

PROPAGATION BY TISSUE CULTURE - WHAT IT'S ALL ABOUT

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Tissue culture is a technique by which small pieces of actively growing parts removed from a donor plant are cultured aseptically in specially formulated nutrient media, under controlled environmental conditions. Depending on the requirements, growth and development of the cultured material is directed into different channels. One important channel is plant regeneration from the cultured material for mass propagation of plants.

What is the Basis for Plant Regeneration?

What makes plant tissues or organs removed from donor plants and incubated under appropriate culture conditions to produce plants? It is a problem in the differentiation of eukaryotic organisms. Answer is not known yet. This capacity to regenerate however, is one of the features which distinguishes plants from animals. In the case of the animal cell, differentiation is final and irreversible; that is, the zygote through a series of developmental stages gives rise to an adult animal. Similarly, plant cells differentiate; the zygote finally gives rise to an adult plant but the process is not final or irreversible. Like in the development of an animal from the zygote, the plant zygote also during its development differentiates into various specialised types to carry out specialised tasks in the plant. Phloem cells, xylem cells and parenchyma cells differentiated for photosynthesis and storage of food reserves are some of the specialised cells in the plant. Unlike the specialised animal cells, these plant cells are capable of dedifferentiating; that is, specialised plant cells could be made to divide and give rise to less specialised types. These dedifferentiated cells could then redifferentiate into other new paths of specialisation. Plant regeneration is one such specialised path. In other words, the specialised vegetative cells removed from the donor plant have the capacity to dedifferentiate backwards into the most embryonic level, that of the zygote and then through redifferentiation, to develop into new plants. This natural capability

to reproduce themselves by asexual means is exploited in propagating plants from excised tissues, organs and isolated cells and protoplasts. This type of plant regeneration is the most spectacular demonstration of totipotency in higher plant cells.

Regeneration of Plants in the Test Tube

Freshly cut, tender plant organs such as shoot apices, axillary buds, leaves, root segments, zygotic embryos and floral buds are used for this purpose. In the laboratory, all manipulations are carried out under aseptic or microbe-free conditions to prevent spoilage of cultures. Firstly, plant materials are disinfected using common disinfectants such as ethyl alcohol, chlorox, bleaching powder, mercuric chloride etc. The type and concentration of the disinfectant and the exposure time are determined through experimentation. Traces of disinfectants left in plant materials are then removed by rinsing in pre-sterilised water by heating to 120°C under a pressure of 15 psi. This material is then cut into small pieces; less than 1 cm in length (called explants) and placed in sterile dishes or vials containing the pre-sterilised culture medium (details given below). Small buds and embryos are cultured intact. Culture containers are carefully closed with sterilised cotton wool plugs or lids (depending on the container type) and incubated under controlled environmental conditions for further growth and development.

The culture medium is usually rich and consists of macro and micro minerals, vitamins, plant growth hormones and a source of carbohydrates, mainly sucrose. If necessary, this medium is solidified using a gelatine-like substance called agar.

Depending on the requirements, other substances such as coconut water, yeast extract etc. are used in the culture medium. pH of the medium is adjusted to between

5.0-6.0. Aliquots of the medium are then dispensed into suitable containers, and disinfected by heating at 120°C under pressure for 15 minutes.

Plant regeneration from the cultured material is a multi-step process. Each step requires different culture conditions and is determined by systematic manipulation of culture medium composition and environmental conditions. Transfer of resulting seedlings from the test tube to soil also involves tedious work and has to be done with utmost care.

Paths of Plant Regeneration

There exists a few paths of plant regeneration from cultured materials (please see figure 1 for details). It is determined by the plant species, type of explant and the culture conditions. The two pathways, direct morphogenesis and indirect morphogenesis are based on the totipotency of plant cells. In the former, the specialised cells of the explant dedifferentiate and then during the redifferentiation process either become embryogenic (produce embryos) or organogenic (produce shoots). The end result in both cases is a complete plant. In indirect morphogenesis, the specialised cells dedifferentiate and divide to form an undifferentiated mass of cells called the callus. These callus cells redifferentiate into embryos (embryogenesis) or shoots (organogenesis). They later develop into complete plants.

Propagation from axillary buds (figure 1) resembles conventional rooting of cuttings, but in the test tube, buds can be induced to proliferate rapidly to produce a large number of buds, within a relatively short period. These buds are then separated and rooted to produce plants.

It is clear from the above description that, during plant development through tissue culture there is no gamete formation or fusion. Hence it is not a sexual process. It is purely a vegetative or asexual process and as such the resulting plants usually resemble the explant-donor or the mother plant phenotypically and genetically. Therefore the technique has tremendous potential in rapid propagation (or cloning) of superior plants. If successfully developed, the technique will be very useful in cloning perennial trees that are difficult or impossible to propagate by conventional methods. So far, cloning by tissue culture had been most successful with plants responding to conventional vegetative propagation meth-

ods. These include some vegetable and fruit plants and many ornamental plants. Perennial trees such as forest and fruit trees and plantation crops are difficult to propagate by conventional methods. Explants from these trees are generally stubborn in culture also. Therefore, with a few exceptions, development of technologies for clonal propagation of selected tree species is still at the experimental stage.

When should Tissue Culture be Utilised for Mass Propagation?

It is important to see whether tissue culture is the answer to the problem at hand. The advantages and disadvantages of tissue culture propagation in relation to the crop(s) to be produced must be considered. In many cases, classical propagation methodologies may be the fastest and least expensive means of overcoming the problem.

For tissue culture to be used successfully on a commercial scale:

1. Multiplication rate of plants from the explant must be rapid.
2. Resulting plants should be uniform not only phenotypically but genetically also. Under certain culture conditions, explants are known to produce abnormal plants in considerable numbers.
3. Large populations of plants should be produced in a relatively smaller growing space and in a reduced time frame (for low cost).
4. Product (plant) should come from superior or elite plants.
5. Establishment of seedlings in the soil should be highly satisfactory.

Finally, tissue culture technology if properly employed, will have a tremendous impact on agriculture and forestry. But development of technologies still seems to be difficult, specially with important tree species. Break-throughs towards plant regeneration have been made but procedures still have a long way to go before they can collectively qualify as a routine tool.

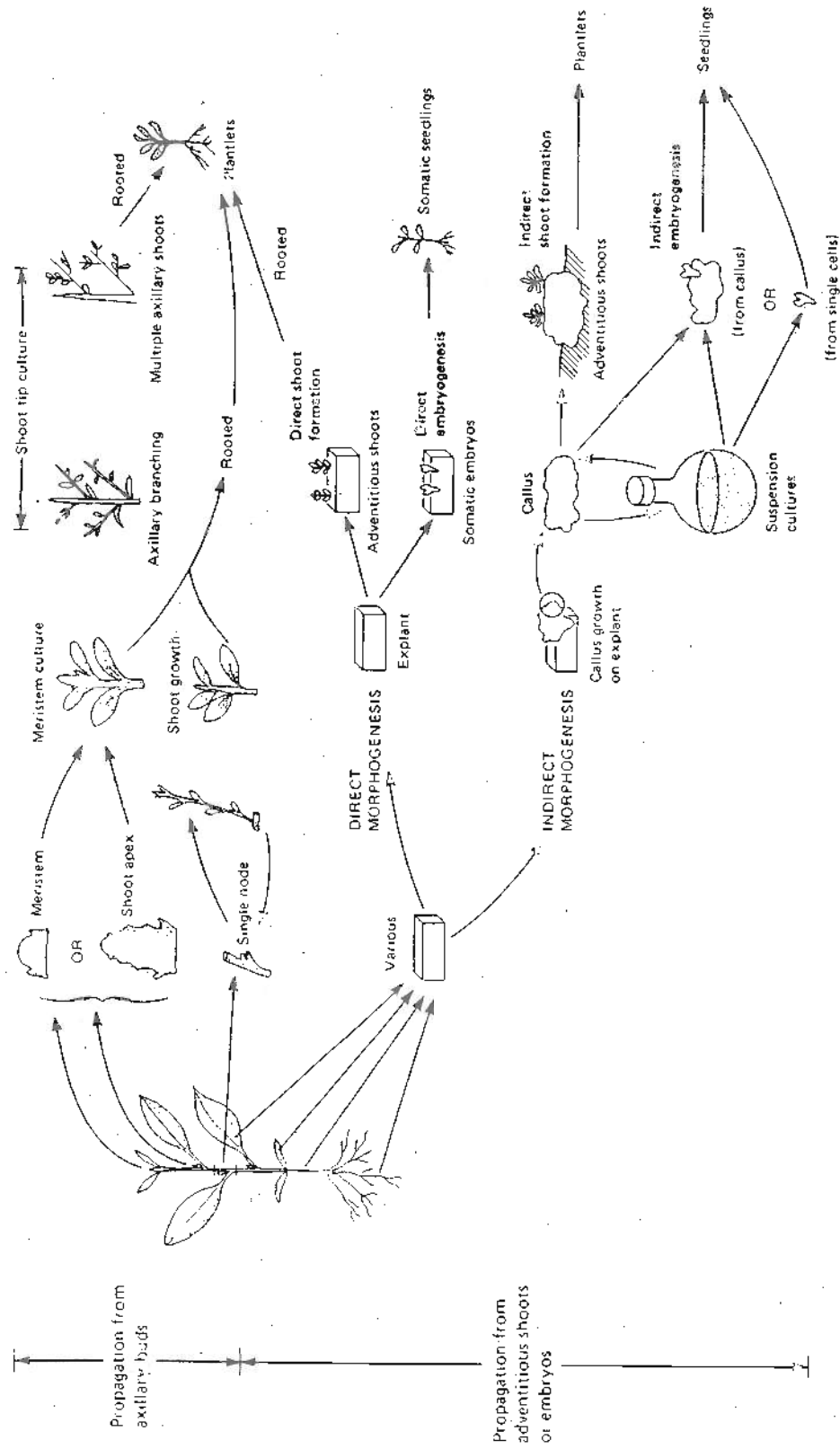


Figure 1 : The principal methods of micropropagation:
Shoots and/or plantlets do not always originate in culture by a single method

Source : Lydiane Kyte (1983)

KILLER MOULDS - A PERSPECTIVE

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Ever since his first appearance on earth, man has been engaged in a never ending struggle with the environment to gather or produce food and, more important, to preserve it from destruction. Next to air which is freely available, food and water invariably have been the two basic requisites of paramount importance for sustenance of mankind. From time immemorial, contamination of his food, water and sometimes air too by chemical pollutants was a serious threat to man. Even today one speaks of man-made pollution of human habitat unavoidably connected with industrial development. Yet, there is another form of naturally occurring chemical pollution of food, beverages and even air that is of biological origin, irrespective of whether a country is industrialised or developing. Man was baffled for centuries by such occurrences the origin of which was not known to him but they almost invariably manifested in the form of mysterious diseases in man himself and also in his livestock causing loss of life.

At a time when science and technology were not developed as it is today, man was compelled to believe that divine involvement was the reason for any mysterious disease outbreak he could not fathom. Micro-organisms or the metabolites they secrete into the environment are the causative agents of many a disease as we know today. But without appropriate technological developments, early man had no means of either discerning most micro-organisms or detecting the presence of their metabolites in particular, for instance in his food. He was aware of the presence of moulds because he could see them growing in his food commodities and he even used some 'mushrooms' as food.

Man's association with micro-organisms extends to antiquity and he was using some of them, for example yeasts (a class of fungi as we classify them today), for leavening of dough before bread making and for brewing of fruit juices or plantcell saps to produce alcoholic beverages like grape wine and cider. He also witnessed the growth

of a host of harmless looking moulds on his food commodities and the damage they are doing by destroying the food itself. When food was scarce he was even forced to eat mouldy grain, nuts or food preparations made out of them. The relationship between such seemingly harmless moulds and the serious diseases they caused in man and his farm animals was beyond human comprehension for centuries. Mysterious diseases in man and his farm animals were known to have existed since medieval times but the significance of moulds as their causative agents was not brought to light until after the second half of twentieth century. With the advancement of science and technology in the fields of microbiology, biochemistry and pathology in particular man attempted to investigate and understand various natural interactions he and his livestock exhibited with other forms of life around them.

An East Anglian turkey farm in Britain lost over 100,000 poultz nurtured in readiness for Christmas festivities as a consequence of mass death due to disease in 1959. This incident triggered off a series of investigations in several countries around the world that would change man's attitude towards moulds in food and feedstuff. The turkey disease in Britain was traced back to the pelleted ration fed to the birds and the incriminated peanut meal imported from Brazil to produce it proved that a mould *Aspergillus flavus* was responsible for the calamity. Further analysis of the peanut meal using Thin Layer Chromatography (TLC) revealed the presence of several new chemical compounds secreted by the mould. The first letters of the name *Aspergillus flavus* of the toxigenic mould involved were adopted in coining a name and these chemicals became known as "Aflatoxins". They exhibited fluorescence when exposed to long wavelength Ultra-Violet radiation and were designated Aflatoxins B and G because of the respective bluish and greenish-yellow colour they emitted when irradiated.

Subsequently, several other *Aspergillus* species and a number of common mould genera were found to pro-

duce similar chemical metabolites which were equally toxic to human beings and farm animals. Some were potential carcinogens. This wide assemblage of toxic chemicals was referred to as "Mycotoxins" purported to mean toxins produced by moulds, and the terms aflatoxin and mycotoxin became synonymous in early 1960. However, the term mycotoxin is a misnomer in its absolute sense because in analogy, phytotoxins and zootoxins mean compounds toxic to plants and animals respectively and one would expect mycotoxins to mean compounds toxic to moulds and not compounds secreted by moulds that are toxic to man and farm animals.

The illnesses caused in man and farm animals by consumption of food infested with toxigenic moulds are known as mycotoxicoses. Mycotoxicoses are of two kinds. Illnesses that occur in subjects due to direct ingestion of mouldy food containing toxins are termed 'primary mycotoxicoses'. It has been observed that farm animals feeding on mycotoxin contaminated feed secrete these toxins in animal products, for instance, cows milk and red meat, and they are detected even in processed dairy products like cheese. Aflatoxin B₁ and B₂ contaminated fodder when eaten by the cow, is transformed and secreted in milk as Aflatoxin M₁ and M₂ respectively in lesser concentrations than the original uptake dosage of B Aflatoxins. Illnesses caused in subjects, specially man, by consumption of animal products contaminated with mycotoxins derived in this manner are referred to as 'secondary mycotoxicoses'. The type of mycotoxicosis developed in a subject could be diverse and is dependant on the toxigenic moulds involved and also on the mycotoxins secreted.

There are only three human mycotoxicoses that are sufficiently substantiated by reasonable evidence to link the disease with the respective suspect mycotoxin. Ergotism, the proximity wasting disease of 9th and 10th centuries, then known as St. Anthony's Fire occurred due to consumption of cereals or bread made from flours of rye and other grains contaminated with resting fruit bodies called sclerotia or ergots of the toxigenic strains of *Claviceps purpurea*. The toxins produced by this fungus are known as ergot alkaloids. They bring about contraction of blood vessels of limbs reducing their blood supply, and if not corrected, the affected tissues die resulting in gangrene and falling-off of limbs. Accelerated pulse, increase of glandular secretions, smooth muscle contractions manifested in uncontrollable spasms and stagger-

ing are the other symptoms of ergotism. Ergot alkaloids also induce vomiting and further, they have contratile effects on uterine blood vessels and musculature of pregnant cows resulting in reduced blood supply to the foetus causing atrophy and subsequent abortion. This latter effect has now been put to good use in giving relief to humans in that some ergot alkaloids are used as labour inducing drugs to ease the labour pains of pregnant mothers at child birth.

The second human mycotoxicosis mostly reported from Russia as early as the 19th century is Alimentary Toxic Aleukia (ATA) or absence of white blood corpuscles (leucocytes) from blood and poisoning of alimentary tract. ATA was associated with the ingestion of overwintered grain, for example wheat, rye, barley, proso millet etc., infected with toxigenic *Fusarium* species *F. poae* and *F. sporotrichoides*. Several steroidal toxins and numerous trichothecene-type toxins have been isolated from infected materials and the current belief is that ATA is caused more likely by the latter. After ingestion of toxin contaminated food, a burning sensation in the mucous membranes of the mouth, throat, stomach occurs followed by inflammation of these regions, with vomiting, diarrhoea and abdominal pain. At the final stages, bleeding of nose, throat, gums and genital tract occurs together with the appearance of bleeding skin rashes. A decrease of white blood cell count and granulocytes occurs, the bone marrow gets exhausted, lowering body resistance to bacterial invasion and if not corrected immediately the patient dies quickly due to a multitude of complications.

The third and widely studied and documented mycotoxicosis is Aflatoxicosis due to Aflatoxins. Contamination of human food and foodstuff by Aflatoxin producing moulds is widespread and the intense carcinogenicity of Aflatoxin B₁, in particular, drew a lot of attention to this single mycotoxin group. It is strongly believed that Aflatoxin may be the causative agent of human liver cancer, as a highly significant positive correlation between the Aflatoxin levels in the diet and the incidence of liver cancer has been obtained from epidemiological studies done in Africa and South-East Asia over the last three decades. In fact, thousands of people all over the world are eating lethal levels of Aflatoxins with their daily diet consisting of naturally contaminated foodstuff, peanut, maize in particular and other grains.

No food or feed could be considered safe from mould infestation because they are prone to natural contamination at one stage or another from origin to consumption. Examination of suspect material would not always show the presence of toxigenic moulds. They may die off leaving behind their deadly secretions. In many cases, one or several toxigenic moulds would grow together or in succession producing the same or widely different mycotoxins into the material and sometimes they are known to produce mixed effects and at other times synergistic effects. Certain toxigenic moulds infect the commodity while in the field viz. maize, wheat and millet. Other commodities are contaminated after harvest or during storage, transportation or processing. If adequate protection is not given, contamination of food could also occur at consumer's home itself, irrespective of whether the food is in storage or (left-overs of) prepared food set aside for later consumption.

Many food commodities have been implicated with mycotoxins. Though not exhaustive they range from cereals (rice, maize, sorghum, wheat, barley, millet, rye, oats); oil seeds (peanut, cotton seed, copra); oil seed meals; crude vegetable oils (peanut oil, coconut oil, olive oil, sunflower seed oil); fruits (apples, figs); apple juice, wine and beer; green coffee beans, cocoa beans; root crops (manioc or cassava, sweet potato); yams and tubers (carrots, radish) onions etc. Milk and cheese at times are found to be contaminated with Aflatoxin M₁. Pork, poultry, beef, dried fish, smoked fish and fish sauce are other products that have been shown to contain mycotoxins.

Since the detection of mycotoxins in commodities, techniques were developed to prevent their incidence and severity of contamination by successful application of improved practices delimiting growth of toxigenic moulds. Man was not destined with control over the climate. Therefore, natural contamination of food commodities by moulds in the field is inevitable. But man has developed techniques to decontaminate and detoxify commodities that were subjected to field and post-harvest mould contamination. The principal aim behind these techniques is to destroy or inactivate the toxins, kill the fungal spores and mycelia - which would otherwise proliferate and secrete new toxins under favourable conditions - leaving behind no toxic or carcinogenic residues in the treated commodity while preserving the nutritive value and acceptability of the product as food.

Mycotoxin decontamination is achieved firstly by physical separation of contaminated material by careful inspection of individual items and manual picking-out of immature, broken or discoloured items and insect damaged items. This is a very efficient method widely applied to food grains, coffee berries, cocoa beans and peanut. Grading by size employing sifters in mechanical separators, and sorting-out of items discoloured by mould infestation using electronic colour sensitive devices are concurrently practised with hand-picking methods. Air classification and floatation techniques also have wide applications. Mould infested and insect damaged crops are virtually lighter than sound material and as such they would either be blown over during air classification or float on the surface of Sodium Chloride solution, the separating fluid generally used in the floatation technique. Ergot sclerotia and aflatoxin contaminated peanuts are efficiently separated by these techniques.

Maize for example can be decontaminated by wet-milling, where starch, oil and other products obtained would be virtually toxin free as 80-90% of aflatoxin originally present is concentrated in the gluten fraction. When oil is expressed from peanuts, over 85% of the aflatoxin present remains in the press cake. This oil can be further decontaminated by passing through a suitable filter and an adsorbant designed for the purpose. The gluten fraction of maize and the peanut press cake with very high aflatoxin levels could be either discarded or diverted to suitable use largely by diluting with toxin free material, in bringing down toxins in the final product to virtually harmless levels.

Inactivation or total degradation of mycotoxins can be achieved by exposure to bright sunlight. If exposed in small bottles or in shallow trays for about one hour during the brightest part of the day, where about 50,000 lux units would be delivered, all remnant aflatoxins in contaminated crude coconut oil, peanut oil etc., can be economically destroyed. This is a very relevant and appropriate decontamination method suitable for developing countries including Sri Lanka.

Mycotoxin contaminated material can also be detoxified chemically. A range of inorganic and organic chemical compounds employed in detoxification trials have not proved to be successful except strong alkaline solutions. Ammonia for instance is the most useful and effective

compound used today in aflatoxin decontamination. Several processes using ammonia for detoxification of aflatoxin have been developed and patented in Europe. In U.S.A. and France, for example, small-scale pilot plants have been built and are operational. Successful transferability of the ammonia process to developing countries remains yet to be studied. Useful detoxification can also be done using chlorine gas which does not need high temperature and high moisture conditions. Though 90% of aflatoxin detoxification occurs here, its usefulness as a practicable process is doubtful because it destroys the organoleptic quality of the detoxified material.

Chemical extraction techniques are available to remove mycotoxins, aflatoxin, in particular, completely from contaminated materials. Ethanol, for example, in an aqueous solvent extraction system has been shown to remove over 95% of the total aflatoxin in peanut meal and cotton seed meal. Acetone, Chloroform and petroleum hydrocarbons in aqueous extraction systems are equally effective. Though efficient, the extraction techniques are uneconomical, firstly because of high costs involved in designing special equipment to remove extraction solvents used, and secondly the resultant detoxified material loses nutritive value appreciably and would only be fit for animal feed at most.

Mycotoxin contaminated materials can also be detoxified by irradiation or heating. Aflatoxins are sensitive to Ultra-violet (U.V.) radiation. Factors like toxin concentration, duration of exposure and the solvent used govern the degradation of toxin in a contaminated product. However U.V. irradiation is not a practicable and worthwhile detoxification technique. Mycotoxins are heat-stable, but if roasted by dry heat at relatively high temperatures close to their melting points - viz. 250°C for aflatoxin-complete degradation occurs. Such an intense heat treatment is disadvantageous because it renders the commodity unacceptable as food due to loss of nutritive value and appearance. However, if heated in the presence of moisture, appreciable degradation of toxin levels occurs at much lower temperatures. Pressure cooking reduces as much as 70% of initial toxin levels. It is a very simple, effective and convenient method to minimise aflatoxin hazards specially in households. Perhaps microwave cooking should prove more promising and effective in this regard.

Pasteurisation, sterilisation, vacuum-packing, canning, cold storage - both refrigeration and freezing- and drying are some other useful techniques in prevention or delaying of mould growth. They give mixed results in that taste and appearance of foods suffer greatly in some cases while other methods are technically and economically not feasible.

Moulds are universally present in or on all food commodities be it grain, nut, fruit or vegetative parts, but toxin production is essentially restricted to a handful of them. At present the three mould genera *Aspergillus*, *Penicillium*, and *Fusarium* are universally considered most significant in toxin production. Among themselves some twenty five toxigenic species/cultivars exist which are known to produce over forty different mycotoxins.

Over a dozen forms of "Aflatoxins" are known today, of which five major forms, B₁, B₂, G₁, G₂ and M₁ are of wide interest. Aflatoxin B₁ and G₂ are detected in food in large amounts world-wide and B₁ is the mostly studied aflatoxin because of its potential carcinogenicity. Moulds of the *Aspergillus flavus* group are responsible for secretion of "Aflatoxins". Several species of *Fusarium* produce the mycotoxin called "Zearalenone". Mycotoxins known as "Ochratoxins A and B", "Penicillic Acid" and "Patulin" are produced by certain *Aspergillus* and *Penicillium* species while certain toxigenic *Aspergilli* also produce the toxin "Sterigmatocystin". The group of chemical compounds known as "Trichothecenes" constituting the largest collection of mycotoxins known amounting to about thirty seven is produced by toxigenic *Fusarium* and *Stachybotrys* species. However, only six trichothecenes have been detected in field infected crops or associated with human and animal mycotoxicoses. Then there are the "Ergot Alkaloids" numbering over twelve produced by *Claviceps purpurea* and *C. paspali*.

Mycotoxin contamination of a commodity could either be a field problem or a storage problem and often a combination of both. Mould growth is a prerequisite of mycotoxin production. However, ability to isolate toxigenic moulds from a commodity does not render it mycotoxin free, because they could remain on the commodity long after the death of the moulds.

Fusarium species and *Claviceps purpurea* of St. Anthony's fire fame are good examples of moulds associated with

mycotoxin production in the field, more abundantly with unusual weather conditions. *Fusarium* invades corn and other cereal grains heavily, prior to harvest. Except for occasional heavy infections of wheat and rye in the field, contamination by *Claviceps* rarely occurs today. *Aspergillus flavus* that held the "storage fungus" tag contaminates food grains, maize for instance, in the field long before harvest. This kind of mycotoxin problems are controllable, by identifying the causative agents and avoiding contaminated material to start with, and then resorting to thorough and appropriate agricultural practices; use of proper and suitable fungicides and insecticides; and above all by developing specific plant varieties resistant to mould attack. Going along these lines of technology, fairly resistant small grain varieties of wheat and rye are being used today in greatly reducing recurrence of Ergotism in Europe. Adopting advanced agricultural practices and making every effort to get the crops out of the field before the onset of European winter and proper preparation for storage, the frequency of Alimentary Toxic Aleukia calamities has been greatly reduced.

However, contamination of commodities due to "storage fungi" is the most significant singular problem prevalent now. In addition to the damage caused by *Fusaria*, *Aspergilli* and *Penicillia* principally, several other mould species *Stachybotrys alba*, *Trichothecium roseum*, *Alternaria alternata* etc., for instance, are commonly encountered toxin producers in food commodities in storage.

Though it is desirable to have a mycotoxin free food rather than a mould free one, it would be more practicable and economical to have a food free of moulds. Mould proliferation and subsequent mycotoxin production can be prevented by controlling invasion and growth of moulds in stored commodities. Mould growth and toxin production in a commodity are promoted by several factors. Moisture content of the commodity, relative humidity of the storage environment, mould spore density, leakage of water or condensation, storage temperature and the influence of rodents, insects and other vermin in causing damage and generation of moisture and biological heat contribute much to enhance mould growth. But moisture, temperature and time of exposure to mould contamination are the most critical environmental factors that influence mould growth and also what could be optimised in controlling invasion.

Mould growth can be effectively controlled by cold storage, but use of low temperatures for large scale storage programmes of agricultural crops is not practicable. Control of available moisture for mould growth is the most economical and practicable approach. Water Activity (a_w) is the entity that denotes available water content for microbial growth. Moulds are capable of growing at very low available water levels and their growth can be successfully arrested below a_w levels of 0.65; $a_w = 1$ meaning the material being saturated with water. Food grains are dried by various techniques, and appropriate drying equipment have been evolved to suit the different needs. Supplemented by relevant and applicable fumigation techniques, the dried commodities are successfully stored under safe moisture levels thereby preventing invasion by moulds. Although removal of excess moisture and storage at optimum temperatures is the general practice, the combination of acceptable minimum moisture levels and the applicable safe storage temperature are considerably different for different commodities, making it a case by case situation. Even under such controlled storage conditions localised moisture and temperature increments can occur due to extraneous factors specially due to insect, rodent and vermin infestations. As such, these should be separately and appropriately dealt with if and when encountered.

Identification and quantification of mycotoxins in contaminated materials are achieved by two basic types of tests namely Chemical Analysis and Biological Assay which supplement each other in complete evaluation of mycotoxins where the identity, concentration, toxicity effects, the dosage response etc., are elucidated. Generally, the analytical procedures for mycotoxin detection follow a common flow pattern involving six or seven steps. The incriminated material is thoroughly mixed to achieve homogeneity and representative sampling is done preferably according to a good sampling plan using an appropriate sampling tool. For solids a sample of 4-5 Kg would suffice, and it is further prepared by repeatedly grinding and sifting through appropriate sieves -No. 14 and No. 20- and then it is thoroughly blended and properly sub divided to give analytical samples (50 g) which are conveniently refrigerated if not tested immediately. Mycotoxins present in very low levels (ppm-ppb) are removed from the analytical samples by an extraction process using a suitable solvent. Many interfering compounds that accumulate are removed at a clean-up operation next. Any oil component present is removed by

a defatting process either at this stage or it could be done prior to the extraction step. The clean-up operation is either a simple liquid-liquid partitioning with a separatory funnel or it could involve column chromatography and divalent metal clean-up procedures. To separate toxins from any remaining interfering compounds, the extract from clean-up is subjected to Thin Layer Chromatography (TLC). Gas-Liquid Chromatography (GLC), Liquid-Liquid Chromatography (LC) and mini column chromatography also can be adopted for this purpose wherever applicable. A detection and quantification step is followed next, to detect and measure the response of the toxins by their fluorescence on TLC plates or in solution; or by U.V. absorption in solution or by a GLC flame detector. At this stage the toxins get separated into their various fractions which need confirmation by a TLC separation in comparison against standard mycotoxin solutions. Mass spectroscopy may be also employed in the confirmation of mycotoxins.

Biological tests are employed for confirmation of mycotoxins and also in the event of chemical analytical difficulties. The standard method employs concentrated extracts of suspect material on day-old ducklings and damage caused to the liver, for instance by aflatoxins, is estimated after 7 days or on death. In other biological confirmatory tests, toxicological effects of the suspect

material on marine borer eggs, zebra fish larvae, brine shrimp eggs or fertile hens eggs are assessed. Rabbits, monkeys, mice, chicken and a host of animal models are also used in biological assays.

Mycotoxin analysis is obviously a very expensive and elaborate procedure, which introduces an exceedingly high risk for the analyst too. Next to operators who handle mycotoxin contaminated produce in trade, mycotoxin analysts are prone to gradual mycotoxin poisoning due to inhalation of toxins present in the aerosols produced during analytical procedures.

For the last three decades more than 50 countries worldwide, including Sri Lanka, have been carrying out extensive or limited studies on mycotoxins in food. It is a common yet a universal problem, imparting heavy financial and material losses to primary producers, middlemen making trade transactions, consumers -human or animal- and the national exchequers of every country. Due to international trade such losses overflow into global levels impairing international food production programmes; commodity and food security arrangements. Though man is powerless to rid his environment of toxigenic moulds, his farsighted objective is to create a mycotoxin-free food production programme universally.

NOVEL STRATEGIES OF INSECT CONTROL : PHEROMONES

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Introduction

Insecticides are designed to combat the attack of various insect pests that cause harm to man. Properties of an ideal insecticide are that it should preferentially attack the target pest species without harming other living organisms or the environment. The insecticides used by us are, however, far from the ideal. Early insecticides, such as Paris Green (1A) and lead arsenate (1B) were highly toxic to insects as well as for man. On the contrary, synthetic organic compounds, eg. organochlorines (2A) and organophosphates (2B), which came to light in the 19th century had low mammalian toxicity and a wide spectrum of insecticidal activity in addition to their cheap cost of manufacture. Thus, these insecticides became popular and were extensively used. They were the saviours of man against killer diseases like Malaria in the Far East during the second world war. Since coming to recognition in the 19th century, the services they have provided to man in the field of agriculture are remarkable.¹ However, the extensive use of the above insecticides over a long period has left us with enormous problems which have been brought to light now. One major problem is the non-degradable nature of these insecticides. They leave toxic residues such as DDD (2A, X=H, the breakdown product of DDT) in the environment. The levels of contamination by this substance have risen to alarming proportions. Due to long exposure to insecticides and their residues, the insects themselves have developed immunity, demanding more toxic insecticides for their control. Thus, synthetic insecticides have become an unwanted friend of man today though their dominant position is not challenged due to lack of alternatives. This situation has nevertheless led to a radical revision of our concepts of insect control during the past few years.

Novel Approaches

The new term "environmentally safe insecticides" which

is in vogue today describes the new trend. The first concept is that of pest management which advocates the suppression of insect populations below the levels of economic injury rather than their destruction with conventional insecticides. Consequently, two possibilities have emerged. A vast amount of insect controlling agents are being sought from plant resources.² A well known example, Pyrethrin (3), obtained from *Chrysanthemum cinerariaefolium* still is one of the highly used plant derived insect controlling agents. Insect anti-feedents, repellents of plant origin and microbial insecticides such as *Bacillus thuringiensis* are some of the possibilities under consideration.

Second approach is the use of insects' physiology and behaviour for their own destruction. Identified in the recent years are the insect hormones (4A), hormone mimics (4B), chemosterilents and insect pheromones (5). These novel compounds generally have the high selectivity against target species and minimum toxicity to other living organisms and the environment.

Insect Pheromones

Insects rely on their keen sense of smell to communicate via a sophisticated chemical language, using as words, specific volatile compounds; a phenomenon recognised in early 1960's. Although other modes of communication such as visual, tactile or auditory signals are important for some insect species, olfaction is often the dominant method. The word insect pheromone³ describes a chemical/s emitted by one member of the species that triggers a response only from individuals of the same species. The most well known are the sex attractant pheromones. Often the female of the species emits a specific chemical/s to announce her availability to her male counterpart. An aggregation pheromone on the other hand may guide hoards of both sexes of a species to a single site

* In the case of sex pheromones, deviations from above have been observed.

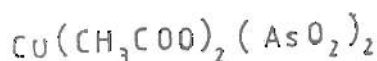
part. An aggregation pheromone on the other hand may guide hordes of both sexes of a species to a single site mass attack on a host plant. Trail pheromones deposited by a foraging ant create a path for other members of that colony to follow towards a food source : an observation we all are aware of. An alarm pheromone is emitted by an insect when it experiences a danger, to alert other members of the colony against possible attack. Nearly 600 pheromones belonging to different species have been identified and among them about 80% fall into the category of sex pheromones.

The fact that specific compounds attract given insects created a practical solution - the basis of insect control by insect pheromones. When an identical chemical message is created for a pest species with synthetic chemicals, members of the same species can be lured into a trap where they could be killed. Thus, the presence of insects in such a pheromone baited trap (Figure I) indicates the presence of that species in that area and the number of insects trapped can be related to population levels present. This is an important information to the

farmer. Guided by this information, the spraying of insecticides can be timed at higher levels of pest populations only, for better results, rather than spraying continuously throughout the pest season. Both the cost of insect control and the environmental damage are minimised as a result. Most of the successful applications of this method are from Lepidopteran sex pheromones such as those of Codling moth, Gypsy moth, Boll weevil, Pink boll worm etc.

Mass trapping is another technique which uses the above basis but a large number of traps are deployed to catch insects in order to bring down pest populations below the levels of economic injury. An alternative approach involves the use of pheromones to disrupt communications between males and females by maintaining fairly high concentrations of pheromones in the atmosphere by spraying methods. Due to the large number of artificial chemical messages, the male is unable to locate the female. Thereby mating is prevented. Thus a reduction of mating in that generation results in low levels of pests in the next generation.

EARLY INSECTICIDES

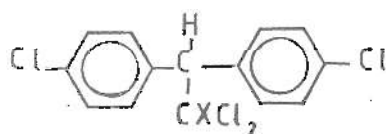


PARIS GREEN (1A)
(1864)

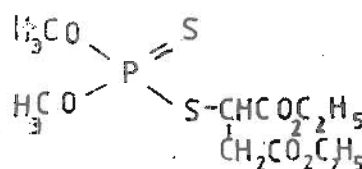


LEAD ARSANATE (1B)
(1892)

SYNTHETIC ORGANIC CHEMICALS

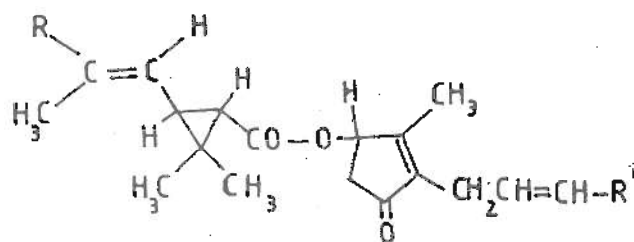


ORGANO CHLORINES (2A)
X = Cl, DDT (1864)
= H, DDD



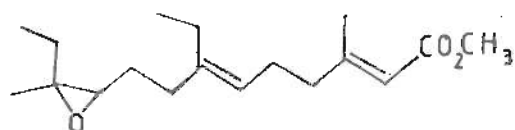
ORGANO PHOSPHATES (2B)
Malathion (1950)

NATURAL PEST CONTROLLING AGENTS



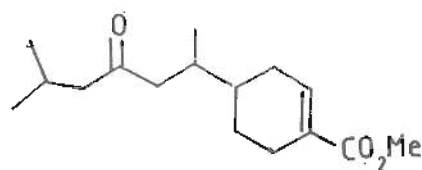
COMPOUND		R ¹	R	
Pyrethrin	1	-CH=CH ₂	-CH ₃	} Pyrethrin (3) (1850)
"	11	-CH=CH ₂	-CO ₂ CH ₃	
Cinerin	1	-CH ₃	-CH ₃	
"	11	-CH ₃	-CO ₂ CH ₃	

INSECT JUVENILE HORMONES



Cecropia JH (4A)

JUVENILE HORMONE MIMICS

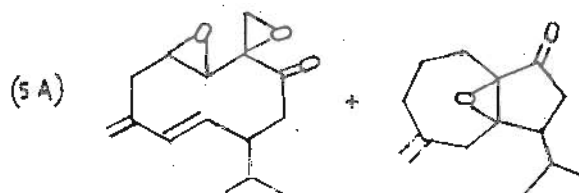


Tuvabione (4B)

INSECT PHEROMONES (5)

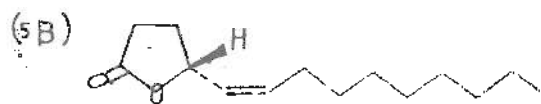
Sex Attractant Pheromones

American Cockroach,
Periplaneta americana



Periplanone - B + A

Japanese beetle,
Papolia japonica

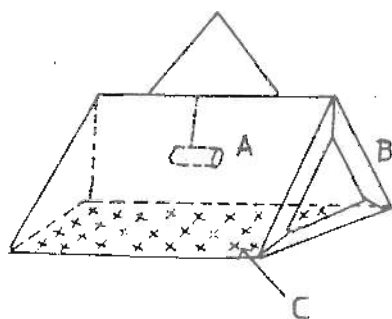
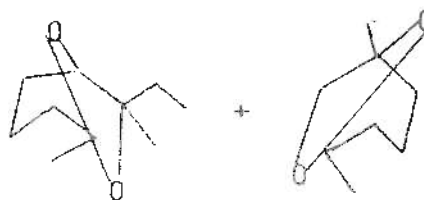


Silk worm moth,
Bombyx mori

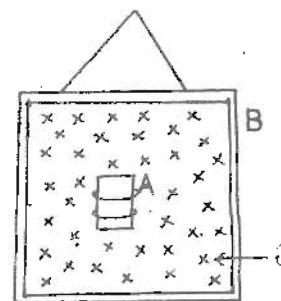


Aggregation Pheromones

Western pine beetle,
Dendroctonus brevicomis



A delta trap

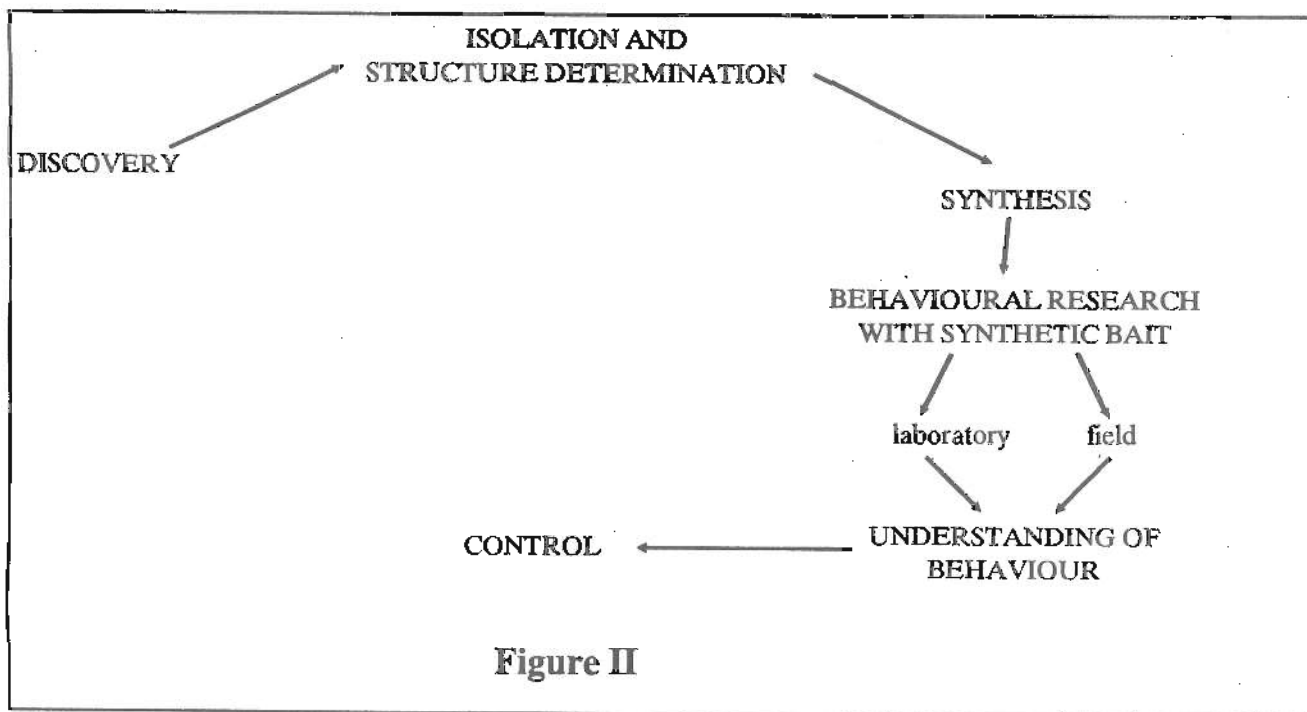


A vertical board trap

A = Pheromone Dispenser B = Plastic body
C = Sticky Surface

PHEROMONE BAITED TRAPS

Figure I



The successful use of pheromones depends on several steps taken before its application. Figure II describes these steps. It is clear that the success depends largely on the correct formulation of the synthetic equivalent of the true pheromone. Although pheromones emitted by insects are sometimes as low as nanogram quantities, owing to advanced methods of isolation and structure determination techniques, correct identification of the natural pheromone has been made possible. Gas Liquid Chromatography, Electro antennography, FT Nuclear Magnetic Resonance spectroscopy and FT Infra red spectroscopy are some of the useful techniques that have been developed. In the case of multi component mixtures the correct proportion of each component is evaluated by sophisticated analytical techniques such as capillary Gas Liquid Chromatography. Subsequently, the synthetic equivalent of the natural pheromone is produced by various synthetic procedures. Reproduction of the correct isomer in high purity is a key factor in success. For example, the Silk worm moth, *Bombyx mori* uses only (10E, 12Z) - hexadecadienol (5C) as its sex pheromone out of the four possible isomers while the male the Japanese beetle, *Popillia japonica* responds to its synthetic pheromone only if traps are baited with the furanone (5B) in 99.95% optical purity. Once the synthetic equivalent is available the bait is prepared by absorbing a predetermined dose onto dispensers such as

rubber septa/polythene vials etc. which release the pheromone for over a period as long as 3-4 weeks. This bait is incorporated in a suitable trap where sticky gum/water/insecticide would act as the killer of the trapped insects. The correct dose of the pheromone, trap design, the position in which it is hung and the trap height are important parameters in a successful application.

In conclusion, pheromones are not panacea and much research remains to be done in the area of field use of pheromones. Although pheromones have brought many benefits to farmers in the developed countries, as it is often the case, the impact they have made on the poorer countries is still rather limited.

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APPROPRIATE BUILDING MATERIAL TECHNOLOGIES

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Housing is a high-priority area for all countries in the world. Population growth, urbanisation and the growth of insanitary dwellings all emphasise the urgency of the problem.

The building materials industry plays a key role in solving economic and social objectives of housing since nearly 50 per cent of the investment in the construction sector is on building materials and components. In order that the housing problems are solved in a practical way, it is necessary to adopt local materials and construction technologies that will minimise the use of scarce national resources such as capital and energy. The local construction industry must recognise that the problems cannot be solved by a quick recipe but instead requires inter-disciplinary interaction among engineers, researchers, architects, planners and builders. A multifaceted approach involving (1) indigenous building materials, technical knowledge and skills (2) promotion of labour intensive technologies and (3) the development of national policies and finance mechanisms is required. Finally, the spectrum of human settlement problems must be addressed ranging from research and development on building materials, development of a viable building industry base, entrepreneurship and social and functional aspects of low-cost housing.

Choice of Building Materials

Cement with its inherent advantages such as better binding properties, early strength development and availability in ready to use condition became the obvious choice during the last few decades. This has led to the decline of the use of traditional materials such as earth, limestone and rubble. The establishment of the steel industry based on imported raw materials too had been contributory to this development.

However, in recent times production of such materials has not kept pace with the increased demand of the construction industry. This has led to the importation of raw materials, products and components resulting in a considerable drain of valuable foreign exchange. Also, the indiscriminate use of new and imported materials with scant attention to their quality, performance and application probably made their optimisation less important. In Sri Lanka, Portland cement is predominantly used in low-strength applications, for foundations, plasters and mortars. This wrong application of Portland cement escalates the cost of construction. The degree to which Portland cement is wrongly applied in construction has reached alarming proportions, and it is estimated that only 20 per cent of the worldwide use of cement requires the strength of Portland cement (Journal of the Network, 1989). Furthermore, some of the imported building materials such as asbestos and corrugated iron roofing sheets are unaffordable to the vast majority of the rural population.

The use of timber adds another dimension to the problem. Our forest resources are dwindling at an alarming rate. But first class timber species are still the prime commodities in construction. The selection and use of secondary and less durable timber species for building are issues of utmost importance. The question that now arises is how appropriate is the choice of building materials.

The Search for a Solution

In order to overcome the problems created by the over-dependence on cement and other imported building materials, the production and application strategy of our building materials need to be redesigned. A number of alternatives exist, some of which have been adopted in

other developing countries where socio-economic conditions are not too dissimilar to Sri Lanka. In summary, these may be grouped as follows:

- (1) Rationalisation on the production and use of cement,
- (2) Production of blended cements with OPC and other cementitious materials produced from clays and agricultural and industrial waste.
- (3) Adoption of other appropriate building materials and construction technologies such as
 - use of bricks and blocks
 - fibre reinforced concrete
- (4) Development of lime-based binders and products

Traditional Materials

The National Building Research Organisation (NBRO) conducted an islandwide survey on rural housing in 1984. This survey revealed that earth (61.8%), burnt clay bricks (16.2%), rubble (8.1%), cement products (4.8%), cabook (4.1%), timber and other materials (4.6%) are the materials used in superstructure walls of rural houses. Earth construction was mainly in the form of wattle and daub (50.4%) with rammed earth (8.4%) and adobe (4.6%) confined to certain areas of the country.

As regards roofing material, cadjan is the most popular with 42.6% of the housing units using it as the prime material. Straw and palmyrah thatching have been used in 4.1% and 1.6% houses respectively. Clay tiles constitute 33.8% of the houses while 10.8% houses are roofed with corrugated sheets (Rural Housing Survey, 1984).

The majority of urban houses have brick walls (82%) tiled roof cover (47.7%) and cement floors (78%) (Housing in Sri Lanka, 1976).

Appropriate Materials-Improvements through R and D

Considerable research and development work has been done in a number of institutions in Sri Lanka (NBRO,

NERDC, IDB, NHDA and some national universities) and abroad to improve the quality of building materials traditionally used in housing construction. Some of the materials developed which are of immediate relevance to Sri Lanka in view of their local availability as well as the relative simplicity and convenience of their processing and application are outlined below.

(1) Lime-Based Cementing Materials

Portland cement could be partially replaced by lime-pozzolana mixtures. Technology for producing these materials is relatively simple, and can be used on a very small scale. The pozzolanas for lime-based cements may be either natural or artificial. The first group includes among others siliceous and opaline shales. It is probable that such materials exist in Sri Lanka. Among artificial pozzolanas the most common is calcined clay. Finally, the ash from burnt rice husks, dried banana leaves and sugar cane stalks have also shown good pozzolanic activity.

The technology used to produce artificial pozzolanas in its simplest form involves activating clay by firing to the appropriate temperature followed by mixing and grinding with lime. The method of manufacture augurs well with the rural development programmes of Sri Lanka.

(2) Soil Stabilisation

Hand operated blockmaking machines which can be fabricated at a cost of about Rs. 7500/= have been developed for the production of cement or lime-stabilised soil blocks (NBRO, NHDA). Cured blocks give a compressive strength of about 2.8 Nmm^{-2} , which is adequate for the construction of most single storey houses. However, greater attention is required in the evaluation of soils for blockmaking.

(3) Blended Cement

Blended cement is a mixture of Portland cement clinker together with active or inactive material ground finely to produce the required cement. This type of cement is being produced in many countries all over the world. Different materials being added are sand, rice husk ash, slag and a variety of industrial wastes. Research is underway at the NBRO.

(4) Secondary Species of Timber

Research on extending the life of non-durable timber species through proper seasoning and chemical treatment have been undertaken at the NBRO. A solar seasoning kiln has been developed in which timber has been successfully seasoned eliminating the use of fuel for steam seasoning.

(5) Coir Fibre Roofing Sheets

The technology of production of coir fibre cement bonded roofing sheets has been perfected in many local institutions (NBRO, IDB, Moratuwa University). The cost effectiveness of the material depends on the condi-

tions of manufacture. The sheets can be used for semi-permanent roofs in the same way as asbestos-cement sheets. The production of sheets on a self-help basis on the site probably makes it a viable product.

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BEE KEEPING AND ITS MANAGEMENT OF COCONUT, RUBBER, CASHEW, DITRUS, GINGELLY, FRUIT, TIMBER AND OTHER PLANTATIONS

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The honey bee (Sinhala: Mee-massa; Tamil: Thenkulavi) has benefited the people of Sri Lanka from pre-Vijayan times; whether one considers its contribution to the nourishment and health of the Veddah peoples already dwelling in the island or to the invaders from the Indian peninsula. While other kinds of related bees in Sri Lanka produce honey (the "danduwel-massa" about 1/2 lb. of honey, and the "bambara" sometimes 10 to 20 gallons per colony), attention is paid mainly to the honey bee owing to the quantity and quality of honey it produces *plus* the convenience in handling.

In early times, a colony of honey bees in the jungle was raided and the combs of honey gathered were chewed along with the grubs, royal jelly and other material they may have contained. This would have supplemented the honey with protein and other nutrients but was a wasteful practice in that the colony was usually destroyed and the finding of the next source of honey was mostly a matter of good luck. A later stage is the use of a clay pot into which a swarm of bees is transferred or to which they sometimes migrate on their own accord. The pot is often placed near the home in order to afford ready accessibility and some measure of protection against bears and against other human honey-gatherers. Combs from these pots or from wild colonies may be squeezed in order to collect the honey in bottles.

The presently widely used wooden hive or "bee-box" has moveable frames on which the bees build their combs of wax. The general design of this wooden beehive with moveable frames resembles that of hives used in Europe and North America but considerable modifications have been made to adapt it to the size and life habit of the honey bee found in Sri Lanka.

The benefit of the provision of moveable frames is that it permits easy handling and management of the colony as

well as efficient extraction of honey from a comb by centrifugation and the return of the comb to the hive for refilling.

Some Benefits of Bee Keeping

Bee keeping is an activity which is particularly suitable for Sri Lanka. While its products can provide food and (or) additional income to those who practise it, it is an environmentally non-polluting activity for which the raw material requirements and persons with at least the basic experience in handling bees are available in most regions of the country.

In other countries, the important role of bee keeping in pollination of fruit orchards and other economic crops is recognised. Indeed, bee keepers may supplement their income by renting out their bee colonies to farmers for the purpose of pollination during the flowering season. The undertaking may often be covered by a legal agreement incorporating safeguards for both parties concerned. In Sri Lanka, in view of the benefits of increased pollination for the agricultural production drive, the State should consider ensuring a reliable and preferably, subsidised supply of bee hives.

Bees' honey, royal jelly, pollen and the venom of bee stings are widely used, locally and abroad, for therapeutic or tonic purposes.

Among the other benefits which accrue from bee keeping and associated activities could be mentioned the income which could be earned by entrepreneurs who are able to set up a reliable supply of stocks of bees, bee hives and other accessories. The need for such a service in Sri Lanka today is evident from the numerous inquiries for these materials received by the Sri Lanka Bee Farmers' Association (SLBFA).

*This article is reproduced from the *Journal of the National Institute of Plantation Management* Vol. 2 No. 1, although it first appeared in 1982, the information contained in the article is still of significance to farming in Sri Lanka.

Some Basic Material Requirements for Bee Keeping (other than suitable plants)

1. *A Stock of Bees*

This may be obtained by capturing a swarm or by preparation from an existing colony by an experienced bee keeper. Purchase of a stock of bees can cost anything from Rs.15/- to Rs.100/- or more. It should be noted here that there can be marked differences in characteristics between colonies of honey bees, e.g. some strains of bees are better honey producers than others or more aggressive than others or more liable to swarm than others.

2. *A Hive*

This should be constructed to the standard specifications of the Department of Agriculture of Sri Lanka. When the author purchased his first hive (made of "Gini sapu" timber), it cost Rs.12/50 but it is difficult today to obtain such boxes for less than Rs.100/-. The Department of Agriculture (Apiculture Research Institute at Bindunuwewa, Bandarawela), has investigated the construction of hives using alternative materials in an effort to reduce costs. The hive should be placed at a convenient height on a stand or other support, with suitable protection against ants, termites and other pests.

3. *Entrance Guard*

This prevents the queen bee from leaving the hive while the new colony is not yet established. This is usually supplied when a wooden hive is purchased.

4. *Wax Foundation Sheets*

These sheets are not essential but are very useful as foundations on which the bees can build their combs. They should be locally produced.

5. *Bee Veil and Gloves*

Many bee keepers do not use a veil or gloves, but wearing them gives confidence to the beginner. Use of these till experience in bee keeping is gained will help the beginner to avoid being distracted from the task he is engaged on. Use of protective clothing can also mask the smell of sweat, which may have an irritant effect on bees. The

Government bee keeping officers may be consulted regarding designs and materials for these and other bee keeping accessories.

6. *Smoker*

A smoker is very useful to quieten the bees when a hive needs to be opened or a colony has to be transferred or otherwise manipulated. Models are available locally from Rs.40/- to Rs.125/- but inexpensive makeshift arrangements to deliver smoke are often to be seen.

7. *Centrifuge*

If only a few hives are concerned, it may be possible to borrow a centrifuge at the time of the honey harvest. A suitable centrifuge could, however, be constructed for around Rs.300/- Rs.400/-.

8. *Record Cards*

For recording the history and performance of colony.

9. Other (non-essential) accessories could include items such as "queen excluders". Some sugar syrup may also need to be fed to a colony occasionally for it to be able to tide over periods of dearth.

Honey

The feeding of sugar syrup to honey bees can result in some of it being stored in the combs. However, this should not, correctly speaking, be called "honey". Another non-floral variety is called "honeydew honey", which is made by honey bees which have collected, principally, the secretions of/on living parts of plants and little nectar from flowers. An example of "honeydew honey" in Sri Lanka is "rubber honey", made by honey bees which have collected (non-floral) secretions from rubber trees. Due probably, to factors of humidity and evaporation, more honey is said to be obtained from rubber trees in wet areas such as Ratnapura than from those in the drier areas.

Floral, nectar or blossom honey is produced by the bee mainly from the nectar of flowers. Monoflora honey (unlike polyflora honey) comes mainly from one kind of plant; the principal type of honey, is often contributed by

its flavour, colour etc., and is investigated by examination of the pollen grains present in it (and which can be used to identify the different plant species).

Pollen

Honey bees collect pollen as well as nectar for the food requirements of the colony. The pollen provides a source of protein and many flowers are visited more for the pollen they provide than for the nectar. The coconut flower is an example of one which is more valuable as a pollen rather than a nectar source; with a further food source being evident from the number of bees which may be observed to visit coconut and other palms which are being tapped.

Bee Flora

Many plants of economic importance (or which are otherwise useful) are visited by the honey bee for pollen, nectar and (or) other secretions. Table 1 gives a selection of some of these plants. It is seen that many intercropped plants (e.g. coconut, coffee and pepper) provide bee forage (bee pasture) so that bees' honey could form a further harvest on the same land, if insecticide usage is not too liberal. The value of ornamental plants, such as the pink antigonon, and others which may not have an economic importance, such as nidikumba, as bee forage should also not be overlooked.

Floral Calendars

Most plants and trees are not in flower continuously throughout the year (the coconut is an exception) but have definite flowering seasons. Figure 1 gives the model of a "floral calendar" drawn up to depict the flowering times of various economic plant species in a hypothetical region. A complete "floral calendar" would, of course, include the time of flowering of the non-economic plant species (often of much importance) in the region. It should also be noted that the optimal foraging range of the local honey bee is up to 1/4 to 1/2 mile so this would constitute the foraging distance in any direction from a given hive, which is of considerable practical importance. It would be helpful, especially, in the present context of agricultural development, if floral calendars (preferably including acreages of plantations) could be drawn up for the different parts of the island and compiled into a (regularly revised) reference work.

Table 1

Some economic and (or) useful plants and trees visited by honey bees for nectar, pollen and (or) honeydew. (As a general rule, a honey bee on a trip for pollen or nectar only visits flowers of a single species).

Ambarella	Indian corn	Parsley
Avocado	Jambu	Passion fruit
Bamboo	Katurumurunga	Peanut
Bandakka	Kitul	Pear
Beli	Kumbuk	Pepper
Brinjal	Lime	Plantain
Broccoli	Mango	Pumpkin
Brussels sprouts	Mangosteen	Radish
Cabbage	Mara	Rambutan
Caraway	Mee	Rape
Carrot	Milla	Raspberry
Cashew	Mint	Rubber
Cauliflower	Mora	Safflower
Coconut	Mukunuwenna	Sorghum
Coffee	Mung	Soyabean
Coriander	Murunga	Strawberry
Cotton	Mustard	Sunflower
Cucumber	Na	Tamarind
Dill	Nadun	Tea
Eucalyptus	Nelli	Teak
Fennel	Onion	Thampala
Gingelly	Orange	Thippili
Goraka	Palm-yrak	Tomato
Guava	Papaw	Veralu

Table 1 : Model "floral calendar" for a hypothetical region for economic plant and tree species

January	February	March	April	May	June
Coconut	Coconut	Coconut	Coconut	Coconut	Coconut
Mango	Mango		Gingelly	Gingelly	Murunga
	Rubber	Rubber		Coffee	
	Teak			Lime	

July	August	September	October	November	December
Coconut	Coconut	Coconut	Coconut	Coconut	Coconut
Murunga	Murunga	Lime	Lime	Maize	
		Orange	Orange	Cotton	
Teak			Coffee	Coffee	Coffee

Bee Keeping on Plantations

For bee keeping on plantations, a source of drinking water for the bees is necessary, but too much feeding of sugar syrup should be avoided in view of the expense involved. The density of colonies (number of colonies per acre) which can be maintained without supplementary feeding of sugar syrup, on a plantation depends on several factors, including the rainfall pattern (which can vary from year to year) and the monthly availability of nectar (or honey dew) and pollen. For example, during "honeyflow seasons" (as appropriate), two or more dozen colonies may possibly be accommodated per acre of eucalyptus or rubber plantations but not more than, say, 3 colonies per acre of coconut. The longstanding interdependence of effective bee farming and agriculture is exemplified by the description that in ancient Egypt bee hives were placed on barges which were moved along the Nile as each fruit orchard or other crop came into flower.

Migratory Bee Keeping

Useful benefits can be obtained from one or more colonies of bees kept on a single plantation throughout the year. In up country areas, about 6 lbs. of honey may be obtained on average per hive per years, although under certain circumstances much higher figures, such as 66 lbs. in one year (Dayaratne, 1968), have been recorded. However, in professional bee farming, enhanced yields of honey are regularly obtained by systematically transporting bee colonies (often hundreds or thousands at a time) throughout the year to each type of bee flora as the "honeyflow" commences. Thus, in the model given in Figure 1, the colonies would be taken from the rubber plantations in April, when the "rubber honeyflow" ceases, and transported to the gingelly area in time to catch this honeyflow.

Such professional type of migratory bee farming would

probably be necessary for the production of adequate quantities of honey for export purposes.

Markets for Honey and Wax

Bees' honey can be readily retailed in Colombo today at around Rs.30/- per lb. and is principally used in Sri Lanka as a food and in the preparation of Ayurvedic medicines. Bees' wax is used in furniture polishing, batik manufacture, etc., and can be sold at anything from Rs.40/- to Rs.120/- per kg. depending on quality etc., while "bambara" wax may fetch a lower price. It should be noted that it can be uneconomic for a bee keeper to sell wax from his hives as bees consume a large amount of honey during the production of wax; so that it is advantageous to extract the honey by centrifugation and to return the combs to the hive for refilling. The SLBFA advises its members on the marketing of their honey while several traders are found in Gabo's Lane and Bankshall Street in the Pettah who deal in bees' honey and wax. Prices can vary widely depending on season, type of honey, and other factors. Imported honey is also marketed, although at higher retail prices than the domestic product. In 1980, the import of 16,605 kg. of bees' honey cost Rs. 352,371/- and that of 1875 kg. of "bees' wax and other insect waxes cost Rs.255,057/- (Customs Returns).

Possibilities exist for developing export markets for Sri Lanka bees' honey. World demand is increasing due, among other factors, to the growing interest in natural and health foods in importing countries. A requirement is, however, that prospective exporter (or exporting group) be in a position to assure regular and fairly substantial quantities of good quality honey for shipment. In addition to analytical quality control for purity, etc., the honey should be permissible under the honey legislation of the country to which it is to be exported. Criteria laid down in the honey legislations can vary significantly from country to country. Further information on export possibilities and the world honey trade is given in publications by the International Trade Centre UNCTAD/GATT (1977).*

Quality Control

The majority of locally produced honey offered for sale in retail quantities has not been subjected to laboratory testing for quality. While the Sri Lanka Standards Insti-

tution has a specification for bees' honey, the large number of small producers who sell a few bottles of (unlabelled) product per year makes its observance difficult. The apparent willingness of some small traders to handle small "one-off" lots of bees' honey offered by unknown vendors further aggravates the problem. One available safeguard is to purchase bees' honey from a supplier in whom one has confidence and here again the SLBFA may be in a position to provide suggestions on request.

Some containers of local honey are seen to contain clean, dry grains of paddy and (or) black pepper corns. These are a traditional addition to enhance keeping qualities during storage.

While a honey may be classed as inferior due to one of a variety of criteria, it may be noted here that a honey may crystallize to give an opaque appearance and somewhat solid consistency without being in any way adulterated. While most consumers in Sri Lanka appear to prefer liquid, clear honeys, in many cases (especially overseas) the granulated varieties are sought after. A honey which has been crystallized can usually be readily clarified by slightly warming, e.g. by standing the container in warm water.

Honey destined for the export market usually has to undergo thorough laboratory testing (e.g. for moisture content, diastase activity, hydroxymethylfurfural content) in addition to examination for acceptability as to appearance, taste etc. A honey exporting country may ship several thousand tons per year and it has been emphasised that there should be consistency between the samples submitted and the qualitative characteristics of the bulk consignments.

The production of honey in other countries is from time to time significantly affected by the spread of diseases in bee colonies and the importation of stocks of bees etc., into Sri Lanka should be avoided to minimise the possibility of introduction of bee diseases and pests into the island.

Bee Diseases and Pests

A number of bee diseases are known (Morse, 1978) but usually the stocks of bees in Sri Lanka are quite healthy. A number of "visitors" such as ants, house lizards, spiders and mice may be found in hives (Gunawardena, 1975)

* Available at the, Sri Lanka Export Development Board, Ramada Renaissance Building, 115, Sir Chittampalam Gardinaer Mawatha, Colombo 2.

A number of "visitors" such as ants, house lizards, spiders and mice may be found in hives (Gunawardena, 1975) but the greatest problem is presented by wax moths. One method of reducing this problem is the regular weekly inspection of the hive, which should include cleaning off the debris found on the bottom board.

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TRADITIONAL CULTIVATION SYSTEMS AND APPROPRIATE TECHNOLOGY

G.K. UPAWANSA

Gannoruwa, Muruthalawa.

The industrial revolution, scientific discoveries and the growth of commercialism have had a profound effect on the transformation of systems of traditional agriculture practised by different civilisations for thousands of years. The theory propounded by Malthus that population growth will outstrip food supply would have acted as a significant catalyst in the search for new and improved methods of food production. However, the most powerful force was perhaps the commercial orientation in agriculture which placed the highest premium on profit maximisation rather than human welfare.

Commercialised industrial production is largely based on supply and demand. Selection of the appropriate technology and labour utilisation are largely governed by profitability considerations. Majority of inputs are physical in nature except in areas of labour and management.

The tremendous success in industrial production had its inevitable impact on agriculture, which came to be organised more and more on the very same lines. The net consequence of these developments has been the birth and establishment, throughout the world, of a system of agriculture heavily dependent on - and specifically appropriate for - heavy machinery, artificial fertiliser, insecticides, weedicides and fungicides. Sri Lanka too was no exception to this phenomenon.

At the very outset, it may be necessary to emphasise one point - that all these developments originally took place in countries in the temperate zone. The new systems may be highly appropriate to those countries - in terms of climatic conditions, soil types, dietary patterns of the population etc. However late it may be, a question can be posed, "How appropriate are they for tropical countries like Sri Lanka?"

Leaving that question aside, let us consider the nature and implications of the new system - beginning with fertiliser usage.

Modern scientific research has identified the macro and micro nutrient and trace element requirements of plants. These are, Nitrogen, Phosphorus, Potassium, Sulphur, Calcium and Magnesium (macro nutrients); Iron, Manganese, Zinc, Copper, Boron and Molybdenum (micro nutrients) and about ten trace elements. However, research work on plants has mainly been concentrated on the major nutrient requirements. Work on micro nutrients and trace elements has been conducted generally in response to specific problems that have come to light (e.g. yellowing of coconut leaves due to magnesium deficiency).

Through research work, macro nutrient and nutrient compounds appropriate for different soil types have been determined and recommended. This procedure is clearly seen in paddy cultivation. Nevertheless, it can be maintained that these findings and recommendations are based on inappropriate concepts and research data. There appears to be two basic defects in this system. Firstly, the concentration on major nutrients and the exclusion of minor nutrients and trace elements. Secondly, most research work is conducted in "special research plots" and not in the actual field.

When the recommendations based on this type of research are followed in the field, it can lead to deficiencies in trace elements and carbonic compounds. Application of nitrogen, phosphoric acid and potash under these circumstances results in chemical imbalances which can have the effect of weakening the plants. Application of nitrogen under nutrient imbalance situations can produce adverse effects rather than benefits.

Environmental pollution stands as clear evidence for the ill effects of artificial fertiliser usage.

Weak plants are highly susceptible to insect and pest attacks. When insecticides are used to eradicate insects, they destroy not only the harmful ones but also the other

insects and useful beings, as well, leading to an imbalance in the living organisms. (The same is true in respect of weedicides). Although a temporary control over the insects can be achieved by this method, the destruction of organic balance leads to a resurgence of the problem on a dangerously large scale after the lapse of some time. The present yields of paddy lands in the Polonnaruwa district are a good illustration of the consequences of plant nutrient imbalance. Under optimum soil and weather conditions, it is possible to secure a yield of about 200 bushels per acre from the modern hybrids that have been introduced in to the Polonnaruwa area. However, today's yields, on the average, do not exceed the 100 bushel per acre level. This is a 50% decline.

The advent of mechanised farming based on the profit motive has led to the development of machine compatible hybrid plants and the substitution of monocrops for mixed farming. Both developing and developed countries have accepted this agro technology as the 'system' - and they appear to be looking down upon the traditional systems of agriculture. Projects were implemented to wean the people away from age old traditions, and the intended objectives seem to have been at least partially achieved. The immediate effect of this transformation is the gradual increase in fuel consumption.

Energy Consumption in a British Farm - (1976)

Input Units		Output units	
Fuel (wood, oil and electricity)	122.2	Food crops	84.4
Fertiliser	83.5	Fruits and vegetables	3.2
Machinery	103.5	Animal products	80.6
Chemicals	8.5		
Buildings	22.8		
Transport	16.3		
Animal food (from outside sources)	53.2		
	-----		-----
TOTAL	414.1		168.2
	== =		== =
		768.2	
Input/Output ratio	= -----	=	40.%
	414.1		

In Africa, where animals are used in agriculture, these ratios stand at 18.5% for cereals and 29.5% for other crops.

Unfortunate and unforeseen symptoms began to appear in countries which could be considered as the earliest converts to this 'new' system. Thousands of acres turned into 'dust bowls'.

Although a handful of persons foresaw the impending disaster, they were labelled as fanatics, but the situation went on changing form bad to worse with commercial agriculture entering the ranks of major sources of environmental pollution. With the realisation of the threat posed by environmental pollution to the survival of life on earth, and the short term and temporary nature of modern technology, the attention of intellectuals, environmentalists and agronomists was drawn towards agriculture. They were motivated to search for alternative agro technologists, and to develop new systems. These systems or technologies are known by several names - organic cultivation, bio dynamic farming, natural forestry farming, energy farming, sustainable agriculture - to quote a few. The number of persons and institutions involved in these new optional systems is on the increase with a proportionate rise in attention and emphasis. As a result of this development, the production and marketing of health foods are under going a rapid expansion in the West, accompanied by a similar output of related publications.

Through these steps, the scientists, environmentalists and related institutions strive to achieve three main objectives. Firstly, the maintenance of a pollution free environment and the preservation of the atmosphere, soil and water on earth in a condition conducive to the life of plants and animals. Secondly, the rehabilitation of arable lands destroyed by modern agro - technology and the utilisation of systems that ensure increasing yields year after year. Thirdly, the protection of life on earth through the world wide application of measures designed to achieve the above objectives. This is not an easy task, because most countries of the world have embraced destructive technologies and alternate, less harmful technologies are not always readily forth coming.

Formulation of alternative solutions through normal research procedures takes a long time. Therefore, many scientists are inclined to believe that better methods

could be found by collection, codification and analysis of technologies used by countries with a history of several thousand years. Practical details of these technologies could be sought from elderly farmers and those living in remote areas. An examination of facts thus collected leads one to conclude that, "the ancient agro production has been based on an environmentally sustainable long term technology."

It is evident that in ancient times agriculture was merely a sub-component of a total system that embraced environment, society, religion and the state - and not a mere production process as practised today. For instance, the chena cultivation was not a destructive system like some of the systems used today. It can be described as an appropriate agro - forestry technology compatible with modern scientific ideas. Large trees were not felled or burnt. What was done was only clearing of the jungle to a degree just sufficient to provide light for growing plants. Thus, only the undergrowth and low hanging branches and creepers were removed. Further, the other elements of chena cultivation, namely, selection of appropriate seasonal crops, land preparation, crop pattern and the adoption of mixed farming display a high level of land management, maintenance of soil fertility and water conservation.

An Intergrated System

Further examination of the above mentioned aspects of the traditional Sri Lankan systems is bound to reveal features that can surprise the modern scientist. Maintenance of eco-systems through appropriate land use, rational water management, optimum utilisation of forest resources; recycling of plant nutrients and the adoption of appropriate crops are some of these notable features. When the insect populations reached epidemic levels due to adverse climatic factors, they were brought under control through mechanical procedures couched in rituals, natural bio chemical agents such as leaves and barks of plants and by crop rotation.

The dry zone of Sri Lanka is served by a network of rivers originating from the central mountainous country and the tanks have been built in a series along the waterways where the overflow from one tank and the connected fields feeds the tank below it. This method of water storage is conducive to soil conservation and prevents

erosion. It also improves the underground water holding capacity.

Forests in the wet zone helped the soil to retain rain water and thereby act as reservoirs. Run off water from the forests and the chenas deposited the top soil and the plant nutrients it carried in the form of sediment in the series of tanks. Annually, during the dry season tanks were dredged and the rich sediment transferred to the paddy fields. Tanks were built in ascending order of size down the stream so that almost all the sediments got collected in the smaller tanks that dried up completely during the dry season facilitating easy dredging. Once the water passed through several such tanks which functioned as effective filters, it was virtually free of all sediment. It is this 'pure' water that entered the larger tanks located further down stream. This explains why the large tanks in Sri Lanka have not completely silted up even after thousands of years.

The farmer in ancient times possessed three types of lands. A chena in the forest, a home garden with useful perennials, vegetables, etc. near the tank and three different types of paddy lands (average mud, medium mud and heavy mud) usually determined by the degree of proximity to the source of irrigation water. The system of chena cultivation, in the manner it was practised then, actually preserved the forest; because the large trees were never felled and removed as is done today. Chena was a source of food, fuel wood, timber, fodder and fertiliser for intensively cultivated paddy lands. It was really a good example of optimum use of resources.

Forest and chena adjoining the village; homes and home gardens adjoining the tank; paddy lands below the tank bund; cultivation based on animal husbandry; rational land use - these were the elements of this integrated system that preserved the environment and ensured ecological balance. Cow dung from the cattle sheds, ash and decaying vegetable matter from the kitchen and home garden were collected and applied to the paddy fields. Fields were kept water logged before ploughing. This ensured a certain degree of weed and insect control, depositing of nutrient rich sediment that came with surface run off in the fields and also facilitated the absorption of nitrogen that came with rain water associated with lightning during the inter monsoon periods. These measures, along with proper cropping systems, ensured the

maintenance of fertility of paddy lands for centuries at a remarkably high level. Brilliant sun light, selected seeds, proper combination of crops and timely sowing of seeds led to vibrant plant growth, capable of resisting insect attacks and disease and ensured high fields.

Another measure that helped in this matter was the crop pattern, that went hand in hand with rainfall. Tradition insisted that there should be specific time lags between one step of cultivation practice and another. E.g. between the first clearing of the forest and the second; between the first ploughing and the second and so on. The whole process of land preparation would have taken several weeks or several months. This practice was definitely helpful for the control of weeds and insects, and special eradication measures were generally not required.

Nevertheless, occasionally, due to adverse weather conditions insect epidemics did occur, and various measures were adopted to keep the insect populations under control. However, the remarkable feature of these measures is the fact that none of them were dependent on toxic agents or were harmful to the environment. Certain insect control methods had religious connotations, while the belief in super natural beings (prevalent among the farmers) also seems to have been exploited in devising insect control measures.

What has to be done today is to examine these measures, identify the underlying scientific principles and use them effectively. A few of these charms and their effective mechanisms are described below;

Whenever there is an attack by "godavella"s (a kind of worm that breeds profusely and noted for voraciousness), a thin disc cut off from the trunk of a banana is struck to a stake and several such stakes planted in the field infected with worms. A few small pieces of ripe fruit (e.g. mango, banana) and a piece of fried dry fish are kept on each disc of banana trunk. In addition, a lamp is also lighted towards the evening. Within a few days, the worm attack subsides.

The probable explanation is that the smell of fried fish, the light of the lamp as well as pieces of fruit serve to attract birds. As soon as they perch on the disc of banana trunk it topples into the field along with the bait. Birds follow the bait and note the worms - the better food.

Once attracted, the flock of birds increases daily - until the entire insect population is destroyed.

In the Kandy district, stakes driven into paddy fields is a common sight. These stakes seem to serve two functions. Firstly, they scare away the rats and non-perching birds that feed on paddy (e.g. paddy birds). Secondly, they serve as perches for nocturnal birds such as owls which feed on rats. However, it may be noted that this practice will be ineffective where there are no nearby forests which provide the breeding ground for nocturnal birds - another instance showing the role of forests in agriculture.

Another 'charm' employed to control insects in paddy fields is to sprinkle a pot of milk and a pot of sand on the court yard near a Bo tree; collect a pot of sand from there and to dash handfuls of sand on to the infected paddy plants. Obviously, this act has the effect of throwing the insects on paddy plants in to the water in the field where they become prey to fish, water snakes and frogs. Again, this method will be ineffective in fields where systemic weedicides and insecticides have destroyed all living beings.

Lighting an oil lamp in the night to attract insects which ultimately end up as prey to aquatic predators is another method. Mechanical methods included the dragging across the paddy plants of winnowing fans and ropes to which latex from jak trees have been applied. In the process, the insects got stuck on the latex. Sprinkling of the juices of certain plants or placing the crushed plants on water ways in the paddy field were among the bio chemical measures employed. These are still practised in some areas.

The brief description given above clearly shows that the traditional systems were neither harmful to the environment nor toxic to man. However, our soil, our environment and ourselves are facing disaster due to the impact of commercialism and the misguided belief in the infallibility of modern scientific systems. Therefore, a determined effort must be made to isolate the appropriate measures from our ancient systems and to introduce them to our farmers before it is too late.

Similar useful measures could be developed from those adopted in foreign countries. Two such examples now in vogue in Thailand are given below.

Use of *Thiththa kinda* - *Tinospora glabra* (Menispermaceae) in the control of insects is one such example. Here, pieces of this plant are anchored to waterways or cut into small pieces and scattered in the field. Paddy plants (before being transplanted) are kept immersed in the water (prepared by keeping 2 kg of this plant in 10 litres of water for 24 hours) for 12 hours. This has been found to be an effective remedy against insect attack.

Margosa is also a widely used insecticide. Powdered seeds of Margosa is applied to the paddy fields in varying doses at different stages of the growth of paddy plants. It has been found to be an effective measure in insect control. Further, the farmers have noticed an improve-

ment in the growth of plants as well as in the quality of paddy. This has been attributed to the ability of margosa to control fungi and fix soil nitrogen.

The main attention in this article was directed towards crop protection because of the prevailing belief among farmers that cultivation is not possible without agro chemicals. Therefore, it should be obligatory on our part to ensure increasing yields in food crops to feed the growing population and the protection of environment and soil fertility through harmless measures as described above. It is also necessary to formulate and discover appropriate new measures and to motivate the people to adopt them.