, branching of the panicle and number of vascular bundles to improve these plant traits.

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TECHNOLOGY NEWS BRIEFS

DEAD BACTERIA CONCEPT SPEEDS GENETIC ENGINEERING APPROVALS

A number of US programs in genetically engineered agricultural treatment systems have received widely publicized setbacks due to public fears of possible health hazards from release of live genetically engineered bacteria into the environment. While that opposition led to federally imposed delays for a number of proposed experiments, Mycgen Corp., San Diego, CA, has quietly gone about developing an alternate concept in which genetically engineered organisms are killed before field tests. Mycogen thus gained EPA approval for field tests of a new biopesticide in 1985, two years before another group plans for live bacteria field tests got the EPA greenlight.

Innovative methods like this are vital for the smaller firms involved in advanced biotechnology as only big chemical companies have the resources to withstand years of delay. The firm's current approach evolved from work begun in 1984 when management realized that EPA was not going to make a fast decision on Mycogen plans to test live, genetically engineered biopesticides. Chemical engineer Andrew Barnes suggested killing the genetically engineered bacteria after they had done the job of producing certain antipest toxins. Company biologists objected that this would destroy the cell wall and deactivate the toxins. To solve that, Barnes and his co-workers developed a process combining heat and chemical treatments that killed the bacteria while cross-linking the molecules of the cell wall. This method turns the dead bacteria into capsules analogous to the gelatin capsules used to protect human pharmaceuticals until they reach the stomach.

The company is pursuing studies of other systems including genetically engineered ones and non-genetically engineered bio-pesticides. Company officials predict that as more groups complete safe tests of live, genetically engineered microbes, EPA approval time for new field tests will speed up. In the future, both live and killed bacteria will find niches. Live bacteria will be more useful on fast-growing plants, such as lettuce. The bacteria will grow with the plant so no reapplication will be necessary.

All these advances are forecast to almost completely transform the pesticide industry. Biotechnology is predicted to eliminate many, if not all, synthetic chemicals over the next few decades.

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Enhancing Sugar Yield Through Intercropping *Rhizobium* Inoculated Legume

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ABSTRACT

Intercropping *Rhizobium* inoculated legumes (soybean and mungbean) significantly increased sugar yield in the ratoon. Incremental benefits due to *Rhizobium* inoculation could be realized via reduced chemical fertilizer application. Inoculation plus 75 kg/ha N gave higher yields than 150 kg/ha N treatment-plot. Furthermore, if the soil acidity built-up as a result of high rate of chemical fertilizer application is imputed, the accrued benefits due to intercropping *Rhizobium* inoculated legumes with sugarcane particularly in the ratoon could be more.

INTRODUCTION

Leguminous crops like soybean and mungbean have been found to be compatible intercrops for sugarcane. It has been shown also that by modifying the row spacing such that the requirements of the main and intercrops are adequately met, the aggregate yield of the farm increased considerably (Mendoza, 1979 and 1985). The role of *Rhizobium* inoculation in the N-fixation of legumes is well established. It had been shown to improved modulation, N-fixation and plant growth (Daff and El Giahmi, 1974; Mosse, 1977). Intercropping legumes with non-legumes has been shown to be complimentary because part of the atmospheric N fixed by the legume is donated to the associated non-legumes (Wahua and Miller, 1978).

This study was conducted to determine the effects of *Rhizobium* - inoculated legumes on the yield and yield components of sugarcane.

MATERIALS AND METHODS

The experiment was conducted at the Central Experiment Station of the University of the Philippines at Los Baños using split-plot design with three replications. The treatments and their corresponding arrangement in a plot are as follows:

Main plot: Thru spacings – 1.25 m and 1.50 m even furrow space, and a double row (2 rows spaced at 0.75 m and 2.25 m) interval space before the next two rows.

Sub-plot: Two fertilizer levels – 75-75-150 and 150-75-200 kg/ha of N, P_2O_5 and K_2O.

Sub-sub-plot: With and without inoculation

PHIL 66-07 was the sugarcane cultivar used while soybean and mungbean were used as test intercrops. Split method of fertilizer application was done. The first was applied at planting time and the second done three months after. No fertilizer was applied to legumes.

All recommended cultural management operations were done during the establishment and growing period of both sugarcane and intercrops. The same operation was applied in the first ratoon. Harvesting of intercrops was done when 80-90 percent of the pods were already dry. In the case of sugarcane, harvesting was done at maturity which was about 11 months after planting. Sugar yield and yield components were recorded during the harvest time.

For the legume intercrops, the following data were gathered: number and weight of modules per 10 plants, and dry weight of 10 plants.

RESULTS

The yield and yield components of sugarcane as influenced by intercropping legumes with and without inoculation are shown in Appendix Tables 1 to 4. Inoculating the legume intercrop did not affect sugarcane yield in the plant crop. However, significantly higher yield were obtained in the ratoon crop as direct effect of *Rhizobium* inoculation (Table 1). In plant crop, 150-75-200 fertilizer level did increase the TC/Ha of sugarcane up to 102.52 in the sugarcane + soybean intercropping system, compared to only 92.08 from the 75-75-150 level. On the other hand, the TC/Ha of ratoon crop was not affected by the level of fertilizer.

Similar effects were observed when the intercrop was mungbean. Plant crop yield was not affected by inoculating the mungbean intercrop but ratoon crop yield was significantly increased (Table 1). This confirms the direct effect of *Rhizobium* inoculated legume intercrop to increase the yield of sugarcane ratoon crop. Also, a fertilizer level of 150-75-200 increased the TC/Ha of plant crop to 95.97 compared to only 85.59 from the 75-75-150 level. The ratoon crop was similarly unaffected as in the sugarcane mungbean intercropping system.

Table 1. PS/HA. TC/HA and PS/TC of plant and ratoon crop as affected by inoculation of the intercrops.*

TREATMENT	PS/HA		TC/HA		PS/TC	
	Plant Crop	Ratoon Crop	Plant Crop	Ratoon Crop	Plant Crop	Ratoon Crop
Soybean						
Without inoculation	144.48a	94.76b	96.49a	48.67b	1.50a	1.96a
With inoculation	144.80a	123.11a	98.11a	68.11a	1.49a	1.83b
Mungbean						
without inoculation	133.81a	94.49b	90.65a	49.59b	1.49a	1.91a
With inoculation	135.37a	126.71a	90.91a	63.59a	1.49a	1.86b

* Treatment means having common letters in each column by crop are not significantly different by DMRT at α = 5%.

Among the 3 factors (spacing, fertilizer level and intercrop legume inoculation) considered in the experiment, only spacing and their interactions did not affect the biological productivity of soybean and mungbean as intercrops for both plant and ratoon crops of sugarcane (Appendix Tables 5 and 6). The number and weight of modules as well as dry matter yield of the intercrop soybean was not affected by the level of fertilizer applied. On the other hand, mungbean exhibited differential response to fertilizer level in terms of module count and weight and dry matter yield. An intercrop for the ratoon crop, its biological productivity was not affected. The higher fertilizer level significantly increased the biological productivity of mungbean when used as intercrop for sugarcane plant crop (Table 2). This shows that for economic reasons the lower level of fertilizer should be used in sugarcane + legume intercropping systems. This is also supported by yield of sugarcane which indicated that only the TC/Ha was increased by the higher level of fertilizer. PS/Ha and PS/TC were not significantly increased by higher fertilizer level (Table 3).

In terms of inoculating the intercrops with *Rhizobium,* generally there was a numerical increase in the biological productivity of the intercrop. For instance, only the number of modules of soybean increased due to inoculation in the ratoon crop + soybean intercropping system. Mungbean nodule count and weight were significantly increased in the plant crop. Dry matter yield was also increased significantly in the ratoon crop but not in the plant crop.

DISCUSSION AND IMPLICATIONS OF THE STUDY

The results obtained in this study complimented the findings of previous studies (Wahua and Miller, 1978; Agboola and Fayemi, 1972; Mendoza, 1979 and 1985). In both legume crop species intercropped (soybean and mungbean) rhizobium inoculation increased the number and weight of nodules and finally plant-drymatter yield. The non-legume crop (sugarcane) was also benefited from the association and the positive increment in yield was evident in the rhizobium inoculated legume intercrops.

The positive benefits of intercropping rhizobium inoculated legumes was evident only in the ratoon (Table 4) but not in the plant crop. This was true for all the spacings used in the study. The other observation is that the benefits of inoculation via enhanced legume N-fixation was higher in the low fertilization rate (75-75-150). There was no considerable increase in yield in the high fertilizer treatment as has been observed also by other workers (Paterno, personal communication).

The other condition why it was only in the plant crop where yield increments were noted can be attributed to the initial condition of the field where the experiment was conducted. First, the lot was formerly used in a fertilizer experiment and it can be inferred that residual fertility provided added nutrients to the experimental plant plus the favorable soil environment as a direct result of thorough land preparation which in turn enhanced mineralization and promoted better root growth.

In the ratoon crop, the soil was compacted. While interrow cultivation was done, this was not comparable to the better soil tilth prevailing in the plant crop.

Rhizobium inoculation was noted to be effective in a less favorable soil environment for plant growth. This was noted again in the current study. What is worth noting is the incremental benefits due to inoculation as shown in the following estimates:

Cost of legume inoculation:

Inoculant	50/ha
Seeds: soybean	30 kg x ₱12 = ₱360.00
Planting	6 md x ₱35 = ₱210.00
Total Added Cost.	₱620.00

If the equation is,

low fertilizer plus Rhizobium inoculation =

high fertilizer less Rhizobium inoculation

Table 2. Total number and weight nodule and drymatter yield of 10 plants of intercrops as affected by fertilizer level of sugarcane plant and ratoon crop.*

TREATMENT INTERCROP	Nodule Count		Nodule wt. (gm)		Drymatter yield (gm)	
	Plant Crop	Ratoon Crop	Plant Crop	Ratoon Crop	Plant Crop	Ratoon Crop
SOYBEAN						
75-75-150	118.72a	122.50a	573.06a	573.06a	59.67a	46.03a
150-75-200	122-45a	121.11a	570.33a	573.33a	52.52a	51.68a
MUNGBEAN						
75-75-150	145.39b	141.00a	465.17b	460.94a	46.84b	51.06a
150-75-200	152.00a	143.67a	486.61a	451.89a	53.42a	52.50a

* Treatment means having a common letters in each column are not significantly different by DMRT

Table 3. Total number and weight of nodules and drymatter yield of 10 plants of intercrops as affected by inoculating the legume intercrop of sugarcane plant and ratoon crop.

TREATMENT INTERCROP	Nodule Count		Nodule wt. (gm)		Drymatter yield (gm)	
	Plant Crop	Ratoon Crop	Plant Crop	Ratoon Crop	Plant Crop	Ratoon Crop
SOYBEAN						
without inoculation	118.22a	119.78b	565.05a	565.05a	49.37a	49.40a
with inoculation	122.95a	123.83a	578.33a	578.33a	53.81a	48.31a
MUNGBEAN						
without inoculation	144.78b	139.45a	463.72b	446.83a	53.20a	48.62b
with inoculation	152.61a	145.22a	488.06a	466.00a	47.06a	54.94a

Treatment means having common letters in each column are not significantly different by DMRT at 5%.

Table 4. Average PS/Ha of sugarcane due to intercrop inoculation in the ratoon crop.

TREATMENTS	INOCULATED INTERCROP	
	SOYBEAN	MUNGBEAN
75-75-150		
without inoculation	98	91
with inoculation	121	120
150-75-200		
without inoculation	92	98
with inoculation	124	133

This means that Rhizobium inoculation can substitute for 75 kg - N/ha.

Assuming further that the cost of Urea -- N is,

$$\frac{₱\ 130}{22.5} = ₱\ 5.78/kg$$

then, 75 kg x P5.78/kg-N = P433.50

However, the yields obtained under low fertilizer plus rhizobium-inoculation were higher than the high fertilizer less rhizobium-inoculation. This merely indicates the positive financial return of rhizobium-inoculation in terms of the following.

a) minimizing cash input to buy fertilizer in the amount of P433.50/ha.
b) obtaining reasonably higher yields through biological means without unduly encountering the acidifying effect of adding chemical fertilizer which requires lime later on.

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Appendix Table 1. PHIL 66-07 of Sugarcane yield components as affected by spacing, fertilizer and intercropped soybean.

Spacing (S)	Fertilizer *F)	Inoculation Mungbean (I)	Plant Height (m)		Stalk Height (m)		Stalk Diameter (cm)		Ave. Stalk wt. (kg)		No. of millable stalks		No. of non-millable stalks (x 1000)		No. of non-millable (t/ha)		Wt. of Tops (t&ha)	
			Plant Crop	Ratoon Crop	Plant Crop	Ratoon Crop	Plant Crop	Ratoon Crop	Plant Crop	Ratoon Crop	Plant Crop	Ratoon Crop	Plant Crop	Ratoon Crop	Plant Crop	Ratoon Crop	Plant Crop	Ratoon Crop
S1.25	75-75-150	without	3.08	2.61	2.37	2.12	2.81	4.18	1.71	1.19	60.22	56.00	10.78	6.44	2.37	1.52	16.84	8.97
		with	3.25	2.67	2.69	2.20	2.80	2.47	1.76	1.11	6.00	69.00	10.78	3.33	2.82	1.26	16.15	11.72
	150-75-200	without	3.30	2.42	2.60	1.91	2.78	2.40	1.72	1.00	73.22	62.11	11.11	16.67	3.68	1.82	18.62	12.72
		with	3.23	2.82	2.52	2.30	2.79	2.57	1.74	1.32	65.67	75.22	8.56	5.89	2.87	1.46	19.17	11.99
S1.5	75-75-150	without	3.14	2.31	2.55	1.77	2.74	2.35	1.72	1.72	57.59	52.87	8.43	20.83	3.44	3.03	12.94	10.90
		with	3.32	2.67	2.62	2.16	2.83	2.49	1.75	1.18	65.83	68.80	7.59	8.24	2.27	1.35	12.64	16.57
	150-75-200	without	3.35	2.40	2.68	1.92	2.76	2.43	1.78	1.05	61.94	44.44	7.78	15.19	2.51	2.19	18.57	9.14
		with	3.30	2.47	2.66	1.95	2.76	2.45	1.80	1.09	62.41	61.20	6.67	10.65	2.02	1.34	11.42	10.27
Double row scheme	75-75-150	without	3.10	2.34	2.38	1.92	2.63	2.42	1.39	1.00	63.61	60.46	16.02	23.52	4.66	10.67	15.71	16.28
		with	2.62	2.54	2.29	2.04	2.70	2.44	1.42	1.08	63.15	71.76	12.13	10.00	2.60	2.46	18.22	13.24
	150-75-200	without	2.93	2.37	2.28	1.95	2.69	5.48	1.44	1.04	71.66	58.15	12.31	24.81	3.69	5.01	21.20	9.52
		with	2.79	2.40	2.18	1.92	2.68	2.48	1.37	1.06	68.24	68.24	85.19	15.97	3.33	2.44	18.27	12.11
ANOVA	F-Test																	
	Spacing (S)		*	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Fertilizer (F)		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	
	S x F		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Inoculation (I)		ns	**	ns	**	ns	*	ns	*	ns	**	ns	**	ns	*	ns	ns
	S x 1		*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	F x I		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	S x F x I		ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns

MODEL: Split – split plot in RCB
ns – non significant

* – significant
** – highly significant

Appendix Table 2. PHIL 66-07 of Sugarcane yield components as affected by spacing, fertilizer and intercropped soybean.

Spacing (S)	Fertilizer *F)	Inoculation Mungbean (I)	Plant Height (m)		Stalk Height (m)		Stalk Diameter (cm)		Ave. Stalk wt. (kg)		No. of millable stalks		No. of non-millable stalks (x 1000)		No. of non-millable (t/ha)		Wt. of Tops (t&ha)	
			Plant Crop	Ratoon Crop	Plant Crop	Ratoon Crop	Plant Crop	Ratoon Crop	Plant Crop	Ratoon Crop	Plant Crop	Ratoon Crop	Plant Crop	Ratoon Crop	Plant Crop	Ratoon Crop	Plant Crop	Ratoon Crop
S1-25	75-75-150	without	3.10	2.51	2.46	2.03	2.93	2.44	1.64	1.09	69.56	74.00	10.33	8.78	2.97	1.36	17.91	12.03
		with	3.04	2.79	2.33	2.29	2.73	2.54	1.59	1.23	63.56	69.67	10.77	6.56	3.78	5.46	17.24	5.74
	150-75-200	without	3.14	2.52	2.49	2.02	2.76	2.37	1.58	1.03	84.00	59.22	12.22	41.78	2.72	1.87	22.31	11.46
		with	3.17	2.77	2.54	2.27	2.76	2.54	1.65	1.27	84.44	83.44	6.11	5.56	1.82	0.86	22.37	11.50
S1.5	75-75-150	without	3.28	2.41	2.71	1.97	2.72	2.45	1.69	1.06	66.57	63.70	6.67	15.19	1.76	2.14	16.37	8.99
		with	3.28	2.58	2.58	2.07	2.85	2.47	1.82	1.169	75.09	69.54	9.81	11.39	2.61	1.60	17.71	14.24
	150-75-200	without	3.10	2.21	2.48	1.80	2.80	2.40	1.75	0.93	68.24	49.81	9.54	26.39	2.39	3.49	19.40	9.40
		with	3.25	2.44	2.62	1.98	2.66	2.44	1.68	1.04	71.39	65.28	7.04	10.00	1.43	1.13	19.38	11.12
Double row scheme	74-75-150	without	3.07	2.37	2.41	1.93	2.75	2.45	1.54	1.04	68.06	53.43	8.61	16.48	2.65	2.23	17.67	12.36
		with	3.21	2.68	2.45	2.17	2.72	2.58	1.50	1.24	60.28	82.87	9.72	8.80	2.27	1.34	16.92	9.61
	150-75-200	without	3.00	2.45	2.38	2.00	2.69	2.47	1.55	1.10	74.63	53.33	8.24	17.87	1.68	3.33	23.85	9.94
		with	3.34	2 60	2.66	2.14	2.77	2.51	1.73	1.21	69.72	71.48	4.54	6.76	1.34	2.10	17.78	12.05
ANOVA	F-Test																	
	Spacing (S)		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Fertilizer (F)		ns	ns	ns	ns	ns	ns	ns	ns	**	ns	ns	ns	*	ns	*	ns
	S x F		ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns
	Inoculation (I)		ns	**	ns	**	ns	*	ns	**	ns	**	ns	**	ns	ns	ns	ns
	S x I		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	F x I		ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns
	S x F x I		ns	ns	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

MODEL: Split – split plot in RCB

ns – non significant

* – significant

** – highly significant

Appendix Table 3. PHIL 66-07 sugarcane yield and yield components as affected by spacing, fertilizer and intercropped soybean.

FACTORS			YIELD COMPONENTS					
Spacing (S)	Fertilizer (F)	Inoculation of Soybean (I)	TC/HA		PS/TC		PS/HA	
			Plant Crop	Ratoon Crop	Plant Crop	Ratoon Crop	Plant Crop	Ratoon Crop
S1.25	75-75-150	without	93.67	56.92	1.60	1.99	148.59	113.39
		with	97.53	61.83	1.67	1.76	160.02	106.76
	150-75-200	without	103.81	52.38	1.44	1.90	149.09	98.42
		with	115.83	74.79	1.51	1.85	144.25	137.38
S1.5	75-75-150	without	98.48	47.23	1.45	1.94	143.02	91.72
		with	100.28	76.653	1.45	1.65	144.25	125.92
	150-75-200	without	98.52	44.36	1.62	2.01	159.53	87.80
		with	102.94	62.61	1.32	1.93	134.25	117.05
Double row scheme	75-75-150	without	83.20	45.44	1.56	1.95	129.53	89.25
		with	79.30	69.40	1.65	1.91	130.87	132.46
	150-75-200	without	101.23	45.70	1.35	1.96	137.22	88.98
		with	92.79	63.60	1.35	1.88	124.63	119.08
ANOVA	F-test							
Spacing (S)			ns	ns	ns	ns	ns	ns
Fertilizer (F)			**	ns	ns	ns	ns	ns
S x F			ns	ns	ns	ns	ns	ns
Inoculation (I)			ns	**	ns	**	ns	**
S x I			ns	ns	ns	ns	ns	ns
F x I			ns	ns	ns	ns	ns	ns
S x F x I			ns	ns	ns	ns	ns	ns

MODEL: Split - split plot in RCB
ns - non-significant
* - significant
** - highly significant

Appendix Table 4. PHIL 66-07 sugarcane yield and yield components as affected by spacing, fertilizer and intercropped mungbean.

FACTORS			YIELD COMPONENTS					
Spacing (S)	Fertilizer (F)	Inoculation of Mungbean (I)	TC/HA		PS/TC		PS/HA	
			Plant Crop	Ratoon Crop	Plant Crop	Ratoon Crop	Plant Crop	Ratoon Crop
S1.25	75-75-150	without	89.00	50.32	1.53	1.87	135.70	93.80
		with	84.90	63.69	1.42	1.82	122.08	113.96
	150-75-200	without	112.83	64.74	1.34	1.94	150.67	125.35
		with	103.44	90.64	1.59	1.82	164.72	161.24
S1.5	75-75-150	without	85.27	43.38	1.52	1.84	128.30	78.26
		with	96.70	71.30	1.39	1.88	133.35	132.19
	150-75-200	without	91.30	44.45	1.29	1.93	116.34	85.12
		with	92.20	62.57	1.41	1.91	129.94	118.60
Double row scheme	75-75-150	without	73.24	50.26	1.64	1.99	120.30	99.94
		with	84.40	63.99	1.57	1.81	130.94	115.20
	150-75-200	without	92.24	46.69	1.64	1.86	151.52	84.48
		with	83.81	62.87	1.57	1.90	131.20	119.09
ANOVA	F-test							
Spacing (S)			ns	ns	ns	ns	ns	ns
Fertilizer (F)			*	ns	ns	ns	ns	ns
S x F			ns	ns	ns	ns	ns	ns
Inoculation (I)			ns	**	ns	ns	ns	**
S x I			ns	ns	ns	ns	ns	ns
F x I			ns	ns	*	ns	ns	ns
F x I			ns	ns	*	ns	ns	ns
S x F x I			ns	ns	ns	ns	ns	ns

MODEL: Split - split plot in RCB
ns - non-significant
* - significant
** - highly significant

Appendix Table 5. Total number and weight of nodules and dry matter yield of soybean as affected by spacing and fertilizer of sugarcane (main crop) and inoculation of soybean (intercrop).

SPACING (S)	FERTILIZER (F)	Inoculation of Soybean (1)	TOTAL NODULE NUMBER/10 PLANTS		TOTAL NODULE WT./10 PLANTS (mg)		DRYMATTER YIELD/10 PLANTS (g)	
			1986	1987	1986	1987	1986	1987
S1.25	75-75-150	without	116.00	117.67	523.00	556.33	47.44	44.93
		with	118.00	122.67	534.67	574.33	54.35	43.70
	150-75-200	without	116.67	116.00	528.33	555.00	48.07	40.83
		with	125.67	117.33	569.33	561.00	54.50	47.93
S1.50	75-75-150	without	118.33	118.00	535.00	561.00	48.80	51.26
		with	118.00	124.00	534.67	579.67	53.23	40.03
	150-75-200	without	120.00	121.00	542.67	567.33	50.60	57.73
		with	128.67	121.00	582.33	568.33	51.84	50.70
Double row scheme	75-75-150	without	119.33	124.00	537.67	577.33	46.91	45.06
		with	122.67	128.67	554.67	589.67	53.28	51.16
	150-75-200	without	119.00	122.00	505.67	573.33	54.41	54.56
		with	124.67	129.33	564.00	597.00	55.65	56.33
ANOVA	F-test							
Spacing (S)			ns	ns	ns	ns	ns	ns
Fertilizer (F)			*	ns	*	ns	*	ns
S x F			ns	ns	ns	ns	ns	ns
Inoculation (I)			ns	ns	ns	ns	*	**
S x F			ns	ns	ns	ns	ns	ns
F x I			ns	ns	ns	ns	ns	ns
S x F x I			ns	ns	ns	ns	ns	ns

MODEL: Split - split plot in RCB
ns - non-significant
* - significant
** - highly significant

Appendix Table 6. Total number and weight of nodules and dry matter yield of mungbean as affected by spacing and fertilizer of sugarcane (main crop) and inoculation of mungbean (intercrop).

SPACING (S)	FERTILIZER (F)	Inoculation of Mungbean (I)	TOTAL NODULE NUMBER/10 PLANTS		TOTAL NODULE MT./10 PLANTS (mg)		DRYMATTER YIELD/10 PLANTS (g)	
			1986	1987	1986	1987	1986	1987
S1.25	75-75-150	without	133.33	145.67	427.66	469.33	45.13	48.94
		with	143.67	135.00	457.33	435.33	43.93	55.08
	150-75-200	without	140.67	130.67	450.00	420.00	53.36	44.63
		with	146.67	131.00	470.00	422.67	48.63	55.81
S1.50	75-75-150	without	138.00	137.67	443.33	438.33	49.23	46.89
		with	153.33	152.00	489.67	486.33	39.30	53.60
	150-75-200	without	156.67	147.33	501.67	472.00	52.33	52.93
		with	160.00	160.67	512.67	514.33	52.60	54.82
Double row scheme	75-75-150	without	148.67	144.00	475.67	462.00	59.20	46.86
		with	155.33	147.67	497.33	474.33	44.23	54.99
	150-75-200	without	151.33	131.33	484.00	419.33	60.93	51.46
		with	156.67	145.00	501.33	463.00	53.67	55.32
ANOVA	F-test							
Spacing (s)			ns	ns	ns	ns	ns	ns
Fertilizer (F)			*	ns	*	ns	*	ns
S x F			ns	ns	ns	ns	ns	ns
Inoculation (I)			ns	ns	ns	ns	*	**
S x I			ns	ns	ns	ns	ns	ns
F x I			ns	ns	ns	ns	ns	ns
S x F x I			ns	ns	ns	ns	ns	ns

MODEL: Split - split plot in RCB
ns - non-significant
* - significant
** - highly significant

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Microbiological and Sensory Quality Attributes of IDLI From Rice With Varying Amylose Content

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ABSTRACT

Idli batters were prepared from rice varieties of varying amylose content. Lactobacilli, yeast, mold and coliform counts were monitored during fermentation. The cooked idlis were evaluated by Filipino and Indian panels.

Results of this study revealed the microbiological quality of the fermenting batters were not affected by the amylose content of the rice used. However, it was found that among the sensory quality attributes evaluated, cohesiveness was found to be correlated with the amylose content of rice used. Filipino and Indian panels evaluated the quality of the idlis in almost the same part of the scale except for flavor wherein the former seemed to notice the sour off flavor which were not detected by the latter. Probably, Indians consider this sour off flavor inherent of idli.

INTRODUCTION

Idli is a small, acidic, leavened and steam-cooked cake produced by lactic acid fermentation of a thick batter made from polished rice and dehulled black gram *(Phaseolus mungo)*, a pulse. The cake is soft, moist, spongy, has a desirable sour flavor and is easily digestable (Ramakrishnan et al., 1979). It possesses a crumb structure resembling that of the bread but much less elastic.

Murthy and Natarajan (1981) observed the presence of lactobacilli and *Leuconostoc mesenteroides* in the idli batter. Presence of yeasts was also reported (Yajurvedi, 1980). However, Pederson (1967) speculated that the presence of yeast in the idli batter is incidental. According to him, it is *L. mesenteroides* that is important in the idli fermentation. Carbon dioxide produced during fermentation acted as the leavening agent.

The dextrans produced by *L. mesenteroides* are important in the retention of gas which upon steaming make idli essentially a leavened sour dough bread (Steinkraus, 1983). This process could be applied in many parts of the world where wheat or rye flour is unavailable. The acidity in idli also makes the rice cake resistant to spoilage. Therefore, this study was conducted to evaluate the microbiological quality of the idli batter and the sensory quality attributes of the resulting idli from rice with different amylose contents.

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MATERIALS AND METHODS

Raw Materials

Four rice varieties with varied amylose content were obtained from International Rice Research Institute (IRRI), whereas the black gram dhal was purchased from India. The rice varieties used included IR8 (high amylose type), IR64 (intermediate amylose type), IR24 (low amylose type) and IR29 (non-amylose type or waxy). The amylose content of each rice variety is presented in Table 1.

Idli Preparation

Formulation used in idli preparation was mainly based on the studies of Joseph et al. (1961). The above formulation resulted in a more desirable batter consistency than the other formulations evaluated.

The formulation used 2 parts rice and 1 part black gram dhal. The rice and black gram dhal were soaked separately in water overnight at the rate of 1:1 (wt/vol) and 1:3 (wt/vol), respectively.

After soaking, rice was blended using the entire quantity of water used for soaking.The water used to soak the dhal was discarded and then water at the rate of 1:1 (wt/vol) was added and then blended. The resulting slurries from each of the blended dhal and rice were mixed thoroughly to form the idli fermenting mixture or batter. Common table salt was added at the rate of 0.8% of the weight of the batter. The batter was then transferred to 1-li beaker, covered with aluminum foil and allowed to ferment overnight (approximately 12-24 hour) at room temperature. An aliquot of this batter was used for microbiological analysis.

The following morning, the batter was mixed thoroughly and portioned into a steamer especially designed for idli. The steamer was made of aluminum and had eight depressions resembling shallow cups. These cups have minute perforations to allow steam to penetrate the sample. The idlis were steam baked for 10 minutes.

Table 1.Percent amylose[1] content of the four rice varieties used in this study.

Variety	Amylose Content (%)
IR8 (high amylose)	28.8
IR64 (intermediate amylose)	21.6
IR24 (low amylose)	17.0
IR29 (non-amylose or waxy)	0

[1]Rice Quality Laboratory, International Rice Research Institute, Los Baños, Laguna. Philippines.

Microbiological Analyses

Lactobacilli

Standard plate count for *Lactobacilli* was carried out using de Man, Ragosa and Sharpe (MRS) agar as suggested by de Man et al. (1960). Suitable decimal dilutions of samples were prepared using 1.0% peptone water. Microaerophilic condition in the plates was maintained by overlayering the inoculated plates with MRS agar. The plates were incubated at 37°C for 3 days. Counts were made only in those plates with 30-300 colonies per plate.

Yeast and Molds

For yeast and mold counts, the samples were plated on Potato Dextrose Agar (PDA). Plates were incubated at room temperature for 3 days and the colonies were counted.

Coliforms

Total counts for coliforms were carried out as per Compendium of Methods for Microbiological Examinations of Foods (1976). Violet Red Bile Agar (VRBA) was used as the plating medium. The plates were overlayed with 3 to 4 ml of additional VRBA to prevent surface growth and spreading of colonies. The plates were incubated at 35°C for 18 to 24 hours. Plates containing 30-150 minute purplish red colonies 0.5mm in diameter or larger surrounded by a reddish zone of precipitated bile were counted.

Sensory Evaluation

The quality attributes of the cooked idlis from the four rice varieties with different amylose content were subjected to sensory evaluation. Evaluation was carried out in individually separated booths. The idlis were evaluated by 10 Filipino panel members (Research staff of the Institute of Food Science and Technology) and by 10 South Indian panel members, the latter being familiar with idli preparation and its taste. Aroma (fermented), density,, coarseness, cohesiveness, off-flavor, fermented flavor and general acceptability were the quality attributes evaluated. All sample were coded with 3-digit numbers and served in random order to each panelist.

Statistical Analysis

Data obtained from the experiments were analyzed by analysis of variance (Snedecor and Cochran, 1967). Where treatments were found significant, Duncan's New Multiple Range Test (Duncan, 1955) was used to locate significant means.

RESULTS AND DISCUSSION

Microbiological Analysis

Lactobacilli

Table 2 shows the *Lactobacilli* present in the batter from each of the rice varieties. It was observed that the *Lactobacilli* counts were low and not significantly different from each other. At the 8th hour of fermentation the differences in *Lactobacilli* counts of the four idli batter were significant, except for IR29 and IR8. Batters from all the four rice varieties were significantly different from each other in the *Lactobacilli* counts at the 12th hour of fermentation. After the 24th hour of fermentation *Lactobacilli* population of batters from IR24and IR 64 were not significantly different from each other but were significantly different in batters of IR29 and IR8.

Table 3 shows the changes in the *Lactobacilli* count of each batter at different fermentation times. In all the idli batters using rice with varying amylose content, it was observed that the *Lactobacilli* population increased many

Table 2 Counts of *Lactobacilli*[1] in idli batters compared among rice varieties of different fermentation times.

TIME (hr)	VARIETIES[2] IR 29		IR 24		IR 64		IR 8	
0	52500	a	4400	a	13400	a	16000	a
4	2925000	a	315000	a	137700	a	332500	a
8	11250000	a	1775000	c	7550000	b	13850000	c
12	89500000	a	13575000	b	84000000	b	19450000	c

[1] N =4. Means within a particular time followed with the same supercripts are not significantly (P = 0.05) different

[2] IR29 = Non amylose
IR24 = Low amylose
IR64 = Intermediate amylose
IR 8 = High amylose

Table 3. **Counts of *Lactobacilli*[1] in idli batters compared within rice varieties at different fermentation times.**

TIME (hr)	VARIETIES[2] IR 29		IR 24		IR 64		IR 8	
0	52500	d	4400	c	13400	c	16000	c
4	2925000	d	315000	c	137700	c	332500	c
8	1125000	c	1775000	c	7550000	b	13850000	b
12	8950000	a	13575000	a	84000000	a	19450000	a
24	6150000	b	8750000	b	6550000	b	835000	c

[1] N = *4.* Means within a variety followed with the same superscripts are not significantly (P = 0.05) different

[2] IR29 = Non amylose
IR24 = Low amylose
IR64 = Intermediate amylose
IR 8 = High amylose

folds with the highest count at 12th hour of fermentation. There was, however, a significant reduction in the *Lactobacilli* population from 12th to 24th hour of fermentation. It appears, therefore, that maximum fermentation time to attain the highest *Lactobacilli* activity for idli regardless of the amylose content of rice used is 12 hours.

According to Wood et al. (1957), a wide range of microorganisms develop in the idli batter initially but eventually the *Lactobacilli* predominate because of the acid produced. Acid production and leavening of the idli (batter volume increase) were known to occur during fermentation (Mukherjee et al. 1965). However, when the batters were analyzed for *Lactobacilli* after 24 hours fermentation, a significant decrease was observed in all batters from different rice varieties. It is apparent that the amylose content of the rice does not affect the growth of *Lactobacilli* in the idli batter during fermentation.

Acidification of the batter with lactic and acetic acids produced by *Lactobacilli,* leavening of the batter with CO_2 produced by yeasts and *Lactobacilli* are the basic biochemical changes that occur in the batter during fermentation. Typical flavor and aroma development in idli can be used to trace biochemical activities of both *Lactobacilli* and yeasts. Some of the chewiness of idli may be due to the production of bacterial polysaccharides by *Lactobacilli* (Steinkraus, 1983). Pederson (1967) reported that mesenteroides, the lactic acid bacterium *L. mesenteroides* produces not only lactic acid, but lesser amounts of lactic acid, ethyl alcohol and carbon dioxide with traces of minor substances, which are all important in imparting the desirable flavor to good idli.

Yeast and Mold

Yeast and mold in the idli batter at different fermentation times are presented in Table 4. Results showed that the counts for yeasts and molds did not vary significantly up to 4 hours of fermentation period of the idli batter from different rice varieties. At the 8th hour, the yeast and mold count from dif-

Table 4. **Counts of yeasts and molds[1] in idli batters compared among rice varieties at different fermentation times.**

TIME (hr)	VARIETIES[2] IR 29		IR 24		IR 64		IR 8	
0	9800	a	3500	a	5200	a	5700	a
4	19400	a	4900	a	62000	a	15600	a
8	63700	bc	5500	c	81000	b	385000	a
12	357500	a	140200	b	340000	a	157500	b
24	855000	b	922500	a	885000	ab	245500	c

[1] N = 4. Means within particular time followed with the same superscripts are not siginificantly (P = 0.05) different.

[2] IR29 = Non amylose
IR24 = Low amylose
IR64 = Intermediate amylose
IR 8 = High amylose

Table 5. **Counts of yeasts and molds [1] of idli batters compared within rice varieties at different fermentation times.**

TIME (hr)	VARIETIES[2] IR 29		IR 24		IR 64		IR 8	
0	9800	c	3500	c	5200	d	5700	d
4	19400	c	4900	c	62000	cd	15600	cd
8	63700	c	5500	c	81000	c	385000	a
12	357500	b	140200	b	340000	b	157500	c
24	855000	a	922500	a	885000	a	245500	b

[1] N = Means within variety followed with the same superscripts are not significantly (P = 0.05) different

[2] IR29 = Non amylose
IR24 = Low amylose
IR64 = Intermediate amylose
IR 8 = High amylose

Table 6. **Counts of coliforms[1] of idli batters compared among rice varieties at different fermentation times.**

TIME (hr)	VARIETIES[2] IR 29		IR 24		IR 64		IR 8	
0	59000	a	1800	a	9500	a	81000	a
4	762500	a	3500	a	76200	a	138200	a
8	48500000	a	342500	c	6175000	b	1490000	c
12	54000000	a	39750000	a	5225000	a	7150000	c
24	810000	a	1345000	a	60000	a	257500	a

[1] N = 4. Means within particular time followed with the same superscripts are not significantly (P = 0.05) different.

[2] IR29 = Non amylose
IR 24 = Low amylose
IR 64 = Intermediate amylose
IR 8 = High amylose

Table 7. **Counts of coliforms[1] of the idli batters compared within rice varieties at different fermentation times.**

TIME (hr)	VARIETIES[2] IR 29		IR 24		IR 64		IR 8	
0	59000	c	1800	b	9500	c	81000	b
4	762500	c	3500	b	76200	c	138200	b
8	48500000	b	342500	b	6175000	b	1490000	b
12	54000000	a	39750000	a	5225000	a	7150000	a
24	810000	c	1345000	b	60000	c	257500	b

[1] N = 4. Means within a variety followed with the same superscripts are not significantly (P = 0.05) different.

[1] IR29 = Non amylose
IR24 = Low amylose
IR64 = Intermediate amylose
IR8 = High amylose

ferent varieties IR24, IR64 and IR8 were significantly different but the counts of IR29 were significantly different from IR24 and and RI64. At the 12th hour of fermentation, there was no significant difference between the counts of IR29 and IR64 and between IR24 and IR8. At 24 hours of fermentation, the yeast and mold population of IR29, IR24 and IR8 were significantly different, but count for RI64 was not significantly different from the count for IR29 and IR24.

Except for IR8, all the varieties showed an increase in the yeast and mold population starting at the 12th hour of fermentation (Table 5). Yeast and mold count of the idli batter from IR8 did not show a definite trend. However, the yeast and mold counts for the other three batters from different varieties increased significantly from 8th hour of fermentation until the 24th hour. The idli from IR8 showed maximum counts of yeasts and molds at the 8th hour of fermentation and decreased significantly as fermentation progressed. Yajurvedi (1980) reviewed the microbiology of idli fermentation in which he established the role of yeasts in idli fermentation. Paderson (1967) believed that the presence of yeasts in idli batter is incidental. However, Desikachar et al. (1960) showed that both yeasts and bacteria participate in idli fermentation using penicillin G and choloretatracyclin as selective inhibitors. Hesseltine and Wang (1980) and Joseph et al. (1961) believed that yeasts and lactic acid bacteria are involved. Pandalai and Kurup (1963) isolated two types of yeasts from idli batter. Murthy and Natarajan (1981) also reported the presence of yeasts besides *Lactobacilli* and *Leuconostoc mesenteroides* in the idli batter.

Venkatasubbaih et al. (1984) have also reported that the yeasts are responsible for the two fold increase in the volume of batter. According to Wood et al. (1975) yeast survives in sour dough type breads as they tolerate acid well.

Coliforms

Table 6 reveals no significant differences in the coliforms till four hours of fermentation in all the 4 rice varieties. At the 8th hour of fermentation, a significant difference in the counts of varieties IR29, IR24 and IR64 was observed the counts of IR8 were not significantly different from IR 24. At 12 hours of fermentation the counts of IR29, IR25 and IR8 were significantly different, but the counts of IR64 being similar to those of IR29. As the fermentation progressed further, there was a significant drop in the coliform population and the 24-hour fermented batter revealed no significant difference in the coliform counts among the different rice varieties.

It was observed that the coliform counts increased from the start of fermentation up to 12 hours of fermentation (Table 7) when all the varieties showed that maximum counts of coliforms. This increase in the estimated number of coliforms could be attributed to the fact that unwashed rice varieties were used for idli preparation in this study The decrease in the coliforms counts observed at 24-hour of fermentation time was not significantly different from the initial counts at 0 and 4 hours batter in IR29 and IR64, and from 0, 4 and 8 hour old batter in IR24 and IR8.

Coliforms in the batter may be helpful in releasing fermentable carbohydrate from starchy substrates thus enabling the *Lactobacilli* and yeast to take over the main fermentation and bring about desirable changes lowering of pH and production of gas (Venkatasubbaiah et al., 1984). Pandalai and Kurup (1963) from their studies of nine samples of rice and grain mixture also observed the presence of a motile gram-negative coliform bacillus.

Sensory Evaluation

Freshly steamed idlis were subjected to Filipino and Indian panel members for sensory evaluation. Results obtained did not show much difference between the evaluation made by two groups of panels. These results were found consistent with those of Oñate and del Mundo (1966a) who conducted studies to determine the difference between a trained and a consumer panel in their ability to assess cooked milled rice and found that the consumer panel gave scores similar to those of Laboratory panel.

Filipino Panel

Mean sensory scores of idlis by a Filipino panel using different rice varieties with varied

Table 8. Mean[1] sensory scores[2] of idli using different rice varieties as evaluated by Filipino laboratory panel.

VARIETY[3]	SENSORY ATTRIBUTE						
	Aroma (Fermented)	Density	Coarse-ness	Cohesive-ness	Off Flavor	Fermented Flavor	General Accept-ability
IR29	4.7 a	4.1 a	3.2 a	5.8 a	4.9 a	3.8 a	2.5 b
IR24	5.4 a	3.9 a	3.8 a	4.8 b	5.3 a	4.1 a	3.5 a
IR64	5.0 a	3.9 a	3.3 a	4.5 b	4.3 a	3.9 a	3.6 a
IR 8	5.0 a	3.9 a	4.0 a	3.5 c	5.1 a	3.9 a	4.3 a

[1] N = 10. Values followed with the same superscripts within a sensory attributes are not significantly (P = 0.05) different.

[2] Range of scores:
- Aroma (Fermented) : 1, not perceptible to 7, very strong
- Density : 1, very tough to 6, very tender
- Coarseness : 1, very coarse to 6, very fine
- Cohesiveness : 1, well separated to 6, very sticky
- Fermented Flour : 1, not perceptible to 7, very strong
- General Acceptability : 1, unacceptable to 6, acceptable

[3] IR29 = Non amylose
IR24 = Low amylose
IR64 = Intermediate amylose
IR 8 = High amylose

Table 9. Mean[1] sensory scores[2] of idli using different rice varieties as evaluated by South Indian panel.

VARIETY[3]	SENSORY ATTRIBUTE						
	Aroma (Fermented)	Density	Coarse-ness	Cohesive-ness	Off Flavor	Fermented Flavor	General Accept-ability
IR29	3.8 a	3.8 a	2.8 b	5.9 a	1.8 a	2.9 a	1.1 b
IR24	4.5 a	4.0 a	3.5 b	3.5 b	2.0 a	3.6 a	4.9 a
IR64	4.4 a	4.4 a	3.0 b	3.2 b	2.0 a	3.0 a	4.5 a
IR 8	4.2 a	3.4 a	5.0 a	3.3 b	1.6 a	3.6 a	4.8 a

[1]N = 10. Values followed with the same superscripts within a sensory attribute are not significantly (P = 0.05) different.

[2]Range of scores:

Aroma (fermented)	:	1, not perceptible to 7, very strong.
Density	:	1, very tough to 6, very tender.
Coarseness	:	1, very coarse to 6, very fine.
Cohesiveness	:	1, well separated to 6, very sticky.
Fermented Flavor	:	1, not perceptible to 7, very strong.
Off-Flavor	:	1, not perceptible to 7, very strong.
General Acceptability	:	1, unacceptable to 6, acceptable.

[3]IR29 = Non amylose
IR24 = Low amylose
IR64 = Intermediate amylose
IR 8 = High amylose

amylose content are presented in Table 8. Results revealed no significant differences among idlis from the different rice varieties for aroma (fermented), density, coarseness, off-flavor and fermented flavor. However, significant differences were observed among the varieties for cohesiveness and general acceptability.

For cohesiveness, varieties IR24 and IR64 (low and intermediate amylose types) were not significantly different. Varieties IR29 (amylose or waxy) and IR8 (high amylose) were significantly different from each other and from varieties IR24 and IR64. IR29 had the highest score for cohesiveness that this particular variety was sticky and IR8 the lowest, indicating the particles were well separated. The score for IR24 and IR64 were almost similar and fell within this range. Perez et al. (1979) have reported that the stickiness of cooked rice determined with an Instron tester has a negative relationship ($r = -0.92$) with amylose content. The results obtained presently conformed to this finding.

Comparison of general acceptability of idlis to the presence or absence of amylose content in rice, irrespective of the percent amylose content, revealed that the non-amylose variety IR29 was found at least acceptable. Scores for general acceptability improves as the amylose content of rice varieties increased; through the differences for general acceptability of idlis among low, intermediate or high amylose rice varieties were not significant. A similar result for puto (Philippine fermented rice cake) was obtained by Sanchez (1975) wherein the amylose content of rice was highly correlated with the general acceptability of puto.

South Indian Panel

Mean sensory scores of idlis by a South Indian panel are presented in Table 9. Results obtained by the Indian panel were almost similar to those obtained by the Filipino panel. Coarseness was the only quality attribute where the results differed between the two panels. The varieties were rated significantly from each other for coarseness by the Indian panel. IR8 variety was rated significantly different from the varieties IR29, IR64 and IR24, which in turn were not significantly different from each other. The highest score obtained by IR8 indicated its fine texture as compared to the other three varieties.

Other characteristics evaluated were aroma (fermented) density, off-flavor and fermented flavor did not differ significantly in the four rice varieties tested. The scores obtained from South Indian panel were almost similar to those obtained from the Filipino panel except for the off flavor. Off flavor had a higher rating (strong off flavor) when evaluated by the Filipino panel compared to the South Indian panel where the scores were low, as the off-flavor was not perceptible This could be due to the fact that idli was a new product to the Filipino panel as they were not familiar with the typical idli flavor, thus, they considered it as an off flavor.

For the cohesiveness IR29 was rated significantly different from IR24, IR64 and IR8, which did not differ significantly from each other. IR29 again was rated the highest score for cohesiveness from the rest of the varieties, indicating its very sticky nature.

As far as general acceptability was concerned idlis from varieties IR24, IR64 and IR8 were not significantly different. Only idlis from IR29 was significantly different from the other three varieties. Here also, IR29 had very low scores as compared to the other varieties indicating that non-amylose rice is not good raw material for idli making.

Based on the above results from sensory evaluation the textural quality of idli is dependent on the amylose content of the rice used. IR8, the high amylose type and IR64, the intermediate amylose type, seemed to result in better texture better, volume expansion and low stickiness compared to the idlis made from low amylose variety IR24 or the waxy variety IR29. Bhattacharaya et al. (1978) found that textural properties of 32 rice varieties (stickiness consistency and viscogram characteristics) correlated well with the insoluble amylose content. As the insoluble amylose increased, the consistency and the setback increased and the stickiness decreased. Sanchez (19745) has reported a good correlation between high amylose content and satisfactory volume expansion. The present results appear to support the view in as much as better idlis were made from high and intermediate amylose type varie-

ties.

Rice with an intermediate amylose content (20 25%) appears to be preferred for preparing fermented rice cake not only because of its soft texture (Jesus, 1965), but also because of the ability of its batter to retain more carbon dioxide during steaming, resulting in a larger volume expansion of the cake. The batters prepared from waxy rice collapsed during steaming. Presumably, rice with an intermediate amylose content provides an optimum amylose/amylopectin ratio with a high enough amylopectin content to allow expansion of the gelatinized starch during steaming, but with an amylose content sufficient to prevent collapse of the expanded cake (Perdon and Juliano, 1975).

SUMMARY AND CONCLUSION

Microbiological evaluation revealed the presence of *Lactobacilli* yeasts and molds and coliforms. At the onset of fermentation, the idli batter had low microbial load. These however increased significantly as fermentation progressed. Maximum population of *Lactobacilli* and coliforms were observed after 12 hours and those of yeasts and molds after 24 hours of fermentation. The highest mean population counts of *Lactobacilli* and yeasts and molds were obtained in the varieties IR29, and IR64 respectively and the lowest mean coliform counts in IR8.

There was not much difference in the evaluation of idlis by Filipino and South Indian panel. Idlis from waxy rice variety IR29 were unacceptable and significantly different from those evaluation by the two panels did not find any significant difference among the rice varieties for aroma (fermented) density off flavor and fermented flavor. However, the attributes of cohesiveness, coarseness and general acceptability were significantly different.

Therefore it can be concluded that 12-hour fermentation of idli batter appeared to be the optimum time for producing idlis with good flavor and spongy texture. High to intermediate amylose rice varieties appeared to be more suitable for idli preparation.

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Effects of Soil Fumigants and Mycorrhizal Inoculants on Early Growth of Benguet Pine *(Pinus Kesiya Royle Ex Gordon)* In The Nursery

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ABSTRACT

Formaldehyde and aluminum phosphide ($AlPO_3$) at different concentrations were used to sterilize Benguet soil. The effects of sterilization of Benguet soil with different concentrations followed by separate inoculation with freeze-dried basidiospores of *Pisolithus tinctorius* (PERS.) Coker and Couch and *Rhizopogon* sp. on the growth of *Pinus kesiya* (Benguet pine) seedlings were studied. Results indicated that the two mycorrhizal inoculants were able to infect the seedlings. Infection intensity was moderate for all treatments except for the unfumigated soil inoculated with *Rhizopogon* sp. which was high. Seedlings inoculated with *Rhizopogon* sp. grown in unfumigated soil had the highest growth. The experiment showed that sterilization by use of fumigants is not necessary unless presence of soil-born pathogens is detected. *Rhizopogon* sp. is recommended inoculant for growing Benguet pine seedlings in Benguet soil.

INTRODUCTION

The first two generations of pine plantations established in a previously natural pine forest area may give high yields but succeeding generations may not meet the desired or expected goal. This maybe due to the loss of nutrients by leaching, surface run-off, soil erosion or by the removal of nutrients during harvest (Marquez, 1980).

Significant economic success from the application of silvicultural measures to increase the production of wood can only be fulfilled if the silvicultural requirements of trees are taken into consideration. Due to the high cost of production, an ailing limited manpower and countryside instability, the result of current government efforts on reforestation do not usually come close to a breakeven economic return.

Minimizing cost of reforestation and maximizing output to achieve satisfactory results are the main targets of the government. Searching for technologies designed to reduce cost of planting stock propagation continuously goes on. Mycorrhizae have been found to be a good alternative to synthetic fertilizers. Fumigants are being used in the nursery for effective and efficient sterilization but not widespread due to its high cost and complexity in its application.

The genetal objective of this study is to

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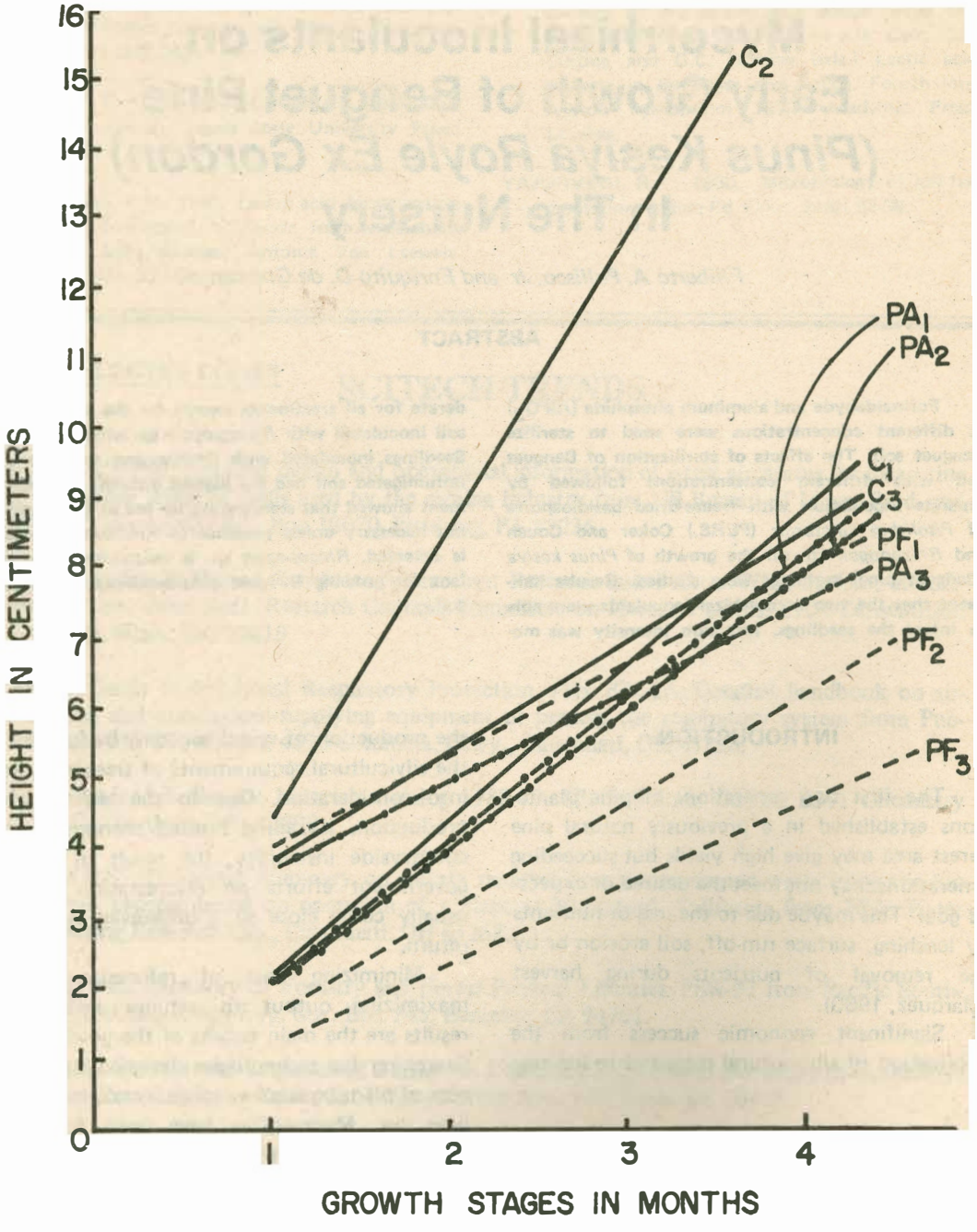

Figure 1. Growth curves of Benguet pine seedlings grown in soil inoculated with *Pisolithus tinctorius* and sterilized by different fumigants.

determine whether fumigants and inoculations of mycorrhizal fungi are necessary in growing Benguet pine seedlings on Benguet soil.

The specific objectives are:

1. To assess the effects of fumigation and root infection of mycorrhizal inoculants on Benguet pine seedlings and
2. To determine the effect of inoculation on the growth of Benguet pine in fumigated and unfumigated soil.

MATERIALS AND METHODS

Basidiospores of *Pisolithus tinctorius* and *Rhizopogon* sp. were the fungal inoculants used in the experiment. They were collected in Pantabangan, Nueva Ecija and Bobok, Benguet, respectively and were freeze dried at about 12-13% moisture content.

The Benguet pine seeds were collected from three (3) pine trees in the vicinity of Atok, Benguet. Two (2) cubic meters of soil were placed in large plastic bags and transported to the nursery of the Foresh Research Institute (FORI) in Mudspring, College, Laguna.

Ninety (90) plastic bags (15.24 cm. x 25.-40 cm.) were filled with unfumigated soil and served as controls. A set was inoculated with *P. tinctorius,* another with *Rhizopogon* sp., and a last set was uninoculated.

Two (2) chemicals were used in sterilizing the soil. The first was formalin and applied at 37%, 74%, and 100%. One thousand ml. of formalin solution was poured in each plastic bag containing 0.27 cu.m. of soil. After sealing with tape, the treated soils were left in the greenhouse for 24 hours after which they were aerated by pouring the contents separately in a wheelbarrow and mixing the soil by hand. The soil was then transferred to the small plastic bags and left in the greenhouse for another twenty four hours.

The second chemical was aluminum phosphide in the form of tablets and applied at 1, 2 and 3 tablets per bag. After dispensing the tablets, the procedures mentioned above were followed. The seeds were soaked in tap water for 24 hours before sown. A 0.01 gram of the basidiospores was introduced in a 2.54 cm. deep depression was covered with soil from the same bag and five seeds were sown in each bag.

Height growth (measured one month after sowing and every 15 days thereafter), % infection intensity, fresh weight and ovendry weight (at the end of the experiment) were the parameters measured. Oven-drying was done at 70°C for 72 hours until the weight became constant.

The experiment was conducted following the randomized complete block design.

RESULTS

1. **Fresh weight of pine seedlings.** The fresh weight of seedlings grown in soil fumigated with $AlPO_3$ and inoculated with *Rhizopogon* sp. decreased as concentration increased, on the other hand, the fresh weight of seedlings grown in soil treated with formaldehyde and inoculated with *Rhizopogon* sp. increased as concentration increased. Statistically, however, the fresh weight of all treated and inoculated seedlings compared to the controls did not show any significant effect.

2. **Ovendry weight of pine seedlings.** Higher concentrations of $AlPO_3$, reduced ovendry weight of seedlings grown in soil inoculated with *Pisolithus tinctorius.* However, for those seedlings grown in soil inoculated with *Rhizopogon* sp. higher concentrations of formaldehyde increased ovendry weight. Statistically, the ovendry weight of all seedlings grown in treated and inoculated soil were not significantly different from each other and when compared with the controls.

3. **Percent mycorrhizal infection intensity.** The degree of infection was moderate for all seedlings treated with the different fumigants and inoculated with the mycorrhizal inoculants. However, the seedlings inoculated with *Rhizopogon* sp. and growing in unfumigated soil had a high infection intensity.

4. **Soil analysis.** Soil treated with formaldehyde became more acidic while soil treated $AlPO_3$ become moderately acidic. The application of fumigants lowered the P content but the overall P content was considered high. Nitrogen and potassium contents were considered low.

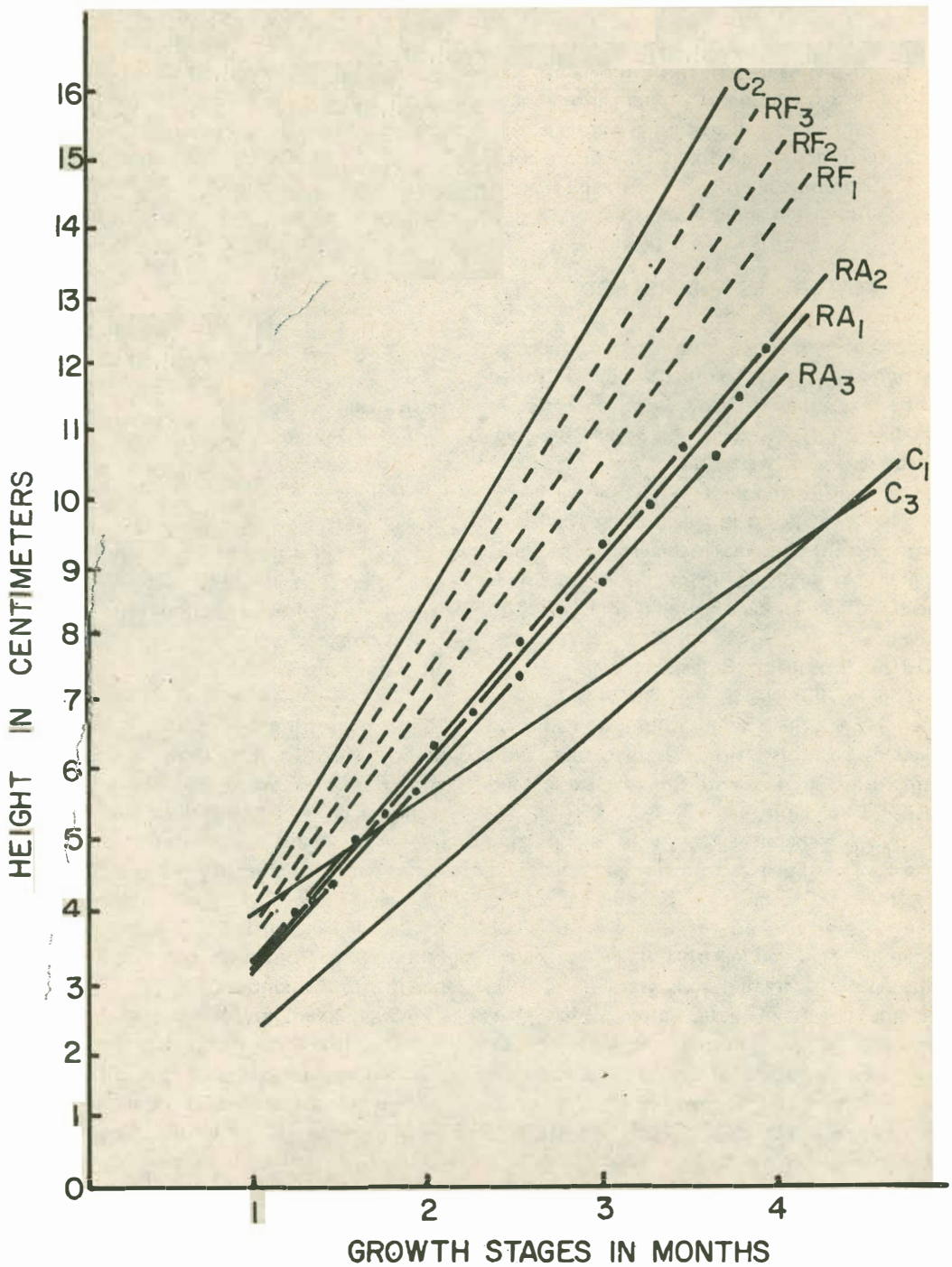

Figure 2. Growth curves of Benguet pine seedlings grown in soil inoculated with *Rhizopogon* sp. and sterilized by different fumigants.

5. **Height growth.** At 4½ months (Table 1) the height growth of seedlings grown in soil treated with the different fumigants and inoculated with *Pisolithus tinctorius* had less than half of the height growths of seedlings in C_2 (Unfumigated, inoculated with *Rhizopogon* sp.). Regression analysis shows that C_2 had the best growth (Figures 1 and 2).

DISCUSSION

The compatibility of the mycorrhizal fungi with the tree species is important with the growth and development of the tree. The growth responses of the pine seedlings grown in inoculated soil with *Rhizopogon* sp. were far better than those inoculated with *Pisolithus tinctorius,* meaning, *Rhizopogon* sp. is more compatible with Benguet pine than *P. tinctorius.* This suggests that the Benguet soil, the Benguet pine seeds and the *Rhizopogon* sp. mycorrhiza collected from the Benguet area, were compatible with one another.

Daft and Nicholson (1969) stated that the development of mycorrhizal roots and their effect on plant growth is greater in soils of low or imbalanced nutrient status. Severe lack of available nitrogen or phosphorus hampers the formation of mycorrhiza as well as growth. Moderate scarcity of one or the other is a condition for infection.

Due to the low nitrogen and potassium content of the Benguet soil, an unbalanced nutrient status might have existed thus making it favorable for mycorrhizal infection.

Overall results indicated that the treatments did not have any significant effect on the infection intensity. Several factors may have influenced the lack of significance of the treatments; the chemicals used might have had a residual effect on the soil preventing a percentage of mycorrhizal spores from germinating. Also, it may be that the chemicals are phytotoxic rendering the seedlings less receptive to intense mycorrhizal infection.

Except for C_2 (unfumigated, inoculated with *Rhizopogon* sp.) which exhibited a higher per cent infection intensity, all the seedlings in the treatments exhibited a moderate infection intensity.

CONCLUSION

Fumigation of Benguet soil is not necessary to attain best height growth of Benguet pine seedlings. Mycorrhizal infection intensity influences the height growth which means that as infection intensity increases height growth also increases. *Rhizopogon* sp. is a better mycorrhizal inoculant for Benguet pine grown in Benguet soil than *Pisolithus tinctorius.*

Table 1. Mean height growth (in cm.) of 4½ month old Benguet pine seedling grown in soil treated with different fumigants and mycorrhizal fungi.

TREATMENTS	REPLICATION			MEAN
	1	2	3	
PF_1	7.5	10.5	8.9	8.97^{od}
PF_2	4.7	8.1	18.8	10.53^{bcd}
PF_3	4.7	8.1	7.7	6.83^{d}
PA_1	7.2	11.7	8.1	9.00^{cd}
PA_2	6.2	13.3	7.8	9.10^{cd}
PA_3	6.1	7.8	7.5	7.13^{d}
RF_1	14.4	16.1	20.2	10.90^{a}
RF_2	23.7	17.5	13.8	18.33^{a}
RF_3	21.9	23.8	14.5	20.07^{a}
RA_1	16.0	19.3	16.7	17.33^{a}
RA_2	11.7	18.7	19.2	$16.53^{a}b$
RA_3	15.0	12.9	15.9	14.60^{abc}
C_1	9.1	11.7	8.6	9.80^{cd}
C_2	13.7	23.7	23.5	20.30^{a}
C_3	7.8	10.6	6.9	8.43^{cd}

Means bearing the same letter superscripts are not significantly different (P 0.05) as based on DMRT.

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COMMERCIABLE TECHNOLOGIES FROM DOST

WATERMELON RIND PRESERVES

Researchers at the Industrial Technology Development Institute, DOST have developed the technology for processing **watermelon rind** into sweetened **preserves** and candies.

In the process watermelon rinds are soaked in lime water for three hours, rinsed with fresh water and then boiled in water for 30 minutes. The rinds are again boiled in a prepared syrup for an hour or until they become transparent and the syrup thickens.

For every eight cups of rinds, two cups of sugar and one cup of water with a little amount of lemon juice are added. Flavors may be added to the syrup.

It costs USD 2 per 8 oz of preserves.

For more information, contact:
MILAGROS RAMOS. Microbiological Research Program. Industrial Technology Development Institute. Department of Science and Technology. Taft Ave., cor Pedro Gil Street Ermita, Manila Philippines.
Telephone: 503-041 Loc. 10

TARO ROOT CULTIVATION

The Philippine Council for Agriculture and Natural Resources Research and Development (PCARRD) offers know-how for commercial cultivation of taro root *(Colocasia Esculenta).*

Taro Root or cocoyam can be cultivated in lowland, upland and waterlogged areas.

In the method, the land is ploughed and harrowed four times before sets prepared from suckers and plants are planted 50 CM apart. The sets consists of upper 1--2 cm tip section of the corms and the first 20--25 CM of the petioles.

Weeding must be done two months after planting. Organic and Inorganic fertilizers can be added for increased production.

Harvesting time in lowland areas varies from 9 to 12 months after planting.

It costs USD 150 to cultivate one hectare of taro. This covers the costs of land preparation, raw materials, equipment and labor.

For more information, contact:
RAMON V. VALMAYOR, Executive Director, Philippine Council for Agriculture and Natural Resources Research and Development (PCARRD), Bureau of Plant Industry Compound, Los Banos, Laguna Philippines.

forestry

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Collection and Germination of Koordersiodendron Pinnatum Seeds

Portia Gamboa Lapitan *

ABSTRACT

Fruits of Amugis *(Koordersiodendron pinnatum)* were collected from trees with yellowish green and yellow pericarp to determine the proper time of seed collection. Results showed that fruits with yellowish green pericarp gave faster rate and higher percent seed germination (100%) than fruits collected with yellow pericarp (28%). These results indicate that seeds of *K. pinnatum* should be collected when their fruits are yellowish green in color.

INTRODUCTION

Maturity of the seed is a very important factor affecting the performance of seeds for germination to occur (Jones and Stodart, 1977), hence mature seeds can be observed to perform better in germination and seed storage than immature ones (Yap 1981, Fables, 1976). This is quite evident in *Shorea talura* seeds which showed a significant increase in percent germination and length of storage with maturation (Sasaki, 1980).

Seeds' susceptibility to fungal attack is also affected to some extent by its maturity. Brown (1986) reported that early harvested seed of *Pinus caribaea* var. *hondurensis* is more susceptible to fungus *Lasiodiplodia theobromae* than those collected later. Mature seeds are better off than immature ones in terms of germination and storage. The collection of seeds, therefore, should be timed to insure that the collected seeds are mature enough to give the maximum number of viable seeds which can be expected to stay viable despite length of storage.

Maturity of the seeds however can be gauged in so many ways. The most practical and commonly used is the color of the fruit which beaks the seeds. Seeds of anabiong (*Trema orientalis),* Benguet Pine *Pinus Kesiya* and some species of Dipterocarps are some examples of seeds which can be certified mature by considering the color of their fruit. Seeds of *Trema orientalis* are considered mature when fruits turn black from green (Lopez 1953). *Pinus kesiya* seeds are mature enough to be harvested when scales of cone start to brown. Seeds of some species of dipterocarps on the other hand like *Shorea sumatrana* will germinate better when collected with pericarp still green. For others best time for collection is when seeds coast are green but with brown wings or when all parts have changed to brown (Yap, 1981). Maturity of the seed as illustrated varies depending upon the species. The color of the fruit when the seed is already mature also varies among tree species. The collector must be able to determine accurately the maturity and quality of the seed. If collection is done too early or too late marked change in germinability of the seed may occur (Brown, 1986).

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It is the objective of this study to determine the appropriate time of seed collection of Amugis *(K. pinnatum)* seeds as indicated by the color of the fruit. This large tree species is a good source of quality lumber.

MATERIALS AND METHODS

Two sets of fruit of *Koordersiodendron pinnatum* were collected from standing trees at Mt. Makiling, College. Laguna. Half of the fruits collected were yellowish green or apple green in color (the color of fruit as it starts turning yellow), while, the other half are yellow in color (the color of the fruit when they are shed from the tree), Figure 1 Initial moisture content and percent viability of the 2 sets of seeds were at once determined. Percent viability was determined using the actual germination test.

Actual germination tests were performed in petri dishes lined with a layer of filter paper and supplied with an initial amount of 15 ml distilled water. Ten randomly selected seeds were sown per dish replicated five times (5X) for each set of seeds. The tests were carried out under an air conditioned laboratory room. A seed is considered to have germinated when the radicle begins to emerge. Number of days to first, 60% and 100% germination are determined and recorded. Likewise, total percent germination is recorded.

RESULTS AND DISCUSSION

Table 1 shows the percent germination of *K. pinnatum* seeds collected when fruits are with yellowish green and yellow pericarp. Seeds in fruit with yellowish green pericarp gave 100% germination for all replicates in contrast to seeds with yellow pericarp which gave an average percent germination of 28%. Analysis of variance of the data revealed statistically significant difference between the two treatments.

Table 2 on the other hand, shows the difference in seed vigor of the two sets of seeds. Days to first germination is earlier for seeds in fruit with yellowish green pericarp (5) than seeds from fruit with yellow pericarp (15.8). It

Table 1. Percent germination of *K. pinnatum* seeds collected when fruits are yellow green and yellow in color.

REPLICATE	PERCENT GERMINATION	
	Yellowish Green	Yellow
1	100	40
2	100	30
3	100	40
4	100	20
5	100	10
AVERAGE	100	28

*Fruits of *K. pinnatum* are one-seeded so that the term fruit is used interchangeably with seed in this paper.

Table 2. Seed vigor and germination of *K. pinnatum* seeds extracted from fruits with yellow green and yellow pericarp.

	SEED VIGOR				
	DAYS TO GERMINATION				
	First	60%	100%	Last	% Germination
Seeds in Yellow Pericarps					
1	12	—	—	20	40
2	17	—	—	17	30
3	10	—	—	20	40
4	20	—	—	20	20
5	20	—	—	20	10
Average	15.8	—	—	19.4	28
Yellowish green					
1	5	12	15	15	100
2	5	12	19	19	100
3	5	12	19	19	100
4	5	12	12	12	100
5	5	12	19	19	100
Average	5	12	16.8	16.8	100

took only 16.8 days for the yellowish green set to complete its germination (100%) while the yellow set required 19.4 days to give a germination of only 28%. These data show that seeds collected when fruits are still yellowish green or apple green in color are much more vigorous than seeds collected when the fruit has already turned yellow in color.

CONCLUSION AND RECOMMENDATIONS

To give the highest percent seed germination in the shortest possible time, the seeds should be collected from the mother tree when they are fully riped or mature. Seeds of *Koordersiodendron pinnatum* should be collected from the tree when the fruit is just starting to turn yellow or when the fruit is yellowish green in color. Seeds collected when they have already turned yellow gave a significantly lower germination of 28% after 19.4 days compared to 100% of yellowish green fruit in 16.8 days.

ACKNOWLEDGEMENT

The author wishes to acknowledge the involvement of the research staff of the Seed Biology Project in the conduct of the study and PCARRD for funding this study under the UPLB-PCARRD Seed Biology of Forest Tree Project.

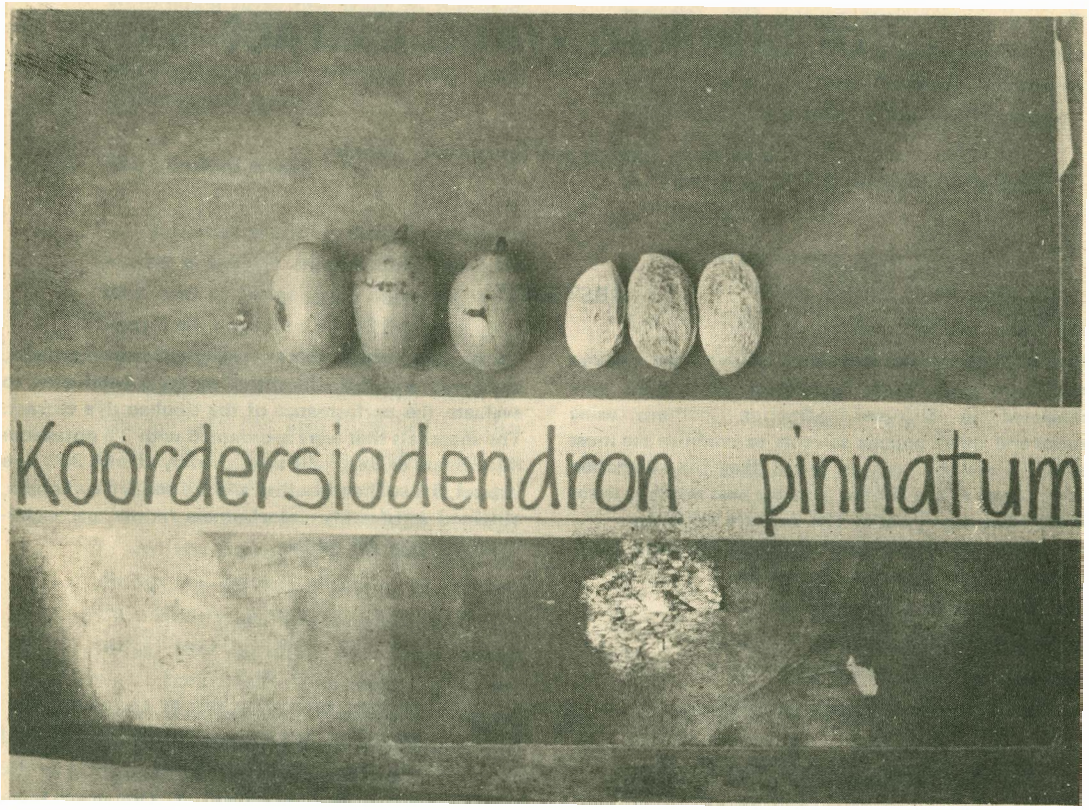

Figure 1 **Seeds of amugis (*K pinnatum*) should be collected from the tree when the fruit has turned yellowish green to yield a significant higher percentage of germination.**

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A Study on the Dyeing Properties of Sibukao Wood Extract

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ABSTRACT

A study on the extraction of dye from sibukao wood was undertaken. Samples of wood chips were subjected to different extraction methods using water and other organic solvents to establish the most effective method. It was found that the extraction using water as the solvent gave the best result in terms of its percentage yield of 12% and dye solubility.

Series of dyeing experiments on silk yarns, abaca fibers and cotton fabrics using different mordants, auxiliaries and dye concentrations were conducted to evaluate the performance of the sibukao dye extract. The materials that were mordanted with 3% potassium dichromate or 3% oxalic acid, then dyed with 30% dye extract, 5 % sodium bisulfite and 1% sodium carbonate, obtained satisfactory colorfastness ratings, particularly on the dyed silk yarns.

INTRODUCTION

A. The Sibukao Tree

Sibukao (*Caesalpinia Sappan,* L.) is a small tree on prolonged exposure to light, air and heat. It attains a height of up to 10 meters and diameter of up to 40 cm. Scattered prickles are present all over the bark including the midrib of the leaf. The leaves are compound with a pair of prickles just underneath the midrib. The leaflets are oblong to oblong rhomboid, very oblique and attached at the lower corner. The apex is generally retuse and the panicles are often as long as the leaves and are terminal. The outer bark is grayish-brown with distinct ridges and many prickles which are removed easily by brushing with the blunted edge of a bolo.

Sibukao grows naturally and abundantly throughout the Panay Island and especially so in Guimaras Island, just off Iloilo City. It has always been the premium firewood for domestic use in the whole of West Visayas area or Region VI. It is also reported to be abundant in the Bicol region. It grows most on hilly areas with clayey soil and/or calcareous rocks at low and medium altitude. It can not tolerate too wet soil conditions and grows best on hillsides where drainage

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is fast after a heavy storm. It is harvested by cutting slightly less than a meter above the ground. Sibukao reproduces by seed and coppice. Profuse coppices grow from the stump within two weeks after the tree is felled. This ability to produce coppices in great abundance does not seem to diminish with age and the sibukao stands continue to regenerate without any help from human agency.

B. The Sibukao Wood

Sibukao wood is straight grained, having a fine to moderately fine texture and is moderately lustrous. It is hard and heavy, very difficult to dry, very refractory and slow to season. It needs protection not only from direct sunlight but also from hot and dry winds. It is lustrous and richly colored making it ideal for violin bows. In addition to its use as a firewood, sibukao is also the source of a red dye and medicine for various ailments of the body. Mature wood is used as house posts, tool handles, and many other small handicraft. The seeds are sometimes powdered and used as a sedative, while the leaves are used to hasten the ripening of fruits.

A red dye (a very important article of commerce), is extractable from the sibukao heartwood. It is a favorite color for textile, paper and food. It has also many applications in other industries.

Most of the red coloring for food is an azo dye. The azo dyes are synthetic and carcinogenic. The coloring has been approved for use in food mainly because there is no available substitute. Thus, there is great need for a red coloring from natural sources to replace the carcinogenic azo-based food coloring.

PTRI EXPERIMENTS ON "SIBUKAO"

For the purpose of producing red dye from "sibukao" wood and establishing dyeing method as applied to silk, cotton and abaca, the PTRI has conducted the following research activities: (1) extraction of dye from "sibukao" wood using water, chloroform and ethanol, (2) use of different mordanting agents at various concentrations, (3) dyeing experiments on silk, cotton and abaca with varying dye concentrations and (4) tests for colorfastness to washing and sunlight of the dyed samples.

A. Extraction of Dye from "Sibukao" Wood

1. Water extraction

   A ten (10.0) gram sample per 400 ml. water was refluxed for five (5) hours. The solution was filtered and the filtrate was evaporated to dryness.

2. Ethanol extraction

   The "sibukao" sawdust wrapped in filter paper was extracted by soxhlet extractor with ethanol (10 g/250 ml solvent) for five (5) hours. The solvent was then evaporated and the residue obtained was oven dried at 90°C.

3. Chloroform extraction

   Follow same procedure as above but use chloroform as solvent.

B. Use of Different Mordanting Agents at Various Concentrations

Different mordanting agents at various concentrations were used on silk yarns, cotton fabric and abaca fibers for color formation. Mordants used were oxalic acid, potassium dichromate, copper sulfate and alum with concentration from 1% -- 10% (based on the weight of material).

The materials were treated with the above mordants at 100°C for thirty (30) minutes at a liquor ratio (L.R.) of 1:30.

C Dyeing Experiments on Silk Yarns, Cotton Fabric and Abaca Fibers with Varying Dye Concentrations

Dyeing experiments with varying dye concentrations ranging from 10% – 40% were conducted on the mordanted silk yarns, cotton fabric and abaca fibers. Dyeability characteristics were evaluated based on colorfastness to light and washing.

1. Dyeing

   Heat mordanted materials at 100°C for one (1) hour with the dye extract and other auxiliaries with L.R 1:30.

2. Test for Colorfastness to Washing and Sunlight

   2.1 Washfastness Test Method used (as described in PTRI Standard)

soap solution	–	5 g/l
liquor ratio	–	1:50
temperature	–	30°C (room temperature)
time	–	30 minutes

   2.2 Lightfastness Test

   The dyed samples were directly exposed to sunlight for 10 hours. The change in color of the dyed samples and the blue wool rating were assessed.

RESULTS

1. Dye extraction

   The best extraction method is water extraction

2. Dyeing application/performance

   The dyeing application for the silk yarns, cotton fabric and abaca fibers adopted in this study and the corresponding ratings are presented in Table 1.

DISCUSSION

The extraction method using water as the solvent exhibited satisfactory result in terms of its percentage yield of 12% and solubility. The dyed silk yarns produced from the best dyeing procedure gave a rating of 4-5 for the washfastness and 4/L5 for the lightfastness indicating a satisfactory result.

It can also be seen from the experiment that the mordants, $K_2Cr_2O_7$ and oxalic acid used can influence the final color shade of the substrate. From the varying dye concentrations (ranging from 10% – 40% tried in the experiment, the 30% dye concentration showed the best dye affinity to the silk yarn.

Application of the dye extract to cotton fabric and abaca fibers was also conducted. The washfastness test of the dyed cotton fabrics and abaca fibers obtained satisfactory ratings, while the lightfastness exhibited poor ratings especially on the dyed cotton fabrics. It was also observed that these materials as compared to the silk yarns have less affinity with the dye extract.

CONCLUSION

Based on the dyeing experiments carried out, it was found that sibukao wood can be utilized as a dye source, particularly suitable to silk materials. However, the red color was not obtained due to the sensitivity of sibukao dye to many factors. Sunlight, heat, oxygen and other chemicals influence the color of the dye. This is considered to be the common characteristics of dyes extracted from wood. This sensitivity may be put to good advantage for developing various shades of the color. It is, however, necessary to establish the chemical structure of the dye itself in order to be

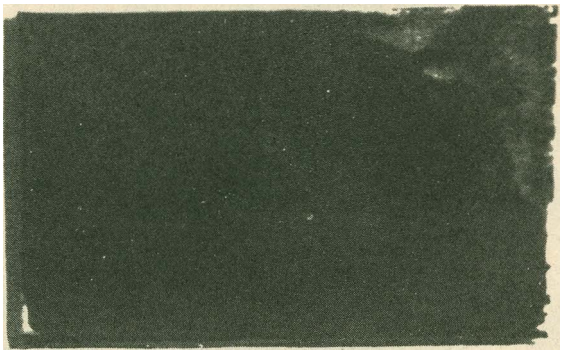
able to take full advantage of its potential. Once the structure is known, it becomes more easy to modify the properties for certain end use.

DYE SOURCE : SIBUKAO WOOD

EXTRACTION : Reflux the sibukao saw-dust in water (10grams per 400ml water) for 5 hours. Filter. Evaporate for dryness.

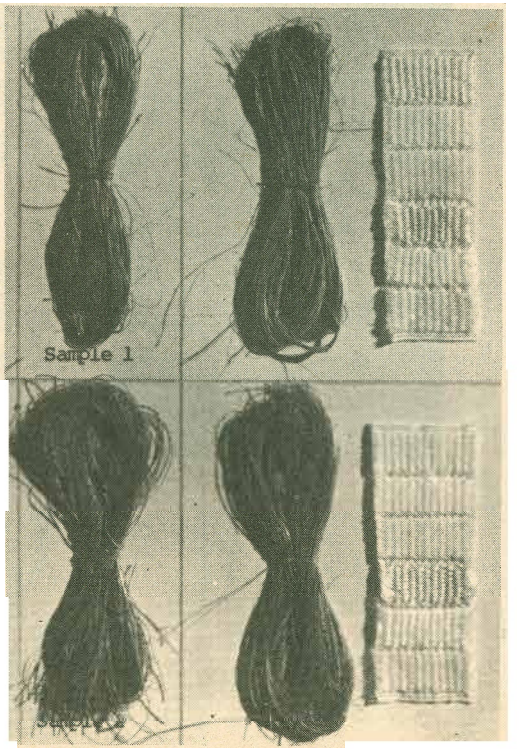
PERCENTAGE YIELD : 12%

DYE EXTRACT



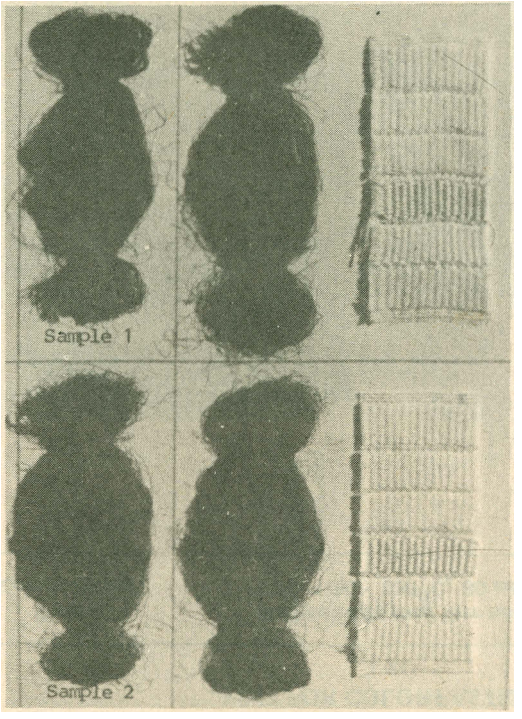
TEST FOR COLORFASTNESS TO WASHING

DYE SOURCE: SIBUKAO WOOD	Dyed (Abaca Fiber	Method: 5.0g soap/liter soln. L.R. 1:50; 30°C; 30 min.	No. 1	STAINING	CHANGE IN COLOR
				Class	Class
Mordanting: 3% $K_2Cr_2O_7$			Acetate	4 – 5	
L.R. 1:30; 60°C; 30 min.			Cotton	4 – 5	
Dyeing: 30% dye 1% Na_2CO_3 5% $NaHSO_3$			Nylon	4 – 5	4 – 5
			Silk	4 – 5	
L R. 1:30; 1 hr. 100°C					
Fixing: 2% Tinofix EW			Rayon	4 – 5	
L R. 1:30; 60°C; 30 min.			Wool	4 – 5	
Mordanting: 3% oxalic acid			Acetate	14 – 5	
L.R. 1:30; 60°C; 30 min.			Cotton	4 – 5	
Dyeing: 30% dye 1% Na_2CO_3 5% $NaHSO_3$			Nylon	4 – 5	4 – 5
L.R 1:30; 1 hr.; 100°C			Silk	4 – 5	
Fixing: 2% Tinofix EW			Rayon	4 – 5	
L R. 1:30; 60°C; 30 min.			Wool	4 – 5	



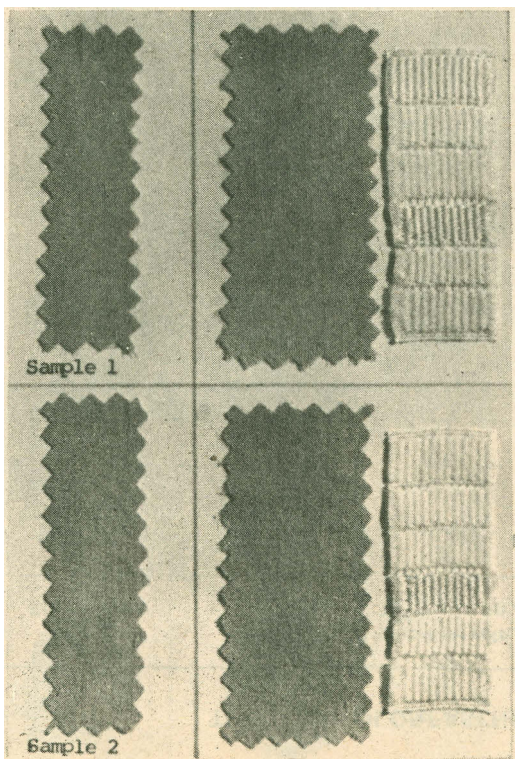
TEST FOR COLORFASTNESS TO WASHING

DYE SOURCE SIBUKAO Wood	Dyed SILK YARN	Method: 5.0g soap/liter soln. L.R. 1:50 ; 30°C ; 30 min.	Multifiber No.1	STAINING	CHANGE IN COLOR
				Class	Class
Mordanting : 3% $K_2Cr_2O_7$			Acetate	4	
L R. 1:30 ; 60°C ; 30 min.			Cotton	4	
Dyeing: 30% dye					
1% Na_2CO_3			Nylon	4	
5% $NaHSO_3$					
L.R. 1:30 ; 1 hr. 100°C			Silk	4	4 – 5
Fixing : 2% Tinofix EW			Rayon	4	
L.R 1:30 ; 60°C ; 30 min.			Wool	4 – 5	
Mordanting 3% oxalic acid			Acetate	4	
L.R. 1:30 ; 60°C ; 30 min.			Cotton	4	
Dyeing : 30% dye					
1% Na_2CO_3			Nylon	4	4 – 5
5% $NaHSO_3$			Silk	4	
L R 1:30 ; 1 hr. ; 100°C					
Fixing : 2% Tinofix EW			Rayon	4	
L. R. 1:30 ; 60°C ; 30 min.			Wool	4 – 5	

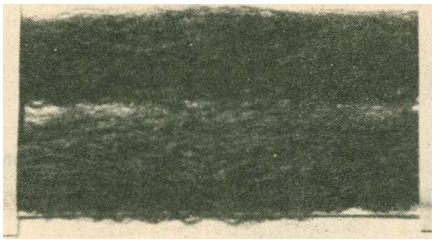


TEST FOR COLORFASTNESS TO WASHING

DYE SOURCE: SIBUKAO WOOD	Dyed Cotton Fabric	Method: 5.0g soap/liter soln. L.R. 1:50 ; 030°C; 30 min.	Multifiber No. 1	STAINING	CHANGE IN COLOR
				Class	Class
Mordanting: 3% $K_2Cr_2O_7$			Acetate	4 – 5	
L.R. 1:30; 60°C; 30 min.			Cotton	4 – 5	
Dyeing: 30% dye 1% Na_2CO_3			Nylon	4 – 5	4 – 5
5% $NaHSO_3$			Silk	4 – 5	
L.R. 1:30, 1 hr.; 100°C					
Fixing: 2% Tinofix EW			Rayon	4 – 5	
L.R. 1:30; 60°C; 30 min.			Wool	4 – 5	
Mordanting: / 3% oxalic acid			Acetate	4 – 5	
L.R. 1:30; 60°C; 30 min.			Cotton	4 – 5	
Dyeing: 30% dye 1% Na_2CO_3			Nylon	4 – 5	4 – 5
5% $NaHSO_3$					
L.R. 1:30; 1 hr.; 100°C			Silk	4 – 5	
Fixing: 2% Tinofix EW			Rayon	4 – 5	
L.R. 1:30; 60°C; 30 min.			Wool	4 – 5	



TEST FOR COLORFASTNESS TO LIGHT (DIRECT SUNLIGHT)

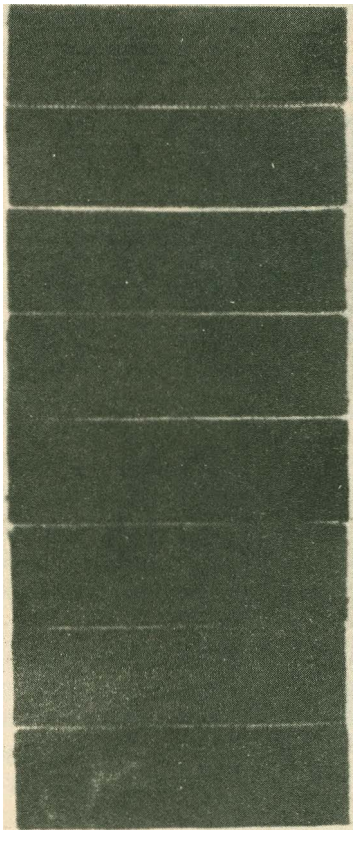


Sample 1

DYED SILK YARNS

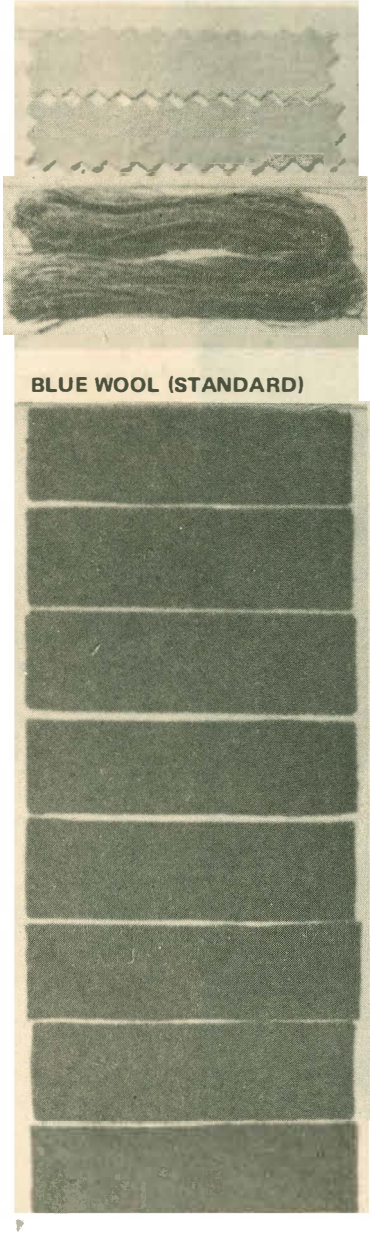
Sample 2

BLUE WOOL (STANDARD)



Total time of exposure : 10 hrs.
Rating : L_5

TEST FOR COLORFASTNESS TO LIGHT (DIRECT SUNLIGHT)



Total time of exposure : 3 hrs.
Rating : L_3

inventions

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An Improved Drafting Triangle

Eliezer N. Arnejo

ABSTRACT

An improved drafting triangle comprising a body shaped as an isosceles triangle and having the two equal sides thereof forming between them a right angle, a hypotenuse making 45° angles with said sides, and rectangular apartures forming angles of 75°, 60°, 15° and 30° with said hypotenuse.

BACKGROUND AND ADVANTAGES

This utility model relates to a simple drafting instrument suitably adapted for use by technical students or whenever simple drafting requirements are to be complied. The state of the art drafting instruments have become highly sophisticated and expensive to such an extent that only big construction firms can afford, least of all, the students. This situation leaves other users without any choice except to use those age-old instruments that have no improvements whatsoever.

The principal feature of this utility model is that the user is able to draw lines at angles different than those which can be drawn parallel to the sides of the triangle.

Various triangles are presently being used in drafting work. These include the 15° – 45° 90° and the 30° – 60° 90° triangles. For most applications, such triangles are satisfactory. However, when it comes to drawing lines at angles other than these specified above, a combination of triangles would be required if one does not use a protractor. This practice is not only time consuming but also requires the procurement of another triangle.

Accordingly, it is the principal object of this utility model to provide an improved drafting triangle useful for drawing lines at angles different than those which can be drawn parallel to the sides of the triangle.

A further object of this utility model is to provide an improved drafting triangle which is convenient for drawing lines as 15°, 30°, 45° 60°, 75° and 90° angles.

Still another object of this utility model is to provide an improved drafting triangle which can be readily manufactured.

These and other objects and advantages of the utility model shall subsequently becomes apparent from the following description taken in conjunction with the accompanying drawing.

Figure 1., the lone figure, is an elevational view of the improved drafting triangle embodying the present utility model.

Referring now to said drafting, there is shown the improved drafting triangle generally designated by numeral 1 comprising a body made of sheet material and having sides 2, 3 and 4 forming an isosceles triangle. The sides 2 and 3, being of equal lengths, form between them a right angle. The remaining side 4, called

hypotenuse in mathematical parlance makes the angle of 45^{o} with sides 2 and 3, respectively. Inscribed in said triangle are rectangular apertures 5, 6, 7 and 8 forming angles of 75^{o}, 60^{o}, 15^{o} and 30^{o} with the hypotenuse, as illustrated. Thus, angles widely used in engineering drawings, may be laid off with the use of the improved drafting triangle of the utility model without using another triangle or a protractor.

The utility model can be manufactured from the transparent plastic materials preferably acrylics using any of the conventional manufacturing process.

It is understood that the foregoing description of the preferred embodiment is for the purpose of illustration only. Other variations or modifications thereof may be resorted to by those skilled in the art without departing from the scope of the utility model as defined in the appended claim.

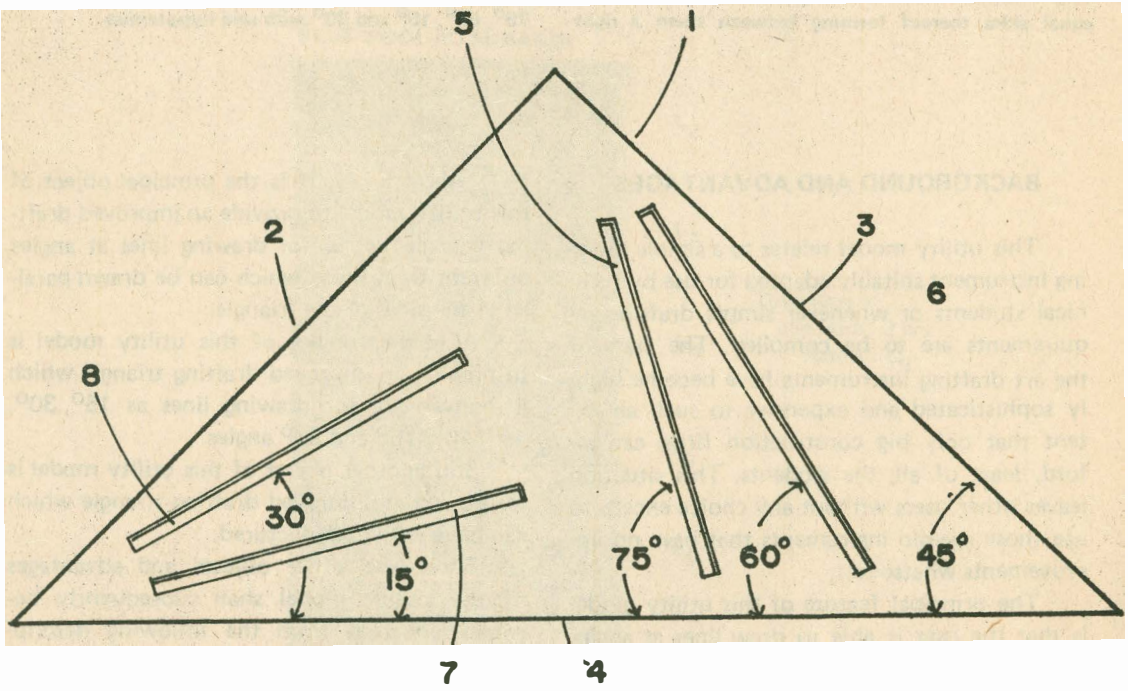

Figure 1. Improved Drafting Triangle. Patent filed on March 24, 1987. Patent No. 6591.

chemical

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Properties and Fatty Acid Composition of Kalamansi Seed Oil and Cottonseed Oil A Comparative Study

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ABSTRACT

A comparative study on the physical and chemical properties of cottonseed (*Gossypium hirsutum*) oil and kalamansi seed (*Citrus microcarpa* Bunge) oil were conducted. % oil content, moisture, acid value, iodine value, saponification value, refractive index and specific gravity were thereby determined. The fatty acid composition was also determined by gas chromatography.

INTRODUCTION

Over a thousand seeds of nuts throughout the world contain fixed or fatty oils. Of these, more than forty of them have been used commercially, but only nine oils dominate over ninety percent of international trade. These oils are those from coconut, palm kernel, palm, soya bean, ground nut, cottonseed, linseed, sunflower and rapeseed (2). Other well-known oils include those from olive, sesame, sunflower, mustard, castor and tung.

In search for other locally available vegetable oils that are of economic and commercial value, ITDI thereon, has identified two important oils, i.e. cottonseed oil and kalamansi seed oil.

Cottonseed *(Gossypium hirsutum)* belongs to the family Malvaceae, while kalamansi seed (*Citrus microcarpa* Bunge) belongs to the family Rutaceae. The families Malvaceae and Rutaceae as found by Hilditch (1) possess the same major component acids: palmitic, oleic and linoleic.

Fatty acid composition of citrus seed oils, such as grapefruit, lime, lemon orange and tangenise seeds have already been investigated by Nordby (7). Results show that the major fatty acids are linoleic acid 36-44%; oleic acid, 19-28% palmitic acid, 20-28%: with linolenic acid, 4.8-11% and stearic acids, 3-4.57%, in minor amounts. The fatty acid composition and other properties of cottonseed oil, on the other hand, is shown in Table 1.

A study conducted by the Pharmaceutical Research Program (PRP), of the ITDI on the production trends of kalamansi shows that the Philippines produced 44,664 metric tons of kalamansi fruit in 1983. The seeds constitute 19% based on the whole weight of the fruit. Kalamansi, however, is primarily used for its juice. Recent researches into its non-traditional uses had been studied at PRP. Essential oil was extracted from its rind. The rind was also utilized for jam and other fruit preserves preparations. Pectin was likewise extracted from the rind, pulp, and seeds.

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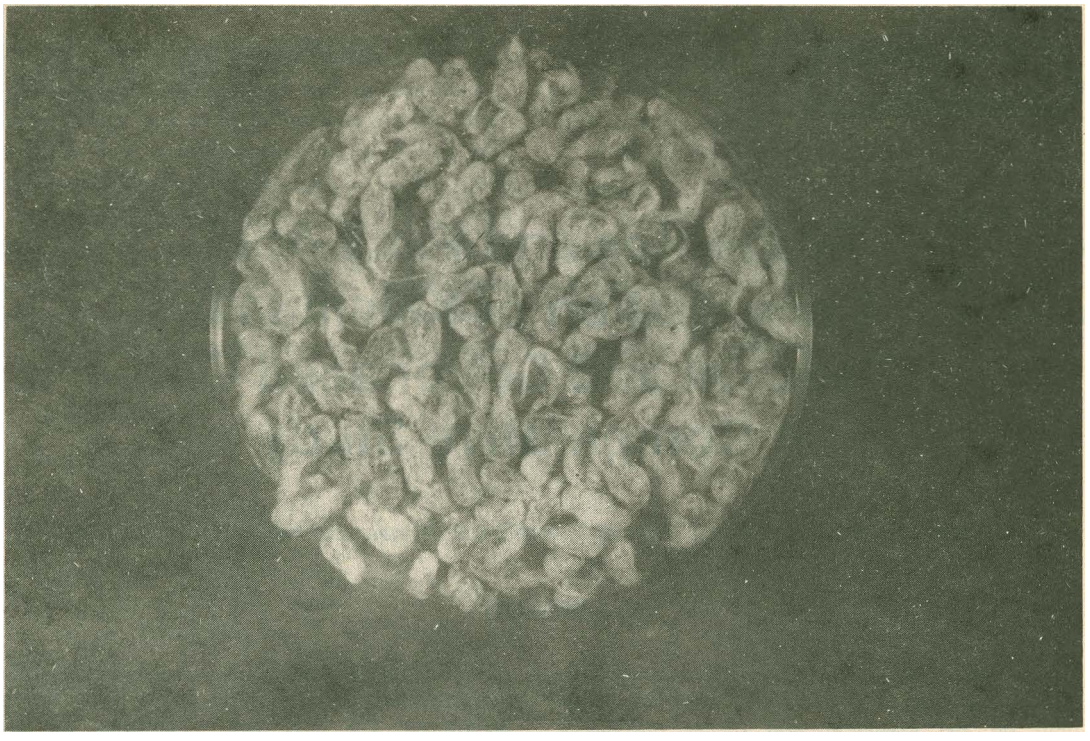

Figure 1. Kalamansi Seeds are effective sources of oil which compare favorably with cottonseed oil.

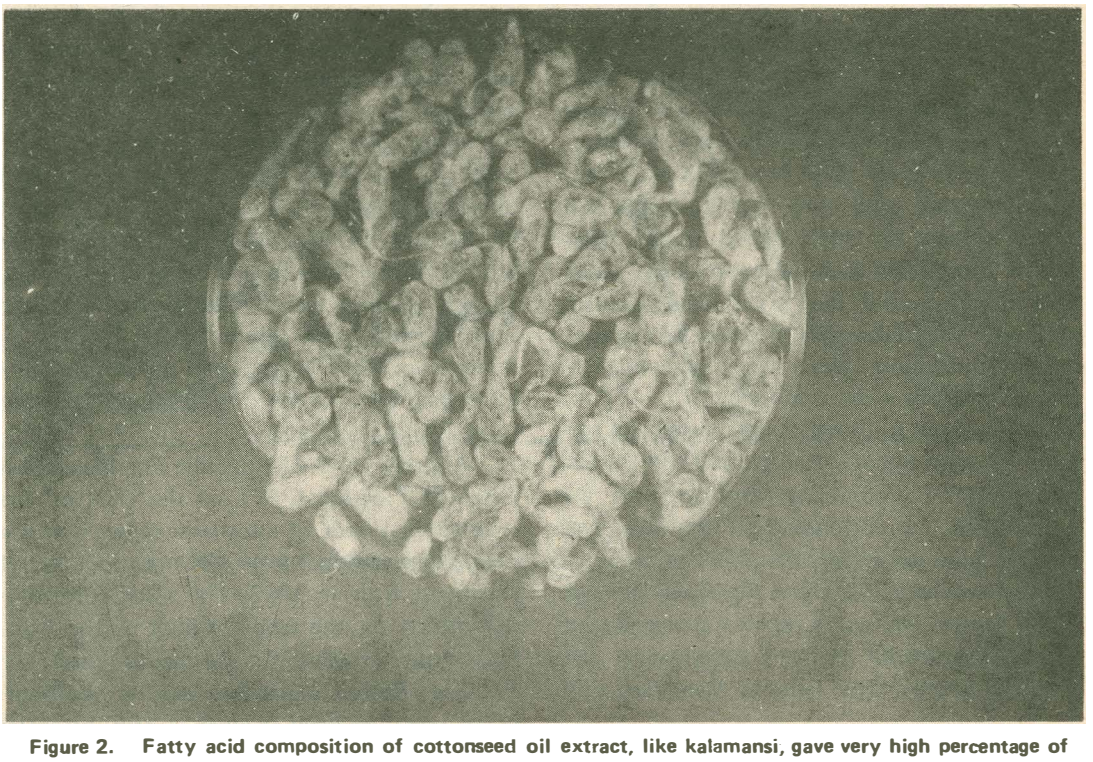

Figure 2. Fatty acid composition of cottonseed oil extract, like kalamansi, gave very high percentage of oleic (average 66%) and palmitic acid (average 25%) acids.

Studies on composition and application of cottonseed oils, on the other hand, is well documented in many fats and oils references (4). The potentials, however, of kalamansi seed oil has yet to be tapped and this study takes a step towards that direction.

Commercial production methods for seed oils, complete with an economic study for small-scale production is given by the Tropical Products Institute (2).

METHODOLOGY

1. **Seed Preparation.** The kalamansi-seeds were soaked overnight in water to recover the pectin adhering to its coat. Then the seeds were air-dried to 7.42% moisture, and dehulled. The cottonseed was first dehulled before air-drying to 6.66% moisture. The dried seed samples were ground to a fine meal using mortar and pestle, and then placed inside the thimbles for soxhlet extraction.

2. **Extraction of oil.** The oils were extracted using a butt-type extraction apparatus connected to a condenser and a soxhlet flask. About 40-45 grams of the ground and air-dried samples were placed in thimbles, and it was extracted with an equal volume or slightly more of petroleum ether (200 mL0 for 16 hours. The flask was heated on a mantle at such a rate that the solvent drops from the condenser on the thimble at a rate of 150 drops per minute. The volume was kept fairly constant by adding enough to make up for any that may be lost due to evaporation. After cooling, the extraction flask was disconnected. The petroleum ether was evaporated with gentle heat to constant weight. The oil was then filtered to separate the fine solid particles.

3. **Analyses of oil samples.**

1. Determination of acid value, saponification value, iodine value, specific gravity and refractive index were all based on AOCS standard methods of analysis.

2. Fatty acid methyl ester composition was determined by gas chromatography.

4. **Esterification.** The oils were first esterified with borontri-flouride-methanol reagent based on AOCS standard method (state method).

5. **Gas-Liquid Chromatography.** Five tenths (0.5) micro liter of the esterified sample was injected into the Perkin-Elmer Sigma 2000 Gas chromatograph equipped with hydrogen flame ionization detector. The 6 ft by 1/8 inch stainless steel column was packed with 8% OV-17 in chromosorb WHP 100/120 mesh. The carrier gas (Nitrogen) flow rate at the column outlet was 35 mL per minute. The column temperature was programmed from 200°C, 220°C and 240°C at the rate of 5°/20 minutes, 10°/5 minutes, and 10°/5 minutes respectively.

The percentage of each fatty acid was obtained by dividing the peak area of the fatty acid by the sum of all the peak areas obtained for the fatty acids.

RESULTS AND DISCUSSION

A fairly high oil yield has been recovered for both kalamansi and cottonseeds which should prove that these oils would become economically valuable in the Philippines. Table 1 shows some of the physical and chemical properties of the oils. Acid values are rather high but these oils are easily refined with alkali. Saponification value of cottonseed oil is lower than the range specified in Table 1. The degree of unsaturation for both oils are almost similar as shown by its iodine value (Table 2) and fatty acid composition (Table 3). Saponification value of kalamansi seed oil, however, is lower than cottonseed oil which shows that the former contains more unsaponifiable matter in the form of hydrocarbons. Having an almost similar fatty acid composition, it is ascertained that both oils belong to the same grade classification of semi-drying oil. This may find many applications for both the edible, cosmetic and plastic preparations.

SUMMARY AND CONCLUSION

The study revealed that the fatty acid composition as determined by gas-liquid chromatography of the oil extracted from the seeds of cottonseed and kalamansi gave very high percentage of oleic (average 66%) and palmitic (average 25%) acids.

TABLE 1. Properties (5) and Composition (6) of Cottonseed Oil

PROPERTY	RANGE OF VALUES
Specific gravity, 25°/25°C	0.916 - 0.918
refractive index, 25°C	1.468 - 1.472
iodine number	99 - 113
saponification number	189 - 198
unsaponification matter, %	not over 1.5
titer, °C	30 - 37
free fatty acids (as oleic, %)	not over 0.25
fatty acid composition, %	
Myristic C_{14}	0.5 - 1.5
Palmitic C_{16}	20 - 23
Stearic C_{18}	1 - 3
Arachidic	0.2 - 1.5
Oleic $C_{18:1}$	23 - 35
Linoleic $C_{18:2}$	42 - 54

TABLE 2. Properties of Kalamansi Seed Oil and Cottonseed Oil

PROPERTY	KALAMANSI SEED OIL	COTTONSEED OIL
oil, %	49.24 - 49.49	35.38 - 38.64
moisture, %	7.42	7.50
acid value	10.22	10.93
saponification value	120.71 - 121.32	148.62-165.02
iodine value	93.54	98.67
specific gravity, 25oC	0.9209	0.9236
refractive index, 25°C	1.4678	1.4687

TABLE 3. Fatty Acid Composition of Cottonseed Oil and Kalamansi Seed Oil as Determined by GLC

FATTY ACID	COTTONSEED OIL	KALAMANSI SEED OIL
C_8 caprylic	0.10	0.09
C_{10} capric	0.09	0.12
C_{12} lauric	0.29	1.97
C_{14} myristic	0.90	2.07
C_{16} palmitic	26.12	24.47
C_{18} stearic	0.33	0.09
$C_{18:1}$ oleic	69.78	63.03
$C_{18:2}$ linoleic	0.45	6.73

In addition to observations of fatty acid content of both oils, chemical studies were also carried out to determine their properties. This included analyses of its acid value, saponification value and iodine value. Results showed that there were no significant difference between the two oils.

The results strongly indicated that kalamansi seeds are effective sources of oil which compare favorably with cottonseed oil. The presence of exploitable amounts of palmitic acid and the occurrence of large percentage of $C_{18:1}$ (oleic acid) would serve as basis in assessing the technical and economic feasibility of adopting this to large scale production.

ACKNOWLEDGEMENT

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Some Factors Influencing Acetic Acid or Vinegar Production Using Coconut Water By Selected *Acetobacter* Strains in Submerged Process

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ABSTRACT

Three selected *Acetobacter sp.* coded as #14-3, # GZ-1 and # 1-8 were used in the determination of some factors affecting acetic acid fermentation by submerged culture process.

The submerged process in shake flask culture gave the highest acetic acid yields of 7.02% by *Acetobacter sp.* # 14-3 using coconut water medium containing 10% alcohol (95% ethyl alcohol) as carbon source, 25% inoculum concentration, 0.472 cell density, pH 4.0 and 300 rpm shaker speed after 7 days of fermentation.

Using sucrose as carbon energy sources an acid yield of 5.10% was obtained in 48-hour old *Acetobacter* #14-3 culture, 25% *Acetobacter* and 10% yeast inoculum, pH 4.0 and 300 rpm shaker speed at 32°C after 7 days of fermentation.

By stirred tank fermentor using *Acetobacter sp.* #1-8 with 10% alcohol and 25% *Acetobacter* inoculum, an acetic acid yield of 5.10% was produced in the 5th day of fermentation at 800 rpm shaker speed and a range of air flow of 0.5 (v/v)/minute.

INTRODUCTION

Vinegar making may play a significant role in the development of the coconut industry in the Philippines because coconut water, a waste product which abounds in coconut region where copra is manufactured, has been proven in previous studies to be an excellent medium for the microorganisms involved in vinegar production. Its value has been greatly enhanced by reports of the presence of a number of growth promoting substances which possess the power of inciting cell division.

Among the Asian countries,the Philippines is the highest vinegar consumer. It is used in all households, restaurants, hotels and pickling plants.Although we are producing vinegar and have other sources such as nipa saps, molasses coconut toddy etc., such production is very limited that some producers resort into adulteration with the synthetic acetic acid (glacial) which is very toxic to the human body.

This study is designed to determine some factors influencing acetic acid or vinegar production using coconut water by submerged process which may be adaptable to the increasing vinegar in this country.

Should the process be successful it will boost the vinegar industry as well as the utilization of exhaustible coconut water in the country. The ultimate outcome of this study would therefore aid in the economic recovery program of the country, and protect the people from the hazard of the dangerous adulterated vinegar with synthetic acetic acid.

Two basic processes for the production of acetic acid/or vinegar are known, the stationary or Orlean or French and the quick generator or submerged methods. In other oxidative fermentation industries, the submerged process has become well established and has shown superiority to the other methods.

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Although several encouraging reports have recently been published no detail reports so far have appeared to determine some factors affecting its production using coconut water by shake flash and fermentor process.

The selected *Acetobacter* strains will be compared on the basis of their respective abilities using two carbon and energy sources either sucrose or ethyl alcohol in shake flash culture and stirred tank fermentor process.

REVIEW OF LITERATURE

Hromatka et al (1949, 1950, 1953) reported several studies on acetic acid fermentation with special emphasis on the submerged fermentation. A submerged vinegar process developed for vinegar production by Hromatka and Ebner in 1951, showed the influence of complete interruption of aeration upon a rate of fermentation and found that 30 seconds was a critical time. The optimal temperature was found by the two authors (1953) to be between 23.3 and 29°C. The influence of temperature was found to be dependent upon the total concentration of acetic acid and alcohol. Acetators made by Heinrich, Frings at Bonn, Germany was developed after years of research by the latter and utilized details of Hromatka and Ebner process.

Several modifications of the basic methods of vinegar manufacture have been made. The most recent modification of the modern generator process consists chiefly in improvements in design and instrumentation. Such improvements are intended to increase the efficiency and ease of operation.

Although not yet of commercial importance, the possibility of oxidizing alcohol to acetic acid by submerged culture of acetic acid bacteria has received some attention. For example, Hromatka and Ebner compared generator oxidation with submerged culture oxidation and found oxygen consumption in generator vinegar production is a linear function in time. In the submerged culture oxidation they found out that acid formation is an exponential function of time. The submerged culture process permitted thirty times faster oxidation of the same amount of alcohol.

Algeir, Withoff, and Hildebrandt have recently undertaken a series of experimental studies of the factors affecting the performance of distilled vinegar generators. They stated that studies of such variables as composition, degree of chlorination, and biological purity of dilution of water, nutrients employed, factors leading to improved aroma, and various other elements involved in vinegar manufacture might be expected to raise production levels and improve the quality of vinegar.

In 1954, Palo and Lapuz have demonstrated coconut water to be an excellent medium in vinegar making. From the viewpoint of microbial nutrition, coconut water contains sufficient sugar to sustain the microbial growth. But from the standpoint of vinegar production, it certainly cannot be considered a complete raw material because it lacks enough sugar from which sufficient alcohol for the conversion of acetic acid is derived. Its deficiency in sugar should, therefore, be supplied in the required amount to make it a good raw material for the production of good quality vinegar as reported by Palo N D. and L. C. Vilela (1967).

MATERIALS and METHODS

Bacterial Strains. The *Acetobacter* strains used in this study were coded: *Acetobacter sp.* #14-3, #1-8 and #GZ-1. The first two were isolated from a rotten chico and mabolo fruits respectively, while #GZ-1 was obtained from an acetozyme sample used in the Frings acetator. These strains were selected from their high acid yielding activity in vinegar production by surface culture process in the Microbiology and Genetics Division, ITDI and the yeast *(Saccharomyces cereviseae)* was obtained from the culture collection laboratory of ITDI.

Culture Media. Stock cultures of the three *Acetobacter* strains were maintained on tomato-peptone-sucrose-salts (TPSS) agar, a nutrient medium developed by Palo and Lapuz (1954) and the yeast *(Saccharomyces cereviseae)* was grown on potato-dextrose agar medium (PDA). The suspension for inoculating the substrates were obtained from cultures grown at room temperature 25-30°C for 24-48 hours.

Determination of Some Optimum Parameters for Acetic Acid Production by Selected Acetobacter Strains.

General Procedure – Fermentation runs were done in shake flask, culture process using an environmental incubator shaker and two-liter stirred tank fermentor. Experiments in shake flask were carried out in sterilized 250 ml capacity erlenmeyer flask containing 50 ml coconut water medium. Sterilization was done by autoclaving for 15 minutes at 15 lbs. pressure. In the two-liter stirred tank fermentation, 1500 ml sterilized coconut water using sucrose or ethyl alcohol as carbon source was used. Acetic acid was computed in percent for titrable acidity.

Comparative Acetic Acid Producing Capacity of the Three Selected Strains, Acetobacter sp. #14-3, #1-8 and #GZ-1 in Submerged Process.

The three strains were compared on the basis of their respective abilities in two sources of carbon and energy using either alcohol (95% ethyl alcohol) or sucrose. Three batches in triplicates of 15% sugared coconut water were inoculated with 25% *Acetobacter* and 10% yeast inoculum and incubated on an incubator shaker with 300 rpm agitation speed at 32°C. Percentage acidity were determined from the first to the seventh day of fermentation.

Effect of Sucrose as the Sole Source of Carbon and Energy on the Selected Acetobacter sp. #14-3 for Acetic Acid Production.

Varied concentration of sucrose 80, 100, 120, 140, 160, 180, and 200 g/1 were prepared using coconut water medium. Seven batches in triplicates were sterilized and after cooling, these were inoculated with 25% Acetobacter sp. #14-3 inoculum with 0.4565 cell density and 10% yeast *(Sacchromyces cereviseae)* and incubated at 200 rpm agitation shaker speed at 30°C.

Optimum Alcohol Concentration (95% ethyl alcohol) as Carbon and Energy Sources for Acetic Acid Production.

Varied concentrations of alcohol: 2, 4, 6, and 8 were incorporated separately in pasteurized coconut water medium. Four batches in triplicates were inoculated separately with three different inoculum of the three *Acetobacter* strains namely: A#14-3, A#1-8, and A#GZ-1 and incubated at 200 rpm agitation speed at 32°C for 7 days.

Effect of Higher Alcohol Concentration (95% ethyl alcohol) in Acetic Acid Production.

Varied higher concentrations of alcohol: 10, 11, 12, 13, 14, and 15 were incorporated separately in the six batches of pasteurized coconut water medium. These batches were inoculated with 25% of *Acetobacter* sp. #14-3 with optical density of 0.6105 and incubated in the incubator shaker at 200 rpm agitation speed at 32°C.

Effect of 10% and 14% alcohol (95% ethyl alcohol) using A#14-3 in Acetic Acid Production.

Concentration of 10% and 14% alcohol (95% ethyl alcohol) were incorporated separately in pasteurized sugared coconut water and inoculated with 25% of A#14-3 with optical density of 0.461 at 200 rpm shaker speed at 32°C.

Influence of 10% alcohol (95% ethyl alcohol) concentration using A#14-3 in Acetic Acid Production.

Two batches of coconut water medium with 10% alcohol were inoculated separately with 25% inoculum of each of the two *Acetobacter* sp. A#14-3 and A#1-8 and incubated at 300 rpm agitation shaker speed at 32°C for 7 days.

pH and Acetic Acid Production.

Acetobacter sp. #14-3 inoculum with 0.584 cell density was inoculated in coconut water medium with 10% alcohol (95% *ethyl alcohol*) and varying pH levels; 2.0, to 6.5 at 0.5 intervals, incubated in the incubator shaker at 200 rpm agitation speed at 30°C.

Optimum Cell Density of Acetobacter strains GZ-1 on Acetic Acid Production.

To determine the optimum cell density of *Acetobacter* #GZ-1 strain, vigorous-growing colonies of the 48-hour old culture was scraped from the surface of the TPSS agar slant medium in roux bottles and transferred into sterile 250 ml capacity erlenmeyer flask containing 100 ml sterile 15% sugared coconut water medium. The inoculum concentration was adjusted to the required cell density of 0.407 and 0.529 at 660 nm. Twenty five per cent (25%) each of the inoculum with the adjusted cell density were inoculated separately into the pasteurized coconut water medium with 10% alcohol (95% ethyl alcohol) concentration and incubated in the incubator shaker at 200 rpm shaker speed at 32°C. Percentage acidity were determined after 7 days of fermentation.

Stirred Tank Fermentor

The three selected strains A#GZ-1, A#14-3. amd A#1-8 were used. Twenty five percent inoculum of each of these strains were inoculated in sterile 1200 ml coconut water medium with 10% alcohol in the two-liter stirred tank fermentor of 800 rpm agitation speed. Air at a flow rate of 0.5 vvm (volume of air per unit fermentor volume per minute) were fed into the fermentor

RESULTS AND DISCUSSION

The three *Acetobacter* sp. coded as A#14-3, A#1-8, and A#GZ-1 strains as shown in plate 1 Figures 1 and 2 were found to produce high acid zone of clearance in TPSS agar medium with 3% calcium carbonate. It can be seen that A # 14-3 showed the biggest zone of clearance followed by A # GZ-1 around the colony. These strains were selected from their high acid yielding capacity using developed fermentation parameters by surface culture method Figure 3 shows that the three *Acetobacter* sp. # 14-3, # 1-8, and # GZ-1 effected highest percent acetic acid yields of 5.10%, 2.58%, and 4.10% respectively in 15% sugared coconut water medium at 300 rpm shaker speed after 7 days of fermentation.

The highest acetic acid producer A#14-3 was used in determining the effect of different sucrose concentrations with a cell density of 0.4565 at 200 rpm shaker speed at 32°C. Figure 4 shows that of the different sucrose concentrations, 14 and 18 percent effected acetic acid yields of 4.47% and 4.38% respectively on the 7th day of fermentation. Based on the above results, it does not follow Cruess (1958) reports that under favorable conditions 50 to 55 parts of acetic acid may be obtained from 100 parts of sugar or approximately 1.26 gram of acetic acid from 1 gram of alcohol. A portion of the sugar is consumed in the production of substance other than alcohol and as food by the yeast and some losses in alcohol and acetic acid by evaporation during the fermentation. However, since our experiments were carried out by simultaneous inoculation of yeast and vinegar bacteria at a shorter fermentation time with varied conditions during investigation, results did not conform with the above findings.

As shown in Figure 5, it is noticeable that A#14-3 towers among the group. Highest acid yields of 4.08%, 3.66%, and 2.82% were produced by A#14-3, A#GZ-1 and A#1-8 respectively in 6% alcoholo (95% ethyl alcohol) concentration on the 7th day of fermentation. It will be noted that although the results were not very high, all these strains favors 6% alcohol concentration at a lower shaker speed of 200 rpm at 32°C. Mori and Terui (1972) reported on the effect of alcohol concentration on *A. rancens* using concentration of alcohol up to 10% , but none of their experiment gave a higher acetic acid yields of greater than 5.5%. According to the authors, the strain of *A. rancens* used was only adapted to a total concentration of 6% and the results obtained were due to the lack of adaptation to a higher total alcohol concentration.

Figure 6 shows the effect of different alcohol concentration in A#14-3 strain using a higher cell density of 0.6105 at a lower agitation speed of 200 rpm at 32°C. Of the different alcohol concentrations 13% gave the highest yield of 5.88% on the 7th day of fermentation. A slight decline was observed on the 8th day.

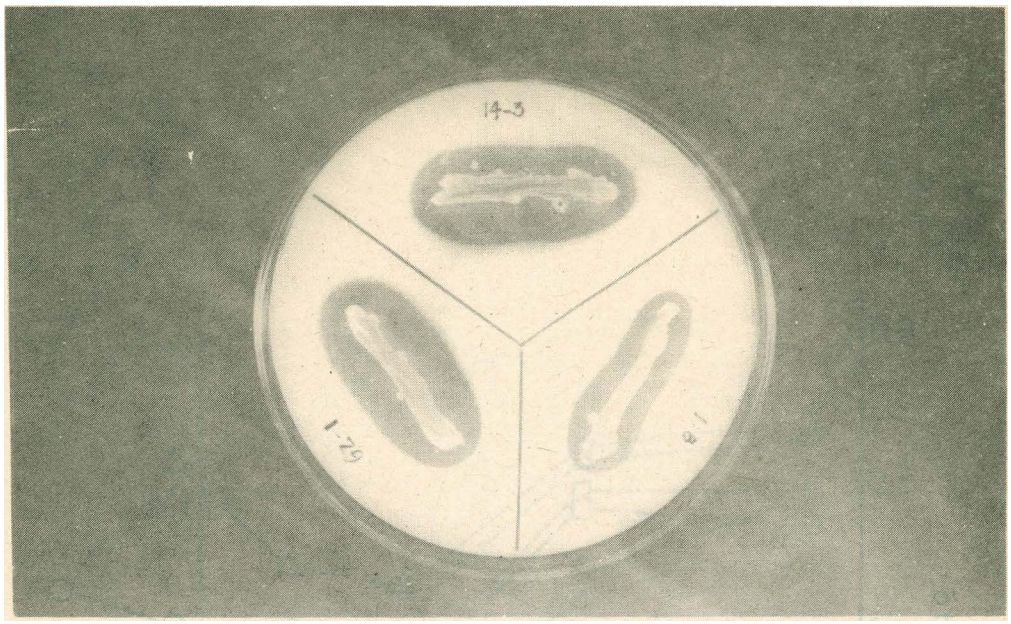

Figure 1. Streak colonies of the three selected *Acetobacter* strains A # 14-3, A # GZ-1 and A # 1-8 from a week old culture in tomato-peptonesucrose-salts agar with $CaCO_3$ with zone of clearance.

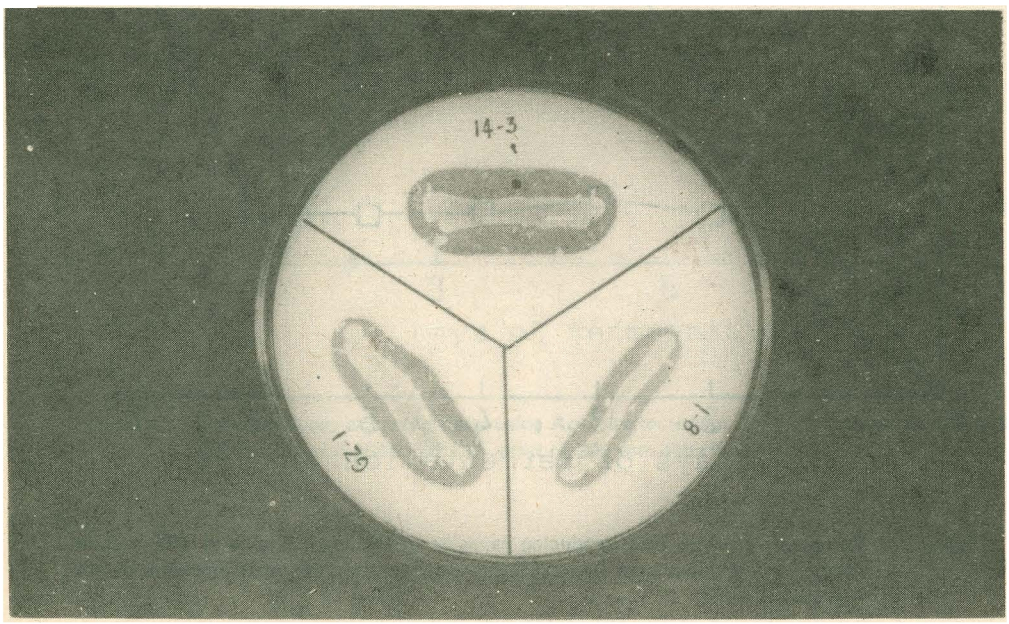

Figure 2. Zones of clearance on the ethanol calcium carbonate agar of the three selected *Acetobacter* strains A # 14-3, A # Z-1, and A # 1-8.

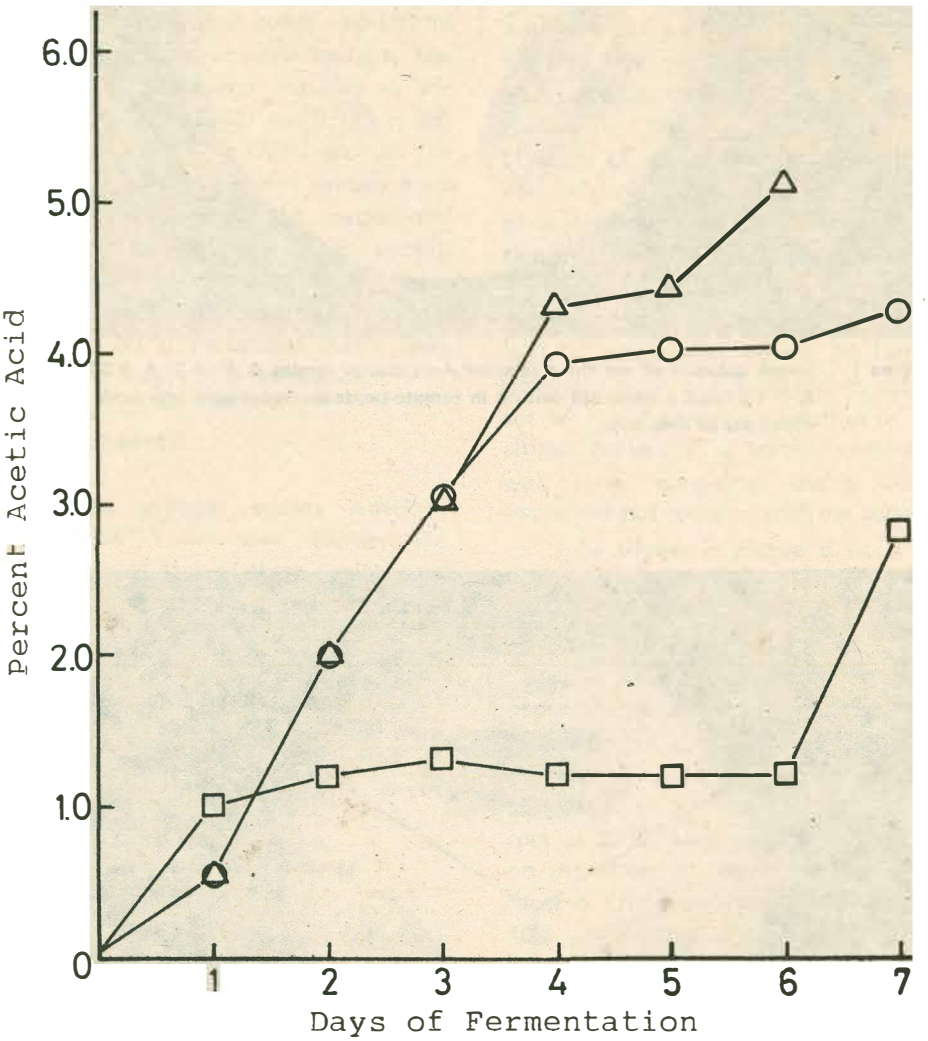

Figure 3. **Comparative acetic acid producing capacity of the three strains in 15% sugared coconut water inoculated with 25% *Acetobacter* and 10% yeast inoculum at 300 rpm shaker speed.**

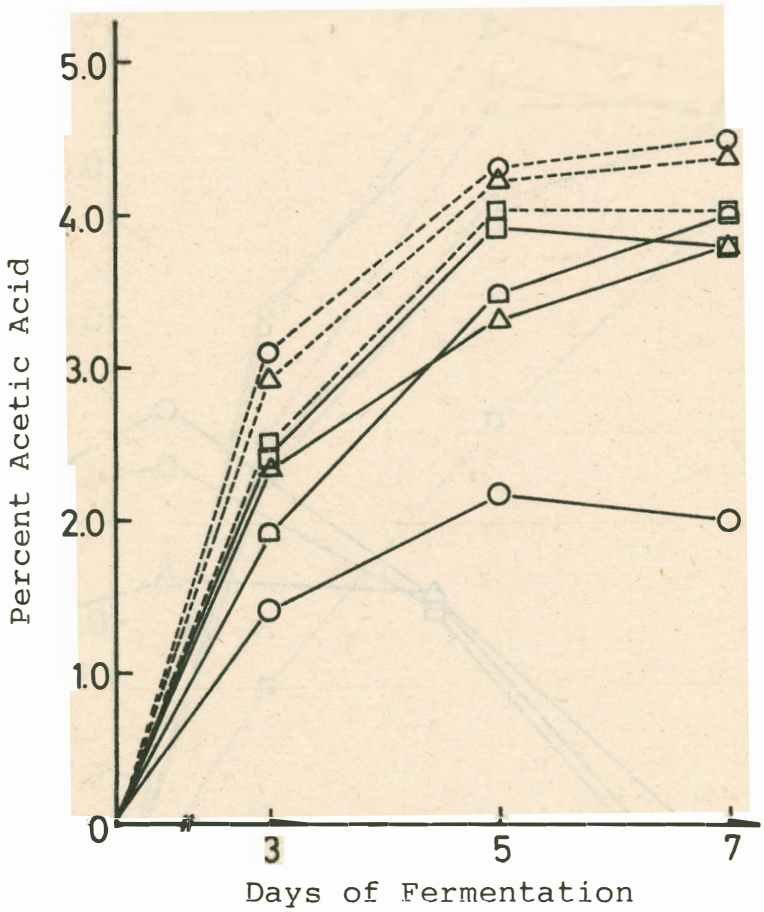

Figure 4. **Different sugar concentrations using *Acetobacter* strain # 14-3 in coconut water with a cell density of 0.4565 and 10% yeast of 200 rpm agitation period.**

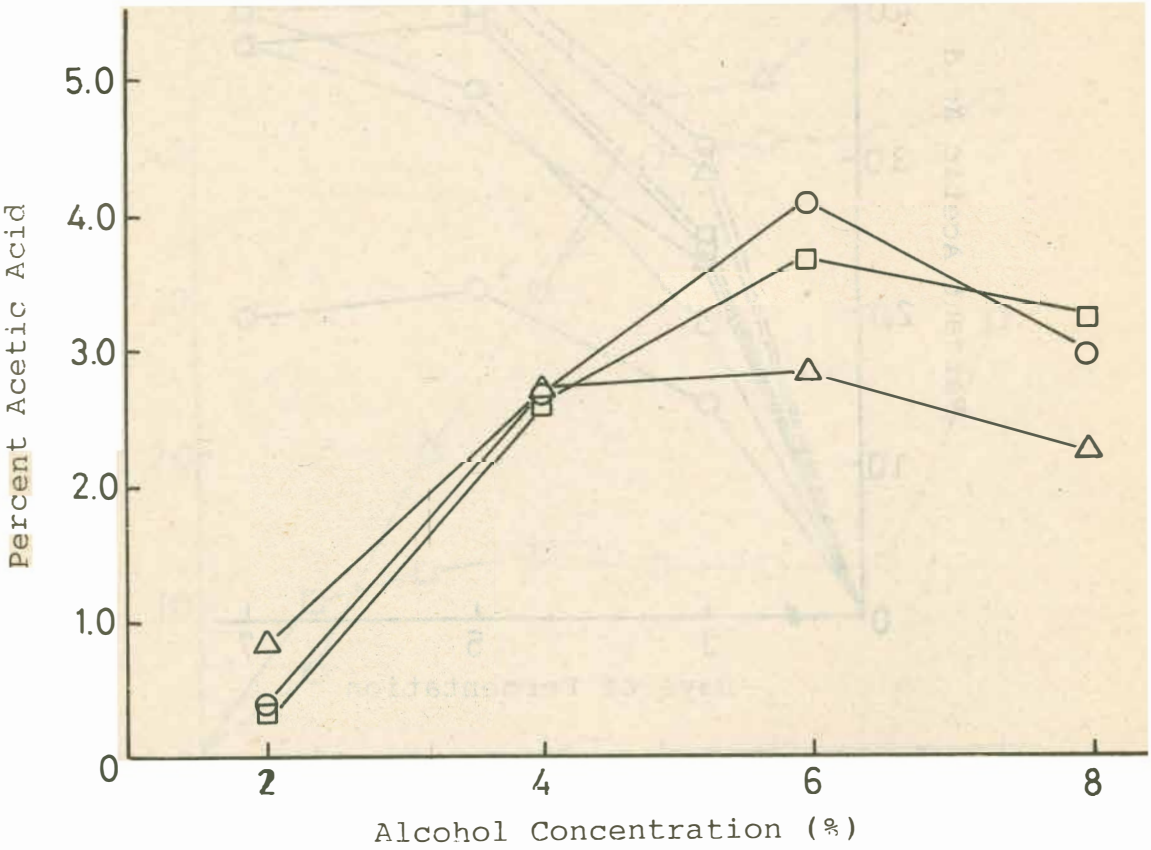

Figure 5. **Effect of different alcohol concentrations in acetic acid production by the three *Acetobacter* strains at 200 rpm agitation shaker speed after 7 days of fermentation.**

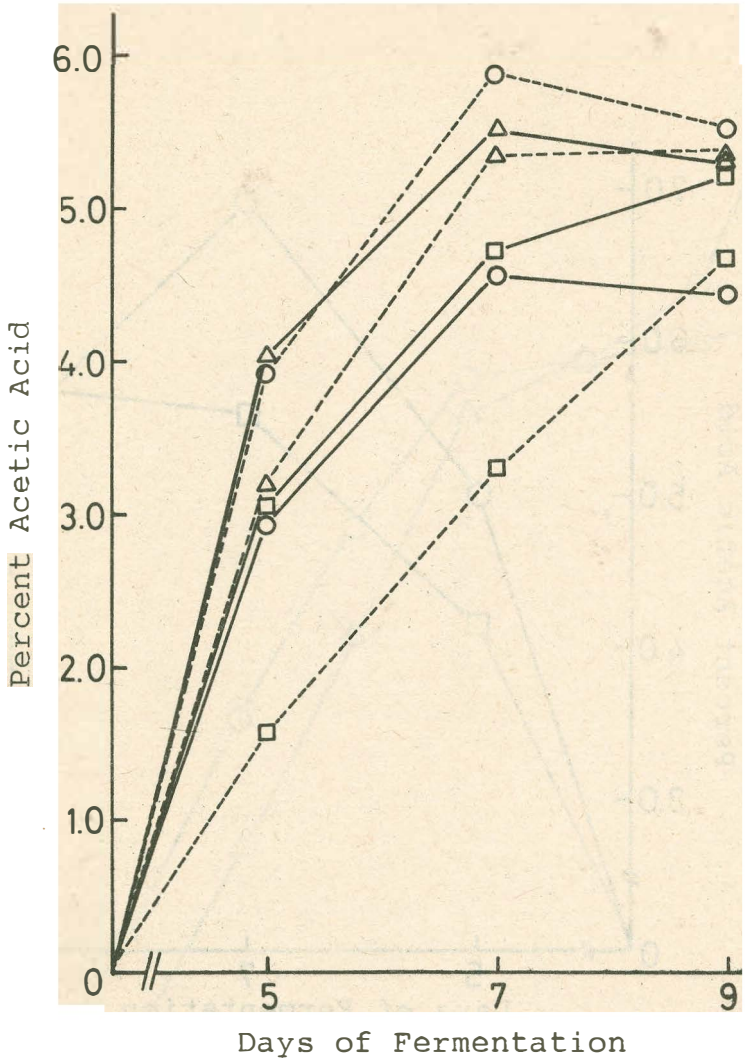

Figure 6. **Effect of different alcohol concentrations using *Acetobacter* strain # 14-3 with a cell density of 0.6105 at 200 rpm shaker speed.**

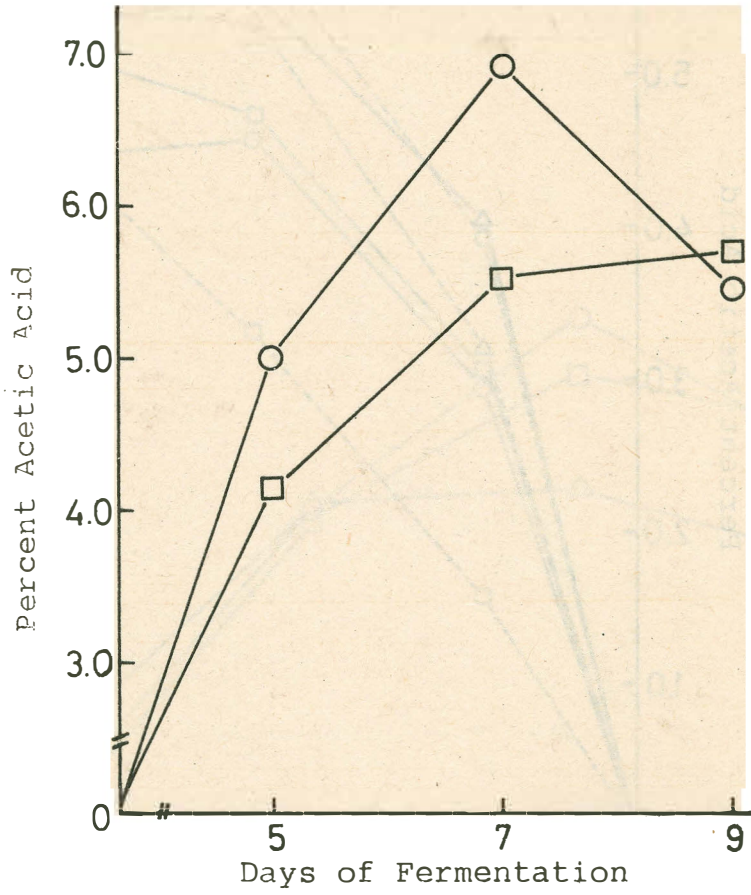

Figure 7. Effect of 10% and 14% alconol concentrations (95% ethyl alcohol) in CW using A #14-3 strain with 0.461 cell density at 200 rpm shaker speed.

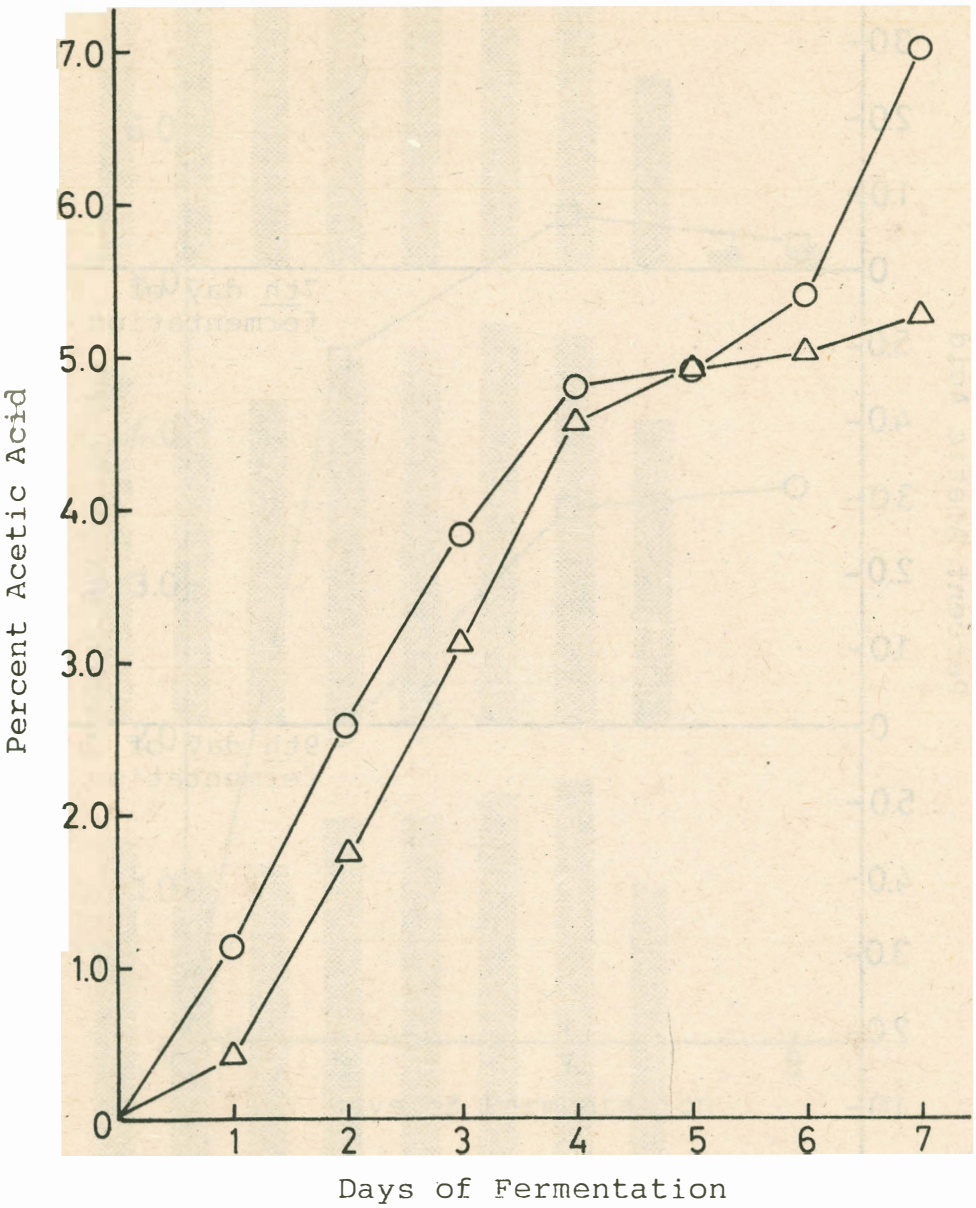

Figure 8. **Effect of 10% alcohol concentration on acetic acid production by *Acetobacter* strain # 1-8, # 14-3, at 300 rpm shaker speed at 32°C.**

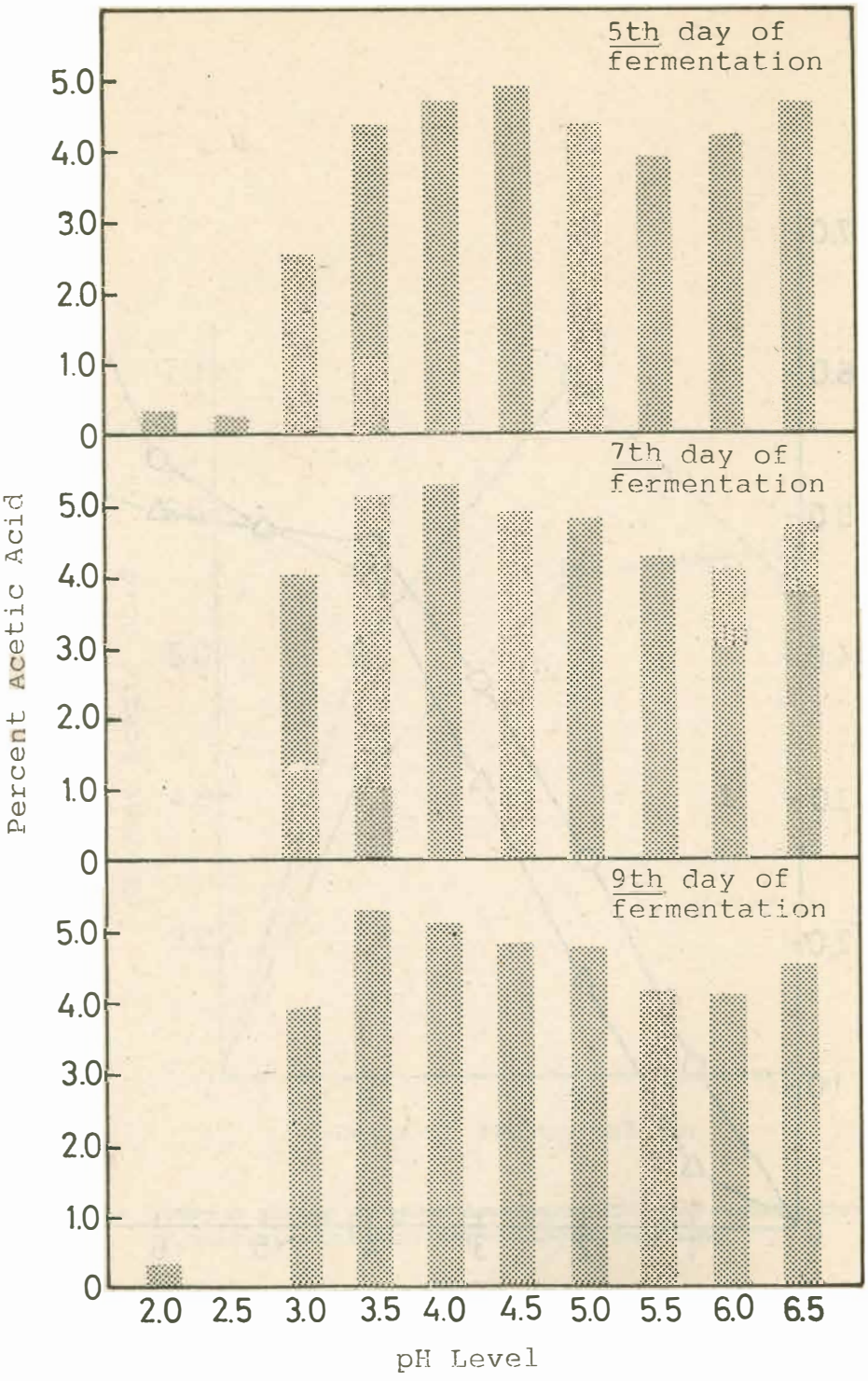

Figure 9. **Effect of different pH levels on acetic acid production using *Acetobacter* strain # 14-3 with a cell density of 0.584 at 200 rpm speed.**

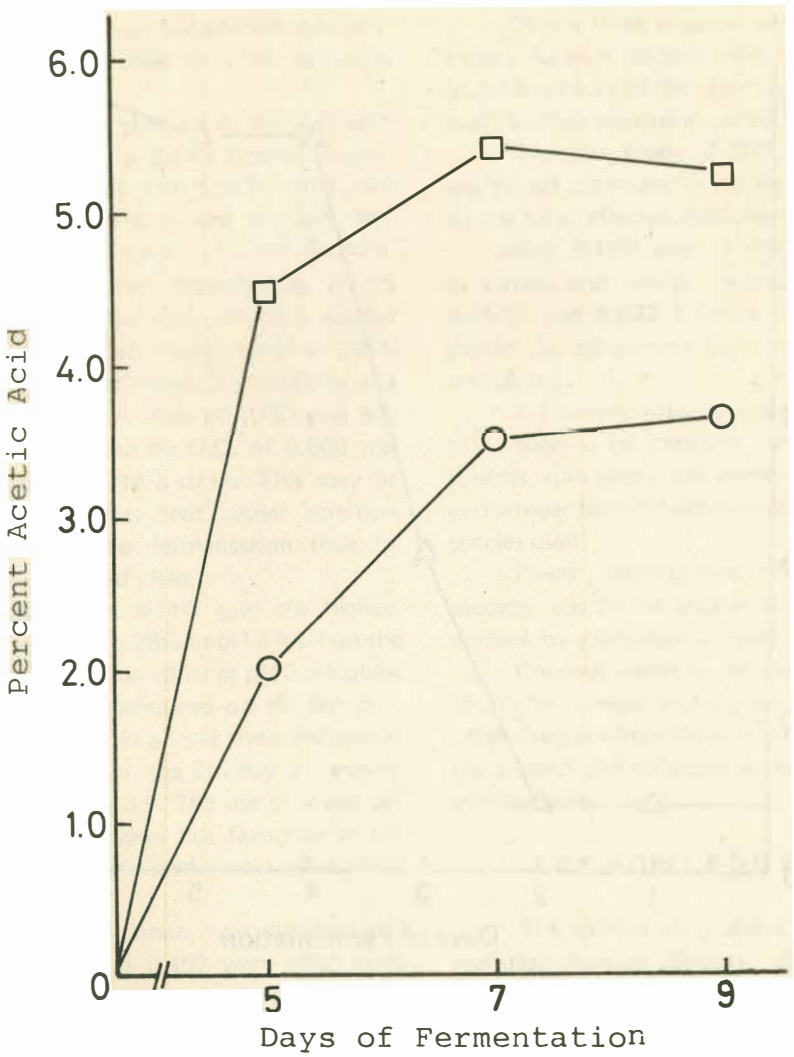

Figure 10. Effect of cell density on the acetic acid production using *Acetobacter* strain #GZ-1 in coconut water with 10% alcohol concentration.

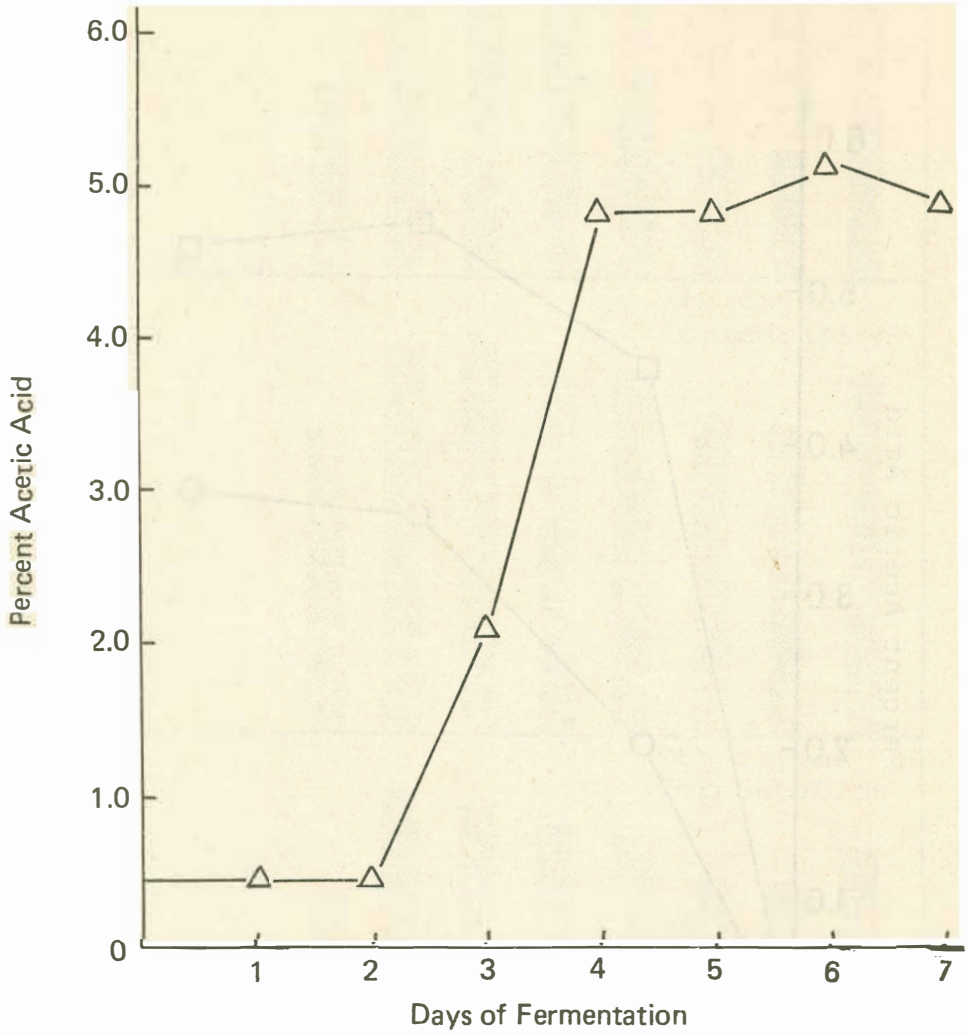

Figure 11. *Acetobacter* **strain # 1-8 in 2-liter fermentor with 10% alcohol concentration at 800 rpm.**

It is noticeable that higher concentration of more than 14% did not favor high acetic acid yields. This corroborates the previous findings of Prescott and Dunn (1949) that using higher alcohol concentration of more than 14%, the zoogleal mat forms with difficulty that the alcohol is incompletely oxidized to acetic acid. It was observed that 10% alcohol gave a lower yield of 4.68% on the 7th day of fermentation which is quite lower than the preceding experiment which may be due to lower agitation speed of 200 rpm.

Figure 7 shows that strain A#14-3 with O.D. of 0.461 in 10% and 14% alcohol concentration yirlded 6.86% and 5.52% acetic acid respectively at a shaker speed of 200 rpm.

The results in Figure 8 further demonstrate the superiority of A#14-3 over A#1-8 strain. At 10% alcohol level, A#14-3 yielded 7.02% acid in 300 rpm shaker speed at 32°C after 7 days of fermentation. It seems that at a higher alcohol concentration of 10% using 300 rpm shaker speed with an O.D. of 0.500 was very favorable to A#14-3 strain. This may be attributed to the fact that higher agitation speed accelerated the fermentation thus increasing the acetic acid yield.

Acetobacter sp. #14-3 gave the highest acetic acid yields of 5.28% in pH 4 level on the 7th day of fermentation while at pH 3.5 highest yield of 5.28% was produced on the 9th day. It was observed that in all pH levels maximum acidity was reached on the 7th day of fermentation except to pH 3.5. The use of lower pH levels of 2.0 to 2.5 were not favorable to the growth of A#14-3 and acetic acid production (Figure 9).

Using A#GZ-1 strain, two different cell density of 0.529 and 0.407 were tried using 10% alcohol concentration. As shown in Figure 10 A#GZ-1 strain favors higher cell concentration of 0.529 yielding 5.4% acetic acid. At lower cell concentration of 0.407 lower yields of 3.5% was produced in 10% alcohol concentration at lower shaker speed on the 7th day.

Of the four fermentation runs in the stirred tank fermentor using the three selected *Acetobacter* strains only A#1-8 was able to complete the fermentation without power interruption yielding an acid content of 5.10 % in 5 days as shown in Figure 11. The two strains suffered power breakdown on the 2nd and 3rd day producing erroneous results. This supports the findings of Hromatka and Ebner (1951) that interruption of aeration upon a rate of 30 seconds affects the acetic acid yield.

SUMMARY AND CONCLUSION

Of the three selected *Acetobacter* strains tested, A#14-3 isolated from rotten chico was found to produced the highest acetic acid yields in shake flask process at varied conditions.

A shaker speed of 300 rpm at 30-32°C was found optimum for acetic acid production by the three selected *Acetobacter* strains.

Using 6-10% alcohol (95% ethyl alcohol) as carbon and energy sources of *Acetobacter* A#14-3 and A#GZ-1 favors higher yields at a shorter period of time from 3 to 7 days of fermentation.

All factors influencing acetic acid production such as temperature, carbon and energy sources, rpm speed, cell concentrations of yeast and vinegar bacteria varies with the *Acetobacter* species used.

Power interruptions of more than 30 seconds was found critical to acetic acid production by submerged process.

Coconut water is the most suitable substrate for vinegar making specifically for distilled vinegar which requires nutrient to support the growth and oxidative activity of the acetic acid bacteria.

ACKNOWLEDGEMENT

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Cough Candy Using Local Plant Extracts

By Minda Flor T. Brandares, Annabelle M. Vuelban and Felicidad E. Anzaldo

ABSTRACT

A caugh candy is formulated using the essential oils of ginger (*Zinigiber officinale Rosc.*) and "kalamansi" (*Citrus microcarpa Bunge*) and extracts from tamarind (*Tamarindus indica* Linn.) and "kalamansi". The product when analyzed chemically yielded a total soluble solids of 95.04% and a moisture content of 4.95% Microbial testing proved its antibacterial activities against test organisms *Escherichia coli* and *Pseudomonas aeruginosa.*

INTRODUCTION

The use of local plant products to substitute for the imported raw materials/ingredients in the manufacture of pharmaceuticals may help contribute to the reduction of prices of drugs which have now become prohibitive. Pulmonary diseases are the number 1 killers in the country at present and to produce a cough medication using extracts from indigenous plants as ginger, tamarind and 'kalamansi" is not only very timely but also contributory towards economic recovery. And cough candy is the dosage form selected because it is not only cheap but is very convenient to administer and is popularly accepted by all ages.

Ginger (*Zingiber officinale* Rosc.) is one of the more popular medicinal plants used locally. Quisumbing (1978) reported that it is very effective for colds, coughs , asthma, dyspepsia and indigestion. By chewing a piece of ginger, discomforts as hoarseness, sore throat and loss of voice are relieved by the agreeable sensation and the production of a copious flow of saliva. Cineol, an active constituent of the volatile oil, is responsible for its stimulating action and its use as antiseptic expectorant (Kirk and Othmer, 1949). It also contains citral which pharmaceutically is said to have an antihistaminic value.

"Kalamansi" (*Citrus microcarpa* Bunge) is also widely cultivated in the Philippines. The juice which is fairly sour is used as a seasoning and for making "ade". It is also sweetened into a concentrate. Valenzuela *et al* (1947) reported that among its various uses, the juice is a good refrigerant (being a rich source of Vitamin C) and a remedy for coughs. The citric acid present in the juice brings out the flavor and gives a "tang" to the drink.

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Its rind is rich with volatile oil which is used as a flavorant.

Tamarind (*Tamarindus indica* Linn.) pulp when boiled with water is an excellent refrigerant. Tamarind water when gargled is useful in healing aphthous sores and sore throats (Quisumbing, 1978). It is also a good cure for complaints of the mucous membrane of the mouth. It contains citric acid which is known to reduce microbial population.

MATERIALS AND METHODS

Ginger rhizomes and "kalamansi" fruits were purchased from Paco market in Manila. Tamarind nectar, ammonium chloride NF, menthol USP and the other ingredients are being sold commercially.

The candy molder was fabricated by a private fabricator through the former Philippine Inventions Development Institute, (Now Technology and Promotion Institute, Department of Science and Technology).

Processing of the Raw Materials

Extraction of the oil from ginger rhizomes —

Sliced fresh rhizomes were extracted of oil by hydrosteam distillation. The oil collected was dried with anhydrous sodium sulfate to remove traces of water and stored in the refrigerator.

Extraction of the "kalamansi" oil and juice —

The essential oil from the rind of "kalamansi" fruits was extracted by hydro-steam distillation. The oil was dried and stored in the refrigerator.

The juice was extracted by ordinary squeezing of the cut fruits.

Formulation

Several formulations were tried to produce a cough candy using the oils extracted from ginger and "kalamansi", tamarind nectar and juice from "kalamansi" Active constituents such as ammonium chloride NF, which is an expectorant, and menthol were added. Leon (1959) formulated a cough drop using essential oils from wintergreen, sassafras and aniseed.

Chemical analysis

The total soluble solids and moisture content of the produced cough candy were determined based on the standard procedures (AOAC, 1945).

Microbial testing

The organisms used to determine the activity of the cough candy were *Pseudomonas aeroginusa* and *Escherichia coli.* The degree and zones of inhibition were noted.

RESULTS

Formulation

After a series of trials, the following formulation and procedure were adopted to produce cough candies using our local plant extracts:

Sugar	-- 80 g	Water	– 25 mL
Glucose	-- 90 g	Kalamansi juice	– 10 mL
NH_4C1	1 g	Tamarind nectar	– 10 mL
Color	– 0.05 g	Ginger oil	– 0.15 mL
Menthol	-- 0.10 g	Kalamansi oil	– 0.15 mL
Powdered	2.5 g	Magnesia powder	– 2.5 g

Place sugar, glucose, water, kalamansi juice, tamarind nectar and food color in a pan on a gas stove and stir until dissolved. Allow the mixture to steam and cover. Boil up to 140°C or until the desired hardness is reached. Then add NH_4C1, mix and fold in menthol crystals and the essential oils (kalamansi and ginger). Pour into previously greased molder. Cool and remove from the molder and blend with a mixture of powdered sugar and magnesia powder. Put on a sieve and pack as desired.

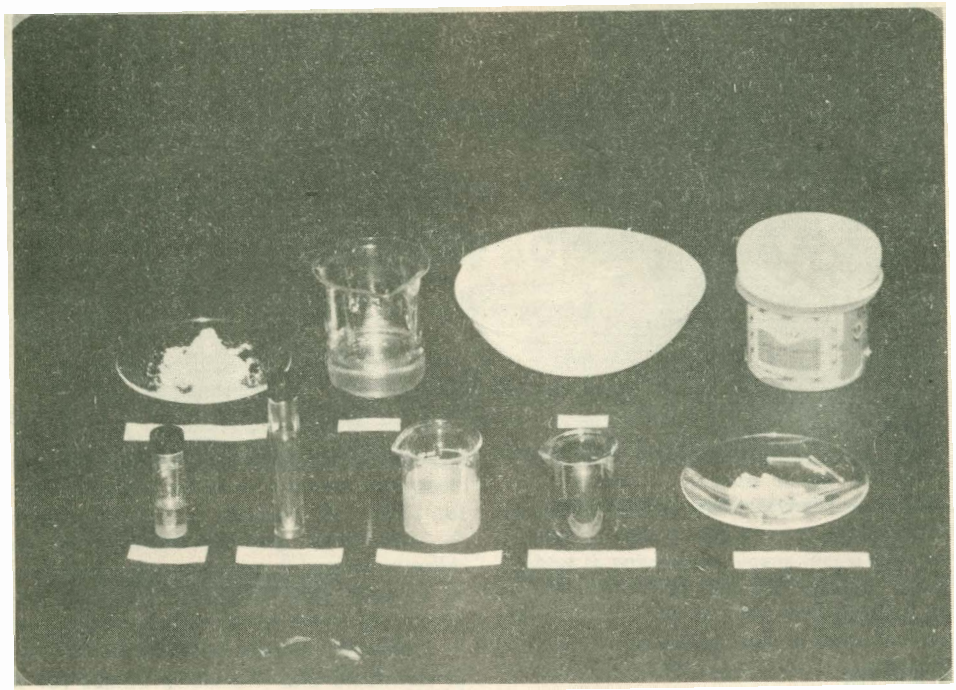

Figure 1. Cough candy ingredients: (From top, left to right) Food color, sugar, glucose, NH_4Cl, menthol crystals, tamarind nectar, kalamansi juice, kalamansi oil and ginger oil.

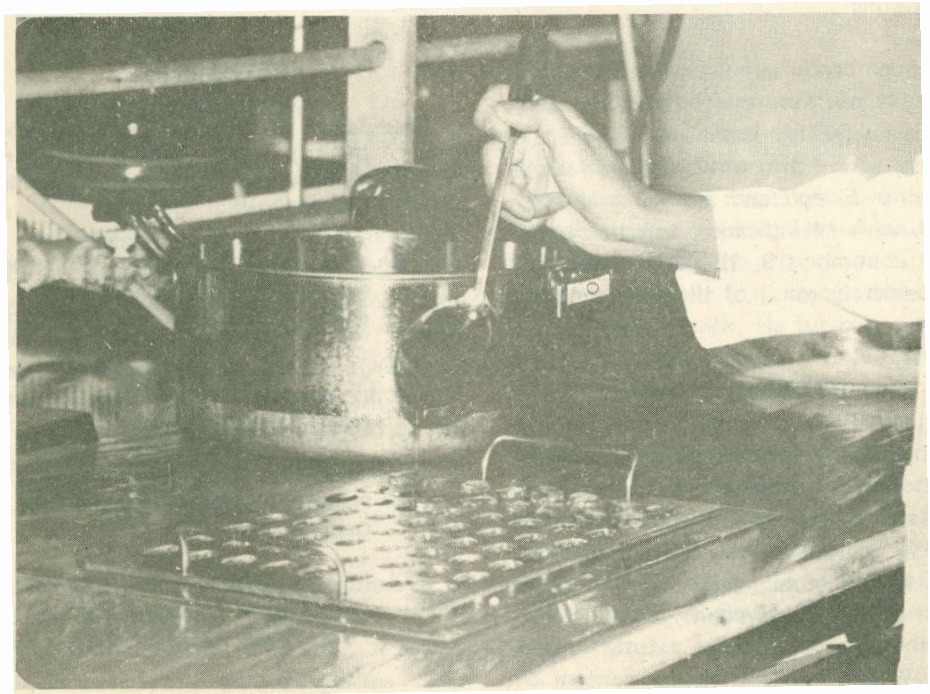

Figure 2. Pouring of cough candy formulation into locally fabricated mold.

Figure 3. The formulated cough candies.

The batch will yield approximately 35-45 candies.

Chemical analysis

The candy sample contained 95.04% total soluble solids which includes its sugar content and a moisture content of 4.95%.

Microbial testing

The cough candy samples when tested microbially gave positive results indicating their activity against the test organisms, *E. coli* and *P. aeruginosa.* A ++ 9 to ++12 reading was obtained against *E. coli* and a ++10 against *P. aeruginosa,* with ++ indicating a partial inhibition and the numbers 9, 12 and 10 representing the diameters (mm) of the halo zones of inhibition.

SUMMARY

A cough candy formulated using essential oils from ginger rhizomes and "kalamansi" rind and extracts from tamarind and "kalamansi" fruits in addition to the other ingredients proved to have microbial action on test organisms *E. coli* and *P. aeruginosa.* On chemical analysis, the product yielded a total soluble solids of 95.04% and a moisture content of 4.95%.

ACKNOWLEDGEMENT

The authors are grateful to Mrs. Teresita S. Bonifacio and Ms. Nelly Balgos for the microbial testing of the formulated cough candies and to Miss Marilyn Wacan for her laboratory assistance. Financial assistance of the RMC Commercial Corporation is also hereby acknowledged.

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aquamarine

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Polyculture Systems Using Groupers *(Ephinephellus tauvina)* and Tilapia *(mossambica)* In Brackiswater Ponds

V. B. Manzano

ABSTRACT

The study was conducted in twenty-one 171 m^2 brackishwater ponds of the Bicol University College of Fisheries from March 3 to October 16, 1986, to determine the optimum stocking combination of tilapia and grouper in polyculture. It consists of seven treatments that were replicated three times in a completely random design. Of the seven treatments, three were monoculture of tilapia (Treatment I - 20,000 tilapia/ha, Treatment II - 25,000 tilapia/ha, Treatment III - 30,000 tilapia/ha), one monoculture of grouper (Treatment IV 1,000 grouper/ha), and 20,000 tilapia plus 1,000 grouper/ha, Treatment VI - 25,000 tilapia plus 1,000 grouper/ha , Treatment VII 30,000 tilapia plus 1,000 grouper/ha).

Polyculture obtained better results for both species. In the monoculture of tilapia, 83-89% of the population were composed of young (less than 20 g). whereas in polyculture, young tilapia was only 3-15% of the population. Grouper reduced young tilapia population by 72 to 76%, thus resulting to higher recovery of the original stock of tilapia and significantly higher ($P < 0.05$) production of marketable tilapia in polyculture. Similarly, grouper obtained significantly higher ($P < 0.05$) recovery, growth, and production in polyculture. Grouper–tilapia ratio of 1:20 proved to be the most effective.

INTRODUCTION

Tilapia mossambica and other species of tilapia are extensively cultured in the Philippines. Intensifying its culture however is limited due to its prolific nature which results to overpopulation of undersized fish. Introduction of piscivorous fish in tilapia pond will not only maintain a desired level of fish population but also will increase the total fish production.

Few studies have been conducted on the use of predator as biological control of tilapia in pond. Cruz and Magisa (1980) evaluated the efficiency of snakehead (*Ophicephalus striatus*) to control tilapia young in freshwater pond. It showed that young tilapia population was totally controlled at stocking rate of 300/ha snakehead plus 10,000/ha Tilapia nilotica. Fortes (1980) established 1:10 tarpon-tilapia ratio in brackishwater ponds. He further suggested an interval of three weeks between stocking of Tilapia mossambica and tarpon to prevent predation on the original stock of tilapia. Recent study on the polyculture of grouper and Tilapia mossambica (Manzano, 1985) disclosed that grouper could be easily reared in brackishwater pond. It attained a survival rate of 82-92% with water salinity ranging from 3-27 ppt. Hence, the practice of many fishpond operators to add about 10 sacks/ha of salt in order to maintain the desired salinity level for grouper (Elizalde and Marcial, 1983) may not actually be required.

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Result showed that the presence of grouper in tilapia pond reduced tilapia population by 327-456% thus increasing the growth of the remaining tilapia resulting to marketable size fish at harvest. Similarly, the presence of tilapia increased the growth and production of grouper.

This study therefore aimed to determine the optimum stocking combination of tilapia and grouper in a polyculture system.

METHODS

Experimental Ponds

Twenty-one 171 m^2 brackishwater ponds were used in this study. Each pond was provided with 10 cm diameter PVC standpipe which served as water inlet/outlet. Every pond was supplied with tidal water through a main water supply canal which was capable of holding water up to 60 centimeters.

Experimental Design and Treatments.

This study consists of 7 treatments that were replicated three times in a completely random design. The treatments were as follows:

Treatment	Stocking rate per hectare	
	Tilapia	Grouper
I	20,000	–
II	25,000	–
III	30,000	–
IV	–	1,000
V	20,000	1,000
VI	25,000	1,000
VII	30,000	1,000

* 1:3 male to female ratio

Pond Preparation

The experimental ponds were repaired, drained, dried and leveled. Nylon screen having a mesh size of 0.5 mm was installed in each opening of the standpipe. Agricultural lime (300kg/ha), tobacco dust (1500kg/ha), and urea (50 kg/ha) were broadcast at the pond bottom. Shelters consisting of 6 stumps per pond were placed and water level gradually raised to about 50 cm before stocking.

Stocking

Tilapia mossambica weighing 8.5-22 g were stocked March 3, 1986. After 53 days grouper fingerlings weighing 2.9-5.9 g were added. This was done to give enough time for tilapia to breed.

Management Techniques

Within the culture period, 16-20-0 was broadcast at 50 kg/ha every three weeks for a total of 10 applications. Salinity, pH, D.O. and temperature of the water were monitored three times a week from 7:00 to 8:00 in the morning (Table 1a). Rainfall readings were taken evryday (Table 1b).

Water freshening was done whenever possible, specifically before fertilizer application and after heavy rainfall.

Harvesting

The grouper and tilapia were simultaneously harvested on October 12-16, 1986. The harvests were classified as to species and size, then weighed and counted. Representative samples were taken from each pond to determine the length-weight relationship.

Maximum size of tilapia that grouper could swallow was determined by force-feeding various sizes of grouper with tilapia that could pass through their maximum mouth opening. The weight, the total length and the body depth of tilapia were measured. For grouper, the maximum mouth opening and total length were determined.

RESULTS AND DISCUSSION

Efficiency of Grouper to Reduce Young Tilapia

Tilapia was classified into two groups. Those below 20 grams or 11 cm total length are the young population. The adult or original stock was more than 20 grams or 11 cm in total length (Table 2).

Table 1a. Ranges of the physicochemical parameters measured during the period of study.

Treatment	Parameters			
	Temp. (°C)	Salinity (ppt)	pH	D.O. (mg/l)
I	26-39	7.5-34	7.9-9.3	2.2-5.4
II	26-39	7.5-34	7.0-9.3	2.0-5.6
III	26-39	7.5-34	6.9-9.5	2.8-6.0
IV	26.39	7.5-34	6.5-9.8	3.2-6.6
V	26.39	7.5-34	6.2-9.1	2.2-5.4
VI	26.39	7.5-34	7.0-9.4	3.0-6.4
VII	26-39	7.5-34	6.2-9.1	1.8-5.4

Table 1b. Monthly averages and ranges of rainfall measured during the period of study.

Month	Average (mm)	Range (mm)
March	5.08	0.40.6
April	2.54	0-15.2
May	3.81	0-30.5
June	3.52	0-25.4
July	4.76	0-63.5
August	6.69	0-45.7
September	1.87	0-18.5
October	10.79	0-36.8

Table 2. Summary of harvest data showing the mean weight recovery and production of grouper, adult tilapia[1], and young tilapia[2] in monoculture and polyculture at different stocking combinations[3].

Treatment	Replicate	ADULT TILAPIA Mean Wt. (gram)	ADULT TILAPIA Prod. (kg/ha)	ADULT TILAPIA Recovery (%)	YOUNG TILAPIA Mean Wt. (gram)	YOUNG TILAPIA Prod. (kg/ha)	Grouper Mean Wt. (gram)	Grouper Prod. (kg/ha)	Grouper Recovery (%)	TOTAL Prod. (kg/ha)
I	1	57.36	432.75	37.5	3.03	380.12	–	–	–	812.87
	2	55.64	507.60	45.3	11.60	406.43	–	–	–	914.03
	3	52.76	533.92	50.2	3.47	522.22	–	–	–	1056.14
	mean	55.25	491.42b	44.3	6.03	436.27a	–	–	–	927.68ab
II	1	42.26	543.86	50.2	6.18	346.78	–	–	–	890.64
	2	58.58	931.58	63.5	4.45	425.15	–	–	–	1356.73
	3	57.37	923.39	36.4	4.45	374.85	–	–	–	898.24
	mean	53.07	666.28ab	50.0	5.03	382.26a	–	–	–	1048.54a
III	1	46.58	735.67	52.6	3.50	512.87	–	–	–	1248.54
	2	42.15	722.22	57.1	3.77	812.28	–	–	–	1534.50
	3	46.08	722.22	52.2	3.32	219.30	–	–	–	941.52
	mean	44.94	726.70a	54.0	3.53	514.82a	–	–	–	1241.52a
IV	1	–	–	–	–	–	162.5	76.02	47.06	76.02
	2		–	–	–	–	114.0	46.78	41.18	46.78
	3	–	–	–	–	–	107.8	88.30	82.35	88.30
	mean		–	–	–	–	128.1	70.37b	56.86b	70.37c
V	1	64.86	804.09	61.6	11.00	6.43	233.3	204.68	88.23	1015.20
	2	51.92	631.58	60.4	8.67	1.75	204.1	202.92	100.00	836.25
	3	41.77	742.69	88.3	7.4	5.62	180.77	137.43	76.47	885.74
	mean	52.85	726.12a	70.1	9.02	4.60b	206.06	181.68a	88.23a	912.40ab
VI	1	49.62	635.67	51.2	11.46	27.48	186.67	163.74	88.23	826.89
	2	47.44	760.23	64.0	7.63	13.45	168.75	157.89	94.41	931.57
	3	40.36	722.22	71.5	8.33	11.69	169.94	169.00	100.00	902.91
	mean	45.81	706.04a	62.2	9.14	17.54b	175.12	163.54a	94.21a	887.12b
VII	1	42.86	771.93	60.0	6.89	15.20	175.0	143.27	82.35	930.40
	2	35.88	921.05	85.6	6.45	18.71	276.89	145.61	52.94	1085.37
	3	56.77	637.43	37.4	5.55	19.30	183.3	128.65	70.59	785.38
	mean	45.17	776.80a	61.0	6.30	17.74b	211.73	139.18a	68.63ab	933.72a

[1] 20 grams and above

[2] less than 20 grams

[3] means in a column having at least one common letter subscript are not significantly different (P=0.05)

Table 3. Tilapia population in monoculture and polyculture at different stocking combinations. [1]

Treatment	Replicate	Adult Tilapia		Young Tilapia	
		Number %	Population	Number %	Population
I	1	129	5.67	2145	94.33
	2	156	20.66	599	79.34
	3	173	6.30	2575	93.70
	mean	153	10.88b	1772	89.12a
II	1	220	18.66	959	81.34
	2	272	14.27	1634	85.73
	3	275	16.04	1440	83.96
	mean	256	16.32b	1344	83.68a
III	1	270	9.73	2506	90.27
	2	293	7.37	3684	92.63
	3	268	19.17	1130	80.83
	mean	277	12.09b	2440	87.91a
V	1	212	95.50	10	4.50
	2	208	98.58	3	1.42
	3	304	95.90	13	4.10
	mean	241	96.66a	9	3.34b
VI	1	219	84.23	41	15.77
	2	274	90.13	30	9.87
	3	306	92.73	24	7.27
	mean	266	89.03a	32	10.97b
VII	1	308	89.02	38	10.98
	2	439	89.78	50	10.22
	3	192	76.50	59	23.50
	mean	313	85.10a	49	14.90b

[1] Means in a column having at least one common letter subscript are not significantly different. ($P < 0.05$)

In monoculture, 83-89% of tilapia population were young. This is significantly higher ($P < 0.05$) than that in polyculture in which young tilapia population was only 3-15% (Table 3). The decrease in the population of young tilapia in polyculture was due to grouper predation. Grouper could swallow tilapia having a body depth equal to its mouth opening. The mouth opening of grouper is 16-20% of its total length. The body depth of tilapia is 29-37% of its total length.

Higher recovery of the original tilapia stocked was obtained in polyculture although no significant difference ($P < 0.05$) existed between treatments. The lower recovery in monoculture could be attributed to population resulting to greater intraspecific competition especially in terms of food (Tables 3 & 4).

Effect of Grouper on the Production of Marketable Tilapia

Highest production of adult tilapia in kg/ha was obtained in Treatment VII (776.67), followed by Treatment III (666.28), Treatment V (726.12), Treatment VI (706.04), Treatment II (666.28), and Treatment I (491.42). Comparing treatments having the same stocking density of tilapia such as Treatments VII and III (30,000/ha), Treatments V and I (20,000/ha) and Treatments VI and II (25,000/ha), showed that the presence of grouper have a direct effect

on the number of marketable tilapia (Tables 2 & 3). Tilapia in polyculture are more plump than those in monoculture (Table 5) because of lesser tilapia population resulting to lower intraspecific competition.

Effect of Tilapia on the Recovery and Production of Grouper

Tilapia significantly benefitted grouper in every aspect. The monoculture of grouper

Table 4. **Intraspecific competition indices of tilapia at different densities in monoculture.**

Treatment with Specifications		Ave. Net Production of 20.000 tilapia (kg/ha)	Intraspecific Competition
I	20,000 tilapia/ha	927.68	
II	25,000 tilapia/ha	838.83	0.10
III	30,000 tilapia/ha	827.68	0.11

Table 5: **Condition factor of tilapia at different stocking densities cultured singly or in combination with grouper.**

Treatment With Specifications		Repli-Cate	Average Length (cm)	Average Weight (g)	Condition Factor
I	20,000 tilapia per hectare	1	11.72	33.68	2.09
		2	12.71	38.61	1.88
		mean	13.15	43.29	1.89
II	25,000 tilapia per hectare	1	13.22	42.03	1.82
		2	12.95	44.22	2.04
		3	14.75	46.25	1.44
		mean	13.64	44.17	1.77
III	30,000 tilapia per hectare	1	12.87	45.07	2.11
		2	14.11	45.45	1.62
		3	13.41	42.10	1.74
		mean	13.46	44.21	1.82
V	20,000 tilapia per hectare plus 1000 grouper/ha	1	13.80	52.13	1.98
		2	14.28	53.60	1.84
		3	13.53	46.67	1.88
		mean	13.87	50.80	1.90
VI	25.000 tilapia per hectare plus 1000 grouper/ha	1	12.38	38.56	2.03
		2	14.20	54.00	1.88
		3	11.19	32.10	2.29
		mean	12.59	41.52	2.07
VII	30,000 tilapia per hectare 1000 grouper/ha	1	12.17	35.79	1.99
		2	13.27	42.35	1.81
		3	12.54	41.71	2.11
		mean	12.66	39.95	1.97

Table 6. Growth rate of tilapia and grouper in monoculture and polyculture at different stocking combinations[1].

Treatment	Replicate	Tilapia		Grouper	
		Ave. Wt. gain (g)	Growth rate per day (g)	Ave. Wt. gain (g)	Growth rate per day (g)
I	1	41.11	0.185		
	2	38.37	0.173		
	3	36.99	0.167		
	mean	38.82	0.175		
II	1	30.46	0.151		
	2	46.03	0.207		
	3	46.02	0.207		
	mean	41.84	0.188		
III	1	38.08	0.171		
	2	20.15	0.091		
	3	26.38	0.119		
	mean	28.20	0.127		
IV	1			158.40	0.937
	2			108.10	0.640
	3			104.90	0.621
	mean			123.80b	0.732
V	1	51.06	0.230	227.40	1.346
	2	38.82	0.175	198.20	1.173
	3	28.27	0.127	177.87	1.052
	mean	39.39	0.177	201.16a	1.190
VI	1	32.82	0.148	180.77	1.070
	2	31.64	0.143	165.85	0.981
	3	25.36	0.114	167.04	0.988
	mean	29.94	0.135	171.22ab	1.013
VII	1	27.86	0.125	172.10	1.018
	2	20.88	0.094	272.79	1.614
	3	42.97	0.194	100.40	1.067
	mean	30.57	0.138	208.43a	1.233

[1] Means in a column having at least one common letter subscript are not significantly different ($P < 0.05$)

Table 7. Interspecific competition indices between tilapia and grouper at different stocking densities.

Specifications			Ave. Net Prod. (kg/ha)		Competition Index
			Monoculture	Polyculture	
A.	Competition of tilapia to grouper				
	1)	1,000 grouper with 20,000 tilapia/ha	927.68	730.72	0.21
	2)	1,000 grouper with 25,000 tilapia/ha	1048.54	723.54	0.31
B.	Competition of grouper to tilapia				
	1)	20,000 tilapia with 1,000 grouper/ha	70.37	181.68	−1.58
	2)	25,000 tilapia with 1,000 grouper/ha	70.37	163.54	−1.32
	3)	30,000 tilapia with 1,000 grouper/ha	70.37	139.18	−0.98

(Treatment IV — 1000/ha) always got the lowest result compared with polyculture (Treatment V — 20,000 tilapia plus 1,000 grouper/ha, Treatment VI — 25,000 tilapia plus 1,000 grouper/ha, and Treatment VII — 30,000 tilapia plus 1,000 grouper/ha).

Highest recovery of grouper was obtained in Treatment VI, 94.21% (Table 2). Similarly, the growth of grouper was higher in polyculture (Table 6). Since tilapia serves as food for grouper, negative results were obtained on the effect of tilapia to grouper (Table 7). This means that tilapia benefits grouper, thus grouper in polyculture are more plump than those in monoculture (Table 8). All these contributed to higher production in polyculture in which Treatment V got the highest (181.68 kg/ha), followed by Treatment VI (163.54 kg/ha) and Treatment VII (139.18 kg/ha).

Total Production

The highest total production in kg/ha was 1241.52 (Treatment III — 30,000 tilapia/ha) followed by 1048.54 (Treatment II — 25,000 tilapia/ha), 933.72 (Tretment VII — 30,000 tilapia plus 1,000 grouper/ha), 927.68 (Treatment I — 20,000 tilapia/ha), 912.40 (Treatment V — 25,000 tilapia plus 1,000 grouper/ha) and 70.37 (Treatment IV — 1,000 grouper/ha).

Higher production in monoculture of tilapia was due to overpopulation, where 83-89% of tilapia. Disregarding the young tilapia which are actually unsaleable, polyculture of grouper and tilapia (Treatments VII, V and VI) got the first three highest production, followed by the monoculture of tilapia (Treatments III, II, and I), then monoculture of grouper (Treatment IV).

CONCLUSION AND RECOMMENDATION

The experiment disclosed that grouper could be used to control the population of tilapia. Feeding on the original stock of tilapia could be avoided by stocking tilapia that are bigger in size than grouper. If tilapia young will serve as the main food for grouper, stocking tilapia ahead of grouper to give them enough time to breed will minimize predation on the original stock as well as provide enough food for grouper.

Of the three stocking combinations that were evaluated, Treatment V (20,000 tilapia plus 1,000 grouper/ha) was found the best.

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Monkey Trading in the Philippines Status and Prospects

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Importance of Nonhuman Primates

Nonhuman primates have been important experimental animals for scientists. During early civilization, Mesopotamians were already using monkey bones in drug manufacture (Morris and Morris, 1966). Particular attention is focused towards the monkey's physiology which closely approximates that of humans. Research areas in reproductive biology, immunology, cardiovascular diseases, genetics, environmental toxicology and organ transplant have been advanced through the use of primates. Since early 1900's the monkeys have been subjected to research on chemical warfare and explosive tests by the military, to the consternation of animal lovers and conservationists. Particularly alarming is the fact that 33-63% of the animals die in transit and in quarantine (Annonymous, 1981). However, there are no records yet on the number of deaths while still being collected in the field, in transit and while being conditioned in holding stations.

Eighty genera and 240 species of living primates are found in the world. Several species are used in experiments such as the rhesus monkey (*Macaca mullata*) which were used in understanding the rhesus factor and also in the manufacture of the polio vaccine.

The demand for monkeys had been dictated solely by their availability rather than their biological characteristics. The following species are currently being used extensively worldwide (Goodwin, 1975): *Macaca mullata, M. fascicularis, M. nemestrina, Saimiri sciureus, Papio cynocephalus, Aotus trivirgatus, Saguinus nigricollis, S. mystax* and *Pan troglodytes.*

World Monkey Trade

The demand for monkey as a laboratory animal is predicted to continue in the next 10-20 years. The intensified research on AIDS (Acquired Immune Deficiency Syndrome) and other diseases of public importance will definitely create a high demand for nonhuman primates. At present, the chimpanzee is the only animal besides man that is known to contract the AIDS virus but likewise, other species of primates will be tested for the disease in the future. The chimpanzee right now is an endangered species costing about $15,000 each in the USA (Annonymous, 1987). At the same time, an interest and demand towards exotic foods does not exempt the monkeys, as what has been practiced in Taiwan, Hongkong and China. Demand for zoo and pet trades is likewise a pressure on the already dwindling nonhuman primate population.

In the USA alone, demand for monkeys exceeds 50,000 per year (Southwick, *et al.*, 1975). Japan imported about 20,000 monkeys in the early 1970's but reduced its importation to

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wards 1980's (Hasan, 1984). Tailing around in the importation trade is Taiwan which imports 5,000 monkeys per year from Asian neighbors but Taiwan was refuted to own one to two laboratories only (IPPL communications, 1986).

India, Bangladesh, Southeast Asian countries Africa and South America are the major monkey exporters in the world. India ranked first in the monkey trade for the last 30 years. Its primary export was the rhesus monkey but in 1955 India imposed a temporary ban which paved the way for the exportation of an excellent rhesus substitute: the crab-eating macaque or *M. fascicularis.* Malaysia, Thailand, Indonesia and Philippines then led the monkey trading until 1978 when India completely banned its exportation. This was precipitated by the wide publicity in the India press about using the rhesus monkey in neutron bomb experiments at the US Armed Forces Radiobiology Research Institute in Bethesda, Maryland (IPPL communications, 1978). Bangladesh also followed suit in 1980. These periods marked the blooming of monkey trading in the Southeast Asian countries. In 1976, Thailand imposed its ban on export of monkeys. Singapore played a key role in the trade by serving as a commercial center for the importation and re-exportation of monkeys. In 1985, Malaysia declared its ban of monkey export.

The fate of *M. fascicularis* in the Philippines and in other southeast Asian countries is therefore at stake. Continuous collection of feral monkeys without replenishment will bring about depletion of the natural population which threatens the species to endangerment. Particularly interesting is the fact that the Philippines now leads in the monkey trade in the world.

Monkey Resources in the Philippines

The crab-eating macaque or Philippine long-tailed macaque *(M. fascicularis)* is the only monkey species in the Philippines. Other nonhuman primates found in the country include *Tupaia palawanensis* (common tree shew), *Urogale everetti* (Philippine tree shrew), *Nycticebus menagensis* (slow loris) and *Tarius syrichta* (tarsier). *M. fascicularis* is also found in Thailand, Laos, Burma, Campuchea, Vietnam, Malaysia and Indonesia (Wolfheim, 1983).

Macaca fascicularis is a small brown monkey with paler underparts and prominent whitist hairs on the face especially on males. Newborns are black with bright pink ears and face. Adult males weigh 5-7 kg, males, 3-4 kg. The average number of young per female per year is 0.6. The species is polygamous or of harem type of breeding and has a longevity of 25-28 years; sexual maturity in males is 4-5 years, in females 3–4 years; gestation is 160 + 17 days (Nazareno, 1986).

Monkey population in the Philippines is still unknown although Indonesia reported a population estimate of 3.7 million *M. fascicularis* (Mackinnon 1986). In Malaysia, the density is estimated at 60 head/km^2 (March and Wilson, 1981) while in Mauritius island, population density was placed from 33-120 head/km^2 (Sussman and Tattersall, 1986).

The population of Philippine nonhuman primates has suffered a lot from losses. Among the causes are over-hunting for food and commercial purposes, human predation in response to crop-raiding and destruction of habitat due to illegal logging and kaingin system.

Role of CITES

The species is listed in Appendix 2 of CITES (Convention on International Trading in Endangered Species of Wild Fauna and Flora) which means that trading is regulated through recommendation of the assigned scientific authority of the country. The scientific authority determines if such exportation will not be detrimental to the survival of the species. The Philippines is one of the signatories of CITES. In 18 August 1981, the Bureau of Forest Development (BFD) was designated as the management authority of CITES.

Between January to June 1982, the USA has received cleared shipments of 1,096 monkeys, 2 black palm cockattoos, 1 eclectus parrot and 1,000 skins of water monitor lizard (IPPL communications, 1983). This shows a very clear violation of agreement of the conven-

Table 1. Monkey exporters in the Philippines, their quota and actual number of head exported in 1986 including fees and taxes paid. (Source: BFD-DENR)

Permittees and Address	Quota	Total No. Exported	Export Value Per Animal	Certification and Inspection Fees
SICONBREC, INC. Makati, M. M.	3,000	3,286	$ 100.00 (uncond. an.) $ 160.00 (cond. an.)	$ 16,430.00
Eugene Del Mundo Tanay, Rizal	1,500	1,105	$ 100.00 (uncond. an.) $ 160.00 (cond. an.)	$ 5,525.00
Alex Lina Ferlite Scientific Makati, M. M.	3,000	1,662	$ 20.00	$ 8,310.00
Marino Keh Iloilo City	3,000	684	S 20.00	$ 3,420.00
A. T. Viri Makati, M. M.	3,000	2,570	$ 80.00	$ 12,850.00
Concha Viri-Hontiveros Parañaque, M. M.	2,000	475	$ 80.00	$ 2,375.00
TOTAL	15,500	9,307	$ 720.00	$ 49,285.00

[1] Certification and inspection fees total 10% of export value (BFD-FAO No. 1-A, Series of 1986); conditioned monkeys command higher value; assume $50 per head as price of monkey.

tion. Trading of Appendix 2 species is only allowed upon certification of the scientific authority which based its recommendation from actual conducted census of the regulated species. There has never been a census of the affected species in the Philippines.

Monkey Trade Status in the Philippines

Currently, six permittees are legally exporting monkeys from the Philippines (Table 1). The Department of Energy and Natural Resources established the quota which ranges from 1,500 to 3,000 head or a total of 15,500 head for 1986. However, the actual number of head exported for that year was 9,307. Quota system or a possible ban for 1988 is still to be determined.

From among the six permittees, only SICONBREC, INC. (Simian Conservation and Breeding Center, Inc.) qualifies as a stockfarm. The other permittees qualify as holding stations only (Rubio, 1986) The basic requirements for stockfarming should be the availability of breeding facilities and attending full time veterinarians. These requirements are fulfilled by SICONBREC, INC.

Most traded monkeys abroad are collected from Mindanao, Visayas and a few other locations in Luzon by professional monkey hunters in the collection sites or in some instances by the natives or cultural minorities. Present monkey price ranges from P50 to P100 de-

pending on size and condition of the animal. Upon reaching holding stations, the animals are observed and conditioned for 1-2 months after which they are dispatched abroad. Monkey exporters sell their monkeys from $50 to as high as $100. For conditioned monkeys or those animals that have stayed in the farm or holding stations and had received medications and had complete historical records, as well as those born in captivity, can command $150-$300 per head. In the USA, further conditioning of the monkey can raise up the price to as high as $600 per head.

The author had visited SICONBREC, INC. in Tanay, Rizal in 1985 and Ferlite Scientific, Inc. in Calamba, Laguna on several occasions. Most of the information here are based on actual observations of the farm and related publications.

SICONBREC, INC.

The center is located on a 14-ha site at Tanay, Rizal about 60 km east of Manila. It was started in 1982. The center is associated with Shamrock Farms Ltd. and the Intersimian Ltd. based in the United Kingdom. There are two general objectives of the center namely: 1) to condition feral cynomolgus monkey to standard comparable with those recommended by the WHO and 2) to establish a large breeding population with the ultimate goal of producing laboratory conditioned animals for breeding stock. The breeding program of the center hopes to establish 7,000 females in harem groups in 1990 and to meet the importer's annual demand of 4,000-5,000 head of monkeys. Five years of continued trapping in the wild is needed to reach such goal (Nazareno, 1986). At present, the center is exporting monkeys to Europe, Australia, USA, Canada, Japan, Taiwan and Korea.

Over a three year period, 25 colonies have been established and 418 young have been weaned. Live birth production rate at SICONBREC,, INC. is 86% (Nazareno, 1986).

The center's supply of monkey comes from Zamboanga where they provided permanent holding facilities. A stabilizing period of two weeks is done on caught wild monkeys, after-which they are transported by air which minimizes stress to the animals. At SICONBREC standardized procedures for the trapped animals are adopted such as tuberculin testing, measle vaccination, antihelminthic and antibiotic treatments, and screening for *Herpes simiae* virus. A conditioning period of six to eight weeks is done at the center.

Since 1986, the center has operated on 30 breeding pens each with a capacity of 25 animals. They have a large and expanding nursery unit to accomodate weaned and growing-on-stock. Two other modern buildings with a holding capacity of 600 animals on individual cages are within the compound. An outdoor communal holding pen with a capacity of 1,000 animals keep the animals before being individually caged in conditioning buildings. All communal areas are provided with cement drainage pipes which serve as escape areas during social dominance displays. A microbiological laboratory is another important structure in the compound which serves mainly to screen stocks within the center. Staff and veterinarian quarters, workshops and guest houses are also within the farm. Fifty people composed the staff, four of which are veterinarians and one is a trained laboratory technician.

With this advanced type of handling of monkeys, SICONBREC, INC. can dictate their price. The cost of conditioned feral monkey is $100/head and $160-$200 per head for captive-bred monkeys. The price is further increased if they are resold to importing dealers which could amount to $500 per head. Annual income of the center is estimated to be about $1 M.

Ferlite Scientific, Inc.

This company is owned and managed by a Filipino businessman by the name of Mr. Alex Lina. The monkey farm was established in 1980 on a seven hectare land in Calamba, Laguna. Most of the animals are housed in two tall buildings which are of communal and semi-communal types. The animals are conditioned in the two buildings from 30-60 days prior to dispatch to individual cages. The sickly and wounded animals are also allowed to recover within these buildings. Later, they are

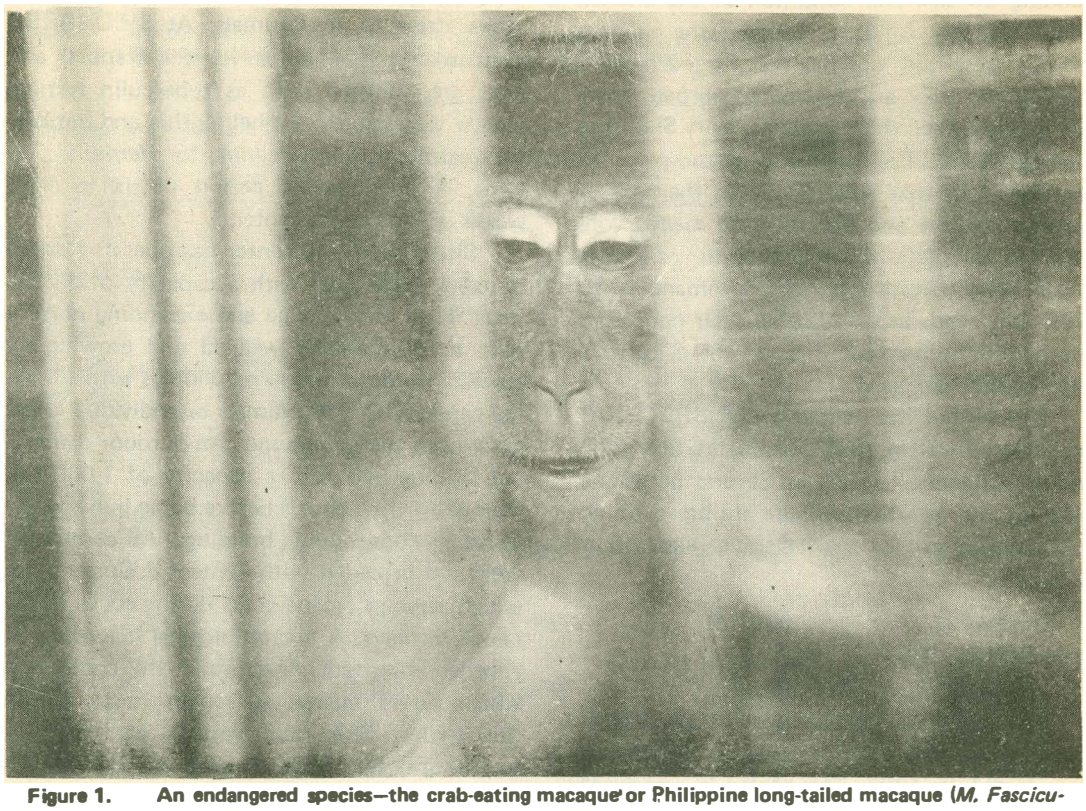
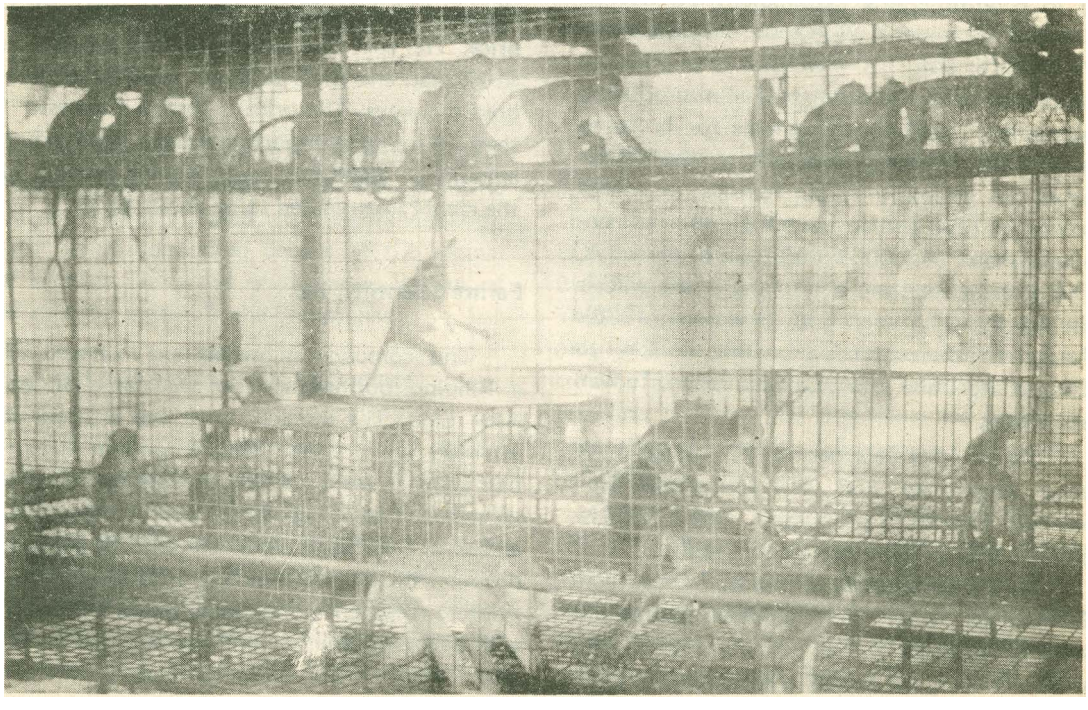


Figure 1. An endangered species—the crab-eating macaque or Philippine long-tailed macaque (*M. Fascicularis*) is the only monkey species in the Philippines. It is a small brown monkey with paler underparts and prominent whitish hairs on the face especially on males.

Figure 2. Monkey population in the Philippines is still unknown.



transferred to individual cages located in two separate buildings. These two buildings have holding capacity of 120 animals and the animals spend 15-30 days here for further conditioning and observation prior to transport abroad. Production of the farm averages one to two shipments per month.

Very recently, the farm has added a large gang-type cage to accomodate up to 500 animals. Another new building was constructed for the individual type of cage. Old buildings housing the individual cages were renovated and extended. Other repairs are going-on and further improvement of the gang-type cages are being planned for anticipated large shipments of monkeys in the future.

Six regular employees work in the farm with their living quarters located right inside the farm. They are assigned to take care and feed the monkeys, maintain facilities and construct transport cages. There is no resident veterinarian in the establishment.

A pair of pig-tailed macaque *(M. nemestrina)* and three offsprings are housed in one of the communal type cages. One young is actually a product of the mating of the female pig-tailed macaque and a male native long-tailed macaque. The owner intends to breed *M. nemestrina* in his farm and later establish a colony. The source of his pig-tailed macaques was Borneo. Mr. Lina is also considering breeding of long-tailed macaque in the future.

Prospects for the Industry

The government has finally realized that collecting and trading of monkeys in the Philippine should be regulated. The regulation will take effect in January 1988. The government will strictly enforce the quota allocation system or impose a ban on export, if necessary. The enforcement will be based on population census of monkeys in the different islands of the country and it is expected to be conducted within this year. Such government action is hoped to regulate the alleged over collection in the wild and establish the population status of monkeys in the Philippines.

There are conflicting reports regarding monkey population in the country. It is claimed that there is an over-abundance in a few localities and rapid dwindling in other locations. An actual population study should end all these allegations.

Population census of long-tailed macaque has never been done in the Philippines. The Bureau of Forest Development programmed a population census in 1981 but due to lack of funds the census was instead reprogrammed in 1983 (IPPL communications, 1982). To this date, however, no census is still being carried out.

As early as February, 1987 BFD had announced its need for program proposal on monkey census. The group of researchers from the UPLB and FORI responded and was eventually given a grant to implement the census. However, since BFD can not provide yet the funds, it was proposed that the group of exporters will be tapped to initially finance the project with the understanding that the exporters will not influence the results and decisions of the research group. The exporters were also encouraged to participate in the field census to give them a chance to check the soundness of the procedures being adopted by the research group.

The program is supposed to be implemented on a sustained basis until such time that population trends are established. BFD later on is expected to implement the census. A training aspect for BFD field personnel is included in the program and later they should be able to carry the work independently. This will facilitate efficient monitoring of monkey population in every regions of the country.

The research group had proposed that five priority areas should be censused. These include Zamboanga del Sur, Zamboanga del Norte, Cotabato, Panay and Negros islands. Census work is expected to start in August of this year.

Recommendations

It is believed that natural populations of *M. fascicularis* in the Philippines are already threatened. As early as 1975, the Institute for Laboratory Animal Resources has already

identified our monkey population was in danger of extinction if massive trades are continued (IPPL communications, 1982). Being one of the important renewable natural resources of the country, *M. fascicularis* populations deserve to be managed carefully.

In order to secure a healthy population of monkeys in the wild, the following recommendations are offered:

1. Implement as soon as possible the census program and determine the quota system or embargo of exportation. Census work should be sustained in order to come up with trends in population. Quota system should be programmed very carefully.

2. Establish preserves or sanctuaries where monkeys and other important wildlife species are protected. Collection of animals should be strictly prohibited in these areas. These protected areas are expected to allow natural regeneration of the species until such time that normal population levels are attained.

3. All exporters should be required to engaged in captive breeding within a period of five years. This should be duly monitored and evaluated by a scientific authority. If they fail to do so, their permits should be cancelled indefinitely until they could show proof of capability to breed monkeys in captivity. Similarly, a policy should be developed and imposed on the breeders to return back 20% of their captive born animals into the wild. This policy will help restore normal population levels in the wild. Again, a duly designated body is needed to authenticate the transplantation:

4. A long-term management plan for monkey population should include research on monkey biology and ecology. Funding of such projects can be generated from collected fees and taxes from the industry. BFD has collected about $50,000 from exporters last year but this amount is supposed to go to the national treasury. There should be a policy to allow certain per cent of the collection to finance research and development projects on monkey. International funding agencies can also be tapped to finance management plans.

5. The government should impose on the exporters strict compliance with the requirements on handling and transporting of monkeys from point of collection to final destination. The exporters should adhere strictly to international standards on cage specifications, in-transit needs and other requirements.

6. The potential of stockfarming of other nonhuman primates as experimental animals and those which can readily substitute for the long-tailed macaque should be considered End-users should consider maximum utilization of available resources such that wasteful consumption is avoided.

7. Review further the monkey trade regulations such as the issuance of collector or export permits, applicant qualifications and capabilities, project plans, taxation schemes and others.

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PUBLICATION CHECKLIST

• **NONDESTRUCTIVE EVALUATION:** *Proceedings of the 16th Symposium on NDE,* available from NTIAC, Southwest Research Inst., Drawer 28510, San Antonio, TX 78284. Documents 42 papers from April 1987 symposium on nondestructive evaluation methods, and R & D results. 387 pp. $90.

• **S & T SWEDEN:** *Reviews of National Science and Technology Policy: Sweden,* available from OECD Publications and Information Ctr., 2001 L St., NW, Wash., DC 20036-4905. Report by OECD panel on programs and policies, with generally high praise for the level of science education and research in a country which "has more to teach than to learn." 112 pp. $13.

• **GEOCHEMICAL ANALYSIS:** *Bulletin 1770,* available from USGS Books, Federal Ctr., Box 25425, Denver, CO 80225. 132 pp. $9.

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• **ENERGY AUDITS:** *Source Book for Energy Auditors, D11: 1987,* available from Svensk Byggtjanst, Box 7853, S-103 99, Sweden. Collaborative effort of nine countries and Commission of European Communities, providing information on energy audits in general, and energy conservation opportunities in multifamily or commercial buildings.

AQUACULTURE DIRECTORY: *Aquaculture: A Guide to Federal Government Programs,* available from Natl. Agricultural Library, Aquaculture Information Ctr., Rm. 304, 10301 Baltimore Blvd., Beltsville, MD 20705. Describes activities and programs supported by government agencies. 34 pp. Single copies free.

SDI/WEAPONS: *Report on Science and Technology of Directed Energy Weapons,* available from American Inst. of Physics, Pub. Sales, 500 Sunnyside Blvd., Woodbury, NY 11797. Report on an American Physical Society Study Group, as prepared for "Reviews of Modern Physics." 300 pp. $20.

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	BLEND	DIESEL OIL	KEROSENE	GASOLINE
Heating Value BTU/LB	19,015	19,500	19,000	20,000
Flammability (Sec) 1.5 ML	36.6	Did not burn alone	45	
Volatility	69.17	19.81	99.97	99.92

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