Journal of Plantation Crops 15 (2): 72-85 December, 1987

Review Article

MICROBIOLOGY OF COCONUT (*COCOS NUCIFERA* LINN.) IN RELATION TO CULTIVATION, PROCESSING, PRODUCT DIVERSIFICATION AND BIODETERIORATION *

GEORGE V. THOMAS, M. V. SHANTARAM[1] and N. P. JAYASANKER[2]
Central Plantation Crops Research Institute, Kasaragod 670 124, Kerala, India

INTRODUCTION

Coconut (*Cocos nucifera* Linn.) is one of the plantation crops grown in India in an area of 1.11 million ha. The palm belongs to the family Palmae. It has a thick and hardy stem, massive crown of leaves bearing flower bunches and nuts at varying stages of development. Besides India, the major coconut-producing countries in the world are Burma, Carribbean Islands, Indonesia, Malaysia, Mexico, Oceanic group of Islands, the Philippines, Sri Lanka, Thailand, the tropical African countries and Florida in the United States of America. India ranks third in acreage and production during 1985–86 was 6620 million nuts. Nearly 60 per cent of coconuts in India is used for culinary-purposes while the remaining is converted into copra for oil extraction. Almost all the plant parts find use in the daily human needs. The leaves are used for thatching houses and stem for building construction. The husk covering the nut yields fibre and is known to the world over as 'coir'. The shell yields activated charcoal. The kernel, is used as copra after drying or the coconut milk is prepared by wet grinding. The coconut oil is used widely for edible purposes and is extracted from the dried copra. The tapped fluid from the flower spathes is the toddy, an alcoholic beverage. The tender nut water is a refreshing soft drink. The pace of modernisation in the post harvest processing sector has been comparatively slow. The situation is attributed to the prevailing inadequacy in the research and development programmes particularly in certain areas. The intention of the present review, however, is only to confine to the microbiological aspects of the coconut palm in relation to growth, productivity, processing, product diversification and spoilage of products such as copra, kernel and oil.

* *Publication No. 634, Central Plantation Crops Research Institute, Kasaragod.*
1 *Department of Soil Science, APAU, Hyderabad.*
2 *CPCRI Regional Station, Kayangulam, Kerala.*

MICROORGANISMS IN RELATION TO PLANT GROWTH AND PRODUCTIVITY

The microbiological activity in the root zone can be directly or indirectly beneficial to the plants. Such an activity can have a profound influence on the nutrition of the plants as also on its productivity. Microbiological investigations in this direction with a relevance to coconut has been done in an exhaustive manner only in this country. Emphasis has been largely given to assess the variation among the root surface and soil microflora in relation to coconut root (wilt) disease prevalent in the southern half of Kerala, India. The pioneering effort was made by Radha and Menon (1954) who have indicated a consistent reduction in the root surface: soil ratio of micro-organisms in root (wilt) affected palms compared to the apparently healthy palms in the disease affected areas. In yet another investigation, Radha and Rawther (1954) demonstrated an increased susceptibility of the diseased palms to environmental factors. A marked variation in the microbial population in the root surface of apparently healthy and diseased palms was observed in a separate investigation (Rawther and Radha, 1963). The ideal canopy of coconut and the limited land utilisation prompted Sahasranaman et al. (1983) to investigate the various aspects of mixed farming with fodder grass and legumes in the interspaces of a heavily root (wilt) infected garden and maintaining milch cows with recycling of cattle waste. Irrespective of the disease conditions, the productivity increased by nearly 28 per cent after a period of five years and there was an increase in the nitrogen-fixing and phosphate-solubilising microorganisms in the root surface of the main crop. The consequent systematic investigations of Potty, (1977), Potty, George and Jayasankar (1977) and Potty and Jayasankar (1983) showed that the population of microorganisms was higher in the root surface of coconut palms, crop mixed with fodder crops irrespective of the disease condition of the palms. The trend was same for indole-producing, nirogen-fixing and phosphate-solubilising organisms. The situation has a positive influence on the growth and productivity of the crop. Antony (1983) on the other hand, investigated the microbiological profiles and activities in the root region of coconut under intercropping with tuber crops. The ability of different green manure legumes to establish in basins under coconut in root (wilt) affected sandy soils has been brought out by Thomas and Shantaram (1984). Among the nine legumes *Pueraria phaseoloides*, *Mimosa invisa* and *Calopogonium mucunoides* yielded 18.43, 17.00 and 14.71 kg of green matter per basin respectively in a period of four months. *In situ* there was a significant increase in the microbial populations as also in the activities of soil enzymes *viz.*, dehydrogenase, urease and phosphatase in the root region of the palms. Green manuring was also effective in augmenting mycorrhizal symbiosis of vesicular-arbuscular type in coconut (Thomas, 1987). In the context, the effort of Lily (1975) has shown the occurrence of vesicular-arbuscular mycorrhizal fungus identified as *Endogone fasciculata* in coconut roots collected from different parts of Kerala State. Ramesh and Rohini Iyer (1979) reported higher numbers mycorrhizal spores in coconut soils where application of fertilizer was not carried out. Thomas and Ghai (1987) observed genotype dependent variation in VA-mycorrhizal colonisation of coconut seedlings. Tall cultivars had greater proportion of roots with mycorrhizal colonisation than dwarfs and hybrids. Another study in root (wilt) affected tract by Thomas (1988) revealed the adverse effect of root (wilt) disease on the mycorrhizal symbiosis of coconut and showed the superiority of intercropping with hybrid napier to crop mixing with cocoa and monocropping systems in increasing the mycorrhizal status of diseased coconut palms. The ability of various bacterial and fungal isolates from different types of coconut soils to solubilise insoluble inorganic phosphate was determined by Thomas, Shantaram and Saraswathi (1985) and Thomas and Shantaram (1986). On the other hand, Nair and Subba Rao (1977 a) did not give any emphasis to the diseased condition of coconut. They have revealed that crop-mixing cocoa *(Theobroma cocoa)* in coconut plantation (in the disease free tracts of Kerala State) improved the microbiological activity in the root region of coconut in respect of auxin producing and phosphate-solubilizing organisms. The bacterium identified as *Pseudomonas* sp. and the fungus *(Aspergillus niger)* were able to solubilise 49 per cent of inorganic phosphate *in vitro* (Nair and Subba Rao, 1977 a, b). In yet another investigation Nair and Subba Rao (1978) further observed a close association of the fungus *Trichoderma lignorum* on the root surface of coconut which possessed an antagonistic activity towards several soil fungi. Rohini Iyer (1981) also reported increase in the population of nitrogen fixers and phosphate solubilizers in rhizosphere of coconut under intercropping with tree spices as compared to that of monoculture. In coconut-based farming systems, Ghai and Thomas (1987) reported the occurrence of *Azospirillum* spp. in coconut and component crops. These studies clearly bring out the contribution of microorganisms in the nutrition and productivity of coconut under varying conditions.

MICROORGANISMS IN PROCESSING AND PRODUCT DIVERSIFICATION

(1) Production of coir fibre in India is in the order of 0.17 million tonnes per annum from about 4000 million coconut husks. The fibre at present is used for the manufacture of yarns, mats, mattings and fancy articles. The two major extraction routes of the fibre are the mechanical defibration process producing brown fibre and the characteristic microbiological retting process producing the white fibre. The production of white fibre is dominant among the two. Fowler and Marsden (1924) were the pioneers to investigate the microbiology of coir retting that has been followed since then from various angles in different investigations. A relevant observation in this regard is that of Varrier and Moudgill (1947) who elucidated the biochemical events leading to the liberation of coir fibre from coconut husk by retting. It is now known that the retting process consist essentially of a microbiological degradation of the pectic substances which hold the fibres together prior to soaking. Several contributions on a systematic manner have emerged on

this aspect from the school of late Professor J.V. Bhat of the Indian Institute of Science. These are essentially of 3 different aspects consisting of the need or otherwise of salinity, involvement of polyphenols and succession of microflora in the process. Investigations of Jayasankar (1966) and Bhat and Nampoothiri (1973) have indicated that under controlled conditions salinity has no influence on the microflora and its activity. Enrichment of the retting liquor with catechol and phenol permitted the development of the yeast *Debaryomyces hansenii* and the bacteria belonging to the genera *Micrococcus* and *Pseudomonas* respectively (Jayasanker and Bhat 1966a, 1966b). As still culture the *Micrococcus* isolates instead of cleaving the phenolic ring, converted the substrate to a melanin-like compound identified as 3, 4 3′ 4′, Tetrahydroxy diphenyl (Jayasanker and Bhat, 1966b). Attempts have also been made to reduce the retting period by crushing the husks before steeping in water (Nagarajan, Sreekumar and Prabhu, 1985) and to develop an alternate retting technology by a closed system in the presence of cellulosic and methanogenic bacteria (Abbasi and Remani, 1985). At the same time coir retting still remains as an art acquired by retters over generations and technological improvements are yet to be applied in the process.

(2) Coconut pith is a byproduct of the coir industry. The soaked husk, after extraction of the fibre, leaves substantial quantities of the pith the disposal of which is a problem. It approximates to wood in its chemical composition and is essentially lignocellulosic. The suitability of the material as a substrate for the cultivation of edible mushrooms has been described. (Anonymous, 1961; Ramos, 1963). Alinea (1968) successfully cultivated *Volvariella volvacea* on coir dust. Iswaran (1972) employed coir dust as a carrier for the preparation of *Rhizobium* inoculants. The bacterium *Rhizobium trifolii* developed well in a mixture of soil and coir dust in equal portions, while Faizah, Broughton and John (1980) indicated the survival of rhizobia in coir dust compost. The liquid endosperm of coconut stimulated the number and volume of nodules of soyabean inoculated with *Rhizobium japonicum* (Iswaran et al., 1970). Potty et al, (1979) employed an enrichment culture methodology and isolated a fungus identified as *Poria ravanulae* which was lignoclastic. However, an appropriate technology for a profitable utilization of coconut pith/coir dust is yet to emerge.

(3) A series of tapping operations in the spathe of the coconut palm result in the oozing of the sap known as 'Toddy' in India and Sri Lanka, 'Tuba' in the Philippines and 'Tuwak' in Indonesia. The sap has ideal characteristics for getting fermented to alcoholic beverage as it contains sugars and has a pH of 4.0. Under the natural conditions, toddy is fermented by the native microflora consisting of yeasts and bacteria. Some of them produce ethyl alcohol while others produce aldehydes higher alcohols and acetic acid. (Naronha, 1972). Yeasts are predominant in the process and are identified as species of *Saccharomyces* (Revestir, 1968). In this connection

Nathanael (1960) observed that fully fermented toddy contains 6.0 to 7.0 per cent (v/v) alcohol. Samarajeeva et al. (1976) suggested that the quality of the alcoholic beverage could be improved by controlled fermentation using a pure culture of yeast as inoculum. An attempt to deodourise and preserve coconut toddy by a process of absorption and pasteurisation has been made by Potty et al. (1978). In spite of similar claims from other countries, an appropriate methodology to preserve unfermented toddy with its natural taste is yet to develop for commercial exploitation.

(4) Fermentation of the coconut sap beyond the alcoholic stage yield acetic acid and the product becomes unpalatable. However, the resultant vinegar is extensively utilised in food processing. Nathanael (1958) standardised the procedure for the large-scale production of vinegar from coconut sap. Tauro, Rao and Johar (1963) described the production of vinegar from sap and coconut water and found that the coconut water, as such, was not suitable for the preparation of vinegar because of its low content of fermentable sugars. On the other hand, much of the vinegar consumed in the Philippines is produced from coconut water. Sugar is added to coconut water to a total soluble content of 15 per cent prior to alcoholic fermentation. The process is hastened by trickling down the alcoholic fermented material on coconut husk or coir impregnated with acetic acid bacteria. In an improved method, fermentation of the sugary coconut water to alcohol is attained in 10–15 hrs by a continuous stirred tank reactor. Vinegar containing 4–6 per cent acetic acid is produced in two-three days by passing the alcoholic solution through selected acetic acid bacteria immobilised in corn cobs in tubular fermentor (Joson 1985). Casyao (1970) compared the effect of three yeast isolates *viz. Saccharomyces cerevisiae* var 'Champagne' 'Magne' and 'Tuba' on the quality of, vinegar from coconut water and found 'Champagne' to be the best on the basis of bacterial load and keeping quality. Gupta, Jain and Shanker (1980) investigated the production of vinegar from coconut sap by submerged fermentation using pure strain of *Acetobacter* and an isolated strain from jaggery and found the suitability of both the strains for an efficient fermentation. 'Nata de Coco', popularly consumed desert delicacy in the Philippines, is the white gelatinous cellulosic material formed by the bacteria *Acetobacter xylinum* on top of coconut water medium containing sugar and acetic acid (Lapuz, Gallardo and Palo 1967). It takes 14–21 days for 'nata' to grow at optimal conditions. Hagenmaier (1977) in a treatise on aqueous processing of coconut has stressed the importance of quality control in such products. Lapuz, Gallardo and Palo, (1962) reported the production of a thermoplastic material derived from the polysaccharide component of a bacterium *Streptococcus crystalloides* in coconut water. The material was suggested as useful in the manufacture of lacquers, paints and other plastic preparations. Arcega (1966) from the Philippines described the production of sauce from a mixture of copra meal and soybean using a strain of *Aspergillus oryzae*. The flavour of the sauce compared well with the soya-sauce. Arguelles, Baens-Arcega and Anglo (1975)

developed a commercial method to prepare crude protease powder by culturing *Aspergillus oryzae* on copra meal. Coconut milk is a liquid extract of grated/communated coconut kernel with or without adding water. Palo et al. (1973) used defatted coconut milk to isolated phycomycetous fungi capable of synthesising enzymes which could substitute rennet for the manufacture of cheese. Fungi belonging to the genera *Rhizopus, Mucor* and *Cunninghamella* occurring in the milk produced milk coagulating enzymes. The potential use of coconut milk as a susbstitute for butterfat in yogurt manufacture was investigated by Davide et al. (1985). The quality of the yogurt prepared from coconut milk–skimmed milk, compared well with commercial yogurt and did not require bacterial starter for the process.

COCONUT WATER AS A SUBSTRATE FOR MICROBIAL CULTIVATION

A number of investigations have revealed the suitability of coconut water to support or stimulate the growth of microorganisms. Coconut water constitutes 26 to 29% of the weight of the coconut and contains approximately 5% total solids including 2 to 3% sucrose. It also contains growth promoting substances required for microbial growth and proliferation. Hipolito (1963) studied the growth characteristics of 76 isolates of yeasts obtained from several fermenting fruit products, in coconut water. The protein content of yeasts varied from 38 to 52 per cent. Hipolito Domingo and Sarte (1965) have patented the process for cultivating the food yeast *Rhodotorula pilimanae* in coconut water. Comparing the suitability of coconut water and diluted molasses for the multiplication of the yeast *Torulopsis utilis*. Minh (1967) observed the superiority of coconut water over molasses. Aliwalas et al. (1968) investigated the various factors affecting the batch fermentation for mass culturing of *R. pilimanae* in coconut water medium. The yeast *Rhodotorula rubra* was reported to grow well in coconut water medium and was suggested for use as an additive in candies, bread and cereals for human consumption (Anonymous, 1972). Paca and Cancio (1973) compared the growth of three yeasts *viz., R. pilimanae, Saccharomyces cerevisiae* and *Candida utilis* in coconut water medium. The growth of *R. pilimanae* was the slowest, but it was most acceptable as a supplement in human foods.

Serrano et al. (1967) measured the growth promoting factors in coconut water in terms of growth response of the mould *Aspergillus niger*. The growth promoting activity was unaffected by the fermentation of coconut water and the factor could be concentrated by extraction with ether, acetone and to a lesser extent with ethanol. The water from tender nuts possessed better growth promoting activity compared to that from mature nuts (Sierra and Velasco, 1976). The production of gibberelin by cultivating *Gibberella fujikuroi* in coconut water was reported by Castillo (1964). Coconut water was reported to be suitable for promoting the multiplication of tobacco mosaic virus (Segretain, 1952). The addition of 10 to 25 per cent volume of coconut water to commonly used bacteriological media nearly doubled the growth of *Staphylococcus aureus*, *Bacillus facalisalcaligenes*, and *B. velihii* (Blauvelt, 1939).

MICROBIAL DETERIORATION OF COCONUT PRODUCTS

The dried kernel of coconut-copra, coconut oil and milk are very much prone to microbial spoilage. The microbial contamination of dessicated coconut has caused considerable concern from the point of view of public health. Ward and Cooke (1932) reported that a gap of more than four hours between the splitting and the commencement of drying, facilitates the activities of bacteria on the wet surface of kernels. They identified the moulds causing deterioration of copra as *Rhizopus* sp., *Aspergillus niger* and *Penicillium glaucum*. Wilson and Mc Kenzie (1955) reported an outbreak of typhoid due to *Salmonella* infection through the consumption of contaminated dessicated coconut. They demonstrated that *Escherichia coli* survived the drying process of coconut for 40 min at 89–93°C. Schaffner et al. (1967) stated that raw unprocessed coconut supported the growth of *Salmonella* which is particularly resistant to dessication and cosidered the contamination to occur due to contact with soil. Meedeniya (1969) studied the cause of contamination of dessicated coconut in the coconut processing mills at Sri Lanka. One of the sources of contamination was found to be the animal excreta in the mill's premises where the nuts were stacked. Two strains of *Salmonella senfienberg* were found to be the most frequent contaminants of dessicated coconut.

Fungi were reported to be the principal agents causing deterioration of copra followed by bacteria. Nathanael (1965) found that the loss due to microbial infection varies with moisture content of dried copra. Subramanyam (1968) recorded the occurrence of *Staphylococcus aureus*, *Bacillus* sp. and yeast at 20–50 per cent moisture content and that the contamination caused discolouration and softening of copra. Susamma, Menon and Thomas (1980) showed that the development of fungal and bacterial populations was influenced by the moisture content in copra, relative humidity, temperature and rainfall. Actinomycetes were not usually encountered in dried copra during storage. A moisture content of 20% was favourable for the incidence of mucorales, while a moisture of 13–17% was highly suited for the growth of *Aspergillus* spp. Only *P. citrinum* could survive on dried kernels having 5% moisture (Susamma, Menon and Alice 1981 b).

The fungus *Penicillium frequentans* was found to cause spoilage of copra even at the low moisture content of four per cent (Nair and Sreemulanathan, 1970). Rao, Sreekantiah and Rao (1971) observed the blackening and spoilage of coconut kernel was invariably due to *Botryodiplodia theobromae* and the infection was reported to occur through the 'eyes' of the coconut. Paul (1969) and Susamma and Menon (1981) isolated a number of fungi and bacteria from deteriorated copra and spoilage by the isolates was confirmed by inoculation tests. The spoilage fungi were listed as *Rhizopus stolonifer*, *R. oryzae*, *Mucor hiemalis*, *Penicillium citrinum*, *Curvularia senegalensis*, *Cochliobolus lunatus*, *Paecilomyces lilacinus*, *Aspergillus oryzae* and *A. fumigatus*. The bacteria causing spoilage was identified as *Bacillus subtilis, Enterobacter aerogenes, Pseudomonas flourescens* and *Sarcina lutea.* Among the actinomycetes the occurrence of *Streptomyces* sp. was recognised but could not be established as a spoilage organism. The changes in aminoacid content of copra due to fungal infection was also investigated by Susamma, Menon and Alice (1981 a).

Mukherji (1967) reported the growth of *Aspergillus niger* in home made coconut oil. Hoover and Gunetileke (1971) found that a species of *Aspergillus* attacked coconut oil and released free fatty acids through the production of a lipase shown to be active in coconut oil. Hoover, Laurentius and Gunetilake(1973) isolated a lipase from a strain of *Aspergillus flavus* which caused biodeterioration of both coconut oil and kernel. The enzyme was purified to increase the activity 100 fold and the properties were studied.

Studies were made to prevent the biodeterioration of copra. Nathan et al. (1980) tested a number of chemicals for preventing fungal infection during the drying of copra and found glacial acetic acid to be effective. Sierra (1971) studied the changes in chemical composition of copra due to contamination by fungi. The quantity of free fatty acids increased with infection whereas the contents of protein and N-free extract decreased.

The development of bacterial flora during storage of coconut products was investigated. Fernandez et al. (1972) resorted to bihourly bacterial counts by plate counting of grated fresh coconut kernel stored for 4 hr at 55°C, 30°C and 10°C. The storage temperature of 55°C, and 30°C favoured rapid multiplication of bacteria in grated coconut. Among the thermophiles, gram-positive long rod shaped bacteria were predominant while gram negative rods were the most predominant among the mesophiles and psychrophiles. Similar studies were also conducted by Fernandez et al. (1970) and Fernandez and Lirio (1970). After 24 hrs storage at 55°C, the thermophiles outnumbered mesophiles while psychrophiles were absent. At 30°C, and 10°C the mesophiles outnumbered psychrophiles and thermophiles were not present.

Thampan (1981) stated that deterioration of copra sets in during drying and storage. Bacterial action is initiated during processing and the severity of subsequent mould infection and insect attack depended upon the extent to which the cell structure has been broken down by the bacteria.

Infection of coconut products by micro-organisms also result in elaboration of toxic substances hazardous to humans and animals. Reports from major coconut growing countries indicated the presence of aflatoxins in coconut products like coconut kernels, copra and coconut oil (Narasimhan, 1968; Arseculeratne and De Silva, 1971; Samarajeewa, 1972; Baur, 1974).

Assay methods used for detection of aflatoxins in groundnut were found applicable to coconut products (Samarajeewa and Arseculeratne, 1974). Narasimhan (1968) detected the presence of aflatoxin in copra by spot flourescence test as well as by flourescence chromatography. A modified method was employed by Baur and Armstrong (1971) who reported the occurrence of aflatoxins B_2, G_1 and G_2. A comparison of the level of the toxin in groundnut and coconut products revealed that contents were low in coconut which was attributed to the drying process with the deposition of smoke in the kernels. (Arseculeratne and De Silva 1971). Subramanyam and Rao (1974) tested a number of oil seeds for their aflatoxin content and found higher concentrations in peanut, castor seed and copra as compared to sesame, saffower, sunflower and niger. Samarajeewa (1972) observed that the fungi associated with aflatoxin production in copra were *Aspergillus flavus* and *A. parasiticus*. Arseculeratne and Bandunatha (1972) recorded significant variations in aflatoxin production by different strains of *A. flavus* isolated from coconut products and cultured on grated coconut. Fandialan and Ilag (1973) further observed that the most favourable temperature for aflatoxin production by fungal isolates obtained from copra was 25°c.

RESEARCH NEEDS

Referring to the health and nutrition of coconut palms emphasis shall be given to inestigations pertaining to vesicular-arbuscular mycorrhizae. Similarly, because of the constant presence of active root matrix of coconut over long years of time, there is the distinct possibility of the development of associative or symbiotic relationships between the roots and the components of soil microflora. The root surface microflora of coconut and their role in biological nitrogen fixation, plant nutrient mobilization and antagonistic phenomenon need detailed studies.

The feasibility of high density multispecies cropping under coconuts is currently receiving attention in this country. In the circumstances, the microbiological changes affecting the growth and the yield of coconut and the associated intercrops have to be monitored. Attempts to pinpoint ideal crops that can proliferate beneficial microorganisms with enhanced activity in the root surface of the coconut, under such situation need attention.

Significant efforts have been made to understand the microbiology and the biochemical events leading to the liberation of coir fibre by the process of retting which consists of soaking coconut husk in water. However, the commercial extraction of white fibre still remains one as an art acquired by retters over generation. It is necessary that technological improvements are attained in this area. Attempts should be made to reduce the period of retting to produce the fibre wherever husks are available by soaking in saline, nonsaline and stagnant water rets. The microbiological process can, no doubt, combine with chemical methods for obtaining quality fibre. It should be the endeavour to come out with an appropriate technology to produce fibre under better hygeinic conditions.

The retting industry produces a lot of organic waste material called the coconut pith the disposal of which is a problem at present. The material is ligno-cellulosic and the bondage as such resistant to microbiological attack. At the same time several organisms particularly fungi are capable of cleaving the ligno-cellulosic bondage. Once this bondage is cleared, it offers great scope for the profitable utilization of both lignin and the cellulosic contents. The possibility of converting this material into 'single cell protein' which can act as base for cattle and poultry feeds by microbial activity is an area that merits consideration.

Very little work has been done in this country on the microbiological aspects of product diversification of the coconut particularly with emphasis on food and beverage processing. The preservation of sweet coconut toddy in its natural form, the utilisation of liquid endosperm (coconut water) as a soft drink and for the commercial production of vinegar are areas worth investigating. The processing of the kernel to acceptable food products similar to sauces, creams, deserts, yogurt etc. needs attention. Needless to emphasise the necessity of undertaking investigations on the post-harvest technology.

REFERENCES

ABBASI, S. A. and REMANI, K. N. 1985. Environmental pollution due to retting of coconut husk and preliminary studies on closed system retting. *Proc. Workshop Coir Research*, Coir Board, Cochin, 1985. pp. 127-134.

ALINEA, C. A. 1968. Growing *Volvariella volvacea* (Bull. ex. Fr.) Sing. on coconut coir dust. BS Thesis, UPCA College, Laguna, Philippines.

ALIWALAS, F. P., HIPOLITO, D. G., ALCAREZ, L. and DOMINGO, J. 1968. Mass culture of *Rhodotorula pilimanae* Hedrick et Burke in coconut water medium. *Philipp. J. Sci.* 97 (1): 57-72.

ANONYMOUS, 1961. How to use coir dust in mushroom culture. *Coco. News.* 6 (17): 1–2.

ANONYMOUS, 1972. Useful yeast from coconut water. *Philipp. Farms. Gard.* 9 (9): 2.

ANTONY, J. 1983. Microbiological activities in the root region of coconut (*Cocos nucifera* Linn.) under intercropping with tuber crops. Ph. D. Thesis, Faculty of Science; University of Kerala (Unpublished).

ARCEGA, L.B. 1966. Feasibility of mold process production of sauce from copra meal and soybean mixture with authentic philippine strain of *Aspergillus oryzae* (Ahlburg) Cohn. *A. J. Agric.* **13** (2): 80-105.

ARGUELLES, A. Ll., BAENS–ARCEGA, L. and ANGLO, P. G. 1975. Preparation of crude protease powder from philippine strain of *Aspergillus oryzae* cultured in copra meal NIST-Annual Report for 1974–75, P. 5.

ARSECULERATNE, S. N. and DE-SILVA, L. M. 1971. Aflatoxin contamination of coconut products. *Ceylon J. Med. Sci.* **20**: 60–75.

ARSECULERATNE, S. N. and BANDUNATHA, C. H. S. R. 1972. Variation of aflatoxin content of culture of *Aspergillus flavus* with duration of incubation in relation to studies on aflatoxin production. *Appl. Bacteriol.* **35**: 43–52.

BAUR, F. J. 1974. Recommended method for aflatoxins in copra, copra meal and coconut. UPAC Tech. Report. 9:8.

BAUR, F. J. and ARMSTRONG, J. C. 1971. Collaborative study of a modified method for the determination of aflatoxins in copra, copra meal and coconut. *J. Assoc. Offic. Agric. Chem.* **54**: 874-878.

BHAT, J V. and NAMBUDIRI, A. M. D. 1973. Effect of some of the environmental factors on quality of coir. *Coir.* **17** (2): 23–25.

BLAUVELT, L. 1939. The use of non cooked, nonsterilised coconut milk as an additional nutrient in culture media. *J. Lab. Clin. Med.* **24**: 420–423.

CASTILLO, A. Y. 1964. Study on the production of gibberellin using coconut water of different concentration as media. BS Thesis, Ad Univ., Manila, Philippines.

CASYAO, J. M. 1970. A comparative study on the effects of three yeast isolates on the quality of vinegar from coconut water. BS Thesis, UPCA College, Laguna, Philippines.

DAVIDE, C. L., PERALTA, C. N., SARMAGO, I. G. and SAMAGO, L. E. 1985. Utilization of coconut milk in filled soft cheese and yogurt production. Proc. Fd. Conf., Feb. 18–25, 1985. Philippine International Convention Center, Manila, Philippines, p. 436.

DEL ROSARIO, R. R. and PUNZLAN, G. C. 1977. Quality control of coconut milk processing, Emulsion stability studies. *Philipp. J. Coconut Studies.* **11** (4): 9–14.

FAIZAH, A. W., BROUGHTON, W. J. and JOHN, C. K. 1980. Rhizobia in tropical legumes –X. Growth in coir dust soil compost. *Soil Biol. Biochem.* **12**: 211–218.

FANDIALAN, I. M. and ILAG, L. L. 1973. Aflatoxin production of *Aspergillus flavus* from rough rice, corn, soybean, peanut and copra. *Philipp. Agric.* **57**. 254–263.

FERNANDEZ, W. L. and LIRIO, V. M. 1970. Thermophilic, mesophilic and psychrophylic bacterial plate count of gata (coconut milk) stored for 24 hrs at 55°C, 30°C and 10°C. *Philipp. Agric.* **54**: 193–201.

FERNANDO, W. L. and ORTEGA, M. O. 1983. Fungi from copra. *Philipp. J. Cocon. Stud.* **8** (16 & 2): 43–45.

FERNANDEZ, W. L., IGNACIO, E. V., LINGA, C. K. and MALAPITAN, A. H. 1970. Bi-hourly, plate count of gata (coconut milk) stored for 24 hr at 30°C, 20°C and 10°C. *Philipp. Agri.* **54**: 202–209.

FERNANDEZ, W. L., CATINDIG, J. M., MASONG-SONG,D.C. and SARAZA, F.J. 1972. Bi-hourly bacterial plate count of grated fresh coconut stored for 24 hrs at 55°C, 30°C and 10°C. *Philipp. Agric.* **56**: 1–8.

FOWLER, G. J. and MARSDEN, P. 1924. *J. Indian Inst. Sci.* **7**: 39.

GHAI, S. K. and GEORGE V. THOMAS. 1987. Occurrence of *Azospirillum* spp. in coconut based farming. *Plant and soil*(Communicated)

GUPTA, R.C., JAIN, V. K. and SHANKER, G. 1980. Studies on production of vinegar from'neera'. *Indian J. Microbiol.* **20**: 119–121.

HAGENMAIER, R. 1977. *Coconut Aqueous Processing.* San Carlos publications, University of San Carlos, Cabu city, Philippines.

HAGENMAIER, R. 1983. Dried coconut milk and other new foods from wet process. *Coconut Today.* **1** (1 & 2): 17–23.

HIPOLITO, D. G· 1963. Strawberry flavoured protein from coconut water–food for millions. *Sci. Bull.* **8**: 60–68.

HIPOLITO, D. G., DOMINGO, J. G. and SARTE, N. M. 1965. High protein yeast, *Rhodotorula pilimanae* Hedrick and Burke from Philippine strawberry fruit. *Philipp. J. Sci.* **94**: 179–187.

HOOVER, R. and GUNETILEKE, K. G. 1971. Spoilage of coconut oil. I. purification of a lipase from a fungus found growing on coconut kernel. *Ceylon Assn. Adv. Sci. Proc.*, 27th Ann. Sess. p. 8.

HOOVER,R., LAURENTIUS, S.F. and GUNETILEKE, K. G. 1973. Spoilage of coconut oil. Purification and properties of a fungal lipase that attacks coconut oil. *J. Am. Chem. Soc.* **50**: 64–67.

ISWARAN, V. 1972. Growth and survival of *Rhizobium trifolii* in coir dust and soybean meal compost. *Madras Agric. J.* **59**: 52–53.

ISWARAN, V., JAYASANKER, N. P., JAUHRI, K. S· and SUBBA RAO, N. S. 1970. Nodulation of soybean as influenced by the liquid endosperm of coconut. *Proc. Indian Acad. Sci.* (Sec. B) **72**: 236-240.

JAYASANKER, N. P. 1966. Studies on aerobic microflora associated with retting of coconut husk. Ph.D. Thesis. Indian Institute of Science, Bangalore.

JAYASANKER, N. P. and BHAT, J. V. 1966a. Mode of attack on phenol by a *Micrococcus* sp. isolated from coir rets. *Can. J. Microbiol.* **12**: 1031-1039.

JAYASANKER, N. P. and BHAT, J. V. 1966b. Isolation and properties of catechol cleaving yeasts from coir rets. *Antonie Van Leeuwenhock J. Microbiol. Serol.* **32**: 125-134.

JOSON, L. M. 1985. Personal communication. National Institute of Science and Technology, (NIST), Manila, Philippines.

LAPUZ, M. M., PALO, M. A. and GALLARDO, E.G. 1962. A study on the industrial application of a polysaccharide produced in sugared coconut water by *Streptococcus crystalloides*. NIST Ann. Rep. 1961-1962. P. 7.

LAPUZ, M. M., GALLARDO, E. G. and PALO, M.A. 1967. The nata organism-cultural requirements, characteristics and identity. *Philipp. J. Sci.* **96**: 91-109.

LILY, V. G. 1975. Note on the development of vesicular-arbuscular mycorrhiza *Endogone fasciculata* in coconut root. *Curr. Sci.* **44** (b): 201.

MEEDENIYA, K. 1969. Investigation into the contamination of Ceylon desiccated coconut. *J. Hyg. Camb.* **67**: 718-729.

MINH, H. V. 1967. Yield of fodder yeast in the fermentation of molasses and coconut water. BS Thesis,UPCA College, Laguna, Philippines.

MUKHERJI,K.G. 1967. *Aspergillus niger* growing in coconut oil. *J. Gen. Appl. Microbiol.* **13**: 407-408

NAIR, S. K. and SUBBA RAO, N. S. 1977a. Microbiology of the root region of coconut and cacao under mixed cropping. *Plant and Soil.* **46**: 511-519.

NAIR, S. K. and SUBBA RAO, N. S. 1977b. Distribution and activity of phosphate solubilizing micro-organisms in the rhizosphere of coconut and cacao under mixed. cropping. *J. Plant. Crops.* **5**(2): 67-70.

NAIR, S. K. and SUBBA RAO, N. S. 1978. Occurrence of fungi in the coconut rhizosphere and rhizoplane and interaction among them. *J. Plant. Crops.* **2**(2): 93-95.

NAIR, M. K. C. and SREEMULANATHAN, H. 1970. Spoilage of copra by *Penicillium frequentans* Westling. *Agric. Res. J. Kerala.* **8**: 125.

NAGARAJAN, R., SREEKUMAR, D. A. and PRABHU, G. N. 1985. Effect of prior crushing on retting of coconut husk. *Proc. Workshop, Coir Research*, Coir Board, Cochin, 1985. pp. 69-75.

NARASIMHAN, M. J. (Jr). 1968. Presence of aflatoxin in copra. *Hindustan Antibiotics Bull.* **11** (2): 104-105.

NARONHA, C. D. 1972. In *Symposium on Alcholic Beverage Industries in India*, CFTRI, Mysore.

NATHAN, H. S., KRISHNANKUTTY, S., JAINAMMA. K. M., KRISHNASWAMY, C., MATHEW, A. G. and SUBRAMANYAN, V. 1980. Control of spoilage during and drying of coconut. *Indian Coconut J.* **11** (6): 4-6.

NATHANAEL, W. R. N. 1958. *Ceylon Res. Inst. Bull.* No. 17.

NATHANAEL, W. R. N. 1960. Chemical and technological investigations on coconut products. *Ceylon Coconut. Q.* **11**: 31-54.

NATHANAEL, W. R. N. 1965. Some aspects of copra deterioration. *Ceylon Coconut. Q.* **16**: 111-120.

PACA, L. G. and CANCIO, J. V. 1973. Utilization of coconut water for the production of food yeast and other mycelial products, NIST Ann. Rep. 1971-72, p. 102.

PAUL, P. G. 1969. Studies on the microbial deterioration of copra and the utilization of certain vegetable oil by fungi. M.Sc. Thesis, University of Kerala, Trivandrum.

PALO, M. A., SANTOS, P. S., PALO, N. D. and FELIX, L. S. 1973. The rennet forming molds in cheese production from defatted coconut milk or gata. NIST Ann. Rep. 1972–73. p. 17.

POTTY, V. P. 1977. Rhizosphere microflora of coconut palm (*Cocos nucifera* L.) in relation to root (wilt) disease. Ph.D Thesis, University of Kerala, Trivandrum.

POTTY, V. P. and JAYASANKER, N. P. 1983. Influence of crop mixing of hybrid napier on the root zone microflora of coconut palm. In *Coconut Research and Development*. Ed. N. M. Nayar, Wiley Eastern Ltd., New Delhi pp. 300–303.

POTTY, V. P., GEORGE, M. and JAYASANKER, N. P. 1977. Effect of crop mixing on coconut rhizcsphere. *Indian Coconut J.* **8** (7): 1–2.

POTTY, V. P., JOSEPH, K. V., MENON, K. S. and JAYASANKER, N. P. 1978. Processing and fermentation of coconut toddy. *J.Food Sci. and Technol.* **15**: 32–34.

POTTY, V. P., JOSEPH, K. V., MATHEW GEORGE and JAYASANKER, N. P. 1979. Microbial degradation of coconut waste materials. In *Proc. PLACROSYM-II*. pp. 93–96.

RADHA, K. and RAWTHER, T. S. S. 1954. Observations on the seasonal variations in the rhizosphere microflora of coconut with special reference to fungi. *Proc. First Conf. Cocon. Res. Workers*, Trivandrum, India. pp. 298-305.

RADHA, K. and MENON, K. P. V. 1954. Studies on the root (wilt) disease of coconut palm. A comparative study of the rhizosphere microflora of coconut from diseased and healthy trees. *Indian Coconut J.*7: 99–106.

RAMESH, C. R. and ROHINI IYER. 1979. Mycorrhiza of plantation crops. In *Proc. PLACROSYM-II*. pp. 99–104.

RAMOS, M. A. 1963. Mushroom from coconut. *Coco News*. **8** (3): 3.

RAO, K. S. N., SREEKANTIAH, K. R. and RAO RAMACHANDRA. 1971. Post harvest infection of coconut kernel by *Botryodiplodia theobromae* and a note on the hydrolytic enzymes secreted by the fungus. *Indian Phytopathol.* **24** (4): 815–819.

RAWTHER, T. S. S. and RADHA, K. 1963. Studies on the microflora of coconut soils. Observations on the microbiological activity of coconut soils with reference to the root (wilt) disease. *Indian Coconut. J.* **16**: 115–126.

REVESTIR, E. M. 1968. Micro-organisms present in freshly gathered tuba. BS Thesis, UPCA College, Laguna, Philippines.

ROHINI IYER 1981. Rhizosphere microflora in crop mixed coconut soil. *Philipp. J. Coconut studies*. **6** (2): 42–45.

SAHASRANAMAN, K. N., PILLAI, N. G., JAYASANKER, N. P., POTTY, V. P., THOMAS VARKEY, KAMALAKSHY AMMA, P. G. and RADHA, K. 1983. Mixed farming in coconut garden-economics and its effect on root (wilt) disease. In *Coconut Research and Development*. Ed N. M. Nayar, Wiley Eastern Ltd., New Delhi. pp. 160–165.

SAMARAJEEWA, U. 1972. Aflatoxins in coconut products. *Ceylon Coconut Q.* **23**: 108–113.

SAMARAJEEWA, U. and ARSECULERATNE, S. N. 1974. Methods for assay of aflatoxins in coconut products. *J. Food Sci. Technol.* **12** (1): 27–31.

SAMARAJEEWA, U., PETHIYAGODA, J. D., ATPUTHARAJAH, and WIJERATNE, N. C. P. 1976. Fermentation characteristics of some yeast types isolated from coconut toddy. In *Coconut Research and Development*. Ed. N. M. Nayar. Wiley Eastern Ltd., New Delhi.

SCHAFFNER, C. P., MOSBACH, K., BIBIT, V. C. and WATSON, C. H. 1967. Coconut and salmonella infection. *Appl. Microbiol.* **15**: 471–475.

SEGRETAIN, G. 1952. Action du coco et de l 'acide naphtalene acetique sur la multiplication d' un virus en cultures da tissues c. r. hebd. *Seanc. Acad. Sci. Paris*. **235**: 1342–1344.

SERRANO, L. B., MINA, P. T., GOMEZ, L. P., SISON (Jr), B. C. and TEN MATAY, A. L. 1967. Studies on the growth factors present in coconut water. *Philipp. J. Sci.* **96**: 229–238.

SIERRA, Z. N. 1971. Proximate chemical composition of copra as influenced by fungal infection. MS Thesis, UPLB College, Laguna, Philippines.