

Chapter 1

ORGANIC AGRICULTURE AND ORGANIC INPUTS

Organic Perspective

“In a world of many choices organic agriculture is a serious option for many farmers, enterprises and consumers. Supporting that choice with credible science can be vital for improving the over all productivity, food security, food sovereignty and environmental impact of agriculture in the country. The challenge lies in creating an environment in which organic is treated as complimentary approach and efforts are focussed on harvesting benefit, organic agriculture can give to a section of the Indian farmers” (Tej Pratap 2006).

After almost a century of development, organic agriculture has been embraced by the mainstream and shows great promise commercially, socially and environmentally. While there is continuum of thought from the earliest days to the present, the modern organic movement is radically different from its original form. It now has environmental sustainability at its core in addition to the founders concerns for healthy soil, healthy food and healthy people. Since the 1970s when organic agriculture re-emerged as an eco-agriculture, institutional strengthening and diversity became a part of the movement. Formation of IFOAM in 1972 indicated that the movement has come of age and that it is going to grow and make a place for itself in over all world of agriculture. Explosive growth of organic agriculture has occurred only since 1990s. In India the movement was initially started by the farming communities and agri-enterprises, it is now being carried forward by several stakeholders, including the Government agencies. Launching of National Program on Organic Production (NPOP) by Ministry of Commerce during 2001 and National Project of Organic Farming (NPOF) by Ministry of Agriculture during 2004 is an indicative of growing awareness for systematic promotion of organic agriculture in the country through Government Initiatives.

Need for Organic inputs while converting to organic

For conversion of a conventional field to organic, first step is to build up the lost fertility of the soil. This can be achieved by complete ban on use of synthetic inputs and increased use of organic and biological inputs. For nutrient management and soil fertility build up crop residue, animal dung, forest leaf litter, bone meal, slaughter house waste, blood meal and green manures are important organic sources. All such organic material needs to be composted properly for appropriate impact. Nutrient value of the raw material and composting methodology determines the quality of produce. Biological resources such as biofertilizers and other microbiological inputs have also attracted lot of attention and are being promoted on large scale. Under National Project on Organic Farming incentives are available for establishment of production facility for Vegetable market waste and ago-waste compost, biofertilizers and vermiculture. Under various other schemes of Central and State Governments assistances are available for setting of vermicompost production facilities.

Basic spirit on use of inputs in organic agriculture

In present day organic farming, stress is given on on-farm management. In this on-farm management nutrient management is looked after by crop rotation, multiple cropping, mixed cropping, incorporation of legumes as intercrops, crop residue management and by use of on-farm made compost. Plant protection is achieved by habitat management, multiple cropping, cropping combinations, crop rotations, release of pest predators and parasitoids and use of botanical and bio-pesticides. The requirement of these inputs is managed by their production at farm with available on-farm resources in the first stage and by purchase from off-farm resources to a limited extent in the second stage.

Organic input agri-business

In promotion of organic farming use of organic inputs has assumed an important position. Contrary to conventional farming where synthetic inputs are used to feed the crop and protect the crop by direct action, in organic farming inputs are used to feed the soil and to create an environment which can collectively keep the pests below economical threshold limit (ETL). In this endeavor although quantity may not be an important issue, but quality of input is of prime importance. In the recent years efforts have been made to promote appropriate production methodologies among farmers for effective conversion of organic waste into nutrient rich compost and for preparing botanical extracts for pest management. Mass adoption of vermicompost technology and use of neem seed kernal sprays by farmers is an indicative of the usefulness of such strategy. But still there is a scope for the entrepreneurs to come forward and establish production facility to produce consistent quality product and made available to farmers at reasonable price. To take the advantage of growing awareness of organic agriculture various types of organic and biological inputs have been launched and are being sold to farmers. Some of such products are the results of research and are being promoted by state agencies also, but some of the products have been launched without much scientific understanding and their quality and usefulness is questionable.

To prevent such unfair practices, awareness among the users is most essential. At Government level some efforts have been made to regulate the production and quality control of some organic inputs. In this, some organic fertilizers and biofertilizers have been covered under Fertilizer Control Order and their standards and quality control parameters have been defined. Manufacture and sale of biopesticides are being governed by the Central Insecticide Act.

The present compilation deals mainly with the production aspects of some important and widely accepted organic and biological inputs.

Government Initiatives for strengthening of organic input industry

Organic inputs such as various types of composts, biofertilizers etc are not only important in organic farming, but are also of prime importance in integrated agriculture with balanced use of fertilizers for sustenance of soil fertility. With the objective of promoting the use of such environment friendly technologies and inputs, Govt of India, under National Project on Organic Farming has initiated an input production promotion scheme. Under this, capital investment subsidy (restricted to specified limit indicated against each component) is provided for the establishment of following input production facilities. This assistance is available for individuals,

registered agencies, private entrepreneurs, companies, cooperative societies, Government Departments, municipalities and other Govt and semi-Govt agencies/institutions.

S.No.	Component	Capacity	Maximum subsidy
1	Vegetable Market waste/Agro Waste compost	100 ton per day	33% of TFO* or Rs 60.00 lakh whichever is less
2.	Biofertilizers and or biopesticides	200 ton per annum	25% of TFO* or Rs 40.00 lakh whichever is less
*TFO = Total financial outlay			

How to avail the facility

Government agencies – All Government agencies such as Corporations, undertakings and Municipalities etc who can not avail loan facility from bank can apply to the DAC or National Centre of Organic Farming, Ghaziabad for direct sanction of subsidy.

Non-Govt and private agencies and individuals – For this category the facility is available in the form of credit linked back ended subsidy. For this the aspiring agency or entrepreneurs need to prepare a project report and apply any scheduled bank for loan. Granting of loan will be subject to terms and conditions of the bank. After the loan is granted and first installment of loan is availed, the bank will apply to NABARD for release of 50% eligible subsidy. On scrutiny and technical evaluation the NABARD may release 50% of eligible subsidy to the loan sanctioning bank. On completion of the project and complete utilization of loan, the bank will again write to NABARD for technical evaluation and release of final amount of eligible subsidy. A three member team comprising of members from Deptt of Agriculture and Cooperation/National Centre of Organic Farming, NABARD and loan sanctioning bank will evaluate the project both from technical and financial point of view. On receipt of recommendations of the evaluation team the NABARD will release the balance 50% of eligible subsidy. The optimum ratio of financing is: 25% promoter's contribution, 50% bank loan and 25% GOI subsidy.

Requirement of registration and license under Fertilizer Control Order

Since April 2006, quality of Biofertilizers such as Rhizobium, Azotobacter, Azospirillum and PSB and organic fertilizers such as City waste compost, vermicompost and press mud are being monitored under the Fertilizer Control Order (Amendment November 2009). All manufacturers, distributors and dealers are required to obtain necessary registration and license from state authorities as per the requirement of the act. Concerned state's Agriculture Department has been delegated with the powers for inspection and issue of registration. All District and sub-district officers of Agriculture Department and Fertilizer Inspectors authorized under FCO shall function as sampling officers. National Centre of Organic Farming, Ghaziabad and its six Regional Centres of Organic Farming at Bangalore, Bhubaneshwar, Hisar, Imphal, Jabalpur and Nagpur have been declared as authorized testing laboratories for biofertilizers and organic fertilizers. States can also develop their own testing laboratories and after fulfilling requisite infrastructure and facilities can notify such testing laboratories under the act through their Gazette Notification.

Chapter 2

BIOFERTILIZER ORGANISMS AND PRODUCTION TECHNOLOGY

Biofertilizers or microbial inoculants are carrier based ready to use live bacterial or fungal formulations, which on application to plants, soil or composting pits, help in mobilization of various nutrients by their biological activity. To ensure a good quality biofertilizer, a formulation should possess following traits.

- a. The product must be carrier based or liquid formulation, capable of holding very high population of specific micro-organisms for sizeable period of time.
- b. In case of carrier based formulations the product should have 30-50% of moisture throughout the shelf life period to sustain microbial population.
- c. For carrier based formulations the microbial population should be in the range of 10^7 to 10^9 cells/gm of moist product. In case of liquid formulations the cell load should be in the range of 1×10^8 to 1×10^{10} during the entire period of shelf life.
- d. It should be free from other contaminating microorganisms.
- e. The microbial strain present in the product should be able to produce adequate nodulation in case of *Rhizobium*, be able to fix at least 10-15 mg of N/gm of carbon source used in case of free living N_2 fixers and be capable of solubilizing significant quantity of fixed soil P.
- f. It should have sufficient shelf life (minimum 6 months for carrier based and 12 months for liquid).

Keeping in view of all these requirements a production technology has been developed which involves 3 steps:-

- (a) Isolation and identification of appropriate strains of targeted microorganisms.
- (b) Up-scaling of microbial biomass.
- (c) Impregnation of carrier with fully grown microbial broth or immobilization of grown cells to obtain liquid formulations

Isolation and identification of strains:

The success of any biofertilizer in the field primarily depends upon the strain of the micro-organisms used in the product. The strain, besides possessing specific attributes (such as host specificity, nodulation potential and N_2 fixation potential) should also have the ability to colonize the soil and rhizosphere, be able to successfully compete with the native soil microorganisms and should have enough capacity to survive in the soil for long time in association with other soil micro-organisms. In India large number of research institutes belonging to Agriculture Universities, Conventional universities, ICAR Institutes and other organizations are involved in isolation and identification of these microbial strains. During the last 50 years large numbers of strains belonging to various micro-organisms have already been identified and are readily available to producers for using them in biofertilizer production. National and Regional Centers of Organic Farming are maintaining such strains for the benefit of producers and are available at nominal cost.

Up-scaling of biomass:

To deliver a very high population of microorganisms in biofertilizers, it is very much essential to cultivate these microorganisms under appropriate conditions to achieve very high population per unit of growing medium. Usually a final cell count of $>10^9$ cells per ml of broth should be achieved. This is being done in laboratories under controlled conditions in small glass containers (for small scale production) or large scale fermenters (for large scale production units).

Preparation of carrier based formulations:

Once the optimum growth of microbial cells is achieved in the multiplication process, it has to be mixed with the suitable carrier material, which can provide ideal home for these micro-organisms for about 6 months to 12 months time. The first step in this process involves the selection of suitable carrier materials. As a result of intensive researches in this field, many materials have been identified as the suitable carrier materials. Among them, peat, charcoal, lignite, charcoal-soil mixture, charcoal FYM mixture, vermiculite and kaolin (mixed with ppt grade silica or hydrogel) have been identified as good carrier materials. Depending upon the availability and cost, different production units are using different carrier materials. For preparation of finished goods the pure bacterial liquid containing very high population of required microorganisms is mixed with the carrier material to obtain moist powdered formulation, which is packed in polythene bags and supplied to the farmers. Depending upon the facilities, mixing of bacterial liquid with carrier material is being done either manually or under complete sterile conditions. Packets prepared by manual mixing method have shorter shelf life (2-3 months), while packets prepared under complete sterile conditions have longer shelf life (6-12 months).

Preparation of liquid formulations:

In this process, in some cases the fermentation is taken up with specialized formulations with cell immobilizers added at different stages of growth. Finally prepared broth with immobilized cells having a cell count of $>1 \times 10^{10}$ is harvested and packed in bottles. In some other cases, the vegetative cells after being cultivated to desired level are converted into cysts or spores. These spores are further treated to keep them in dormant position. Final preparation is packed in bottles. If sterile conditions can be arranged then instead of bottles plastic pouches can also be prepared.

Biofertilizer organisms

Azospirillum

Distinguishing characters

Plump, slightly curved and straight rods, about $1.0 \mu\text{m}$ in diameter and $2.1\text{-}3.8 \mu\text{m}$ in length, often with pointed ends. Intracellular granules of Poly- β -hydroxy-butyrate present. Enlarged pleomorphic forms may occur in old alkaline cultures or under conditions of excess oxygen. Gram negative or gram variable. Motile in liquid media by a single polar-flagellum. On solid media at 30°C numerous lateral flagella of shorter wavelength are also formed. Fixing nitrogen microaerophilically. Grow well aerobically also in the presence of combined nitrogen such as ammonium salt. Possess mainly a respiratory type of metabolism with oxygen or nitrate as the terminal electron acceptor, but weak fermentative ability may also occur. Under severe nitrogen limitation nitrate is converted into nitrite or to nitrous oxide and nitrogen gas. Optimum temperature is $35\text{-}37^\circ\text{C}$.

Growth in different selective media

In MPSS broth :- grow as plump, slightly curved rods and straight cells having a diameter of up to 1 μm . Many cells have pointed ends. In semisolid nitrogen free malate medium (Nfb):- *A. lipoferum* develops predominantly into pleomorphic cells within 48h. *A. lipoferum* cells grow as elongated cells (.4 - 1.7 μm x 5 to over 30 μm long), which are non motile and have an S shape or helical shape. These forms eventually seem to fragment into shorter ovoid forms. *A. brasilense* grow mainly as motile vibroid cells. Non-motile enlarged pleomorphic forms (C forms) may also occur, especially in older cultures, on the surface of nitrogen free agar media. Pleomorphism in both the species is probably because of the alkalinity of the malate medium due to the oxidation of malate. Pleomorphism fails to occur when the organisms are cultured in semi-solid nitrogen-free glucose medium, which does not become alkaline. In N-free malate semi-solid media nitrogen fixation occurs only under microaerobic conditions. This is mainly because of the lack of oxygen protection mechanism for the nitrogenase. The best way to obtain N_2 dependent growth is by culturing the organisms in semisolid nitrogen free media (Nfb media) under an air atmosphere at 35-37°C. After 3-4 days of incubation the growth will appear as thin veil or disc (called pellicle) few to several millimeter below the surface of the medium at a point where the rate of diffusion of oxygen into the medium corresponds to the respiration rate of the organisms so that no excess oxygen remains in the solution. As the bacteria multiply the disc of growth migrates closer to the surface, until finally it is just below the surface

On BMS agar :- On BMS agar (potato-agar) solid media after 1-2 weeks of incubation at 33-35°C, colonies of azospirilla are pink, opaque, irregular or round, often wrinkled and typically have umbonate elevations. Pigmentation is best on BMS agar incubated in light. Some strains of *A. brasilense* form colonies that have a very deep pink colour

Azotobacter

Distinguishing characters

Large ovoid cells 1.5 - 2.0 μm or more in diameter. Pleomorphic, ranging from rods to coccoid cells. Occur singly, in parts or in irregular clumps and some times in chains of varying lengths. Do not produce endospores but form cysts. Gram-negative. Motile by peritrichous flagella or non-motile. Aerobic but can also grow under decreased oxygen tension. Water soluble and water-insoluble pigments are produced by some strains of all species. Chemoorganotrophic, using sugars, alcohol and salts of organic acids for growth. Nitrogen fixer. Generally fixes non-symbiotically at least 10 mg of atmospheric nitrogen/gm of carbohydrate (usually sucrose) consumed. Molybdenum is required for nitrogen fixation, but may be partially replaced by vanadium. Nonproteolytic. Can utilize nitrate and ammonium salts (all but one species) and certain amino acids as sources of nitrogen. Catalase positive. The pH range for growth in the presence of combined nitrogen is 4.8 - 8.5, the optimum pH for growth and nitrogen fixation is 7.0 - 7.5. Occur in soil and water. One species occur in association with plant roots.

In N-free medium with glucose as carbon source, the young cells of different species are remarkably similar in appearance, mainly rods with rounded ends. 1.3 - 2.7 μm in diameter and 3.0 - 7.0 μm in length. In older cultures the cells tend to be ellipsoidal, chains and filamentous forms become more common and metachromatic and

sudanophilic granules are observed. *A. paspali* produces long filamentous forms even in young cultures, which differentiate this species from others. In peptone yeast extract agar media all members of the genus produce distorted cells. Cysts are formed in older cultures grown with sugar as the carbon source. To induce cyst formation a medium containing butane-1-ol (n-butanol) can be used. The cysts may be distinguished from an endospore by its characteristic structure: a central body surrounded by a cyst coat, consisting of an exocystorium and an exine. Unlike a spore the cell inside the cyst coat is similar to the vegetative form and there are no cytological changes in the cell prior to its germination. During germination, the cyst exocystorium is ruptured at one point and the cell which emerges may already be in dividing state.

On nitrogen-free agar medium with sugar as the carbon source, colonies appear with in 48 hr at 30°C and reach a diameter of 2-6 mm in a week. The colonies are generally smooth, glistening, opaque, low convex and viscid; however, colonial variations may occur. For *A. vinelandii*, smaller variant colonies may appear due to decreased production of extracellular polysaccharide. *A. armenicus* some times produce translucent colonies and *A. paspali* forms undulate edged and unevenly convex colonies with a dull or rough surface. On sucrose or raffinose agar the production of diffusible homopolysaccharides, results in formation of a diffused halo around the colony, is species dependent. *A. vinelandii* and *A. paspali* do not form diffusible homopolysaccharides.

Rhizobium

Distinguishing characters

Rods 0.5 - 0.9 x 1.2 - 3.0 µm. commonly pleomorphic under adverse growth conditions. Usually contain granules of poly-β-hydroxy butyrate which are refractile under phase contrast microscopy. Non-spore forming, gram negative, motile by one polar or sub-polar flagellum or two to six peritrichous flagella. Aerobic possessing a respiratory type of metabolism with oxygen as terminal electron acceptor. Often able to grow well under oxygen tensions less than 1.0 kPa. Optimum temperature 25-30°C. Optimum pH 6-7. Colonies on Yeast-extract mannitol agar are circular, convex, semitranslucent, raised and mucilaginous, usually 2-4 mm in diameter with in 3-5 days. Pronounced turbidity develops after 3-5 days in agitated broths. Chemoorganotrophic, utilizing a wide range of carbohydrates and salts of organic acids as carbon sources, without gas formation. Cellulose and starch are not utilized. Produce an acidic reaction in mineral salts medium containing mannitol or other carbohydrates. Growth on carbohydrate media is usually accompanied with copious extracellular polysaccharide slime. Ammonium salts, nitrate, nitrite and most amino acids can serve as nitrogen sources. Peptone is poorly utilized. Casein and agar are not hydrolyzed. Some strains require biotin or other water soluble vitamins. 3-ketoglycosidase not produced. The organisms are characteristically able to invade the root hairs of large number of legumes and incite production of root nodules, wherein the bacteria occur as intracellular symbionts. All strains exhibit host range affinities mean they are able to incite nodules only on some selected leguminous roots. The bacteria in nodules present in pleomorphic forms as bacteroids, which are involved in fixing atmospheric nitrogen.

Further Descriptive Information

In young cultures the cells are short rods, but in old cultures or under adverse environmental conditions the cells are commonly pleomorphic. Under unfavourable conditions sometimes become swollen, either globular, ellipsoidal, club shaped or branched. The unfavourable conditions inciting such deformities may include extremes of temperature and pH, low oxygen tension and low concentration of calcium or magnesium in the medium or excessive amounts of various amino acids, certain alkaloids, glycosidase, dyes or antibiotics. Excess of yeast extract in the medium can also incite cell distortions. Granules of poly- β -hydroxy butyrate are common in old cells and on staining appear as coloured bands. Within root nodules, the pleomorphic bacteroids do not contain polyphosphate inclusions and usually no glycogen granules, but may have large concentrations of poly- β -hydroxy butyrate. All strains produce water soluble extracellular polysaccharides, the principal constituent of which is acidic heteropolysaccharide. All strains grow rapidly on a mineral salt medium containing yeast extract and any one of a wide variety of carbohydrates, particularly, mannitol, glucose, arabinose, fructose, galactose and sucrose. Best growth is achieved mainly with mannitol and glycerol. Acid is usually produced from carbohydrates to a moderate degree and is best estimated by incorporation of bromothymol-blue indicator in the medium. Dextrin is rarely utilized. Intermediates of tricarboxylic acid cycle can be utilized as sole carbon source, provided the basal medium has sufficient Ca^{+2} and Mg^{+2} . Most strains lack the ability to absorb congo-red from the Yeast-extract mannitol, agar medium containing Congo-red dye. This results into colourless, white or faintly pink colonies, whereas contaminant colonies are often deep red or of other colours. Temperature range is highly strain dependent and ranges from 4 - 42.5°C; however growth at 4°C is rare, and only *R. meliloti* can grow at 42.5°C. The temperature optima for large number of strains is 28-32°C and maxima at 38°C. The pH range for the genus is 4.5 to 9.0 depending upon strains. *R. meliloti* is the most alkali tolerant species. *Rhizobium* strain are weakly proteolytic, but most strains produce a slow digestion in litmus milk, forming an upper clear "serum zone", usually with a slight alkaline reaction or no change. *R. meliloti* strains tend to produce acidic reaction.

Phosphate solubilizing microorganisms

Phosphate solubilizing microorganisms include bacteria, fungi, actinomycetes and yeast, capable of dissolving inorganic phosphates. They can grow in medium having tricalcium, iron and aluminium phosphates, hydroxyapatite, bone meal rockphosphate and similar insoluble phosphate compounds as the sole phosphorus source. These microbes not only assimilate phosphorus but in their presence a large portion of soluble phosphate are released in quantities in excess of their own requirement. The most efficient phosphate solubilizing bacteria belong to genera *Bacillus* and *Pseudomonas*, though species of *Brevibacterium*, *Corynebacterium*, *Micrococcus*, *Sarcina* and *Achromobacter* have also been reported to be active in solubilizing insoluble phosphates. The reported *Bacillus* sp include *B. bravis*, *B. cereus*, *B. circulans*, *B. firmus*, *B. licheniformis*, *B. megaterium*, *B. mesentericus*, *B. mycoides*, *B. polymyxa*, *B. pumilus*, *B. pulvifaciens* and *B. subtilis* isolated from the rhizosphere of legumes, cereals like rice and maize, areca nut, palm, oat, jute and chilli. Similarly the reported *Pseudomonas* species include *P. striata*, *P. cissicola*, *P. fluorescens*, *P. pinophilum*, *P. putida*, *P. syringae*, *P. aeruginosa*, *P. putrefaciens* and *P. stutzeri* isolated from root zone of plants fertilized with fresh organic matter, rhizosphere of leguminous and cereal crops, desert soils, antarctica lake, rhizosphere

of brassica, chickpea and soybean. In addition *Erwinia* spp *Escherichia freundii*, *E. intermedia*, *Serratia phosphaticum* and *Xanthomonas* spp have also been found to be equally efficient in solubilizing insoluble phosphates.

The most efficient phosphate solubilizing fungi belongs to genus *Aspergillus* and *Penicillium*. The common species belonging to the genus *Aspergillus* include *A. niger*, *A. flavus*, *A. nidulans*, *A. awamori*, *A. carbonum*, *A. fumigatus*, *A. terreus* and *A. wentii* obtained from root nodules of legumes and rhizosphere of maize, soybean, chilli, tista soils, acidic lateritic soils and compost. A few species of *Cephalosporium*, *Alternaria*, *Cylindrocladium*, *Fusarium*, *Paecilomyces fuisporus*, *Penicillium digitatum*, *P. simplicissimum*, *P. aurantiogriseum*, *Rhizoctonia* sp, *Sclerotium rolfsii* are also good solubilizers of insoluble phosphate. A few species of yeast viz. *Torula thermophila*, *Saccharomyces cerevisiae* and *Rhodotortula minuta* have also been reported to solubilize inorganic phosphates.

Biofertilizer Production Technology

Facilities required for a biofertilizer production unit

Building and infra-structure

Requirement of the cover area and type of construction depend upon the size of the unit and the total likely out put. But usually it varies from 4500 sq. ft (minimum) to >10,000 sq. ft.

The building should have following working area

1. **Main working laboratory** - With sanitary fittings for sinks (water supply and drain) and three phase power supply line for incubators, refrigerator and other equipments.
2. **Incubation/ transfer room** - preferably fitted with window mounted air conditioner for working comfort. The room should be free from dust and contamination. All windows should be sealed. A provision for an exhaust fan should be kept duly protected with cloth filter. Coolers or fans should not be used in this room. The entry should be through double doors. This can be arranged by making a small cabin at the entrance. The person entering the transfer room will first open the door and enter into the preparatory cabin. Makes himself hygienic, wear sterile apron, cap and gloves and then enter into the main room through other door.
3. **Incubation/Fermentation room** - Depending upon the total output the size of the room can vary from 200 - 400 sq.ft. As the room must have effective temperature control system therefore its location is very important. In areas where summers are very hot then ensure that, it's none of the walls and roof is exposed to the sun directly. It should have enough of space to accommodate 2-4 big fermenters (100-200 lit cap.), 25-40 seed fermenters and a small rotary shaker. For effective temperature control the room should be fitted with 2 adequate capacity air conditioners and 2-3 heat blowers with thermostatic control. If room size is above 500 sq. ft then instead of window mounted air conditioners, split type air conditioners should be preferred with in-built heating system. The room must have at least two air conditioners to ensure temperature control even in the event of one being closed for repair or maintenance. Fermenter room should have necessary pipe fittings for water inlet, drain, cooling water and broth line etc. Bigger fermenter can be cooled or heated by the supply of cold or hot water through their jacket or through

their cooling coils. In such cases they can be kept in separate fermentation room without temperature control system.

4. **Media preparation room** - This room should be divided into two, parts. In one part all the essential chemicals, balances and media preparation tanks/tubs are kept, while in other part autoclaves, ovens and distillation apparatus are to be kept. The room should have adequate power supply and water supply provision for autoclaves ovens and other equipments. Media preparation room should be attached to the washing room from internally as well as from outside for cleaning of glassware etc.
5. **Chemical store**
6. **Bottling/packaging room** - This room should also be provided with air conditioning for working comfort. As the packaging and bottling is to be done under complete sterile conditions the coolers and fans etc can not be used. This room, in its other requirements is very much similar to the transfer room.
7. **Carrier/raw material store**
8. **Finished goods store** - To keep the biofertilizers under optimum temperature this room is also to be provided with appropriate cooling system.

Other infra-structural requirement

1. 3-phase electric connection
2. Access to clean soft water
3. Proper drainage facilities for disposal of contaminated material and discarded broth
4. Proper connectivity to road for easy transportation of raw material and finished goods.

Essential equipments, glassware, plastic ware for general laboratory working and quality control

a. Equipments

- | | |
|--|---------|
| 1. Portable vertical autoclave (approx 300 x 450 mm chamber size) | one |
| 2. Hot air oven 2.5 - 4 cft | one |
| 3. Distillation apparatus preferably Barndtsted type, cap. 2 lit/hr | one |
| 4. Balance 10 gm to 5 kg capacity | one |
| 5. Chemical precision balance 0.01 to 100 gm capacity | one |
| 6. Refrigerator 165 lit to 290 lit | one |
| 7. BOD Incubator 9 cft (5 - 50°C) with small orbital shaker platform on lower shelf and two stationary platform | one |
| 8. Laminar air flow work station, working table size 3' x 2' | one |
| 9. Rotary shaker (capable of holding 25, flasks) | one |
| 10. Binocular research microscope with phase contrast attachment (MOST IMPORTANT) having turret condenser and matching phase objectives of 10x, 40x and 100x magnification, 10x wide field eye pieces and telescopic centering eyepiece. | one set |
| 11. Small oil free vacuum and air compressor pump with necessary vacuum and pressure gauges | one |
| 12. Air conditioner for transfer chamber (1.5 ton) | one |
| 13. pH meter (portable or pen type) | one |
| 14. Spectrophotometer/ colorimeter (Optional) | one |
| 15. Vortex mixer/ cyclo mixer | one |
| 16. Magnetic stirrer | one |

17.	Variable volume micro-liter pipettes,	0.01 - 0.2 ml	one
18.	- do -	0.2 - 1 ml	one
19.	- do -	1.0 - 5.00 ml	one
20.	- do -	2.0 - 10 ml	one

b. Glassware (all Borosilicate glass)

1.	Test tubes 15 x 150 mm	100 no
2.	Test tubes 18 x 150 mm	100 no
3.	Mc Cartney bottles 15 ml (JSGW make)	100 no
4.	Petri dishes 100 x 17 mm	50 pairs
5.	Conical flasks 100 ml, 250 ml & 500 ml	24 each
6.	Pipettes 1,2 and 5 ml capacity (B type)	6 each

c. Laboratory Plastic-ware

1. Test tube stands
2. Measuring cylinders, 0, 50, 100, 50 and 1 liter cap.
3. Beakers (TPX or PP), 250, 500 and 1000 ml
4. Microtips of appropriate sizes for microliter pipettes
5. Microtip boxes of matching sizes
6. Utility trays. Pipettor stand

d. Additional items required for media preparation

1. Large plastic buckets of 50-100 liter capacity
2. Absorbent/non-absorbent cotton
3. Glass wool
4. Thread balls
5. Aluminium foils
6. Thick brown paper sheets for sterilization
7. Electric heaters/hot plates/LPG stove

e. Equipments and other items for mass multiplication and fermentation

1.	Large vertical autoclave or Horizontal autoclave. Size as per requirement	one
2.	Small mother culture fermenters 1-2 lit cap.	10 no
3.	Portable seed fermenters of 10/20 or 30 lit cap.	10-30 no
4.	Stainless steel fermenters 100 - 300 liter capacity	2-4 no
5.	Air compressor (preferably oil-free type) with storage tank and automatic pressure regulator, 250-500 liter capacity or If oil-free type is not available then oil-bound compressors can also be used, but in such cases oil-cum-moisture trap should be used.	1 no.
6.	Air conditioners- Depending upon the room size and requirement it can be two 1.5 ton window AC, or 2.0 ton capacity split type AC's. Always install two AC's instead of one to ensure continuous running, even if one is stopped for repair or maintenance.	2 no.
7.	If feremeters are of higher capacity i.e. > 100 liter then install water cooling tower of appropriate capacity for cooling the fermenters or install thermostatically controlled hot and cold water circulation system	1 no.
8.	Thermostatically controlled heat blowers or heat convectors for areas	3 no.

where winters are chilly.

9. Peristaltic pump for dosing the fermenters. Continuous flow type cap. 10 lit liquid/hr.

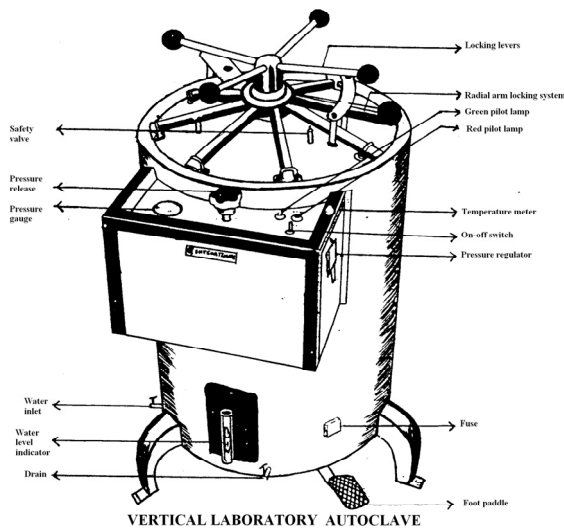
1 no

f. Equipments/ machines for finished goods preparation and packaging

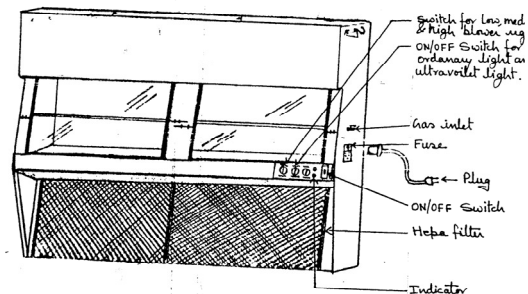
i. For carrier based inoculant

1. Small top loading dial type or counter weight type balances
2. Polythene heat sealers
3. Measured volume, broth injecting machine
4. Appropriate sized vertical or horizontal autoclave
5. Label printing machine
6. Polypropylene bag for carrier sterilization
7. LDPE printed bags for finished bags
8. Gummed plastic tape and tape dispensers

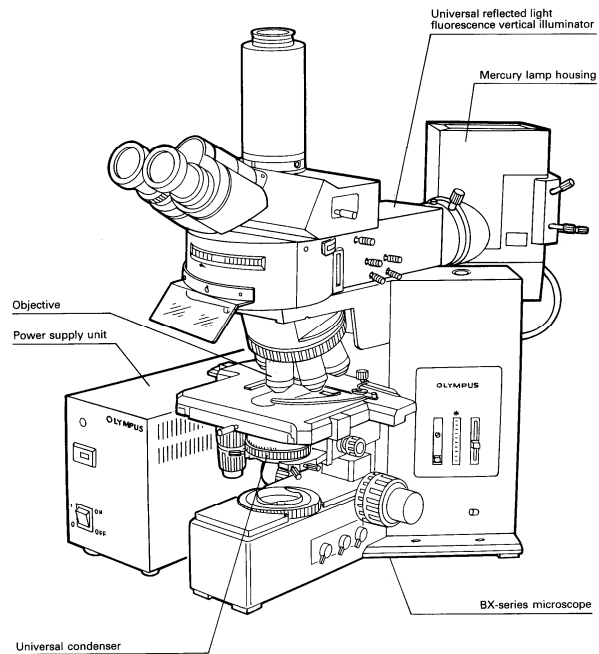
If the volume of the production is quite high, then the entire process can be made automatic by installing form-fill-and-seal machine. Such machines are combination of liquid filling and powder filling units and are installed in a completely sterile room. In such machines sterilized carrier powder is fed from one feeder, while broth is fed directly from fermenters at another point. While in operation, the machine first makes a bag, in which a measured amount of carrier is poured followed by measured volume of broth and bag is sealed. Mixing of the contents is done by gently beating the bag with the help of paddles, while moving over a conveyor belt. Liquid inoculants can be filled in bottles with the help of automatic bottle filling machines under sterile air flow.



Vertical Autoclave



Laminar Air Flow Work Station



Phase Contrast Microscope

Mass multiplication of organisms

In biofertilizer production the specified bacteria are serially up-scaled in a step-by-step process from a slant to bulk fermenters. Various steps of up-scaling are as follows:

Slant

Test for purity

Small conical flasks of 100-250 ml cap.

Small mother culture fermenters of 1- lit cap.

Check for purity

Portable seed fermenters

Check for purity

Large stainless steel fermenters

Check for purity

Release for production

Basic requirements of biofertilizer organisms for rapid growth

Almost all the microorganisms being used in biofertilizers such as *Rhizobium*, *Azotobacter*, PSB etc are aerobic and are basically surface grower. *Azospirillum* is although a microaerophile in N-free conditions but can grow aerobically under constant supply of nitrogen. Optimum temperature requirement for different bacteria although range between 28 to 35°C but it is bacteria specific. Rhizobia usually require a temperature range from 28 - 30°C. *Azotobacter* and PSB (*Pseudomonas* and *Bacillus*) grow well at 30 - 32°C, while *Azospirillum* grows well at 34-35°C. Ambient temperature requirement can be achieved by maintaining appropriate temperature in the incubation room. Besides temperature, second most essential requirement for fast multiplication of these organisms is continuous supply of oxygen for respiration and continuous change in surface area. Both these requirements can be accomplished by either continuous shaking of fermentation vessels (small to medium sized flasks kept on rotary shaker) or by bubbling sterile air at reasonable rate through the broth (as in fermenters). The rate of aeration is usually kept at about 10 liter of air per liter of the broth per hour.

Inoculating liquid broth in small flasks from slants

For mass multiplication of microorganisms their biomass is upgraded serially from slants to big fermenters. The first stage in this process is of the preparation of mother culture. For this proceed as follows:

1. Prepare appropriate liquid medium in small conical flasks of 100 or 250 ml capacity duly stoppered with cotton plugs. The flasks should be filled with only 50% of their capacity.
2. Autoclave at 121°C for 20 min.
3. Allow to cool the medium. Transfer these flasks and slants of the required bacterial culture in transfer chamber or laminar-air-flow cabinets.
4. Transfer a loopful of bacterial growth from slants to the conical flasks and replace the plugs.
5. Incubate at appropriate temperature on rotary shaker for 5-10 days, under continuous shaking.
6. Check for purity before using this culture as mother culture

Inoculation of liquid media with specified quantity of liquid inoculum

For serial up-scaling next step is transfer of liquid mother inoculum to either small seed fermenters or to higher capacity conical flasks or to aspirator bottle fermenters. For inoculation of 10-20 lit capacity seed fermentors mother cultures should be prepared in 1-2 lit capacity aspirator bottle fermenters with outlet tube at the base. Such bottles can be inoculated from their top mouth by opening the plug inside the transfer chamber.

Small glass fermenters and seed fermenters

Once the mother culture is ready in small flasks, the next step is to cultivate the organisms in small seed fermenters. Depending upon the requirement and facilities different types of seed or portable fermenters are available. Fermenters are basically air-tight vessels with arrangement for sterile venting for oxygen supply and agitation. Most of the small fermenters are autoclaved by placing them in appropriate sized autoclaves. Some models of small portable fermenters are shown below:

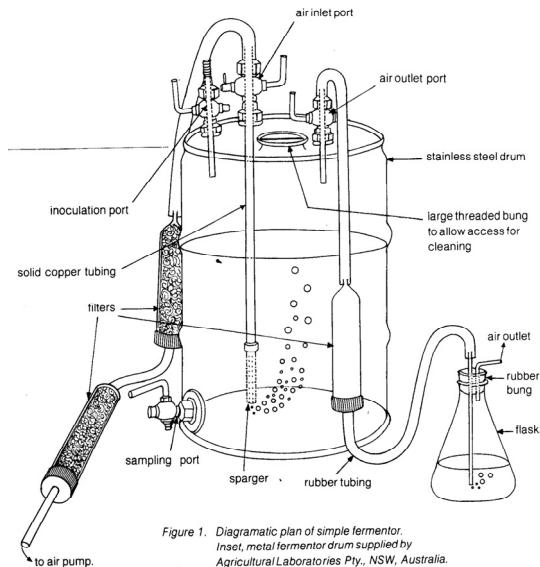


Figure 1. Diagrammatic plan of simple fermenter.
Inset, metal fermenter drum supplied by
Agricultural Laboratories Pty., NSW, Australia.

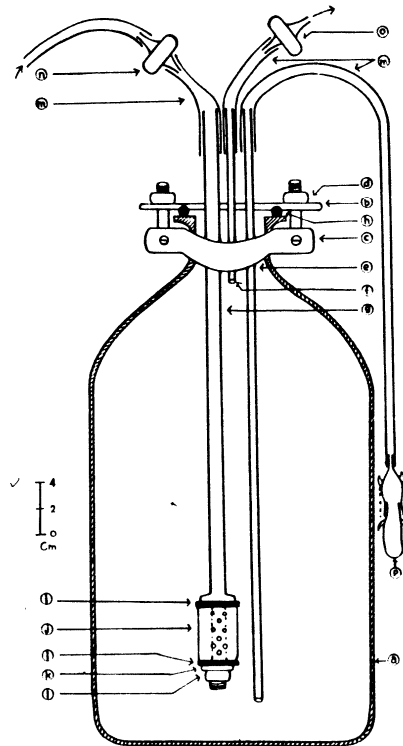


FIG. 1 MODIFIED PORTABLE FERMENTOR

Portable Stainless Steel Fermenter 30 lit cap

Glass Fermenter of 10 li Cap

Evaluation of growth and quality checking

Depending upon the type of organism, quantity of inoculum, rate of aeration and temperature conditions, fermentation period varies from 48 hrs to 7 days. Most of the slow growing rhizobia at 1% inoculum level takes 7-8 days to achieve a population level of 1×10^9 cell/ml, while fast growing rhizobia require 3-5 days. *Azospirillum* takes 2-4 days to achieve a cell count of 5×10^9 cell/ml. Phosphate solubilizing bacteria such as *Bacillus polymyxa* and *Pseudomonas striata* takes 3-5 days while *Azotobacter* in N-free media takes -7 days to achieve an optimal cell count.

Using sterile techniques, take out a small sample for purity checking and for evaluation of total cell count. First sampling should be done after 24-48 hrs of inoculation for fast growers and after 48-72 hrs for slow growers. Repeat sampling after another 24 to 48 hrs of first sampling. Perform following tests as per the standard procedures:

- Check for pH
- Check for contamination by gram staining
- Check for total cell count with Halber or Petroff Hausser counting chamber
- Measure optical density by spectrophotometer
- Samples should also be tested randomly, for confirmation by total viable cell count method on their respective agar media.

- If the product of these fermenters is to be used as inoculum for large SS fermenters then wait till the results of total viable cell count on agar plates are available.

Producing inoculum in large steel fermenters

For production of bacterial broth in mass scale for commercial production, stainless steel fermenters of capacity ranging from 100 lit to 2000 lit are being used. All steel fermenters are essentially steel pressure vessels, capable of tolerating a steam pressure of up to 45 psi with provisions for cooling coils, air spargers, air outlet, inoculation, sampling and drain ports and pot for cleaning and filling etc. All the ports are guarded with Teflon seal ball valves. 100-300 lit capacity fermenters are usually not provided with mechanical agitation system while fermenters of capacity above 300 lit are provided with mechanical stirrers. In biofertilizer industry although, different sizes of fermenters are in use but non-agitated fermenters are most popular because of their being less prone to contamination. Higher capacity fermenters with mechanical stirrers are not very much successful and tend to get contaminated, usually in case of rhizobia. All manufacturers in Australia use 100-300 lit capacity non-agitated fermenters. Agitated fermenters of capacity above 500 lit to 1000 lit installed by NifTAL at their Asia centre, Bangkok, Thailand were finally discarded mainly because of their contamination problems. 1000-2000 lit capacity agitated fermenters being used by different manufacturers in the country are also not free of contamination problems. The designs of a fermenter, found very effective is given below.

Inoculation level and incubation time

Inoculation levels are usually kept at 0.1 to 1.0% providing 10^6 to 10^7 cells/ ml of liquid media. The mean generation time (time required to double the cell number - MGT) will be greatly affected by the stage of the growth of starter culture and the resultant lag phase, temperature of incubation, availability of nutrients, aeration and off-course the size of inoculum.

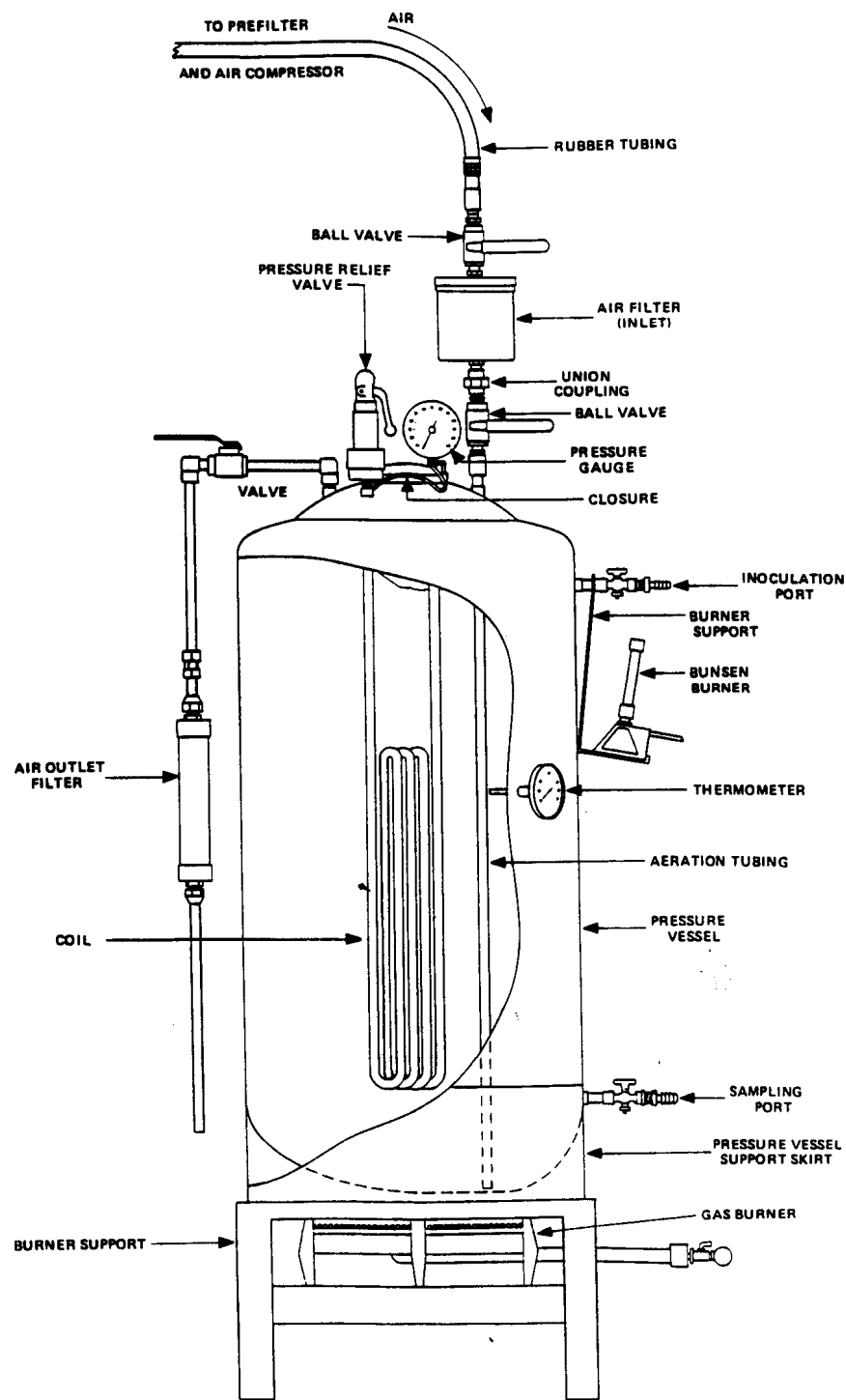
The following minimum batch time will be necessary for a starter consisting of 1×10^9 viable, actively growing cells/ml to provide a finished broth of 5×10^9 cells/ ml.

Type of bacteria used	Time required to achieve full growth when provided with inoculum at	
	1%	5%
Fast growers having MGT of 2-4 hr	25 - 52 hr	18 - 36 hr
Slow growers having MGT of 6 - 12 hr	78 - 156 hr	54 - 108 hr

In higher capacity fermenters 5-6% inoculum is usually preferred over 1%, which is reached by progressive scaling up through flasks, small mother culture fermenters and seed fermenters.

For seed fermenter an inoculum level of 0.1 to 1.0% should be preferred. Low inoculum levels extend the incubation time and allow sufficient scope for the contaminants (if any) to grow (contaminants usually grow very fast and are visible

through microscope with in 12-18 hrs of inoculation) and allow the manufacturer to complete checks for their purity, before it is harvested for further use.



Stainless Steel Fermenter 100 lit Capacity (NifTALType)

Storage of finished broths should be kept at minimum, but if it is unavoidable, cultures should be stored in the same vessel at 4°C. This can be done by closing all the valves of fermenter, except air outlet valve and by passing chilled brine through cooling coils or through the jacket.

PREPARATION OF INOCULANT

After the microorganisms have been cultured in liquid broth with a final population level $>5 \times 10^8$ (for *Azotobacter*) to 5×10^9 /ml (in case of *Rhizobium*, *Azospirillum* and PSB) of the media, they are mixed with carrier materials. Mixing of broth with carrier material is a very important stage, as the required population level of bacteria can not be maintained at significantly high level in liquid broth. At ambient temperature, in liquid broth, the growth sooner or later enters into death phase and total count per milliliter decline drastically after 7-10 days of stationary phase, unless stored at low temperature (4°C).

Carrier material carries the appropriate load of microorganisms for a significant period of time (shelf life) and provides all the necessary conditions suitable for their survival and growth. The carrier material should be suitable not only for microbe's survival, but also be ideal for seed treatment and be able to stick to the seed coat in required quantity. Although, during the last 30 years, various carriers have been identified by different workers and are being used by different manufacturers, but among them most popular ones are peat, peat soil, lignite and charcoal (burnt wood coal).

Ideally a carrier material should meet the following requirements.

1. The material should be finely ground to allow thorough mixing with other component and be compatible with its final use. More than 75% of the material should pass through the 75 micron IS sieve.
2. The pH should be 6.5 to 7.0. If not within the range, should be suitably amended with pH correcting materials. Peat and lignite are usually acidic and needs pH correction by the addition of precipitate grade calcium carbonate.
3. Should possess good moisture holding capacity. Ideally a carrier material having moisture holding capacity in excess of 100% (capable of holding equal or more amount of water by its weight) is considered suitable.
4. The carrier material should be sterilizable to favour survival of the desired microorganisms.
5. Should be free of toxic materials.

Sterilization of carrier material

In India most of the manufacturers use unsterile carrier and mix with pure broth. This leads to incorporation of unspecified number of contaminants. When the packets are stored at temperatures below 25°C, these contaminants do not pose much threat to the inoculant quality for 3-4 months. But when the packets are exposed to temperature ranging from 28-32°C (which is very common and is generally prevailing in India), the contaminants grow rapidly along with the biofertilizer organisms and their population becomes at par with biofertilizer organisms within 2-3 months time. In such cases due to high contamination load, the inoculants become ineffective within 2-3 months. The worst scenario will be when packets are exposed to temperatures

in excess of 34°C. At this temperature, even a brief exposure of 5-6 days is enough to spoil the quality of the inoculant.

Under such circumstances it is very essential that all inoculants be prepared with sterile carrier material under complete sterile conditions. A sterilized carrier can only be expected to remain sterile if it is retained in the same container, in which it is sterilized. Under such situations, the carrier is sterilized in the packets itself.

Sterilization of carrier bags

For sterilization of carrier in bags of appropriate sizes, first calculate the quantity of carrier to be inoculated with pre-determined quantity of the broth to obtain the final weight. Suppose if the final package is to be 200 gm and the moisture holding capacity of the carrier is 120%, the requirement of the carrier and broth will be about 110 gm carrier and 90 ml broth. Therefore if the above formulae holds true then for 200 gm pack size fill each carrier bag with 110 gm of carrier material. Charcoal powder easily absorbs the liquid after sterilization and charcoal filled-inoculated bags require very little manipulation for dispersal of liquid between its particles. Lignite being rich in waxy contents is difficult to mix and usually require lot of manipulation in the form of smudging for mixing the contents. A mixture of charcoal and lignite can be a suitable answer to the problem. Ratio is to be worked out with some experimentation. Usually 25-30% charcoal mixed with 75-70% lignite serves the purpose.

Polypropylene bags of about 180-200 gauge are suitable containers for autoclaving. HMHDPE bags can also be used, but usually HMHDPE bags develop micro-holes during autoclaving.

Fill appropriate quantity of carrier material containing approximately 10% moisture (on dry weight basis) and heat seal the bags with a cotton wick at one end to allow the steam and air to escape. Keeping a cotton wick prevent the bursting of bags in autoclaving. For sterilization, carrier bags placed in used gunny bags and loosely tied at the mouth serves as good sterilization containers. Specially prepared metal or wood containers having holes all around the walls can also be used as sterilization containers. Sterilization is accomplished at 121°C for 1 hr. After the completion of withholding time, keep the autoclave on very-slow exhaust and let the contents cool overnight. take out the sterile packets/bags only after cooling. Remove the cotton wick and seal the hole by heat sealing. Such autoclaved bags can remain sterile for long periods and to save the time, during seasons, sterile bags can be prepared well in advance of production season, during off-peak periods.

Inoculation of broth in carrier bag

To prepare the inoculant packet a measured volume of fully grown broth having a total cell count $> 5 \times 10^9$ cells/ ml is to be injected aseptically in to the carrier bags.

Methods of Application

Selection of biofertilizers:

While going in for the use of biofertilizers, it is essential to select the right combination. For increased availability of nitrogen and phosphorus, always use nitrogen fixing biofertilizer and phosphate solubilizing biofertilizer (PSB) together in equal quantities.

(a) **For pulses and legume oil seeds** - like moong, urad, lentil, pea bengal gram, arhar, cluster bean, groundnut, soybean, berseem, leucern and all types of beans and other legumes and pulses.

***Rhizobium* + PSB in equal quantities to be used only as seed treatment**
Remember *Rhizobium* should be specific to that crop/plant.

(b) **For all nonlegume crops-** such as wheat, rice, maize, bajra, oats, barley, mustard, sesame, niger, onion, potato, sugarcane, cotton etc and all types of vegetables and fodder crops. Plantation crops like banana, citrus, pomegranate, coconut, coffee, tea, rubber, mulberry etc.

- In light textured soils such as sandy loam, loam or sandy type with low moisture holding capacity - **use *Azotobacter* + PSB in equal quantities.**
- In heavy textured soils such as clay-loam or clay type with high moisture holding capacity including submerged or waterlogged soils – **Use *Azospirillum* + PSB in equal quantities.**
- If the soil is medium loam with moderate moisture holding capacity and pH more acidic – **use *Azotobacter* + *Azospirillum* + PSB in a ratio of 1:1:2 respectively.**

Method of application:

Biofertilizers can be applied to different crops and plants by three different ways.:

1. **Seed treatment** Suspend 200 gm each of nitrogen fixing and PSB in 300-400 ml of water and mix thoroughly. Pour this slurry on 10 to 12 kg of seed and mix by hands, till all the seeds are uniformly coated. Dry the treated seeds in shade and sow immediately. For acidic and alkaline soils it is always advisable to use 1 kg of slacked lime or gypsum powder respectively for coating the wet biofertilizer treated seeds.
2. **Seedling root dip treatment:** - Suspend 1 to 2 kg each of nitrogen fixing (*Azotobacter*/*Azospirillum*) and PSB into just sufficient quantity of water (5-10 lit depending upon the quantity of seedlings required to be planted in one acre). Dip the roots of seedlings in this suspension for 20-30 min before transplanting. In case of paddy make a sufficient size bed (2mt x 1.5mt x 0.15mt) in the field, fill it with 5 cm of water and suspend 2 kg each of *Azospirillum* and PSB and mix thoroughly. Now dip the roots of seedlings in this bed for 8-12 hours (overnight) and then transplant.
3. **Soil treatment:** - For soil treatment depending upon the total number of plants per acre 2-4 kg of *Azotobacter*/*Azospirillum* and 2-4 kg of PSB are required for one acre. Mix two types of biofertilizer in 2-4 liters of water separately and sprinkle this suspension on two separate heaps of 50-100 kg of compost. Mix the two heaps separately and leave for incubation overnight. After 12 hours, mix the two heaps together. For acidic soils mix 25 kg lime with this mixture. In plantation crops apply this mixture at the root zones by dibbling. In some field crops the mixture is broadcast evenly in the moist field and mixed with soil just before sowing. In sugarcane the biofertilizer manure is to be applied in furrows near the root zone, after 30-40 days of planting and covered with soil. In potato it is to be

applied after 20 days of planting or at the time of earthing-up operations. In case of sugarcane and potato, if setts/tubers are not treated with plant protection chemicals then biofertilizer compost mixture can be applied in furrows immediately before planting

A KEY TO BIOFERTILIZER USE

For Crops	Biofertilizers recommended & Doses
1. Pulse crops like moong, urad, arhar, cowpea, lentil, pea, bengal gram, all beans, ground nut, soybean, leucern, berseem and other legume crops.	<i>Rhizobium</i> 200 gms + PSB 200 gm for every 10 kg of seed as seed treatment.
2. All nonlegume crops like wheat, seed sown upland paddy, barley, maize, cotton, sorghum, bhindi, mustard, sunflower, niger etc. and other non legume crops taken by direct seed sowing.	<i>Azotobacter</i> 200 gms + PSB 200 gms for every 10 kg of seed as seed treatment.
3. Jute	<i>Azospirillum</i> 200 gms + PSB 200 gms for every 10 kg of seed as seed treatment.
4. Vegetables like tomato, brinjal, chilli, cauliflower, cabbage etc. and other transplanted crops.	<i>Azotobacter</i> 1kg + PSB 1 kg for one acre as seeding root dip method.
5. Lowland transplanted paddy.	<i>Azospirillum</i> 2 kg + PSB 2 kg for one acre as seedling root dip for 8 -12 hours.
6. Potato, ginger, colocassia, turmeric and jhum paddy.	<i>Azotobacter</i> or <i>Azospirillum</i> 4 kg + PSB 4 kg/acre mixed with 100 - 200 kg compost and applied in soil.
7. Standing plantation crops like tea, coffee, rubber, mulberry and fruit trees.	2 - 3 kg <i>Azotobacter</i> / <i>Azospirillum</i> + 2-3 kg PSB mixed with 200 kg Compost for one acre and applied as soil treatment. This treatment is to be done 2 to 3 times a year with a gap of 4- 6 months
8. Sugarcane	5 kg <i>Acetobacter</i> mixed in sufficient water for setts dipping treatment

Chapter 3

ORGANIC FERTILIZERS AND COMPOST PRODUCTION TECHNOLOGY

COMPOSTING NEED & TECHNIQUES

Introduction

Organic materials like crop residues, leaf litter, green weeds, and byproducts of agro-industries, urban and rural habitation wastes and marine biomass are important source of nutrients for plants. The natural processes involving the flora and fauna of soil and water recycle these waste materials to replenish carbon, nitrogen, phosphorus and sulphur through biological and chemical cycles. Organic materials are recycled and converted into compost/ farmyard manures. Animal wastes like cattle dung, goat dung, poultry manure are also composted and used as Biomanures.

Principle and Techniques

Process of composting

The biodegradation process is carried out by different groups of heterotrophic microorganisms like bacteria, fungi, actinomycetes etc. Organic materials undergo intensive decomposition under thermophilic and mesophilic conditions in heap, pits or tanks with adequate moisture and aeration and finally yield a brown to dark coloured humified material, called compost.

Factors controlling the process of composting

Composting process and quality of compost is mainly controlled by the following factors.

- 1. C/N ratio of organic materials:-** The conversion of organic material into manure is essentially a microbiological process and influenced by the proportions of carbonaceous and nitrogenous materials that are present in the organic wastes. To start with, microorganisms need carbon for their growth and nitrogen for protein synthesis. It is observed that C/N ratio of 30 (26-40) of raw material is most favourable for efficient composting. When the organic materials are poor in nitrogen, i.e. C/N ratio is wide such as in cereal residues of Wheat, Paddy, Jowar, Bajra, Maize, sugarcane trash (C/N ratio 60-90), stalks of cotton, jute and sawdust (C/N ratio <100), microbiological activities diminishes, as they do not get sufficient amount of nitrogen. Consequently several cycles may be required to degrade carbonaceous materials and this may prolong the period of composting. On the other hand, if C/N ratio is low i.e. less than 30, the proportion of nitrogen is in excess of the requirement of microorganisms, consequently, the process of decomposition is faster and inassimilable nitrogen is lost in the form of ammonia gas. In case of preparation of bio-manure from cattle dung and night soil, 20-40% nitrogen is lost in the form of ammonia during composting process. Therefore, it is extremely essential to mix high C/N ratio materials with low C/N ratio materials to adjust the C/N ratio of raw materials between 30-40 to control the loss and retain important plant nutrients in the final manure, influencing the quality of manure.
- 2. Shredding and blending of raw materials:-** The raw materials used for composting should be shredded and blended properly before composting. The

- most desirable particle size for composting is less than 5 cm; small size raw materials become more suitable to bacterial attack and microbes get more surface area to work. But in any case it should not be less than 2 cm to avoid compaction.
- 3. Moisture:-** The moisture of decomposing organic materials should be maintained around 50-55%. During the period of decomposition by aerobic mode, in higher moisture (80-90%) conditions, oxygen gets dissolved in water and renders the conditions anaerobic, which results in bad odour and incomplete decomposition. Moisture percentage below 30% reduces microbial activity and slows down the composting process. However, in prepared or mature compost, 10-15% moisture should be maintained for retaining the microbial population of compost.
 - 4. Aeration:-** It is essential to maintain proper aeration in composting unit, whether pits, heaps or tanks. Therefore, the breadth of tank/pit/heap should not be more than 5 feet and depth/height should not be more than 3 feet. The ideal size of the unit can be 12'x5'x3'. The length of the composting unit can be as per the availability of raw materials, but it should not be more than 20 ft.
 - 5. Temperature:-** Aerobic decomposition of organic material is an exothermic reaction. In aerobic process, 484-676 Kcal energy is released per glucose molecule. Therefore, temperature of the compost pile increases to 55°C-65°C after 2-5 days of composting. High temperature is essential for the destruction of pathogenic microorganisms and weed seeds. Decomposition is faster at thermophilic stage, where thermophilic microorganisms replace mesophilic microorganisms. The extent of rise in temperature in compost depends on the type of material being composted and on the size of heap or pile of raw materials. High temperature of compost heap or pile indicates that biodegradation of organic materials is in progress and ensures good quality of compost, free from pathogenic microbes and weed seeds.
 - 6. pH:-** The initial pH in compost heap is generally slightly acidic, around 6.0. The production of organic acids during the early stages of composting renders further acidification (pH-4.5-5.0); but as the temperature increases; it turns to slightly alkaline pH (7.5-8.5). pH of mature compost is 7.5 to 8.5. (Gaur *et al* 1984).

Some Common methods of composting

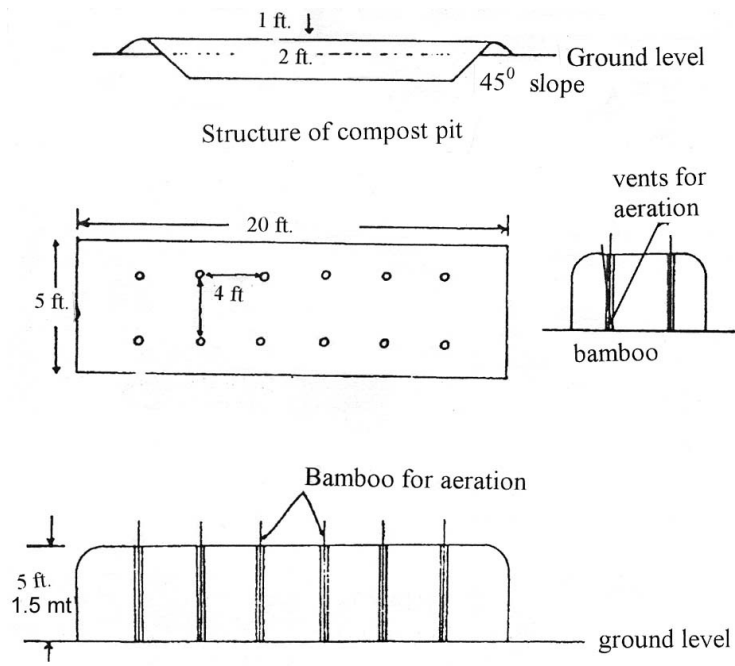
Compost can be produced in heaps or pits or aerated tanks (NADEP compost). But in all methods, sufficient aeration and moisture must be maintained. It is recommended that in summer, compost must be prepared in pits to avoid moisture loss and consequent delays in composting; while in rainy season, compost must be prepared above ground in heaps or tanks to avoid water accumulation. Compost units should be in shade (under tree) to avoid direct influence of rain, sunlight or extremes of temperature.

1. Indore method

Sir Albert Howard in Indore systematically developed Indore method. In this method, (i) a pit of 9'x5'x3' size is prepared, (ii) it is partitioned into 3 equal parts of which, two parts are filled and third part is left empty for turning, and (iii) input matter includes dry and green agricultural waste, grasses, etc. soaked with water and cattle-dung slurry followed by cattle-dung and soil layer 1" to 2" thick. After filling, the tank is sealed with 3" thick layer of soil covered with cowdung and mud plaster. The process is accelerated by turnings (Fig 1), whereby aeration, mixing of composting materials and moistening (if necessary) is done. This causes more or less total disintegration of matter, yielding brown homogeneous manure in about three months. Under this

process of decomposition, losses of organic matter and nitrogen are heavy amounting to 40 to 50 per cent of the initial and the manure resembles the traditional farmyard manure in appearance and properties.

This method of manure preparation, however, involves a considerable labour in building up the heap/pit to proper shape and periodical turnings rendering it impracticable and expensive, where large quantities of materials have to be processed. However, turnings of the biomass are not necessary; decomposition can go on to the desired extent, without turning if adequate moisture level is maintained. The average composition of manure prepared by the Indore method has been found to be having 0.8 % nitrogen, 0.3-0.5% phosphate and 1-1.5 % potash.



Design of Compost heaps in Indore Method

2. Fowler's method of activated composting

Fowler worked out the process of activated compost in which fresh raw materials are incorporated in an already fermenting heap so that the established and large microbial population brings about quicker decomposition. The process is useful particularly where offensive materials like night- soil are to be quickly and effectively disposed off.

3. NADEP Method of Composting

This method is developed by an old Gandhian worker of Maharashtra (from Pusad), called Narayan Deorao Pandharipande and therefore derives its name abbreviated as NADEP, to the method of composting.

a. Construction of tank:- NADEP compost is prepared in an aerobic tank made up of bricks and cement. The size of the tank is 12'x5'x3'. All the four walls of NADEP tank are provided with 6" vents by removing every alternate brick after the

height of 1 ft. from bottom for aeration. Tank can be constructed in mud mortar or cement mortar (Fig. 2).

First Filling

Before filling, the tank is plastered by dilute cattle dung slurry to facilitate bacterial activity from all four sides. It is then filled in definite layers consisting of the following sub-layers.

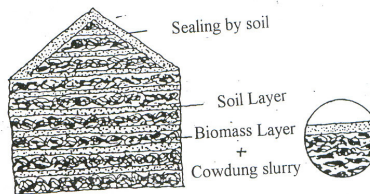
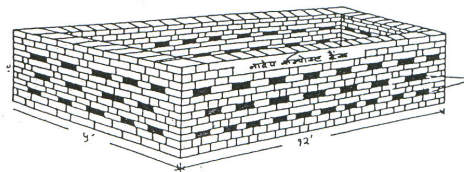
Sub-layer -1:- 4 to 6" thick layer of fine sticks or stems of tur stalk / cotton stalk (This is provided for the initial layer only to facilitate aeration), followed by 4 to 6" layer of dry and green biomass.

Sub-layer-2:- Approximately 4 kg cattle-dung is mixed with 100 litres of water. This slurry is sprinkled thoroughly on the agricultural waste to facilitate microbial activity. This slurry is used only as a bacterial inoculum in this method.

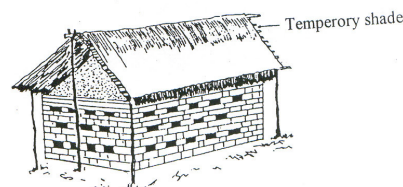
Sub-layer-3:- Approximately 60 Kgs of soil is sprinkled uniformly over the biomass layer. Addition of soil serves three purpose (1) retention of moisture (2) soil micro-flora helps in biodegradation and (3) it acts as buffer and controls pH of media during decomposition.

In this way, approximately 10-12 layers are filled in each tank. The tank is filled approximately 1.5' above the height of the tank. After filling the tank, biomass is covered with 3" thick layer of soil and sealed with cattle-dung and mud plaster. After 15-30 days of filling ,the organic biomass in the tank gets automatically reduced to 2 ft. At this time, without disturbing the initial sealing layer, tank is refilled by giving 2-3 similar layers over it and is resealed. After this filling, the tank is not disturbed for 3 months, except that it is moistened at intervals of every 6-15 days according to the weather conditions.

From each NADEP tank, approximately 2.5 tonnes of compost is prepared within 90-120 days.



Filling of NADEP Compost tank



Completely filled NADEP tank

(a) Innovation in 'NADEP' Technology (I.I.T. Delhi)

In states like western U.P., Haryana, Punjab, large amount of dung is easily available whereas availability of surplus agroresidues / biomass at the household level is limited due to socio-cultural reasons. Also, it is difficult to fill and seal the tank within 48 hrs. Hence, acceptability of 'NADEP' Technology remained very low. Based on the research work carried out at I.I.T. Delhi, a new method was developed through optimizing the ratio of substrates, filling duration, additional alternative modes of aeration to the microbes etc.

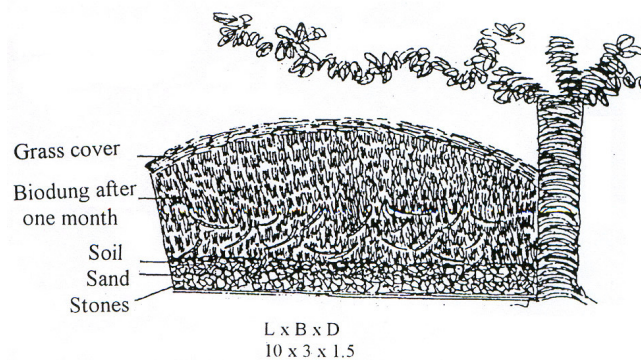
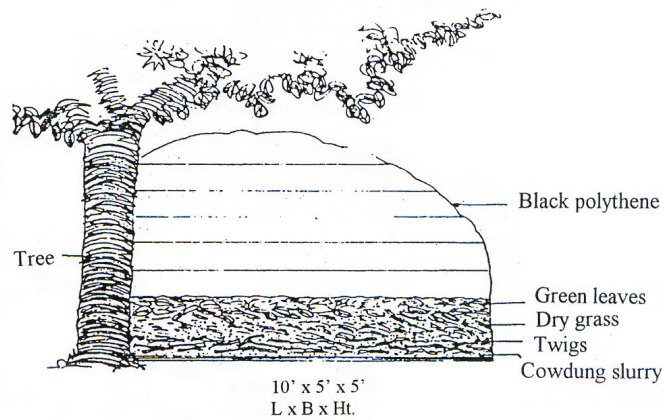
- Thus in the new method, filling is done systematically as mentioned earlier but the raw material for composting is taken in the following ratio.
- 20% biomass/agro-residues and house hold ash, 50% dung and 30% soil. Filling period can be extended up to 4-5 days
- Biggest advantage of this method is that amount of water required is very less and optimum moisture in the tank is maintained without spraying extra water. In summer the tank needs to be protected from direct sunrays using suitable shade
- If suitable space for tank construction (from the angle of making holes for aeration) is not available with the family, then tank can be constructed using support of one / two walls already built. Under such a situation, adequate aeration can be achieved by inserting a few bamboos (having holes) in the composting mass

4. Biodung compost technique

The biodung manure preparation method was developed at Centre of Science for Villages, Wardha and is based on the bioconversion of green biomass, mainly monsoon weeds hedge plants and leaves of fast growing trees which are not commonly consumed by the farm animals. Biodung is a scientific method of composting in which organic biomass is soaked with cattle-dung slurry and polythene coverage over biomass provides optimum conditions for temperature, moisture and aeration for microbial activity.

Methodology

Biodung manure is made above ground surface heaped under tree shade. It does not require any special structure like pits or tanks and mainly require agricultural field biomass, monsoon weeds and leaf litter as raw material. It requires very less cattle-dung (1 to 2% of the weight of the biomass, in case of green biomass; while it is 10-20% in case of dry biomass), which is used as a microbial inoculum. Initially biomass is piled up in systematic layers, roughly each layer of the thickness of 6"-9" containing organic biomass (dry agricultural waste, green biomass, leaf falls, cutting of hedge plants and tree leaves like that of *Leucena leucocephala*). The biomass layer is well soaked in water and cattle dung slurry or biogas slurry and the heap is covered with black polythene sheet (Fig.3). Two turnings are given to this heap at the interval of 15 days and 50-60% moisture is maintained in the heap. Polythene coverage helps in maintaining moisture and temperature (50-60°C) in the biodegradation of heap during the initial period of 10-15 days. This helps in the activity of thermophilic microorganisms and to destroy the weed seeds and pathogenic microorganisms.



Fully decomposed dark colored compost is prepared in 50-60 days called biodung. It has C/N ratio of 15-20 and N from 0.8 to 1.2%, depending on the raw biomass used. The total recovery shall be 50% if prepared from green biomass, while 60-70% if prepared by using a mixture of dry and green biomass. (Priti Joshi, 2001). After 30 days partially decomposed organic matter from this process can be used as vermi-feed for vermicomposting.

5. Vermicomposting

The term "vermicomposting" means the use of earthworms for composting organic residues. Earthworms can consume practically all kinds of organic matter and they can eat as much as their own body weight per day. The excreta or "casting" of earthworms are rich in nutrients (N,P,K and Mg) and also in bacterial and actinomycetes population. The collection of vermicast along with microbially degraded organic compost is called vermicompost.

Requirements for production of earthworms for vermicomposting

To produce vermicompost enough earthworm population is required and as a corollary, their multiplication on a large scale is essential. To achieve the goal of economic multiplication of earthworms, it is necessary to fulfill the following basic requirements.

- a. **Selection of suitable earthworm species:-** Among the 3000 species of earthworms so far identified in the world, only a few species are known to be used for economic multiplication of earthworms for vermicomposting. The species identified for multiplication & vermicomposting are (i) *Eisenia foetida* (ii) *Eudrilus*

eugeniae and (iii) *Perionyx excavatus*. The first two are exotic and last one is indigenous. These species are most suitable because these are (a) prolific breeders with high multiplication rate, (b) having short life cycles with less mortality and (c) voracious feeders. They are easy to handle, having 1 to 1.5 years longevity, sturdy and survive very well throughout the year under varying weather conditions. Such species are economically feasible for vermicomposting and are easily available.

b. Suitable and adequate food:- Any well-decomposed food of any organic waste in adequate quantity having C/N ratio of 20 to 40 can form feed for earthworm. If the C/N ratio is less than 20 it can be used directly as manure.

c. Adequate moisture:- Earthworms cannot survive without moisture. Water is one of the most important requirements. Earthworms contain 85% water in their body and hence constitute the basic need. Respiration is done through the body wall, it is kept moist. Much water is lost from the body through urine. Thus, more than 35% water must be present in the earthworm feed for proper growth. Earthworms do not have any protective body cover and they have to keep the body surface moist as the body wall serves as respiratory organ. They will be constantly releasing mucus through the dorsal pores to keep the body wet. Therefore it is essential to maintain 60% Moisture in the medium (one must feel the wetness in the material). Excess moisture or water stagnation creates anaerobic conditions in the medium and thus deters the growth of earthworms and also the quality of compost.

d. Suitable temperature:- The temperature limit of the earthworm feed should be in the range between 20°C to 35°C. The high temperature > 45°C results into desiccation of the body and moisture stress and temperature below 0°C stops earthworm activities.

e. Protection from light:- Earthworms are nocturnal in habit and are hence active during night. They are injured and may be killed by exposure to light and are specially affected by ultra-violet wavelength. It is advisable to provide shade to the vermicomposting structures as earthworms are photonegative. They avoid day light and thus they are active during night. To increase their activity all through the day and night, it is essential to reduce the light intensity in the structure. (UV light will not affect the epigeic earthworms as they are pigmented). It is the heat factor of day light which is deleterious rather than the light intensity.

f. Suitable pH:- For effective multiplication of earthworms, pH of the feeding material should be at neutral level i.e. 7.0. The earthworm population is severely affected if the pH of the feed material is <4 & >9. Normally a pH range of 6.0 to 8.5 in the feed mix is suitable for the activity of earthworm. At the two given extremes, there may be slight reduction in food consumption and compost production. To get an ideal pH within this range, it is essential to use green matter along with dry biomass and regulate the moisture in the medium.

g. Location for earthworm multiplication:- Suitable place for multiplication should be under shade. Earthworms can be multiplied very well in pit and raised beds or on heap of 2' height filled with ready food of decomposed or partially decomposed organic waste.

h. The compost pit:- Compost pit of any convenient dimension can be dug in the backyard or garden or in a field. The most convenient pit of easily manageable size is 2m x 1m x 0.45m. [A tank may be constructed with brick and mortar with proper water outlets or a plastic crate 60 cm x 30 cm x 30 cm with holes drilled at the bottom or empty wooden crates (drilled wood boxes) or well

rings of 75 cm dia and 30 to 45 cm height can also be used with slight modification in the thickness of layers used.

Preparation of organic biomass for earthworms feeding

Earthworms are very sensitive to temperature; they cannot resist temperature beyond 35°C. Any biomass, dry or green, generates heat while decomposition and the temperature of the heap increases beyond 40^o-50^oC. Therefore, it is very essential to predigest the organic biomass before it is used as a vermifeed. It can be digested in heaps, pits or tanks. It is preferable to decompose organic biomass by using biodung technique (described on page 14) after 30 days when organic biomass is partially digested after two turnings and its temperature comes down to 25 - 30°C, it can be used for vermicomposting. Similarly, fresh cattle-dung also can not be used for vermicomposting as the generation of ammonical gases and high temperature of cattle-dung heap becomes lethal for earthworms. Thus, cattle-dung heap of the size 3m x 1.5m x 1.5m shall be prepared in shade and about 50 - 60% moisture should be maintained in the heap for about 30 days. This heap also should be turned at least twice at the interval of 15 days. After 30-40 days when the temperature of the heap is reduced to 25-30°C, this predigested or partially digested cattle-dung should be transferred to vermibed.

Preparation of vermibed

Like temperature, earthworms are also very sensitive to light. Therefore, shade (either tree shade or artificial tin shade) is must for vermicomposting. Vermibed of the size 3m x 0.9m x 45cm can be prepared under shade. The breadth of bed should not exceed 1.2 m and depth or height should not exceed 45cm to avoid compaction and heat generation from the organic matter. The bottom layer of vermibed should be loosely lined with brick pieces, pebbles or twigs to facilitate aeration and avoid compaction. At the bottom layer dry and hard agriculture biomass should be given. To save from red ants, lining of wood or charcoal ash also can be given over vermibeds. Similarly covering the bed with neem leaves or other dry biomass like wheat straw paddy straw or dry grass also can help in protecting worms from red ants. This basal layer makes the housing for earthworms. After this, approximately 9" - 12" thick layer of half decomposed biomass over the basal layer of vermibed is made. Water is sprinkled over this layer to maintain moisture. 2000 earthworms are inoculated in one bed. Three common varieties used for vermicomposting are (1) *Perionyx excavatus* (2) *Eudrilus eugeniae* and (3) *Eisenia foetida* of which *Perionyx excavatus* is a local variety, while *Eudrilus eugeniae* and *Eisenia foetida* are exotic varieties. In a vermibed single/mixture of varieties can be used for vermicomposting.

Earthworms when released into the vermibed or tanks containing half decomposed organic biomass, enter in to it on their own and feed on the material, layer after layer and release their excreta on the surface. Earthworms keep moving down wards as most of the material at the top is converted into their cast. The process of vermicomposting i.e. conversion of partially decomposed organic matter to fine granular vermicompost takes 40-45 days. Every kg of earthworms feeds on 5 kgs of waste with 40 to 50% moisture per day. In this way, with the help of earthworms, composting can be carried out with minimum cattle-dung. Use of different materials like green biomass, fibrous material, dry leaf litter and animal dung in combination

results in the recovery of good quality of compost. In the end, compost recovery will be around 50 to 60% of the original material both by weight and volume.

Compost preparation by using local earthworms

Beside exotic varieties, vermicompost can also be prepared by using local epigeic varieties of earthworms like *Perionyx excavatus*. These epigeic earthworms can be collected from moist and cool places like tree shades, irrigated orchards, kitchen drainage systems and near cattle sheds, where availability of moisture and organic matter is found throughout the year. However, epigeic earthworms can be collected in the rainy season. After collection these earthworms can be transferred to vermibed as described earlier and vermicompost can be harvested after 40-45 days.

Harvesting of Compost

As soon as vermicast is collected on the top layer of vermibed, regular watering should be stopped. Due to loss of moisture from the surface and lack of feeding material earthworms will move downward. After 2-3 days, small heaps of compost are prepared on the vermibed and kept open. This facilitate earthworm to move downwards. Vermicompost is then harvested from the surface and stored in shade. Fresh feeding material is added in the vermibed. After 2-3 days the harvested vermicompost is sieved through 4-5 mm sieves. If the vermicompost contains many cocoons or juveniles or subadults, then compost is watered and covered with grass mulch. To collect small worms from vermicompost small balls of wet cattle-dung are prepared and they are buried at several places in the compost. As markers small pieces of sticks to identify the buried dung can be fixed. It is left for 15 days. After 15 days these balls of cattle dung are collected. Small earthworms juveniles, subadults or other escaped cocoons are all aggregated in the balls of cattle dung. They can be easily separated from compost.

Biomass production of Earthworms

The biomass production of exotic earthworms like *Eudrilus eugeniae* and *Eisenia foetida* may lead to a level of 40 to 90 folds in a period of 3-6 months with adequate space and food. For example a tank of the size 60 x 45 x 60 cm can hold a population of 1000 to 1500 adult *Eudrilus eugeniae*, three thousand to five thousand *Eisenia foetida* and *Perionyx excavatus*. The growth rate and reproduction of earthworms is controlled by population density. In case of *Eudrilus eugeniae*, earthworms remain small in size and produce less number of cocoons when they are crowded. *Perionyx excavatus* and *Eisenia foetida* can withstand the population pressure (density pressure) but *Eudrilus eugeniae* cannot. Thus frequent harvesting of earthworms is essential to bring down population pressure. It has been observed that addition of wheat bran; gram husk or grain powder and even neem cake increases or stimulates the reproductive potential of *Eudrilus eugeniae*.

Predators and Parasites of Earthworms

Although vermicomposting is very simple technology, care has to be taken to save earthworms from predators. A large number of invertebrates that predate upon earthworms are giant flat worms, the carnivorous slugs, the carabid and staphylinid beetles and the centipedes. The carnivorous earthworms like *Agastrodrilus* species is reported to feed upon earthworms. Besides these, frogs, toads reptiles, rodents, badgers, foxes, moles and birds are the main vertebrate predators (Fig. 4). Therefore the composting units either vermibeds or vermitanks should be well

covered with wire mesh or with thorny leaves to protect from predators. Kitchen waste, half decomposed organic materials as well as cattle-dung attract red ants. These ants feed on cocoons and young earthworms. A native decoction which contains a mixture of 20 liters of water, 100 gms chilli powder, 100 gms turmeric powder, 100 gms salt and little soap powder is prepared and sprinkled over the bottom layer and corners of vermibed to save earthworms from red ants. In some cases where vermicompost is produced at commercial level vermicompost shed is surrounded by narrow water channel, which protects the vermibeds from red ants. Besides this, for household level Vermicomposting, the wooden crates, baskets or small tanks used for vermicompost should be kept at 2 feet height from the ground level and should be covered from top to protect from predators.

Recommended dosage

Vermicompost is used like any other manure (a) 100 g of vermicompost for a pot containing 8 to 10 kg soil (b) 1-10 kg. of vermicompost per tree, depending on the size of tree, (c) 2000 kg vermicompost per acre of land, (d) Regular watering and mulching of the land is important, (e) No chemicals should be sprayed over the compost pit and (f) sprays of extracts from plant origin are recommended, only if necessary for plant protection.

6. Four tank system

Based on the biodung and vermicompost technique, a new system has been designed by the author for continuous compost production, using cattle dung produced daily from the cattle sheds, grasses such as weeds, leaf litter and other farm waste collected daily. In a 4 tank system, a big tank of the size 3.6m x 3.6m. x 75cm. (l x b x h) is made under a tree shade. It is then divided into four equal parts with 9" brick wall construction in such a way that this partition wall contains small vents to facilitate aeration and migration of earthworms from one tank to another tank (fig.5). It is designed for the small and marginal farmers, who possess 2-3 cattles and collect approximately 20-30 kg of waste daily from cattle waste or farm waste. The method of filling 4 tanks is summarized in table below. In this way, the cycle continues and approximately 500 kgs of compost is prepared every month after 3 months, yielding 2-3 tons of compost annually. This method is suitable for epigeic earthworms specially *Eudrilus eugeniae* as they can not resist population pressure and have tendency to migrate

Period	Tank	Process
0 - 30 days	Tank - 1	Collection of biomass and cattle dung
30 - 60 days	Tank - 1	Biodung preparation
	Tank - 2	Biomass collection
60 - 90 days	Tank - 1	Inoculation of earthworms
	Tank - 2	Biodung preparation
	Tank - 3	Biomass collection
90 - 120 days	Tank - 1	Vermicompost ready, migration of earthworms from Tank-1 to Tank-2
	Tank - 2	Vermicomposting initiated
	Tank - 3	Biodung preparation
	Tank - 4	Collection of biomass
120 - 140 days	Tank - 1	Harvesting of compost and afterwards collection of fresh biomass

Tank - 2	Vermicompost preparation and migration of earthworms into tank-3
Tank - 3	Vermicomposting initiated
Tank - 4	Biodung preparation

Observations after 4 months

It was observed that after 4 months (120 days) of initial feeding in tank-1 (vermibed preparation) approximately 400 kg of fresh vermicompost was harvested from this tank. The earthworm count in this tank was increased to five fold. The total earthworm population taken after 4 months was 12.5 times more than that of the initial count. After next 30 days 300 kg. of compost was harvested from tank-2 . In this way in one year approximately 2.5 tons of compost can be harvested annually from a 4 tank unit.

Viability of biodung and 4 tank method

Biodung method is user friendly to farmers. Biodung is better than FYM i.e. effect of 5 ton biodung is comparable with the effect of 10 ton farmyard manure (FYM) in yield as well as nutrient status and microbial population of residual soil. From the 4 tank system approx. 300 kg of compost can be prepared per month upto 12 months which is approx. 2.5 tons in the first year and 3.5 tons from the second year onwards. From agriculture waste also approx. 2.5 tons of compost can be prepared. In this way 5 tons compost can be prepared in a year, which is sufficient for 1 hectare of land for 1 year. This shows that if properly managed a small farmer having 2.5 acre land can produce the compost required for his land from the biomass resources available on his farm, which will convert his farm organic and save his expenditure on chemical fertilizers and pesticides, thus increasing his profit.

7. Phospho-compost

Phospho-compost is an organic and natural manure. It is produced from crop residues, cattle dung, urine and other similar organic matter. These organic residues are mixed with phosphorus-rich rock phosphates or pyrite and enriched with phosphate solubilising microbes. This enables the non-solubilised nutritional factors like phosphorus to get solubilised, which can easily be taken up by plants from soil.

Method of phospho-compost production

Since long, farmers have been using compost in agriculture; but the traditionally prepared compost is very low in nitrogen and phosphorus, which may amount to the extent 0.5% and 0.25%, respectively. Phospho-compost contains these ingredients 2-8 times more. The Method of phospho-compost production is as follows.

- i. **Pit size:-** Generally, pits of the size 2.5 m x 2.5 m x 1.0 m are dug. The size can be varied on the basis of the availability of organic matter. Pits should be dug at an elevated place, where rainwater does not enter and stagnate. If the pits are made impermeable (RCC) nutrient loss may be avoided
- ii. **Filling of the Pits:-** Organic matter or crop residues are layered at the bottom for 3-4 inches over this rock phosphate, cattle dung, soil and saprophytic inoculum is sprinkled in the form of suspension. Then, a second layer of organic matter is added. To bring about uniform exposure of organic matter to the microbial inoculum, small heaps of organic matter is mixed thoroughly with inoculum before adding to the pit. In this way, alternate layers of organic matter and microbial

suspension are put to fill the pit completely. While filling the pit, organic matter, cattle dung, soil and compost are added in the ratio of 8:1:0.5:0.5,. The whole mixture contains 12.5% rock phosphate and saprophytic microbes are used @ 0.5 kg/MT of organic matter. To prepare nitrogen-rich phospho-compost, pyrite @ 10% w/w and nitrogen @ 1% w/w is used. It is especially monitored that organic matter contains 50-60% moisture. After filling the pit completely with organic matter, it is covered with either plastic sheet or mud.

iii. Aeration of the Pit:- For appropriate microbial action, content of the pit has to be given 3-4 turns in every 15 days. Prior to turning adequate water should be sprinkled on the decaying organic matter, so that 60-70% moisture is maintained. In this way, phosphorus-rich well-ripened (applicable to crops in farm) phospho-compost will be ready within 3-4 months.

The manure can be collected and stored in shed. The final compost to be stored must have 12-15% moisture level. It can be then packed into 50 kg polypropylene bags and stored. Such ready phospho-compost can be used as phosphoric manure.

8. Biogas plant slurry

Anaerobic digestion of raw animal dung by microbes in the biogas plant offers more advantages in improving the manurial value of the slurry as compared to the manurial product of aerobic decomposition. An aerobic decomposition of organic matter results in about 30 to 50 percent loss of nitrogen, whereas there is almost complete conservation of nitrogen in anaerobic digestion (Chawla 1984). During anaerobic bio-digestion; about 15 to 18 percent of the total nitrogen is converted into ammoniacal nitrogen as a main source of soluble nitrogen. It is therefore, necessary to take precaution for proper storage of slurry and also during its application to soil to reduce the loss of ammoniacal nitrogen. Improved methods of dehydration of BDS are therefore, recommended over sun drying. (Kate - 1988, 1996). In addition to its application in farms for improving soil fertility and obtaining higher crop yields. The bio-digested slurry has recently been put to multiple utilities like soil amelioration, cultivation of aquatic biomass, organic hydroponics, mushroom cultivation and earthworm rearing, etc.

All chemical elements except carbon, hydrogen, oxygen and sulphur contained in animal dung are conserved in bio digested slurry (BDS), which is reported to be rich in plant nutrients-both macro and micro nutrients-compared to FYM (Kologi et al, 1993) It is also reported that regular use of organic manure can postpone deficiency of zinc and other trace elements in soils, where high yielding varieties are grown (Khandelwal, 1984). Improved soil conditions due to addition of BDS prevents weed growth, helps in retention of soil moisture, increases soil micro-flora, and protects soil from erosion.

Chapter 4

BIODYNAMIC PREPARATIONS IN ORGANIC FARMING

Introduction

Since time immemorial, it is believed that stars, sun and moon play very vital role in every sphere of our life, environment and climate. Agriculture was also believed to be highly affected with the position of these astral bodies. Realizing the potential of these astral and ethereal powers, various biodynamic preparations have been developed for soil fertility build up and pest control. These biodynamic preparations help in restoration of soil's lost fertility by initiating certain processes that leads to gradual buildup of soil health. Some of the biodynamic preparations can also be used as prophylactic agents to combat the menace of insects and diseases. Biodynamic preparations on being applied, initiate specific natural processes making the soil sufficiently sensitive to react to and absorb the incoming stream of life from the cosmos. These preparations are not food for the plants, but they facilitate the effective functioning of etheric forces. They are also not the usual compost starters, but can stimulate compost organisms in various ways. In short they are biologically active dynamic preparations which help in harvesting the potential of astral and ethereal powers for the benefit of the soil and various biological cycles in the soil.

So far 9 biodynamic preparations have been developed, arbitrarily named as formulation 500 to 508. Out of these, formulation-500 (cow horn compost) and formulation-501 (horn-silica) are very popular and are being used by large number of organic farmers. Formulations-502 to 507 are compost enrichers and promoters, while formulation 508 is of prophylactic in nature and helps in control of fungal diseases.

Biodynamic formulation-500 (BD-500)

As per the established norms of Indian Mythology and the promoters of biodynamic process "Rudolf Steiner" while cow-dung is full of astral and ethereal powers; the cow-horn shell has the potential to absorb astral powers. In this formulation the inherent potential of these two components is harvested in making a biologically active formulation.

Method of preparation

1. **Selection of cow-horn** – Cow-horn can be obtained from skeletons of dead cows. Cut the horn from the base and take out the shell by removing the internal contents. Wash it thoroughly and dry in sun till there is no smell. The horn should be preferably from a cow which has gone through at least 2-3 cycles of lactation. The horn shell should not have any hole or crack. If the horn is painted then remove the paint with the help of kerosene or petrol.
2. **Selection of cow-dung** – Collect fresh cow dung from a healthy lactating cow, which is being fed on green fodder. Ensure that the cow was not provided with any hormone or medicine during last 15 days.
3. **Preparation of pit** – In a good fertile upland soil dig a pit of about 40 cm deep. Spread 5cm layer of good top soil. Length and breadth of the pit can be kept as per the requirement.

4. **Preparation of formulation** – Whip the fresh cow dung to prepare a thick smooth paste. Fill the empty horn shells with this paste. Ensure that the shells are completely filled and there is no air bubble or space left. Now place these horns in the pit in upright position with the pointed closed end of horns facing upwards. Fill the pit with good fertile soil and compost mixture (25 : 1) till ground level. Put four pegs or bamboo sticks at four corners of the pit for identification. The soil of the pit is to be kept moist for all the time. If required sprinkle water at repeated intervals.
5. **Time for burying and digging of pit** – As per the Indian moon calendar "*Kwar Navratra*" (October-November) is the most ideal period for placing the dung filled horns in pits. The horns are kept buried for approximately six months and are taken out during "*Chaitra Navratra*" (March-April).
6. **Collection and storage** – Dig out the horns at appropriate time and take out the BD-500 compost. The compost should be moist and should have a pleasing smell. Store the compost in earthen pots till its use. For proper storage ensure that all the time the compost should remain moist and there is enough space for aeration through its lid. Keep the earthen pot in cool place or keep it half buried in moist soil under shade.
7. **Application** - BD-500 can be used in a crop twice, first dose is to be applied a day before sowing and second dose after 20 days of seedling emergence. For best results it should be applied close to full moon days. BD-500 applied during low-moon or no-moon days will not be that effective.
8. **Method of application** – Mix 30 gm of BD-500 in about 13 lit of rain or fresh tube-well water. Stir the solution with hand for one hour. The stirring is done in one direction until a funnel is created in water, reaching to the bottom of the container, then the direction is reversed abruptly. Again a funnel is created and direction is reversed abruptly and so on for one hour. Apply this suspension with the help of Knapsac sprayer. In the absence of a sprayer the solution can be spread with the help of a whisk broom. BD-500 water suspension should be used within one hour of its preparation. The best time of application is close to sunset. BD-500 application encourage the growth of beneficial microorganisms and earthworms, promote rooting process and harvest terrestrial forces for better crop growth and increased biological activity in the soil.

Biodynamic formulation 501 (BD-501)

In this formulation fine powder of quartz silica is filled in empty cow-horn shells and kept buried in soil for six months during hot summer season. BD-501 is used as foliar spray and is known to be promoting photosynthetic activity of the plants, resulting into better growth of the plants and better quality of grains and fruits.

Method of preparation

1. **Preparation of silica powder** – Collect quartz crystals. It is a natural mineral and is mined out from quartz rocks. Break large pieces into small crystals. Remove stones, sand and other unwanted material. With the help of a pulverizer make a fine powder of about 250-300 mesh (like talcum powder).
2. **Filling of horn shells** – Mix silica powder with rain or fresh tube-well water to make a soft and loose dough. Fill this dough in empty horn shells. Keep the horn shells in upright position (with pointed ends facing floor) for about 2 hours. Drain out excess water which comes on top of silica and fill the horns with fresh dough till top. Burry the shells in pit as described in BD-500 process.

3. **Time for burying and taking out** – Opposed to BD-500, the silica filled horns are buried during March-April (*Chaitra Navratra*) and taken out during Oct.-Nov. (*Kwar Navratra*)
4. **Collection and storage** – At appropriate time dig out the horns and collect powdered formulation of BD-501. Dry it in sun and store in glass bottles or ceramic containers. BD-501 containers should be stored in dry, well aerated and sunny place. It should not be stored in cool and dark place.
5. **Method of application** – 1gm BD-50 is sufficient for one acre. Mix 1gm BD-501 in 13 lit of water and mix by whirling for one hour as per the method described in BD-500. Apply this suspension in the field as fine mist spray. A knapsack sprayer with very fine nozzle can serve the purpose. While spraying hold the nozzle up in the air to create a mist or cloud. This will facilitate the uniform spread of silica particles on leaf surfaces. BD-501 should be applied in early morning hours when there is mild breeze. BD-501 is to be applied first at 3-4 leaf stage followed by two more application at an interval of 30 days. BD-501 also acts as prophylactic agent and helps in prevention of many fungal diseases such mildews and blights.

Other biodynamic preparation

Besides 500 and 501 there are seven other biodynamic preparations having numbers from 502 to 508, but as their method of preparation is not in consonance of Indian traditions, they are not very popular in India and are not being used in large scale. Their methods of preparation in brief are as follows:

BD-502 – Moistened *yarrow* (*Achillea millefolium*) blossom gathered in spring, are packed into the bladder of deer stag or hart. The bladder is hung into the sun over the summer and buried into good soil over the winter. The contents, dug up in the spring, will aid the compost to regulate potassium and sulphur processes.

BD-503 – Chamomile blossoms (*Matricaria chamomilla*) gathered in the summer are moistened with chamomile tea and stuffed into the small intestine of a freshly butchered cow, made into little links of sausages and buried into good humus soil in the fall. The burial place should be close to melt water flow of snow after the winter. This preparation helps regulate the calcium processes of compost.

BD-504 – Stinging nettle (*Urtica dioica*) is buried in the soil for one full year, enclosed in a mantle of peat moss. It aids in humification of the compost.

BD-505 – Scrapings of the outer rind of Oak bark (*Quercus robur*) are placed in the skull cavity of a domestic animal such as sheep or goat and buried in fall in ground that has water percolations through it (such as below leaking drain pipe). The contents are used in the spring. This preparation works on calcium processes and contributes to making plants disease resistant.

BD-506 – Dried flowers of Dandelion (*Taraxacum officinale*) gathered in spring are moistened and folded into the mesentery (membrane that holds intestines) of a cow. This is buried in soil until the spring. It helps to regulate the silica processes in relation to the potassium processes.

BD-507 – Extract the juice of Valerian (*Valeriana officinalis*) flowers by squeezing. The juice is diluted in rain water and sprayed on the compost pile. This preparation regulates the phosphorus processes in the compost.

Although, the method of preparation of these compost BD formulations are difficult, but they are required in very small quantities and can be stored in glass containers for long periods. Once prepared can be used over large number of compost piles. A tea spoonful of each (502-507) will suffice for a normal garden compost pile of 3 m³. On a compost pile poke 5 holes of about 30-40cm deep as shown in the figure below and stuff each with tea spoonful of formulation 502 to 506. Formulation 507 is stirred in a bucket of water and uniformly spread over the entire compost pile.

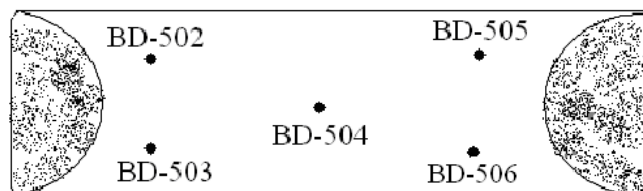


Fig. 7 Compost pile showing location for placement of different BD-formulations

BD-508 – Fresh tissue of horse tail plant (*Equisetum arvense*) is made into a tea by boiling with water for 20 min. Filtered tea can be stored in glass bottles and diluted at the time of use. This formulation is used as prophylactic agent against mildews, blights and other fungal disorders.

Cow-pat Pit (CPP)

Prepare a brick lined pit measuring 90 x 60 cm and 30 cm deep without any lining in the bottom. Mix 60 kg fresh cow dung with 200gm crushed and powdered egg shells and 300 gm basalt dust (or blue granite dust or bore well soil). Mix thoroughly to obtain smooth paste. Fill the mixture in to pit up to 12 cm height. Dog 5 holes in the paste and put one teaspoon full (3 gm each) of preparation 502 to 506 in each hole. Preparation 507 is mixed with water and half is poured in one hole and half sprinkled over the entire surface. Cover the surface with wet gunny bag.

After four weeks, aerate the dung by turning it with the help of a fork. Smooth out again and cover. Thereafter turn every week. CPP compost will be ready in 12 weeks time.

CPP can be used in various ways depending upon the requirement and crop/plants. Use 100 gm CPP/acre, mix with BD 500 or 501 and use as spray. CPP can be used as soil inoculant (@ 2 kg/acre) mixed with composts. CPP can also be used as foliar spray (@ 5kg/acre) right from the beginning of crop to up to fruit/pod formation stage with an interval of 7 to 15 days. CPP can also be used as paste on stem of fruit trees. CPP can also be used as inoculant to biodynamic composts in place of 502 to 507.

Chapter 5

EM – TECHNOLOGY IN ORGANIC FARMING

What is EM

EM or Effective Microorganisms is a consortium culture of different effective microbes commonly occurring in nature. Most important among them are : N₂-fixers, P-solubilizers, photosynthetic microorganisms, lactic acid bacteria, yeasts, plant growth promoting rhizobacteria and various fungi and actinomycetes. In this consortium, each microorganism has its own beneficial role in nutrient cycling, plant protection and soil health and fertility enrichment.

Benefits of EM use

- Improve seed germination, seedling emergence, growth of plants, flowering, fruiting and ripening of grains and fruits.
- Improves photosynthetic potential.
- Increase tolerance in plants against pest attack.
- Improves physico-chemical and biological properties of soil.
- Help in control of soil borne pathogens.
- Interdependent biological activity of different EM organisms creates a congenial environment for growth and spread of soil's flora and fauna. They also promote the growth and colonization of VAM, which further help in plant growth promotion.
- Help in quick degradation of organic matter. With the use of EM the requirement of compost can be reduced or dispensed with. Just recycling of crop residue with EM can give similar results as with good compost. This saves lot of labour and space required for compost preparation.
- Improves soil biota and makes the soil soft and porous

How to use EM

Application of EM in agriculture involves four steps as follows:

- Procurement of primary EM- available in market
- Preparation of secondary EM – to be carried out by the farmer
- Appropriate dilution of the secondary EM solution
- Application to plants, soil and organic matter as spray

Preparation of secondary EM solution

Depending upon the requirement and its end use, various EM formulations have been developed. Even among one formulation depending upon the place and climatic conditions some variations have been incorporated and recommended by promoting institutes and agencies. Some of the widely used and popular formulations are described below. Water used in all formulations should be either rain water or fresh tube-well water. Tap water is not to be used.

1. **EM-1 formulation-** This formulation is used for seed treatment, soil enrichment and for spray in field after the emergence of seedlings.
 - Dissolve 5 kg jaggary (chemical free) in about 100 lit of water
 - Add 5 lit of EM

- Mix thoroughly and pour into a plastic carboy. Seal the carboy and allow to ferment for 7 days
- Dilute this solution in a ratio of 1:1000 and spray over soil or crop residue. For seed treatment soak the seeds in this diluted solution.

2. EM-5 for control of insects and pests –

- Dissolve 100gm of jaggary in 600 ml of water
- Add 100 ml each of natural vinegar, wine or brandy and EM
- Mix thoroughly and transfer the contents in a plastic bottle or carboy and seal the container.
- To increase the potency few cloves of garlic and chilly paste can also be added to this suspension before sealing the container.
- Allow the contents to ferment for 5-10 days under shade.
- Release the gas daily
- Within 10 days the EM solution will be ready for use. This can be stored up to 3 months at normal room temperature in a cool and dry place.
- Dilute the contents in a ratio of 1 : 1000 and apply as foliar spray with the help of a sprayer.

3. Fermented Plant Extract (FPE) – In this formulation fresh green weeds are fermented with EM to obtain a fermented plant extract.

- Grind 2.3 kg of fresh green weeds to a coarse paste.
- Dilute with 14 lit of water⁵⁹
- Dissolve 42 gm of jaggary in some water and mix with weed suspension.
- Add 420 ml of EM
- Transfer the contents to a plastic drum and with the help of a thick plastic sheet cover the drum and tie with a rope.
- The drum should be filled up to the top, leaving very little space for air.
- Fermentation and gas formation process will start slowly.
- Mix the contents at repeated intervals.
- Finished FPE having a pH of 3.5 with pleasing smell will be ready in 5-10 days time.
- Filter the solution through a cloth and collect the filtrate.
- For spraying on soil dilute the FPE in a ratio of 1 : 1000 with fresh water.
- For spraying on crops dilute FPE in a ratio of 1 : 500.
- Spraying should be done after germination of seeds in early morning hours once or twice a week.

4. EM-Bokashi – Bokashi is a type of compost prepared by fermentation of waste organic matter with the help of EM. Bokashi is mainly used for improving the fertility status of soil and for enhancing the degradation of crop residue.

- Collect sufficient quantity of different organic matter (such as rice bran, fish meal, animal waste etc) equivalent to 150 lit drum volume.
- Mix 150gm of jaggary and 50 ml of EM in 15 lit of water.
- Mix this solution with organic waste thoroughly in such a way that entire contents get uniformly moistened.
- Transfer the contents in a plastic bag and seal the bag.

- To ensure the anaerobic conditions put this bag into another polythene bag and seal
- Allow the contents to ferment for 3-4 days in a cool shade place
- Bokashi will be ready after 4 days.
- This can be used immediately.
- In plastic air tight bags Bokashi can be stored up to 6 months.

How to use Bokashi – Bokashi can be used directly as compost in poor fertility soils. It can also be used along with the crop residues. For 0.1 ha mix 100-150 kg Bokashi with sufficient quantity of finely chopped crop residue. Spread this mixture over 0.1 ha area and mixed with soil a day before sowing. Spraying of 5-10 lit of 1:500 diluted simple EM-solution over this mixture can further boost the degradation process. By using Bokashi+crop residue+EM-solution the requirement of compost can be dispensed with. This can save lot of labour, time and space required for compost process.

Application of EM formulations

At the time of land preparation – Dilute 5-10 lit of simple EM solution in 50-100 lit of water and sprinkle/spray over 0.1 ha of land, when soil is wet a day before sowing.

For seed treatment – Soak seeds for 5-6 hrs in 1 : 100 fold diluted EM solution and sow immediately.

As foliar/ soil spray – After seedling emergence, 1 : 1000 diluted EM solution or FPE should be sprayed at the rate of 500 lit per ha, 4-5 times at an interval of 7-10 days. In fast growing crops such as vegetables, spraying should be done twice a week. In transplanted crops 1 : 500 diluted FPE can be sprayed after 5 days of transplanting @ 750-1000 lit per ha.

For soil enrichment – For every 0.1 ha mix 100-150 kg Bokashi with crop residue and mix with soil just before sowing. Simple EM solution @ 5-10 lit can also be used as spray over this residue-Bokashi mix. Spraying the soil with 5-10 lit of FPE mixed in 500-1000 lit of water per ha also add to the fertility of the soil.

Chapter 6

MASS PRODUCTION TECHNIQUES OF BIOCONTROL AGENTS

Usually chemical pesticides are applied for the control of pests as they are considered to be most effective and dependable. However their indiscriminate use has resulted in several problems such as growing resistance in pests to pesticides, resurgence of outbreaks, toxic residue in food, water, air and soil, elimination of natural enemies and disruption of ecosystem. If their indiscriminate use is not checked then their continued use may result into irreparable damage to the ecosystem and environment.

In view of this use of biocontrol agents and Biopesticides are gaining importance as supplementary source of pest management tools in agriculture, forestry, horticulture and in public health programmes. Increased emphasis is being given by the Government agencies, non government agencies and pesticide industries to promote the use of Biopesticides. In organic farming use of bio-control agent and biopesticides are emerging as most viable pest management strategy. The mass production / multiplication techniques of important biocontrol agents like *Trichogramma*, *Chrysoperla*, *Trichoderma* and Nuclear Polyhedrosis Virus (*Spodoptera* and *Helicoverpa*) are described here.

Mass production of Trichogrammatid egg

Large numbers of species, sub species and strains of trichogrammatids are distributed throughout the world, parasitising eggs of over 200 insect species belonging to 70 families and 8 orders in diverse habitats from aquatic to arboreal. In India about 18 *Trichogramma* species are recorded, of which *T. chilonis*, *T. japonicum* and *T. achaeae* are widely distributed and are key mortality factor for many crop pests. These parasitoids attack eggs of many lepidopterous pests such as sugarcane borers *Chilo* spp. and *Scirpophaga excerptalis*; tomato fruit borer, *Helicoverpa armigera*; cutworms *Agrotis* spp; cotton bollworms, *Pectinophora gossypiella*; *Earias* spp; maize stem borer *Chilo partellus* etc. Angoumois grain moth *sitotroga cerealella* is used as factitious laboratory host for mass production of trichogrammatids in USA, USSR and in many European countries. However, in India rice grain moth, *Corcyra cehalonica* is used as the laboratory host.

Description

The trichogrammatidae represent a large group of minute parasitic wasps. Size varies in length from 0.40 – 0.70 mm and width across head 0.15 – 0.25 mm. This group can be identified by tarsi which is 3 segmented, broad fore-wing, pubescence in row or lines marginal and stigmal veins forming a single curve. The genus *Trichogramma* was erected by Westwood in 1833 with *T. evanescens* designated as type species.

Pigmentation in *Trichogramma* varies only to relatively limited extent from species to species. Three major categories can be considered when dealing with cultures namely, a light coloured group of which *T. chilonis* is member, a moderately pigmented group of which *T. fasciatum* is typical and a dark group of which *T. japonicum* is representative. However, these are supplementary characters only.

Present knowledge suggests that *Trichogramma* species can be identified by male genitalia and about 36 biparental species are classified into 9 groups.

Biology

Egg period of trichogrammatids lasts 16-24 hours, larval period 2-3 days, prepupal period 2 days and pupal period 2-3 days. Total development is completed in 8-10 days during summer months and 9-12 days during winter months. Genus *Trichogrammatids* takes 1-2 days extra than *Trichogramma* to complete development.

Facilities Required for mass production

For rearing of *Corcyra cephalonica*

- Working tables
- Slotted angle iron racks
- *Corcyra* rearing boxes
- Oviposition drums
- Trays
- Aluminum cups
- Scissors and brushes
- UV chamber
- Hot air oven
- Refrigerator
- Air conditioners
- Exhaust Fan
- Vacuum pump
- Measuring cylinders
- Glass/plastic tubes
- Cotton wool
- Honey
- Formalin
- Crushed sorghum grains

For rearing of *Trichogramma*

- Working tables
- Fluorescent tube light (15 watt)
- Scissors and brushes
- *Corcyra* eggs
- 'Tricho' cards
- Polythene bags (25 x 20 cm)
- Clips
- Honey
- Culture board with 3 cm diameter holes
- Glass tubes (15x3 cm)

For shipment

- Knife, scissors
- Cardboard cartons
- Thermocole Sheets
- Brown paper sheets
- Gum paste
- Shipping labels

Steps Involved in Production

1. Procure sorghum with bold white grains meant for human consumption. The sorghum should not be treated with insecticides (to test this a sample containing 100 gm. from each bag is crushed and 20-1st / 2nd instar *Corcyra* larvae are allowed to feed for 2-3 days to find out whether the sorghum has previously been treated with any of the insecticides. The conclusion could be drawn based on the mortality of the larvae)
2. The required quantity of the sorghum is milled to make 3-4 pieces of each grain.
3. The sorghum is heat sterilized in oven at 100°C for 30 minutes.
4. The crushed sorghum seeds are sprayed with 0.1% formalin. This treatment helps to prevent the growth of moulds as well as increases, the grain humidity to the optimum (15-16%) which was lost due to heat sterilization.
5. Air dry the sorghum.
6. Pour 2.5 kg Sorghum in each box.
7. To start with, infest 600 boxes (containing 2.5 kg. of sorghum/box) with 300cc of *Corcyra* eggs, and secure the lid for about 30 days. Later on infest at the same ratio on 45th, 90th, 135th, 180th and 225th day. On 270th and 315th day infest only 200 boxes.
8. Keep the first lot of 600 boxes in racks and close the lid (follow the same procedure for subsequent lots).
9. On 40th day the moths start emerging and the emergence continues for two months, 10 to 75 moths, emerge daily, peak of moth emergence is between 65th and 75th days.
10. Collect the moths daily and transfer to the specially designed oviposition cages. Moth emergence reduces after 100 days of initial infestation and boxes are re-used after cleaning.
11. The eggs are collected which pass through 15, 30 and 40 mesh sieves, and run over a slope of paper to eliminate dust particles.
12. The eggs are treated with UV rays (15 wt. UV tube for 45 minutes at a distance of 2 feet) to prevent hatching.
13. The egg are glued to 'Tricho' cards of 15cm x 10cm which are prepunched to obtain 8 pieces of 4cm x 3cm leaving uncovered space at one end to facilitate stapling. The eggs are exposed to adult *Trichogramma* in the ratio of 8: 1 for 24 hours. In case if cards in polythene bags are exposed, the egg to parasite ratio should be 30: 1 but in this method the females are allowed to parasitise till they die. After parasitisation, 6 day old parasitised egg cards are prepared for shipment/field release. A pair of cards is stapled in such a way that the eggs do not touch each other. Twenty 'Tricho' cards are packed in each polythene bag. In polythene bags a strip of wood wool coated with concentrated and dried honey is placed inside the box before closing, so that if adult parasitoid emerges in transit its saliva will come into contact with strip and this will facilitate feeding. 2, 4 and 6 days old parasitised *Trichogramma* eggs (417 for moth crop, 625 for maize and 1250 for cotton) could be packed in perforated capsule, the perforations will permit the *Trichogramma* to emerge but prevent the predator attack. Ordinary corks are bored with cork borer to makes a cavity for eggs and covered with small piece of mesh ensure emergence of adult *Trichogramma* but not the entry of predators. Such corks containing eggs are dispersed in the field randomly as are the capsules mentioned above.
14. For field release, select 40 spots per ha and ensure required quantity of eggs placement. Ensure to place 417 eggs for most crops, 625 for maize and 1250

for cotton each of 2, 4 and 6 days old parasitised eggs at weekly interval till the availability of hot eggs in the field. The initial release could however be decided by putting up pheromone traps/visual observation for the target pest.

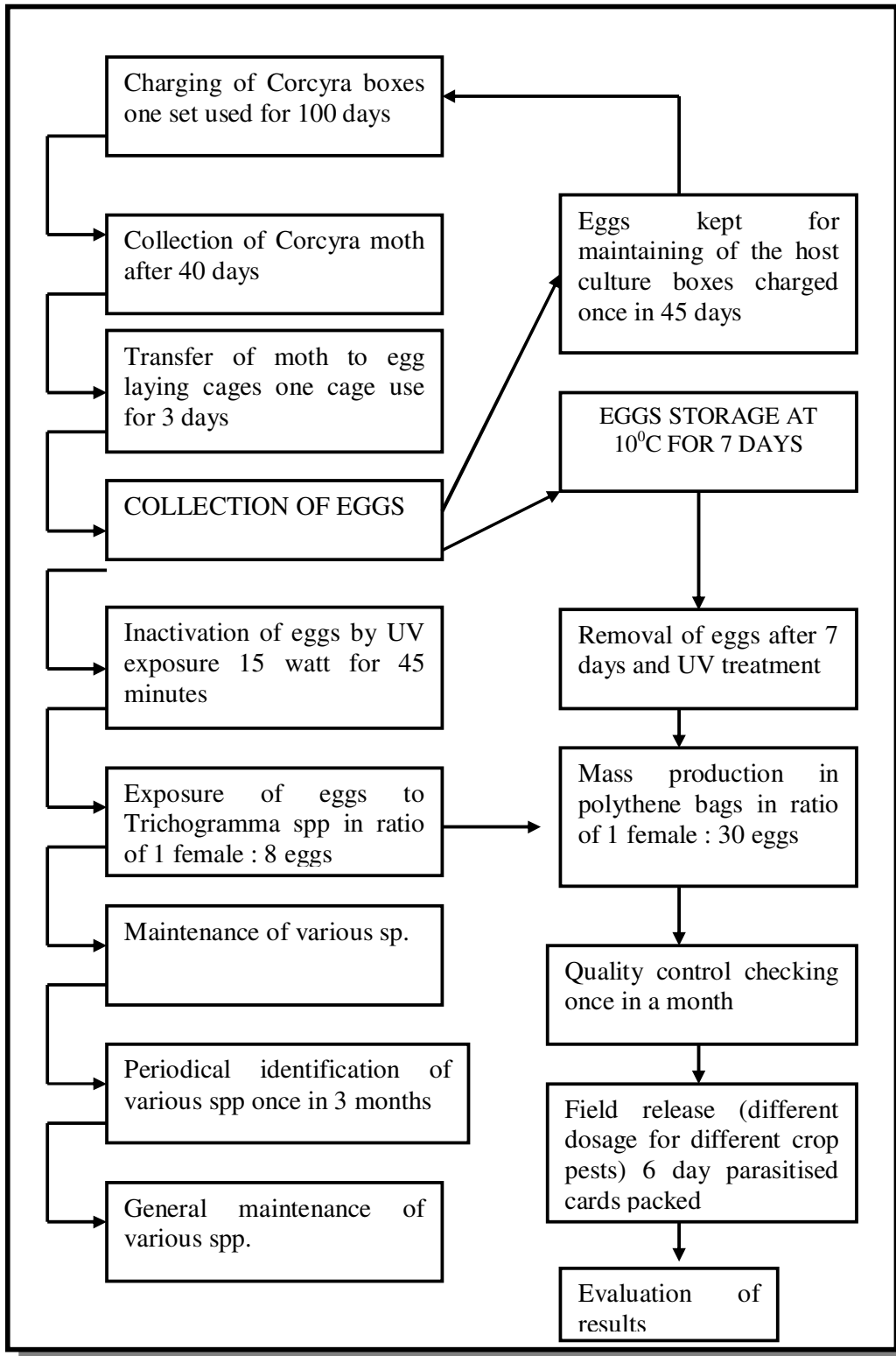
Precautions

It is advisable to observe following precautions during packaging and release for better results.

1. 'Tricho' cards should be packed keeping parasitised surface on inner side.
2. Emergence date should be specified on cards for the guidance of the user.
3. Cut pieces of 'Tricho' cards should be stapled on the inner-side of the leaf to avoid direct sunlight.
4. Card pieces should be stapled in morning hours and just before emergence to avoid predation.
5. Corks containing 'Tricho' eggs should be randomly dispersed. The emergence mesh should be glued properly.
6. In case adult *Trichogramma* is released. The farmers should open the bag after 8 days from the date of egg parasitisation. Move along the rows and go on trapping the bag.
7. Refrain from using pesticides in the field where *Trichogramma* are released. If need arises use selective / safer pesticides. Ensure that pesticides are used 15 days before or after *Trichogramma* release.

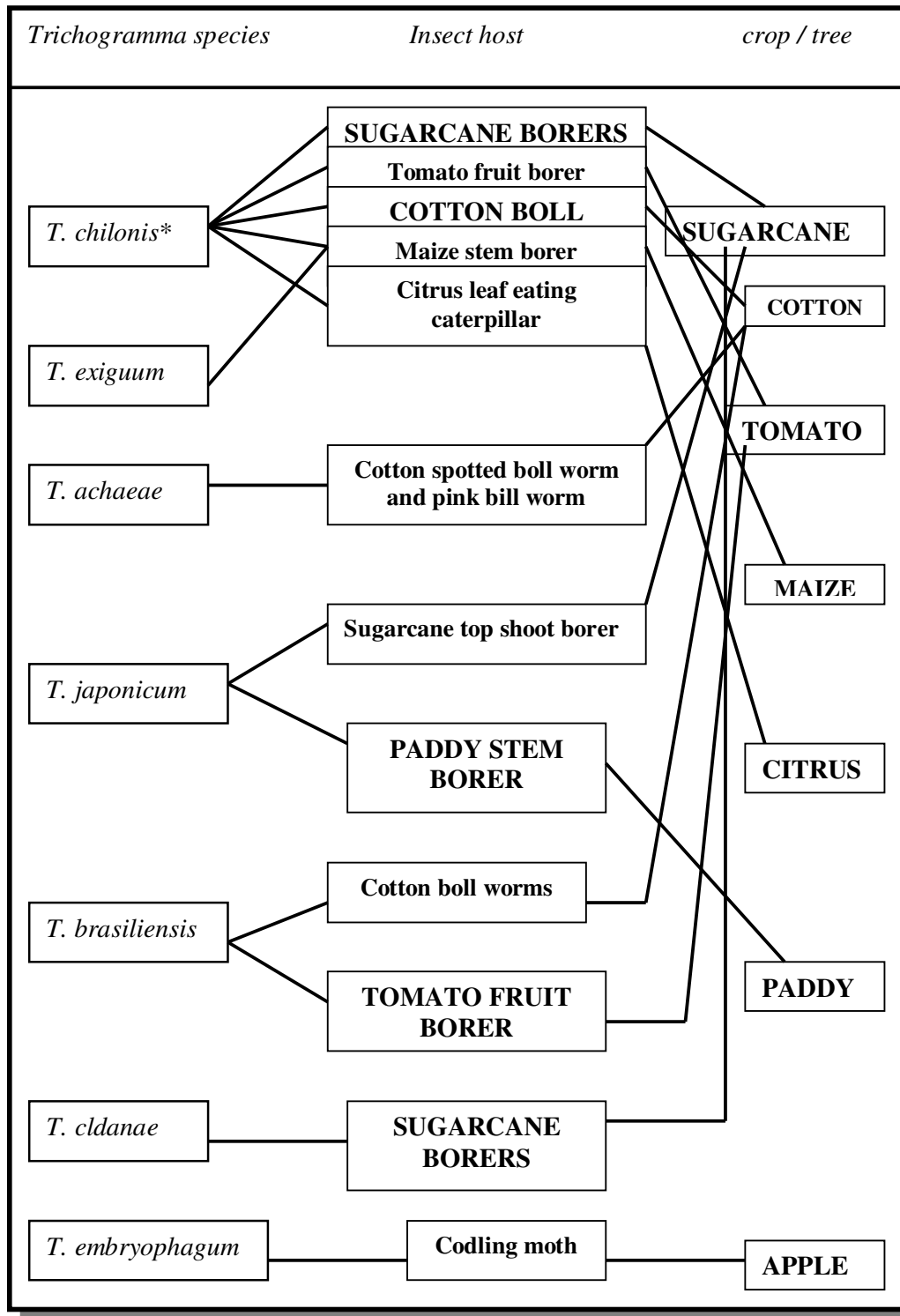
FIELD USE – RELEASE, FREQUENCY

1. Sugarcane borers Early shoot borer <i>Chilo infuscatellus</i>	<i>Trichogramma chilonis</i> sugarcane strain, 4-6 releases @ 50,000/ha at 10 days interval starting from 45 days after planting.
Top shoot borer <i>Scirpophaga excerptalis</i>	<i>Trichogramma japonicum</i> sugarcane strain @ 50,000/ha. 4-6 release at 10 days interval with observation of the pest, usually 60 th day onwards.
Stalk borer <i>Chilo auricilius</i> Internode borer <i>Chilo sachhariphagus indicus</i> Gurdaspur borer (<i>Acigona steniellus</i>)	<i>Trichogramma chilonis</i> sugarcane strain 8-10 release @ 50,000/ha at 10 days interval starting from 90 th day onwards.
2. Cotton bollworms Old world bollworm <i>Helicoverpa armigera</i> Pink bollworm <i>Pectinophora gossypiella</i> Spotted/spiny bollworms <i>Earias</i> spp.	<i>Trichogramma chilonis</i> or <i>Trichogramma achaeae</i> cotton strain @ 1,50,000/ha. from 45 th day onwards 6 weekly releases. Moths can be monitored by pheromone traps.
3. Maize stem borer <i>Chilo partellus</i>	<i>Trichogramma chilonis</i> @ 75,000/ha. 6 releases from 45 th day onwards at an interval of 10 days.
4. Tomato fruit borer <i>Helicoverpa armigera</i>	<i>Trichogramma brasiliensis</i> 6 releases @ 50,000/ha. from 45 th day onwards at weekly interval.
5. Paddy stem borer <i>Tryporyza incertulas</i>	<i>Trichogramma japonicum</i> @ 50,000/ha /week; with the appearance of the pest or 30 days or after transplantation, 6 release to be made in one season.



Schematic presentation of Trichogramma production and release procedure.

TRICHOGRAMMA SPECIES USE IN INDIA



* Different strains are use for different crop pests.

II MASS PRODUCTION OF CHRYSOPID PREDATORS

In recent year use of green lacewings is being recommended in integrated pest management. In India, 65 species belonging to 21 genera have been recorded from various crop ecosystems. However, some species are distributed widely and are key natural enemies for soft bodies' insects. Amongst them *Chrysoperla carnea* and *Mallada boninensis* and are the most common.

The green lacewing is being mass released in the field for the control of aphids, white flies, mealy bugs and eggs and young larvae of lepidopterous pests. It is being mass produced primarily on the eggs of rice grain moth *Corcyra cephalonica* in India. For mass production of *Chrysoperla*, an efficient rearing technique is required.

Artificial diets for rearing larvae are available, but they cannot be used efficiently until encapsulated in an artificial egg.

Description

Chrysopids are generally green in colour, size varying in length for 1.0 – 1.3 cm and width across head 1.0 – 2.0 mm. The head of adult chrysopidae has no ocelli but the compound eyes are prominent. The vertex of the head is often slightly raised. The antennae are long, multi segmented and filiform varying in length from about half to as much as twice the fore wing length. Many species have characteristic markings on various parts of the head which have been widely used in identification. The legs are generally long and slender with 5-segmented tarsi. The wings are large and broadly oval. Hind wing is often narrower. They have rich and regular venation giving the family its common name 'green lacewing'. The membrane is transparent, some species have brownish making, and veins are usually green. The abdomen is 9-segmented in both sexes. In males, abdomen is narrower and tapering whereas in females it is bulged and 2-3 times broader than males.

Biology

The eggs are stalked and green in colour. The length of the eggs in various species ranges between 0.7 to 2.3 mm and that of the stalk between 2 to 26 mm. The eggs are laid singly or in clusters. Eggs turn pale whitish and then black before hatching. Egg period lasts 3-4 days. The larvae are white in colour on hatching. The larva has 3 instars which are completed in 11-13 days. The larva spins a cocoon from which the adult emerges in 5-7 days. Adults on emergence mate repeatedly. Generally preoviposition period lasts 4-6 days. Adult start laying eggs from 4th day onwards and peak egg-laying period is between 9-15 days after emergence. The male longevity is 10-12 days and female can live upto 35 days. Fecundity is 300-400 eggs/female.

Facilities Required

Rearing of *Chrysoperla* spp. requires one room of 6x6m maintained at 27±1.0°C 70% R.H. and constant light of normal illumination levels supplied by fluorescent tube.

1. For handling of adult

- Slotted angle iron racks
- Adult oviposition cages (75 x 30 x 30 cm)

- Weighing balance
- Scissors and brushes
- Cotton wool
- Tissue paper and sponge
- Fructose
- Protinex
- Honey

2. Larval rearing facility

- Larval rearing facility
- Plastic leaves 60 x 22 cm with 2.5 cm square cubical cells
- Acrylic sheets to cover the leauvers
- Aluminum trays
- Plastic containers 27 x 18 x 6 cm or one liter capacity wide mouthed jars.
- Forceps, brushes and scissors
- Glass vials (3 x 1 cm)
- Organdie
- Brown paper roll
- Cotton wool
- Gum paste
- Honey
- Corcyra egg

Steps Involved in Production

1. 3000 adults are kept in oviposition cage, measuring 75x30x30 cm. The sides of the cage are lined with smooth nylon wire mesh (not preferred for egg laying) but the sliding top cover is fitted with black cloth. To prevent damage to the eggs the top is sliding over a comb fitted on both the sides of cage. The top is opened every day starting from 4th day onwards and dead adults are removed every alternate day. The adults in oviposition cage are fed daily on swabs (kept in plastic plates) of:
 - Drinking water
 - 50% honey
 - Diet consisting of Protinex 40 gm + fructose 70 gm dissolved in 250 ml of drinking water.
 - Castor pollen
2. 24 hour old eggs are dislodged from the black cloth top cover of oviposition cage by gently working with a piece of sponge.
3. In first step of larval rearing, three day old chrysopterid eggs are mixed with 0.6 cc of *Corcyra* egg (The embryo of *Corcyra* eggs are inactivated by keeping them at 2 feet distance from 15 watt ultraviolet tube light for 25 minutes) in a plastic container (27 x 18 x 6 cm). On hatching the larvae start feeding. On 4th day the larvae are transferred to 2nd step individual rearing in 2.5 cm cubical cells of plastic leauvers. Each leauver can hold 192 larvae. 0.3 cc *Corcyra* eggs are provided in all the cells of each field salt shaker. Leauver is secured on one side by organdie or brown paper sheet and after transfer of larvae it is covered with acrylic sheet and clamped.

Subsequently 1.3, 1.3, 2.6, 2.6 and 2.6 cc eggs on 5th, 7th, 8th, 10th and 12th days are provided for ensuring complete development of the larvae in each of the leauvers. One 2m x 1m x 45cms angle iron rack can hold 100 leauvers containing 19,200 larvae.

4. Cocoons are colleted after 24 hours of formation (when they get hardened) by removing organdy or paper from one side. Adults are some times allowed to emerge in leauvers and colleted against glass window panes by suction.
5. One set of leauvers remain in use for 13-15 days. After utilization leauvers are cleaned sterilized and reused.
6. For field release 3 days old eggs which are about to hatch are mixed with *Corcyra* eggs before sending for shipment.

Precautions

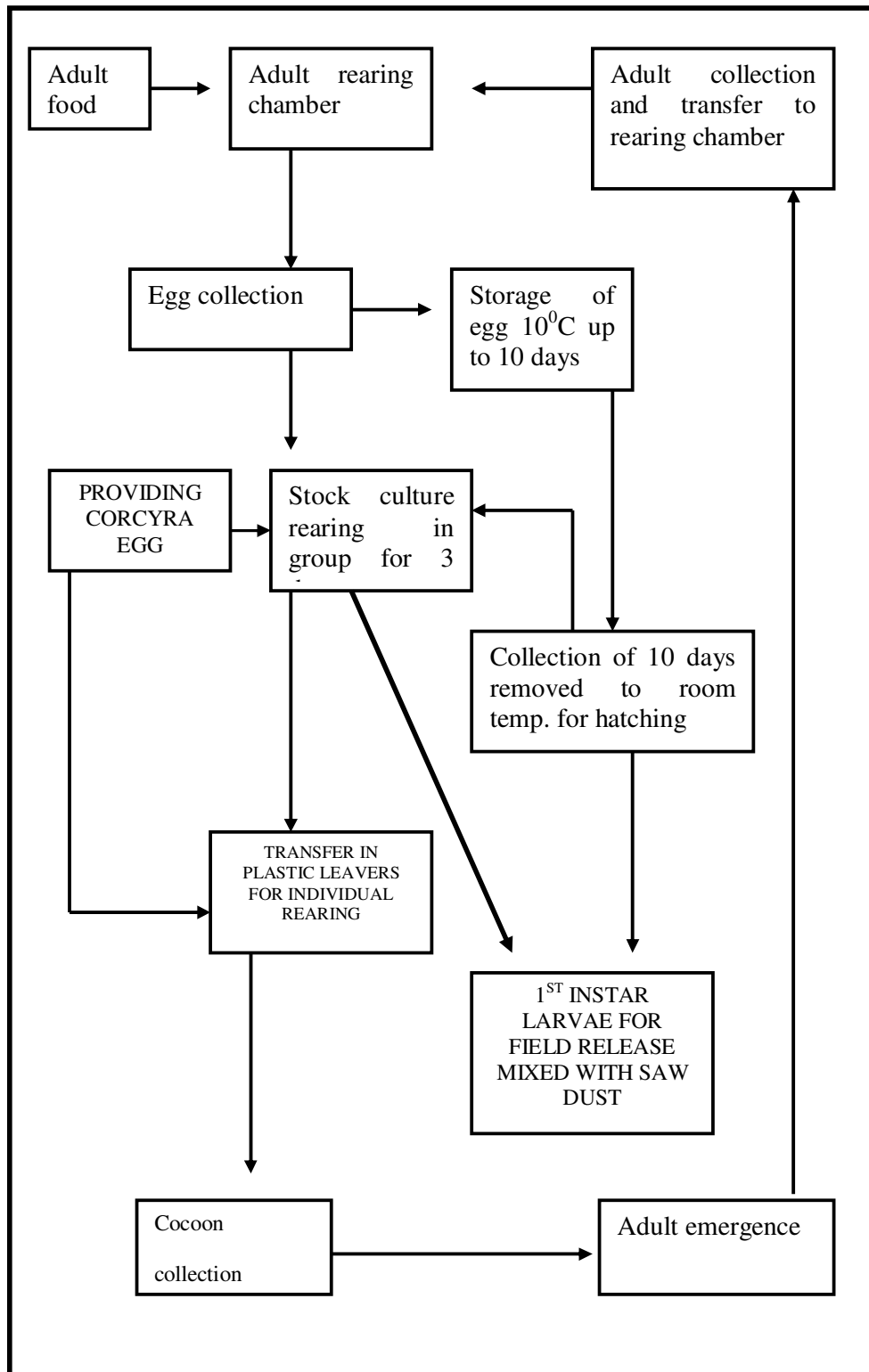
Following precautions must be observed before sending shipment and for effective utilization of Chrysopids.

1. 3 days old chrysopid eggs which are about to hatch should be packed in plastic jar with *Corcyra* eggs, paper strips be provided to minimize contact between chrysopid larvae.
2. To avoid cannibalism only 2-3 days old larvae are released in the field mixed with saw dust.
3. Releases should be made in early hours in the morning to allow larvae to settle on crop canopy.
4. Chrysopid larvae should be released in recommended numbers on crops but on fruit crop, release should be on infested plants.
5. Do not use pesticides in the field where predators are released. In case need arises use selective/safer pesticides after waiting for at least 1-15 days before making the release.
6. Do not release Chrysopids in egg stage as they may be parasitised in the field by egg parasitoids.

FIELDS USE – RELEASE AND FREQUENCY

Normally chrysopids are recommended for use against different crop pests @ 100,000 1st instars larvae/hectare, 4-6 larvae/plant or 10-20/fruit plant. Depending on situation, 2 releases are recommended.

<p>1. Cotton Old world bollworm <i>Helicoverpa armigera</i> Spotted/spiny boll worms <i>Earias</i> spp Pink bollworm <i>Pectinophora gossypiella</i> White fly <i>Bemisia tabaci</i> Aphid - <i>Aphis gossypii</i></p>	<p><i>Mallada boninesis</i> or <i>chrysoperla carnea</i> @ 1,00,000/ha twice during the season with a gap or 15 days.</p>
<p>2. Tobacco Aphid <i>Myzus persicae</i> Tobacco caterpillar <i>Spodoptera litura</i> White fly <i>Bemisia tabaci</i></p>	<p><i>Chrysoperla carnea</i> @ 100,000/ha or 6 larvae per plant twice during the season with an interval of 15 days.</p>
<p>3. Sunflower Head borer <i>Helicoverpa armigera</i> Aphid <i>Aphis</i> sp.</p>	<p><i>Chrysoperla carnea</i> @ 100,000/ha twice during the season with an interval of 15 days.</p>
<p>4. Groundnut Aphid <i>Aphis craccivora</i></p>	<p><i>Chrysoperla carnea</i> @ 100,000 1st instar larvae/ha twice during the season with an interval of 15 days.</p>



Schematic presentation of *Chrysoperla carnea* production procedure

III Production of Biocontrol, Agents for Plant Pathogens

Introduction

Root-rot of pulses and oilseed is a serious disease in rain fed pulses and oilseeds. Chemical control of this disease is not economical and effective since seed treatment with chemicals can give protection only in the early stages of crop growth up to 15 days. But the disease manifests severely in crops around 45 days of age. The continuous use of chemicals has deleterious effect on the beneficial microorganisms in soil, in addition to creating residue problems. Under these conditions the bio-control fungi and bacteria can be effectively used for the management of root-rot diseases. The biocontrol agents multiply in soil and remain near the root zone of the plants and offer protection even at later stages of crop growth.

Preparation of Media

Fungi may be grown in liquid media, either on the surface of the liquid or under constant shaking, throughout the medium. They can also be grown on various solid media. The compositions of various media are given below.

i. Selective media for *Trichoderma* (Elad and Chet, 1983)

Magnesium sulphate	0.2 g
K ₂ HPO ₄	0.9 g
Ammonium nitrate	1.0 g
Potassium chloride	0.15 g
Glucose	3.0 g
Dexon 60 WP (or) Apron (metalaxy1)	0.3 g
PCNB	0.2 g
Rose Bengal	0.15 g
Chloromphenicol	0.25 g
Agar	15.0 g
Distilled water	1 lt.

ii. Potato Dextrose agar (Fungi)

Potato	200 g
Dextrose	20 g
Agar	15 g
Distilled water	1 lt.

(Boil the potato and take the extract)

Isolation of *Trichoderma* from Soil

Procedure

- Collect the soil sample from the field, mix and make into fine particles. Collection of soil sample should be made in the root zone and 5-15 cm depth. Wherever possible, soil samples should be collected from the rhizosphere.

- Ten gm of soil – sample is taken in a measuring cylinder and made up to 100 ml with sterile distilled water. Shake well (1:10).
- Take one ml from this and transfer to 9 ml of sterile water in tube (1:100)
- Make serial dilutions by transferring 1 ml of the suspension to the subsequent tubes to get 1 : 10,000 dilution
- Transfer one ml of the desired soil suspension to sterile Petri plate.
- Pour the melted and cooled *Trichoderma* selective medium in the same Petri plate.
- Rotate the plate gently and allow it to solidify.
- Incubate at room temperature and observe for the development of colonies after 4 to 5 days.

Observations

Trichoderma colonies on the selective medium will be white initially and later on turn to green.

Count the number of colonies developing in individual plates.

Mass multiplication of *Trichoderma* sp.

Vrious methods used for mass multiplication of antagonistic fungi are given below:

i) Wheat bran medium (Henis et al., 1978)

- Autoclave 100 gm wheat bran + 20 ml tap water at 121°C (15 lb pressure) for 1 hr on two successive days.
- Pour conidial suspension of antagonistic fungi
- Incubate for 1 week under light
- Add 150 gm of this culture to 1 sq.m. of soil.

ii) Wheat bran: Saw dust medium (Elad et al., 1980)

- Take 3 parts of wheat barn + 1 part of saw dust + 4 parts of tap water in Polypropylene bags.
- Sterilize at 121°C for 1 hr on two successive days
- Inoculate antagonistic fungi
- Incubate in illuminated chamber at 30°C for 14 days
- Mix the inoculums to the soil @ 150 g/sq.m.

iii) Liquid fermentation method

- Autoclave 30 g molasses + 5 g baker's yeast + 1 lt. of water at 121°C for 1 hr.
- Inoculate with a mycelial disc of antagonistic fungus
- Incubate for 10 days

- Mix 500 ml fungal biomass along with medium with 1 kg talc powder.
- Air dry and add carboxy methyl cellulose as a sticker @ 5 g/kg of product.
- Use the product for seed treatment at 4g/kg. The product can be stored for four months. This product should contain a minimum spore load of 2×10^6 cfu /g.
- If fermentor is not available, inoculate the medium in conical flasks containing not more than 100 ml.
- Talc based Trichoderma formulation is used as seed treatment for the control of root rot disease of pulses, oilseeds, cotton etc. This product can be treated on seeds and can be sown immediately. The antagonist around the seeds were found to colonize the rhizosphere region and protect the crop against root rot pathogen,

IV MASS PRODUCTION OF NUCLEAR POLYHEDROSIS VIRUSES

Insect viruses occur naturally and induce diseases in Lepidoptera, Coleoptera, Diptera and several smaller groups. Many of viruses are closely related to the viruses which are pathogenic to man, domestic animals and a wide range of invertebrates and plants. Only the viruses in the baculovirus group have no such dangerous relationships and they have a very narrow host range. In the baculovirus group, research aimed at insect pest control is generally confined to nuclear polyhedrosis viruses (NPVs) and granulosis viruses (GVs). In our country, work has been conducted on the use of NPVs for tackling two major polyphagous pests – *Spodoptera litura* and *Helicoverpa armigera*.

1. *Spodoptera litura* Nuclear Polyhedrosis Virus (S-NPV)

Spodoptera litura which is a polyphagous pest is also known as tobacco caterpillar. It is a serious pest of tobacco nurseries. *Spodoptera litura* is also sporadic pest of cauliflower, cabbage, castor, cotton, groundnut, potato and Lucerne. It has been recorded from over 100 cultivated and wild host plants. It causes serious losses to several crops when outbreaks occur. If suitable measures are not adopted, the pest is capable of defoliating entire nurseries. Also, the pest could be carried away along with infested seedlings to the planted crop where the larvae could continue to carry out their damaging spell.

The *Spodoptera litura* nuclear polyhedrosis virus (S-NPV) is specific and infects only *S. litura* cell. It can be successfully multiplied on its host and utilized in the field. It is necessary to multiply *S. litura* larvae continuously for production of S-NPV.

1.1 Production procedure for *Spodoptera litura*

The culture of *S. litura* is initiated by collecting eggs from the field of castor, cauliflower, Lucerne or tobacco etc. These field collected eggs are reared in isolation to eliminate the emerging parasitoids and diseases, if any.

The culture can also be established by collecting the gravid females from the light traps. Once the pure culture is established the mass production is commenced from the first established laboratory generation.

Pairs of newly emerged moths of *S. litura* are placed in well ventilated plastic container (20 x 15 cm). The inner walls of the container are lined with paper to obtain eggs. The bottom of the container is lined with sponge covered over by blotting paper. The moths are provided with 50% honey solution and water on two cotton swabs placed in small plastic cups. The eggs which are generally laid in batches on the paper are cut out. Freshly laid egg masses are sterilized by dipping in 10% formalin for 30 minutes, washed in running water for 30 minutes, dried on blotting paper and kept for hatching in sterilized glass vials (20 gm capacity).

The freshly laid eggs can also be surface sterilized in 0.05 per cent solution of sodium hypochlorite for 5 minutes. These eggs are washed several times in running tap water to remove the traces of sodium hypochlorite. The traces of sodium hypochlorite could be neutralized by dipping the eggs in 10% sodium thiosulphate solution and again the eggs are washed thoroughly under running tap water. The surface sterilized eggs are kept in plastic tubes (7.5 x 25 cm) on moist tissue paper for continuing the stock culture. After 3 days the newly hatched larvae are transferred to bouquets of castor leaves (the leaves are surface sterilized with sodium hypochlorite solution, rinsed in sterilized water and dried before making a bouquet) and kept in a plastic container. The pupae are collected 3 days after all the larvae enter the sand. The pupae collections are surface sterilized using sodium hypochlorite solution and rinsed in sterilized water. The excess moisture is later removed by utilizing a blotting sheet on which the pupae are gently rolled. The pupae are sexed and kept on a lid over a wet sponge in adult emergence cage (22 x 15 cm). After 10 days, freshly emerged males and females are collected from their respective emergence cages.

1.2 Production of *Spodoptera litura* Nuclear Polyhedrosis Virus (S-NPV)

From the stock culture of *S. litura*, 90% of 7-9 day old larvae (4th instars; head capsule width 1.1 mm) are used for S- NPV production and the remaining 10% for continuation of laboratory culture. The larvae are collected and starved for 8 hrs. S-NPV suspension (10⁸ POBs / ml) is prepared in 250 ml of water (with teepol) in a bottle. The larvae are exposed to S-NPV infection by dipping the clean castor leaves in S-NPV suspension, for 15-20 minutes and providing them to the larvae for two consecutive days. Thereafter, the larvae are fed on healthy (not treated with S-NPV) leaves (the petiole of which is dipped in water for maintaining the leaf turgidity and freshness) for the remaining part of their life. Fresh leaves are provided every day for the larvae. The larvae could also be exposed to S-NPV infection by transferring them in to semi-synthetic diet treated with S-NPV suspension.

The viroed larvae show characteristic symptoms within 4-5 days of infection. They start dying from 7th day onwards and are placed @ 300 per container containing drinking water and are allowed to putrefy for 3 days. The S NPV infected larvae could be easily distinguished by the pinkish colour on the

under surface of their skin which turns to white with the accumulation of POBs (on death of infected larvae). The skin ruptures and the white liquefied body contents ooze out. The larvae are ground and filtered through muslin cloth. The virus is allowed to settle in sufficient water for about a week. The supernatant is now carefully removed and the polyhedra are suspended in water. Further purification can be done by centrifugation at 500 rpm for 5 –10 minutes and the pellet containing only tissue debris is discarded. When POBs settle at the bottom, the supernatant fluid is discarded. The collected POBs are further purified by high speed centrifugation at 2500 rpm for 5-10 minutes. The white preparation of POBs is finally obtained. The pure POBs suspended in water are counted through modified Neubauer haemocytometer. The count is expressed on larval basis as well as on per unit of larval weight basis. The POBs are dried over calcium chloride or by acetone precipitation and formulated by adding permitted spreaders / wetting agents.

1.3 Field application and impact assessment

The S - NPV @ 250 larval equivalent (LE) is mixed in 125 liters of water, 1% crude sugar and 0.01% Teepol per ha of nursery and sprayed in the evening hours. The virus is sprayed 3 times at fortnightly intervals; the first spray is applied as soon as the young larvae are recorded in the nursery or 3 weeks after planting. Its subsequent sprays could be altered with 2% neem seed kernel sprays. Castor is used as a trap crop around nurseries. S-NPV could be sprayed on castor as soon as the eggs start hatching and the spraying could be repeated at weekly intervals.

On other crops, the first spray is applied as soon as the eggs start hatching and subsequent sprays at 7 – 10 day intervals. The dosage varies from 250 LE to 450 LE/ha. Before applying in the field, 200-400 liters of water, 1% crude sugar and 0.01% Teepol is added. The quantity of water added is reduced to 1/4th in case power sprayer is used for spraying.

2 *Helicoverpa armigera* Nuclear Polyhedrosis Virus (H-NPV)

Helicoverpa armigera is widely distributed in India and attacks a variety of cultivated and wild plants throughout its distribution range. It is a serious pest in the states of Karnataka, Maharashtra, Andhra Pradesh, Tamil Nadu, Orissa, Punjab, Gujarat and Uttar Pradesh. It is a serious pest on commercial crops like cotton; pulses like red gram and Bengal gram; vegetables like tomato okra and dolichos; oilseeds like sunflower and safflower and cereals like sorghum and maize.

H- NPV is a highly infective microbial Biopesticides which can be used for management of *H. armigera*. It is derived from naturally diseased larvae of *H. armigera*. It exhibits high level of infectivity against *H. armigera*.

H- NPV is produced in vivo on *H. armigera*; hence the production of the host insect is essential.

2.1 Production procedure for *Helicoverpa armigera*

The culture of *H. armigera* is initiated by collecting the adults in light traps (inclusion of adults collected in light traps to the laboratory culture also helps

to increase the vigor of the culture). *H. armigera* larvae could also be collected on a large scale from its host crops in endemic areas for initiating the culture. Nucleus culture can also be obtained from the established laboratories. The material thus obtained is reared in laboratory in aseptic conditions and the healthy progeny is selected and established.

The production plan starts with the availability of 550 pairs of adults every day which will yield 22,000 eggs daily. The adults are kept @ 100 pairs in each oviposition cage. Each cage consists of a cylindrical iron frame (50 cm height x 30 cm diameter) having two rings and with a white or black cloth enclosing the frame. A circular plastic mesh (on which cotton swabs soaked in water and honey solution are placed in small containers) rests on a support 5 cm above the base of the frame. The cloth cover is open at both ends with a 20 cm vertical slit in the centre which can be closed with a zip or cloth clips. The cloth cover enclosing the frame is tied with rubber bands at both ends. It is placed on an enamel or aluminium tray (40 x 40 x 5 cm) with a 3 cm thick sponge at the bottom soaked in water. Even in summer months, the temperature inside the cage is maintained at 26°C and humidity at 60 – 90%.

The eggs are laid all over the inner surface of the cloth cover. The egg cloth is removed daily. This cloth is surface sterilized in 10% formalin for 10 minutes, the eggs could also be surface sterilized using 0.2% sodium hypochlorite solution for 5 – 7 minutes and treated with 10% sodium thiosulphate solution to neutralize the effect of sodium hypochlorite, rinsed in distilled water five times for about 10 minutes and eggs collected using a washing machine. The eggs are later placed on paper towel under laminar flow hood for drying. The dried cloth pieces containing eggs are kept in 2 liter flasks containing moist cotton. Flasks are plugged with cotton wrapped in muslin cloth and the bottom of the flask is wrapped with aluminum foil.

***Helicoverpa armigera* larval rearing on semi-synthetic diet.**

The larvae of *H. armigera* can be reared on a chickpea based semi synthetic diet. Composition of the semi-synthetic diet and diet preparation are detailed under:-

The composition of diet for rearing larvae is as follows:

No	Item	Quantity
A	Chickpea (gram) flour	105.00 gm
A	Methyl para - hydroxy benzoate	2.00 gm
A	Sorbic acid	1.00 gm
A	Streptomycin sulphate	0.25 gm
A	10% Formaldehyde solution	2.00 ml
B	Agar-agar	12.75 gm
C	Ascorbic acid	3.25 gm
C	Yeast tablets	25 tablets
C	Multivitaplex	2 capsules
C	Vitamin E	2 capsules
-	Distilled water	780.00 ml

- 390 ml of water is mixed with fraction 'A' of the diet in the blender which is run for two minutes. Fraction 'A' and 'C' are mixed and the blender is run

again for 1 minute. Fraction 'B' is boiled in the remaining 390 ml water, added to the mixture of A and B and the blender is run for a minute. Formaldehyde solution is added in the end and the blender is run again for a minute

- The diet is poured as per the requirement either on the nylon mesh for rearing 5-7 days old larvae or in tray cells for rearing the older larvae or poured into sterilized Petri plates and allowed to solidify. The diet can be stored in the refrigerator for up to 2 weeks. For preparing large quantities of diet, the quantity of diet ingredients to be used should be calculated accordingly and industrial type warning blenders could be used.
- The positively phototropic larvae are removed from the top of the aluminum foil wrapped flasks with a fine sterilized camel hair brush and then transferred to the diet. 220 larvae are transferred to diet impregnated on nylon mesh and placed in 25 x 14 x 11 cm ventilated plastic containers or sterilized glass vials. 100 such containers are maintained daily for 5 – 7 days. A total of 800 (700 + 100) containers are required. Multi-cellular trays with semi-synthetic diet could also be used for rearing a large number of larvae.
- Starting with 22,000 eggs, the total number of larvae available is 20,900 considering an estimated 5% mortality in egg stage. Considering 10% mortality up to first 5 – 7 days, the total number of larvae available for transfer to the trays will be 18,810, out of which 80% will be utilized for virus production i.e. 15,048 and 20% for continuation of host culture i.e. 3762 larvae.
- Diet requirements for the young larvae up to 5 – 7 days at 2 gms / larva will be 4.18 kg.
- Diet requirement for 15,048, 5 – 7 day old larvae to be utilized for *Ha* NPV production at 4 gms / larva will be 6.02 kg.
- Diet requirement for 3762 five to seven days old larvae for continuation of host culture at 6 gms / larva will be 2.26 kg.
- Daily average diet required for rearing the field collected larvae for augmenting the nucleus stock will be about 1 kg.
- Twenty per cent of larvae, which are sent to host culture units, start pupating when they are 18 – 19 days old and the pupae are completely formed within 2-3 days. The pupae are harvested from the diet and are surface sterilized using 0.2% sodium hypochlorite solution, washed and neutralized with 10% sodium thiosulphate solution, washed thoroughly with distilled, sterilized water and dried by rolling over blotting paper. The male and female pupae are separated out and placed in separate small containers, which are placed over moist sponge in adult emergence cages similar to oviposition cages.

- The egg, larval, pupal and adult stages of *H. armigera* last 3 – 4, 18 – 20, 7 – 8 and 7 – 9 days, respectively. The oviposition period of the females is about 5 days.

2.2 Production of *Helicoverpa armigera* Nuclear Polyhedrosis Virus (H-NPV)

For H-NPV production, diet used for rearing *H. armigera* is poured at 4 gm / cell and the diet surface is uniformly sprayed (just enough to cover the diet surface without drenching it) with virus prepared in sterilized distilled water at 18×10^6 POBs /ml. 80 per cent of the total 5 – 7 day old larvae are utilized for H- NPV production and the remaining 20% are transferred into trays where 6 gm diet / larva is provided (for continuation of the host culture).

The trays are incubated at 26°C for 7 days. In case of virus infected larval trays, the diseased / dead larvae are harvested after 7 days and subsequently macerated in mixers / blenders in sterilized distilled water.

The product is standardized with regard to the number of POBs per ml in terms of LD₅₀ with 95% fiducial limits. The POBs can be stored in distilled water and packed in plastic cans / bottles with proper instructions provided on the containers. The virus produced will be sufficient to apply 3 times in about 70.77 ha. Chickpea crop at 250 larval equivalents / ha/ spray in a season. One larval equivalent is equal to 6×10^9 POBs or their equivalent in activity.

2.3 Field application and impact assessment

H-NPV is utilized for the suppression of *H. armigera* on chickpea, pigeon pea, field bean, cotton, sunflower, and tomato. Three to four sprays of H-NPV 250 LE (larval equivalents) / ha. (1 LE = 6×10^9 POBs) are particularly effective on chickpea. Crude sugar 0.5% + groundnut oil cake 0.5% are found to increase H-NPV – caused insect mortality by 40 to 60% on chickpea.

Chapter 7

NEEM IN PEST MANAGEMENT

Neem in pest management

Extensive research over the past years has proved that neem products are the most potent growth regulators and feeding deterrents ever assayed. They repel or reduce the feeding of many species of pest insects as well as some nematodes. In fact, neem is so potent that a mere trace of its presence prevents some insects from even touching the plants. Unlike chemical insecticides, neem compounds work on the insect's hormonal system, not on the digestive or nervous system and therefore do not lead to development of resistance in future generations. The neem compounds belong to a general class of phyto-chemicals called 'limonoids'. The limonoids present in neem make it a safe and effective insecticide, pesticide, nematocide, fungicide making it a valuable and versatile input for crop protection. The most significant limonoids found in neem with proven ability to block insect growth are: azadirachtin, salanin, meliantriol and nimbin. Azadirachtin is currently considered as neem's main agent for controlling insects. 'It appears to cause 90% of the effect on most pests. It does not kill insects – at least not immediately – instead it both repels and disrupts their growth and reproduction.

Action of Neem on insect pests

Various neem extracts are known to act on various insects by :

- Disrupting or inhibiting the development of eggs, larvae or pupae.
- Blocking the molting of larvae or nymphs
- Disrupting mating and sexual communication
- Repelling larvae and adults
- Deterring females from laying eggs
- Sterilizing adults
- Poisoning larvae and adults
- Deterring feeding
- Blocking the ability to "swallow" (that is, reducing the motility of the gut)
- Sending metamorphosis awry at various stages
- Inhibiting the formation of chitin.

All these effects listed above are not equally strong or certain. Blocking the larvae from molting is considered to be neem's most important quality, which can be used to eliminate many pest species. Neem products are harmless to most insect eaters, humans and other mammals. In spite of high selectivity, neem derivatives affect ca. 400 to 500 species of insects belonging to *Blattodea*, *Caelifers*, *Dermaptera*, *Diptera*, *Ensifera*, *Hetroptera*, *Hymenoptera*, *Isoptera*, *Lepidoptera*, *Phasmida*, *Phthiraptera*, *Siphonoptera* and *Thysanoptera*, one species of *ostracod*, several species of mites, and nematodes and even noxious snails and fungi, including aflatoxin-producing *Aspergillus flavus*.

Neem is quite effective against armyworm, one of the most devastating pests of food crops in the western hemisphere. Azadirachtin in extremely low concentrations – a mere 10 mg per hectare – inhibits the pests. Neem extract is useful against leaf miner, a serious pest. Neem seed extract works as well as available commercial synthetic pesticides. It has been approved by the US Environmental Protection

Agency for use on leaf minors. Neem is extremely useful as an anti-feedant and ovipositional repellent for protection of crops like tobacco, groundnut, cotton and sweet potato from the damages caused by tobacco caterpillar or tobacco cutworm, a serious polyphagous pest of several crops in India.

Neem products are quite effective against the larvae of a number of mosquito species which stop feeding and die after treatment. At present developing countries use expensive imported pesticides to control mosquito population. These countries can save a lot of money by using locally available simple neem products which are equally effective.

Experiments have shown that neem is also effective against fruit flies. Med fly, one of the most damaging horticulture pests, can be controlled by spraying neem solution under fruit trees. Neem has an advantage over the currently used pesticides. Whereas the conventional pesticides kill fruit flies as well as their internal parasites, neem products on the other hand, leave the biological-control organisms unaffected; they only kill fruit flies. This reduces, in fact, eliminates adverse, unintended effects. Neem is useful against gypsy moth, a pest which is causing severe damage to forests in parts of North America. Laboratory tests have shown that a very low concentration application of neem seed extract formulation, approved by the US Environmental Protection Agency, can kill gypsy moths.

As mentioned above, neem products can influence about 400-500 insect species. So far effects of neem products have been studied on some of the important insects, which cause severe damage to crops and animals. As can be seen from the discussion above, it is now established that neem and its products are highly effective against many pestiferous insects.

Methods of preparation of Neem extracts

Neem has attracted world wide attention in recent decades mainly due to its bioactive ingredients that find increasing use in modern crop and grain protection. Described below here are some easy methods by which the Neem extracts can be prepared by the farmers.

The highest concentrations of bitter component are found in the neem kernel. Neem kernel is a valuable source of major limonoids responsible for pest control. Hence good quality of neem fruit is essential for production of high quality neem extract. It is therefore essential to follow scientific practices in neem fruit collection and depulping.

Neem fruit collection and depulping

Neem fruit collection

It is necessary to cover the ground below neem tree with cotton or jute cloth, or shade net to avoid contact of neem fruits with soil. It will also facilitate the collection of fruits. Only yellowing and ripe fruits are to be collected for processing. Being rich in carbohydrates neem fruits get attacked by fungi when they come in contact with soil. Such fruits may get infected with toxins developing fungus and may damage the quality of the final product prepared from these fruits. Hence it is strongly recommended to avoid contact of neem fruits with soil. As the fruit ripens during rainy season they must de-pulped as early as possible. Avoid storage of fresh/wet

fruits in the plastic bags. Use bamboo baskets or jute bags for storage.

The effect of neem on some major pests are given below

Pest	Mode of Action
Desert Locust	Neem oil causes solitarization of gregarious nymphs at 2.5 l/ha. They became solitary, lethargic, almost motionless and highly susceptible to predators like birds
Cockroach	Neem seed extracts kills young cockroaches. Adults inhibited from laying eggs
Green Leaf Hoppers	Inhibits feeding
Brown Plant Hoppers	Reduction in survival, affect the development of nymphs to adults stage, oviposition deterrent, sterility, repellent, mating failure
Mosquito	Throwing crushed neem seeds in ponds prevent breeding, affect larvae
Mexican Bean Beetle	Retard growth, inhibit feeding, disrupt molting
Khapra Beetle	Inhibits feeding, disrupt molting, toxic to larvae
Bean Aphid	Reduces fecundity, disrupt molting
Diamond Back Moth	Strongly suppresses larvae and pupae, retard growth, Inhibit feeding
Pink Boll Worm	Retard growth, Inhibit feeding
Army Worm	Retard growth, Repel adult, Inhibit feeding, Disrupt molting, Toxic to larvae
Mealy Bugs	Repels, Inhibit feeding
Rice, Cowpea and Boll Weevils	Inhibit feeding, Disrupt growth, toxic
Cabbage loopers	Inhibit feeding
Rice gall midge	Toxic
Gypsy Moth	Retard growth, Inhibit feeding, Disrupt molting
Leaf Minor	Larvae unable to molt, Retard Growth, Inhibition Feeding, Toxic
Fire ant	Inhibit feeding, Disrupt growth
Fruit flies	Repellent, (100 % control by neem spay under tree)
Nematode	Inhibit hatching, prevent second stage juvenile(neem cake)
White fly	Repellent, growth retardant, feeding inhibitor
Sorghum shoot fly	Feeding inhibitor
Spotted cucumber beetle	Growth retardant, feeding inhibitor

Depulping of Neem Fruits

Depulping is a process to remove seed coat and pulp from the neem seed. It is done either manually by hand or by using mechanical depulper. In manual de-pulping the ripe neem fruits are rubbed between palms in a bucket of water and the fruit is washed till the seed and pulp separate. Use clean water for de-pulping. After thoroughly de-pulping and cleaning, dry the neem seed in shade keeping in thin layers. Select the place with good aeration. Do not dump the fruit or seed in a heap. Provide protection from direct rains. Dried neem seeds (up to 11% moisture) can be stored in a jute gunny bags or bamboo baskets. Do not store in plastic bags as it

may damage the quality of seed. Keep the neem seed in a cool and dry place. If processed properly these neem seeds can be stored for 6-12 months. It is recommended to use neem seed for preparation of extract or oil extraction after 3 months and before 8 months of storage. The highest concentration of limonoids and oil is found during this period.

Neem Kernel Aqueous Extract:

The simple method of Neem Kernel Aqueous Extract preparation consist of following steps –

- Take dried neem seed. Decorticate (Removal of seed coat) it with the help of mortar and pestle or any mechanical decorticator. Clean the neem kernel and seed coat mixture by winnowing seed coat.
- Weigh 1 kg of clean neem kernel and make powder of grain size like fine tea powder. It should be pounded in such a way that no oil comes out. Soak in about 10 lits of clean water. Add 10 ml of neutral pH adjuvant (mixture of emulsifier, spreader etc.) and stir the mixture. Finely ground soapnut powder is known to make a good natural emulsifier. Keep the mixture overnight and filter it on the next day with clean muslin cloth. Add fresh water to the residue and repeat the extraction 2-3 times. Use spent residue as manure.

Spraying of NKAЕ

The spraying of 1.25% to 5% (Neem Kernel wt. Basis) of NKAЕ is recommended on the crops. The use is recommended as a preventive at lower concentration and protective at higher concentration i.e. upto 5 %. Use the spray solution on the same day. Spraying should be done in the low intensity of sunlight, preferably in the afternoon. The effect of the NKAЕ remains for 7-10 days. Care should be taken to cover the entire plant foliage with NKAЕ solution.

Neem Leaf Extract:

For 5 litres of water, 1 kg of green neem leaf is required. Since the quantity of leaves required for preparation of this extract is quite high (nearly 80 kg are required for 1 hectare) this can be used for nursery and kitchen gardens. The leaves are soaked overnight in water, crushed and the extract is filtered. The extract is beneficial against leaf eating caterpillars, grubs, locusts and grasshoppers. Add emulsifier to the extract as explained in kernel extract.

Neem Cake Extract:

100 gm of Neem cake is required for 1 litre of water. The Neem cake is put in a muslin pouch and soaked in water. It is soaked overnight before use in the morning. It is then filtered and emulsifier is added at 1ml per litre of water. It can then be used for spraying.

Neem Oil Spray

15-30 ml Neem oil is added to 1 litre of water and stirred well. To this emulsifier is added (1ml/1litre). It is very essential to add the emulsifier and mix properly. This should be used immediately before the oil droplets start floating. A knapsack sprayer is better for Neem oil spraying in preference to a hand sprayer.

Precatutions for using Neem Extracts/Formulations

Spraying should be undertaken in the morning or late in the afternoon. Insects lay eggs on the underside of the leaves. Hence it is important to spray on the underside of the leaves as well.

Caution

The active principles of Neem are destroyed by

- Heating and boiling the extract- do not boil the mixture
- Acidic or alkaline pH emulsifier- use neutral pH emulsifier
- Ultraviolet rays of sunlight – Spray during moderate sunlight,
- Hydrolysis of water- use aqueous extract on same day

Neem against non-pest insects

Research in recent years has shown that neem is quite effective against non-insect pests also. Threadworms are among the most devastating agriculture pests. These nematodes are very difficult to control. Use of synthetic nematicide is not desirable as they cause toxic effects. Research has shown that these pests are susceptible to neem products. Certain limonoid fractions extracted from neem kernels are providing active protection / defence against root-knot nematodes'. Water extracts of neem cake are also nematicidal. Neem cake is already being used on commercial basis by cardamom farmers in south India.

Fungi attack plants and trees in numerous ways and forms. They cause massive damage to important crops such as wheat, rice and corn. Several tests have demonstrated that neem acts as a fungicide. On being applicable, it would have enormous positive effect on agriculture, environment and food supply with highly valuable effects like reducing poverty and increasing production on a global scale. Some tests have shown unusual and promising results. Neem-leaf extracts failed to kill the fungus *Aspergillus flavus* but completely stopped it from producing aflatoxin. This is important because aflatoxin is a powerful carcinogen that is causing increasing concern regarding the world's food supplies.

Against storage pests

Postharvest losses are notoriously high in developing countries. Worldwide annual losses in storage goes up to 10% of all stored grain, i.e. 13 million tons of grain lost due to insects or 100 million tons to failure to store properly. One of the traditional uses of neem in Asia has been for controlling pests of stored products. Farmers usually mix neem leaves with grain before keeping it in storage for several months. Neem leaves, oil or extracts acts as repellent against several insects such as weevils, flour beetles, bean-seed beetles and potato moths. Treatment of jute sack by neem oil or azadirachtin-rich-products prevents the penetration of pest like weevils and flour beetles. Neem oil destroys bean-seed beetles (bruchids) – a variety of insects mostly attacking legumes – at the egg-stage itself. A mixture of neem leaves with clay and cow-dung develops pest resistant property so it can be used to make bins for storage of grain.

While neem treatments cannot completely replace chemical pesticides use in storage, the amount of pesticide needed could be reduced, thereby decreasing the pesticide load in food grains. With proper timing and innovative methods of application, their use could be integrated in stored product management.

Chapter 8
SOME INNOVATIVE FORMULATION
(Developed by farmers and different NGOs for growth promotion and plant protection)

For soil enrichment and growth promotion

Sanjivak

Used for enriching the soil with microorganisms and quick residue decomposition.

- Mix 100-200 Kg cow dung, 100 Lit cow urine and 500 gm jaggary in 300 lit of water in a 500-lit closed drum.
- Ferment for 10 days
- Dilute with 20 times water and sprinkle in one acre either as soil spray or along with irrigation water.
- Used as soil application either by sprinkling or by applying through irrigation water. Three applications are needed one before sowing, second after twenty days of sowing and third after 45 days of sowing.

Jivamrut

- Take 100-lit water in barrel and add 10 kg cow dung plus 10 lit cow urine.
- Mix well with the help of wooden stick add two kg jaggary and two kg gram or any pulse flour mix this solution well with wooden stick.
- Keep this solution for fermentation for 5 to 7 days. Shake the solution regularly three times a day.
- Used as soil application either by sprinkling or by applying through irrigation water. Three applications are needed. One before sowing second after twenty days of sowing and third after 45 days of sowing.

Amrit Pani

- Mix ten kg cow dung with 500 gm honey and mix thoroughly to form a creamy paste.
- Add 250 gm of cow desi ghee and mix at high speed. Dilute with 200-lit water.
- Sprinkle this suspension in one acre over soil or with irrigation water.
- After thirty days apply second dose in between the rows of plant or through irrigation water.

Concentrated Organic Manure

Fermented concentrated organic manures are very popular in Japan and are being used mainly to compensate the loss of nutrients during switch over period from inorganic to organic.

Materials Required

- Rice bran 10 parts
- Fish meal 1 Part
- Oil cake 1 part
- Egg shell 1 percent of total weight
- Rock phosphate 1-5%
- Molasses and

- Undisturbed forest soil for microorganisms on mixed microbial inoculants

In place of fishmeal, bone meal, blood meal, slaughterhouse refuse also can be used. In place of forest soil wide range of microorganisms from different sources can also be used. Mixture of at least 10-12 different types of decomposing bacteria, Fungi and actinomycetes, which are available commercially, can also be used. Partly decomposed material from a compost pit mixed with sour milk or curd, fermented coconut milk etc can also be used as microorganisms source.

Process

- Mix all the contents except molasses in the appropriate proportion as shown above. Dilute molasses with water (1:500). Add molasses water in the mixture to obtain 50-55% moisture.
- Keep the contents in a container or make a heap on cement floor and cover with polythene sheet.
- Give first turning after 24 hrs
- Thereafter turn the mixture twice a day.
- Maintain the temperature of the mixture below 40-45°C
- Compost will be ready with in 4-5 days.

At the end of 4-5 days, there should not be any foul odour. If there is foul odour, it means that some thing has gone wrong. In that case thoroughly turn the mixture three times a day. The mixture will become healthy and foul odour will disappear. If foul odour still persists then inoculate the mixture with fresh formulation of microorganisms and turn three times a day. Compost will be ready in next 2-3 days.

Concentrated chicken manure

Mix dry chicken manure, crushed oil cakes, some fresh ashes and rock phosphate in a ratio of 10:10:2:2. Grind the mixture to a fine powder. Excellent concentrated manure is ready. Depending upon requirement and soil conditions, the ratio can be altered. In acidic soils some lime can also added to this mixture.

Cow Urine

Cow urine alone is also a good liquid fertilizer and can be used directly for spraying the crop. Dilute 1 lit of cow urine with 100 lit of water and use it as foliar spray. For one acre of crop 200 lit of such dilute suspension will be sufficient. This can be used in any crop in all the seasons.

Vermiwash as growth promoter

Vermiwash alone or mix with cow urine is also an excellent growth promoter. Dilute one liter of vermiwash or 0.5 lit of vermiwash + 0.5 lit of cow urine in 20 lit of water and use as foliar spray. Three to four applications are needed for excellent results.

FOR SEED TREATMENT

Bijamrut

Mix cow dung 5 kg, cow urine 5lit and cow milk 1 lit with 250 gm lime in a drum with 100 lit of water. Keep the solution overnight. Sprinkle this solution over seeds for treatment. Dry the seeds and sow.

For plant Protection

Dashparni extract

Crush following plant parts in a 500-lit drum

- Neem Leaves 5 Kg
- Vitex negundo leaves 2 Kg
- Aristolochia Leaves 2 Kg
- Papaya (Carica Papaya) 2 Kg
- Tinospora cordifolia leaves 2 Kg
- Annona squamosa (Custard apple) leaves 2 Kg
- Pongamia pinnata (Karanja) leaves 2 Kg
- Ticinus communis (Castor) leaves 2 Kg
- Nerium indicum 2 Kg
- Calotropis procera leaves 2 Kg
- Green chilly paste 2 Kg
- Garlic paste 250 gm
- Cow dung 3 Kg
- Cow Urine 5 lit
- Water 200 lit

Crush all the ingredients and ferment for one month. Keep the drum in shade and covered with gunny bag. Shake regularly three times a day. Extract after crushing and filtering. The extract can be stored up to 6 months and is sufficient for one acre.

Panchgavya

- Cow dung slurry 4 Kg
- Fresh cow dung 1 Kg
- Cow Urine 3 lit
- Cow milk 2 lit
- Curd 2 lit
- Cow deshi ghee 1 kg

Mix all the ingredients thoroughly and ferment for 7 days with twice stirring per day. Dilute 3 lit of Panchgavya in 100 lit water and spray over soil. 20 lit panchgavya is needed per acre for soil application along with irrigation water. Panchgavya can also be used for seed treatment. Soak seeds for 20 min before sowing.

Enriched Panchgavya (Dashgavya)

- Fresh cow dung 1 Kg
- Cow Urine 3 lit
- Cow milk 2 lit
- Curd 2 lit
- Cow deshi ghee 1 kg
- Sugarcane juice 3 lit
- Coconut water 3 lit
- Banana paste of 12 fruits

Method of application same as Panchgavya above

Some broad spectrum botanical pesticides

Neemastra

- Crush 5 kg neem leaves in water
- Add 5lit cow urine and 2 kg cow dung
- Ferment for 24 hrs with intermittent stirring
- Filter squeeze the extract and dilute to 100 lit
- Use as foliar spray over one acre
- Useful against sucking pests and mealy bugs

Brahmastra

- Crush 3 kg neem leaves in 10 lit cow urine
- Crush 2 kg custard apple leaf, 2 kg papaya leaf, 2kg pomegranate leaves, 2 kg guava leaves in water.
- Mix the two and boil 5 times at some interval till it becomes half
- Keep for 24 hrs, then filter squeeze the extract. This can be stored in bottles for 6 months
- Useful against sucking pests, pod/fruit borers.
- Dilute 2-2.5 lit of this extract to 100 lit for 1 acre.

Agneyastra

- Crush 1 kg Ipomea (besaram) leaves, 500 gm hot chilli, 500 gm garlic and 5 kg neem leaves in 10 lit cow urine.
- Boil the suspension 5 times till it becomes half
- Filter squeeze the extract.
- Store in glass or plastic bottles
- Useful against leaf roller, stem/fruit/pod borer
- 2-3 lit extract diluted to 100 lit is used for one acre.

Formulation – 1 for wide range of leaf eating and sucking pests

- In a copper container mix 3 kg crushed neem leaves, 1kg neem seed kernel powder with 10 lit cow urine and ferment for 10 days. Boil the suspension to half and filter
- Suspend 500 gm garlic paste and 250 gm chilly paste in 1lit of water separately and keep over night.
- Next day mix all the three solutions and filter
- Dilute to 200 lit with water and use as foliar spray over one acre.

Formulation – 2 for wide range of leaf eating and sucking pests

- Suspend 5 kg neem seed kernel powder, 1 kg Karanja (Pongamai) seed powder, 5 kg chopped leaves of neem and 5 kg chopped leaves of besharam (Ipomea) in 10-12 lit of cow urine in a 200 lit drum and fill with water
- Ferment for 10 days.
- Distil the suspension.
- Distillate can be used as pesticide. Distillate obtained from above quantity can be diluted to 200 lit for use over one acre.

For nematode control in turmeric (Innovator- K.M.Chellamuthu, Tamilnadu)

- Ground all the ingredients to fine paste and mix with 150-lit water, Apply over soil of about one acre after 120 days of planting.
- Mix ginger 250 gm, Chilli 250 gm, Nirgudi leaves(*Vitex negundo*) 1.0 kg, garlic 500 gm, Aloe Vera 500 gm, neem seeds 1 kg and *Cleodendron inerme* 1 kg.

For insect control in paddy (Innovator- K.M.Chellamuthu, Tamilnadu)

- Ground to fine 1 kg each of *Vitex negundo*(Nirgudi), *Cleodendron inerme*, Aloe Vera and neem seeds. Dilute with 100 lit water and use as foliar spray over 1 acre.

For control of sucking pests on cotton, castor and green leafy vegetables (Innovator- Rajnikant Bhai Patel of Gujrat)

- Crush 3 kg fresh leaves of Black Veldi (a croton sp) in 20-lit water and boil till the volume reduced to 5 lit. Filter and use as foliar spray.
- Spray three to four times with a gap of 10 days.

Control of prodenia and Heliothis larvae (Innovator- Rajnikant Bhai Patel of Gujrat)

- Mix crushed 4 kg Aloe Vera (Korfad), 500 ml neem oil, 500 ml tobacco decoction in 20 lit of water. Boil till the volume is reduced to 5 lit.
- Filter, dilute with 100-lit water and use as foliar spray. In one sprayer use 100-150 ml of extract in 15 lit of water.
- Repeat spraying after 10 days.

For control of Army worm, Aphids, Cotton semilooper, Green leaf hopper, Mites, Powdery mildew, Pulse beetle and Rice weevil

- 1 kg Turmeric+ 4 liters of cows urine with 20 liters of water

For control of American ball worms, Aphids, Pulse beetle white fly etc.

- 2 kg ginger paste and 30 12/13/06 Spray the filtrate in half acre
- Ginger, Garlic and chilly extract
- Make 500 gm garlic paste in 100 ml kerosene+100 gm chilly paste in 50 ml water+100 gm ginger paste
- Add all the paste in to 30 liter of water along with emulsifier.
- Spray in the field over half acre.

For control of aphids and beetles

- Custard apple seed Powder is insecticide, and anti feed ant. It is contact poison to flies, aphids and several beetles.
- Take 500 gm in 2 liters water and boil till 500 ml solution remains. Mix it with 15 liters of water and spray over the crop.
- 2 kg Custard apple leaves fresh juice in 500 ml water +500 gm of chilly water extract + 1 kg Neem seed extract in 2 lit of water.
- Dilute it with 60 lit.

Some other Natural Pest control Techniques –

For fungal disease control

- A mixture of ash (2-3 kg) and 1 liter of castor oil is spread on a seed bed of a size of about 100m². The application is repeated 2-3 times at intervals of 7-10 days. This provides protection against soil borne diseases in tobacco nurseries.
- A mixture of 2 kg of turmeric powder and 8 kg wood ash is used as dust over leaves for treatment against powdery mildew.
- Ginger powder at 20 gm/lit of water and sprayed thrice at interval of 15 days can also effectively check the incidence of powdery mildew and other fungal diseases.
- Handful of slaked lime applied at the base of tomato plant can combat damping-off disease.
- Cattle and goat urine have fungicidal properties. Two cups of cattle urine with 5ml peppermint oil and 10 lit of water can be used to control fungal diseases on grapes

For termite control

- Mixture of lime and sulphur forked into the soil discourages termite attack.
- Wood ash heaped around the base of the trunk prevent termite infestation in coffee bushes and date palms.
- Repeated pouring of cattle urine diluted at 1:6 with water in termite holes helps in keeping their spread under control.
- Mixture of cattle dung and red coloured clay with water is coated on the trunk and large twigs at the onset of monsoon when termite damage is severe. Fresh and young grafts are can also be coated with it to protect them from termites.

Supli (*Mundulea suberosa*) a wonder insecticidal value plant for control of leaf eating, sucking and fruit/shoot borers of vegetables

- Crush one kg green leaves of supli in a pot with 10 liters of water.
- Boil the mixture till it is reduced to half.
- After cooling dilute it to 100 liters with water for spraying over one acre.

Flyash-based herbal pesticides against pests of rice and vegetables – Farmers can make their own 'Flyash (FA)-based Herbal Pesticides' (FHP), which include FA + neem seed kernel 10% dust, FA + eucalyptus 10% dust, FA + vitex leaves 10% dust, FA + ocimum leaves 10% dust, FA + acorus leaves 10% dust, FA + pepper 10% dust and FA + chilli 10% dust and FA + turmeric 10% dust. These eight FA based herbal pesticides showed efficacy in thwarting various groups of pests infesting rice and vegetables. Among them, FA + turmeric 10% dust and FA + neem seed kernel 10% dust were found to be the most effective against large numbers of insect pests, including *Epilachna* on brinjal and *Spodoptera* on okra, followed by FA + vitex 10% dust and FA + eucalyptus 10% dust and FA + ocimum 10% dust.
(Source -Sankari and P. Narayanasamy, Current Science, Vol. 92, No. 6)

Crop Growth Promoter (KAMAAL 505) cum pesticide formulation – Innovation of Shri Iswarsingh Kundu of District Kaithal, Haryana. Prepare two different solutions by

boiling the ingredients in equal quantity viz., **solution A** [comprising of neem (*Azadirachta indica*) leaves, aak (*Calotropis gigantea*) leaves, dhatura (*Datura metel*) leaves and bhang (*Cannabis indica*) leaves] and **solution B** [comprising of tobacco powder, chiraita (*Swertia chiraita*), Kutki (*Picrorhiza kurroa*), bawachi (*Psoralea corylifolia*), tamarind pulp, red chilli powder and reetha (*Sapindus trifoliatus*)]. These two solutions are then mixed to a base comprising of neem oil, tobacco powder and reetha (soapnut).

Agrochem a Herbal insecticide –Innovated by Sri Dhyaneswar Patil Science teacher in Janata high school, Shindkheda, Dhule is effective against all harmful insects. The basic ingredients and method of formulation are:

- 50gm datura leaves + 50gm chandrajyoti (*Jatropha grandulifera*) + 50gm *Ipomeae fistula* leaves + 50gm tobacco + 50gm varul (*Garuga pinnata*) leaves + 50gm neem seeds + 50 gm neem leaves + 100gm jowar tillers and 100gm congress grass.
- Mix all these ingredients and crushed well to prepare a paste (A).
- Four ingredients namely 3 litres water, 50gm wavding (*Embelia ribes*) powder, 150ml kerosene and 5-teaspoonful soap powder (Nirma) are heated together till it boils and 200 ml of cow urine is added to prepare a solution (B).
- Both A and B are mixed and kept for 5 days in a vessel.
- After 5 days, the mixture is filtered and stirred well for 5 minutes to get the final formulation.
- Before use, one part of the formulation is mixed with 100 parts of water, mixed well and then sprayed.
- The formulation can be stored for indefinite period of time and has no expiry period.

Use of Clerodendrum, Aristolochia, Azadirachta and Enicostemma to control cotton pest - Farmer Shri Jadubhai Savaliya from Bhavnagar district has developed a formulation that has proved to be highly effective in the control of insect-pest attack and also disease infestation in the cotton crop.

- Formulation comprises of leaves of Arni (*Clerodendrum phlomidis*), kidimari (*Aristolochia bracteata*), mamejavo (*Enicostemma littorale*) and neem (*Azadirachta indica*).
- The leaves are crushed in water and juice is extracted after filtering through fine cloth.
- 200- 500 ml of this extract is diluted to 15 lit water for spraying. About 30 liters of the filtrate is required for effective control of pest and disease in one Bigha of land (800 sq mt).

Formulation for control of pest in cotton – Innovation of Shri Naranbhai Solanki of Bhavnagar, Gujarat is highly effective against the pest complex that is reported to cause damage to the plant and economic yield of cotton crop.

- The formulation contains Kuvar or kuvar Pathu (*Aloe vera*), karyatu (*Canscora diffusa*), kokadva of Karen (*Nerium indicum*), 'kadi jeeri' (*Nigella sativa*), custard apple (*Annona squamosa*), kothimada (*Cucumis callosus*), mamejavo (*Enicostemma littorale*), inner bark of neem 'limdo' (*Azadirachta indica*), 'marchi' (*Capsicum annum*), roots of 'saragvo' (*Moringa oleifera*) and washing powder.

- The ingredients are collected and soaked overnight in 20 liters of water.
- 200 -250 ml of the filtrate is dissolved in 15 liters of water to spray.
- The filtrate is sprayed twice or thrice depending upon the insect attack and prevalent weather conditions.

Use of Tamarind and lemon for pest control –Banidanbhai a small farmer of village of Tamilnadu innovated a solution for hairy caterpillar in castor crop.

- Mix 500 ml juice of tamarind with 500 ml juice of lemon in 15 litres of water.
- This mixture is then sprinkled over the infested crop in 0.25 ha.
- Almost complete control of hairy caterpillar in castor crop can be achieved.

Phytopalm-Herbal pesticide against Coconut mites - Phytopalm, is a herbal pesticide made from 10 herbals to fight Eriophyde mites and other sucking insects in coconut crop. The extract of 10 herbs – Kolingi (*Tephrosia pupurea* (L.) Pers), Notchi (*Vitex negundo*), Lantana, Vinca Rosea, Pongamia, Anona, Turmeric (*Curcuma longa*), Neem (*Azadirachta indica*), Garlic (*Allium sativum*) and *Cassica auriculata* – is also useful in private gardens. (Innovator - Mr. Louis, Director of Centre for Innovation and Transfer of Technology, Kanyakumari – Nagercoil, Tamilnadu).

Mukkadaka decoction to control hoppers in paddy - Mr. B.S. Dinesh of Shimoga, Karnataka, has innovated an effective bio-control measure against hoppers and other insect pests by using a decoction from a local herb Mukkadaka (*Lasiosiphon eriocephalus* - a common plant in the locality which is very bitter and found to cause burning even if a small amount of extractant falls on the skin).

- To one kg Mukkadaka leaves add 10 liters water and boil.
- The solution is filtered and diluted with water in 1: 10 ratio and sprayed twice, once during nursery stage and another after transplanting of paddy.
- The decoction is also effective against crabs.

Control of leaf curl disease in brinjal – Popatbhai Jambucha, a farmer of Village Mathavada, Gujrat and an assistant teacher of agriculture at Lokshala (secondary school), Kalasar uses marine algae for control of leaf curl disease in brinjal.

- The spirogyra obtained from the sea is dried, preserved and used when required.
- A mixture of two kilograms of dried spirogyra, three kilograms of wood burnt ash, five liters of cow urine and forty liters of water are kept in a drum for 12 hours.
- The mixture is then filtered through muslin cloth and sprayed on the affected crop with the help of pressure sprayer pump.
- Time of spraying is in the morning and evening.
- Spraying is taken up at an interval of 10 days for as long as the crop is in the field. By spraying this, 30% leaf curl disease can be controlled. Spraying also results in better plant growth, colour and it helps to control the growth of aphids and cures withering of flowers. Better productivity can also be achieved by using the method. This method is successful in chilly plants also.

Botanical growth promoter and pest control formulation - A decoction of 'sothukathali' (*Aloe vera*), 'neem', 'tulsi' (*Ocimum tenuiflorum*), 'nayuruvi' (*Achyranthes aspera*) and *Aristolochia bracteata* leaves is made in boiled water. The

decoction is mixed with water (100 ml decoction per litre of water) and sprayed on the tomato and citrus crop. This prevents pest and disease attack in both the crops. It reduces flower shedding and increases the yield in citrus. (NAPDB: Aloe vera has antibacterial and antifungal properties. It is also reported to inhibit the growth of *Cuscuta reflexa*. (Ref: Chauhan JS et al 1989 Indian J. Exp. Bid. 27 10:877-884)

Termite management - `Akada' (*Calotropis gigantea*) plant material, 8-10 kg, is soaked in water for at least 24 hours then filtered. This liquid is poured on termite-infested soil. Farmers evaluate the effectiveness by placing pieces of wood at various points in the field. If the wood remains pest-free for one week then the treatment is judged effective. (Innovator from Vill:Choryana Muvada, PO:Sandasal, Tal:Savli, Baroda, Gujarat. Reference from Honey Bee, 3(3&4):17, 1992).

Caterpillar control in Cotton - Caterpillar infestation can severely damage a cotton crop. Reportedly the latex of `Akda' (*Calotropis gigantea*), when diluted with 15 parts water and sprayed on the crop, effectively controls the pest within three days. The new growth after treatment is also free from infestation. (Innovation from Vill:Khagiyali, Tal:Sihor Bhavnagar Gujarat Reference from Honey Bee, 3(3&4):17, 1992) .

Management of nursery bed pests - To control nursery bed pests such as white grubs, termites, and others-soak 0.5-1 kg of `Arithas' (*Sapindus emarginatus*), in one litre of water overnight. Crush the softened nuts, filter the pulp through cotton cloth strain, then pour the filtrate on the soil. (Innovator - Dhandhalya Bhargav K Vill:Gunjur, Tal:Muli Surendranagar, Gujarat, Reference from Honey Bee, 3(3&4):17, 1992).

Chapter 9
PANCHGAVYA
AN EFFECTIVE ON-FARM INPUT
FOR ORGANIC FARMING

Panchagavya, an organic product has the potential to play the role of promoting growth and providing immunity in plant system. Panchagavya consists of nine products viz. cow dung, cow urine, milk, curd, jaggery, ghee, banana, Tender coconut and water. When suitably mixed and used, these have miraculous effects. Mix 7 kg cow dung and 1 kg cow ghee thoroughly both in morning and evening hours and keep it for 3 days. After 3 days mix 10 lit cow urine and 10 lit water and keep it for 15 days with regular mixing both in morning and evening hours. After 15 days mix Cow milk - 3 liters, Cow curd - 2 liters, Tender coconut water - 3 liters, Jaggery - 3 kg and well ripened poovan banana – 12 nos and panchagavya will be ready after 30 days. All the above items can be added to a wide mouthed mud pot, concrete tank or plastic can as per the above order. The container should be kept open under shade. The content is to be stirred twice a day both in morning and evening. The Panchagavya stock solution will be ready after 30 days. (Care should be taken not to mix buffalo products. The products of local breeds of cow is said to have potency than exotic breeds). It should be kept in the shade and covered with a wire mesh or plastic mosquito net to prevent houseflies from laying eggs and the formation of maggots in the solution. If sugarcane juice is not available add 500 g of jaggery dissolved in 3 liter of water.

Physico-chemical properties of Panchagavya revealed that they possess almost all the major nutrients, micro nutrients and growth hormones (IAA & GA) required for crop growth. Predominance of fermentative microorganisms like yeast and lactobacillus might be due to the combined effect of low pH, milk products and addition of jaggery/ sugarcane juice as substrate for their growth. The low pH of the medium was due to the production of organic acids by the fermentative microbes as evidenced by the population dynamics and organic detection in GC analysis.

Table 1. Physico chemical and biological properties of Panchagavya

Chemical composition		Microbial Load	
pH	5.45	<i>Fungi</i>	38800/ml
EC dSm ²	10.22	<i>Bacteria</i>	1880000/ml
Total N (ppm)	229	<i>Lactobacillus</i>	2260000/ml
Total P (ppm)	209	<i>Total anaerobes</i>	10000/ml
Total K (ppm)	232	<i>Acid formers</i>	360/ml
Sodium	90	<i>Methanogen</i>	250/ml
Calcium	25		
IAA (ppm)	8.5		
GA (ppm)	3.5		

Lactobacillus produces various beneficial metabolites such as organic acids, hydrogen peroxide and antibiotics, which are effective against other pathogenic

microorganisms besides its growth. GC-MS analysis resulted in various compounds of fatty acids, alkanes, alconol and alcohol group (Table 2).

Recommended dosage

Spray system - 3% solution was found to be most effective compared to the higher and lower concentrations investigated. Three litres of Panchagavya to every 100 litres of water is ideal for all crops. The power sprayers of 10 litres capacity may need 300 ml/tank. When sprayed with power sprayer, sediments are to be filtered and when sprayed with hand operated sprayers, the nozzle with higher pore size has to be used.

Flow system - The solution of Panchagavya can be mixed with irrigation water at 50 litres per hectare either through drip irrigation or flow irrigation

Seed/seedling treatment - 3% solution of Panchagavya can be used to soak the seeds or dip the seedlings before planting. Soaking for 20 minutes is sufficient. Rhizomes of Turmeric, Ginger and sets of Sugarcane can be soaked for 30 minutes before planting.

Seed storage - 3% of Panchagavya solution can be used to dip the seeds before drying and storing them.

Table 2. Organic composition of panchakavya

Sl.No.	Fatty acids	Alkanes	Alconol and Alcohols
1.	Oleic acids	Decane	Heptanol
2.	Palmitic acid	Octane	Tetracosanol
3.	Myristic	Heptane	Hexadecanol
4.	Deconore	Hexadecane	Octadeconol
5.	Deconomic	Oridecane	Methanol, Propanol, Butanol and Ethanol
6.	Octanoic		
7.	Hexanoic		
8.	Octadeconoic		
9.	Tetradecoic		

Table 3 Periodicity of use

1.	Pre flowering phase	:	Once in 15 days, two sprays depending upon duration of crops
2.	Flowering and pod setting stage	:	Once in 10 days, two sprays
3.	Fruit/Pod maturation stage	:	Once during pod maturation

Effect of Panchakavya

Leaf - Plants sprayed with Panchagavya invariably produce bigger leaves and develop denser canopy. The photosynthetic system is activated for enhanced biological efficiency, enabling synthesis of maximum metabolites and photosynthates.

Stem - The trunk produces side shoots, which are sturdy and capable of carrying maximum fruits to maturity. Branching is comparatively high.

Roots - The rooting is profuse and dense. Further they remain fresh for a long time. The roots spread and grow into deeper layers were also observed. All such roots help maximum intake of nutrients and water.

Yield - There will be yield depression under normal circumstances, when the land is converted to organic farming from inorganic systems of culture. The key feature of Panchagavya is its efficacy to restore the yield level of all crops when the land is converted from inorganic cultural system to organic culture from the very first year. The harvest is advanced by 15 days in all the crops. It not only enhances the shelf life of vegetables, fruits and grains, but also improves the taste. By reducing or replacing costly chemical inputs, Panchagavya ensures higher profit and liberates the organic farmers from loan.

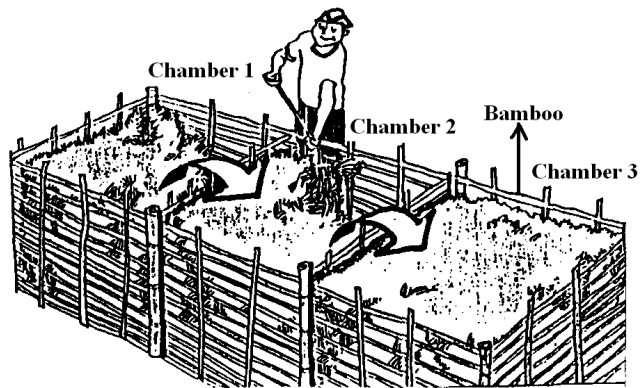
Drought Hardiness - A thin oily film is formed on the leaves and stems, thus reducing the evaporation of water. The deep and extensive roots developed by the plants allow to withstand long dry periods. Both the above factors contribute to reduce the irrigation water requirement by 30% and to ensure drought hardiness.

Time of application of Panchakavya for different crops

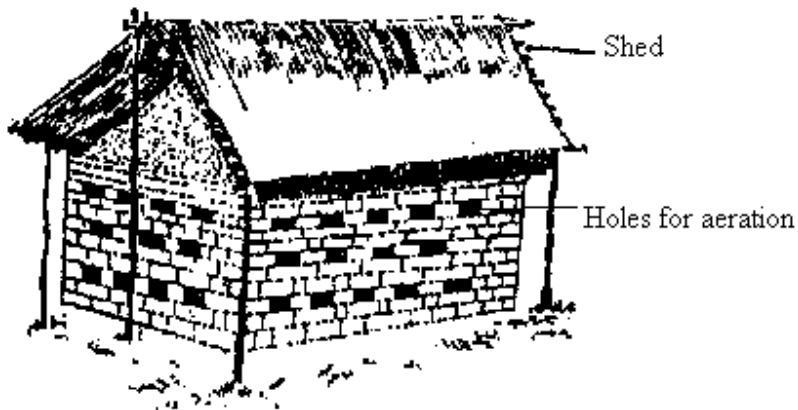
Crops	Time schedule
Rice	10,15,30 and 50th days after transplanting
Sunflower	30,45 and 60 days after sowing
Black gram	Rainfed: 1st flowering and 15 days after flowering Irrigated: 15, 25 and 40 days after sowing
Green gram	15, 25, 30, 40 and 50 days after sowing
Castor	30 and 45 days after sowing
Groundnut	25 and 30th days after sowing
Bhendi	30, 45, 60 and 75 days after sowing
Moringa	Before flowering and during pod formation
Tomato	Nursery and 40 days after transplanting: seed treatment with 1 % for 12 hrs
Onion	0, 45 and 60 days after transplanting
Rose	At the time of pruning and budding
Jasmine	Bud initiation and setting
Vanilla	Dipping setts before planting

(Abstracted from TNAU Agritech Portal,
http://agritech.tnau.ac.in/org_farm/orgfarm_success%20stories.html).

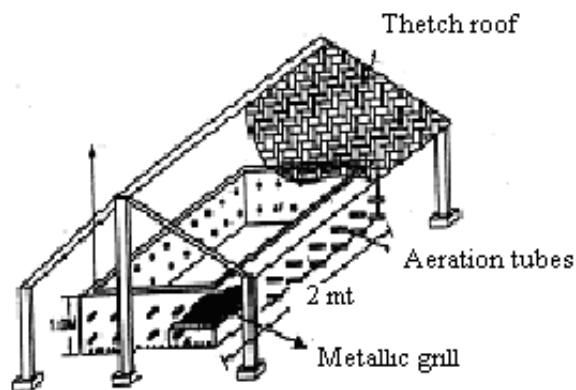
Various Compost Production Facilities



Three Compartment Compost Production Unit



NADEP composting unit



LARGE VERMICOMPOSTING BED