III. BIOPESTICIDES

Introduction

The high intensity of chemical pesticide application has become a serious cause of concern in recent years. Although the use of pesticides is comparatively small in India, the damage caused by them to the environment and health is already evident. (see chapter I). Furthermore, the current trends suggest that the use of chemical pesticides is likely to continue to increase in the near future. In the circumstances, there is a growing need to promote the use of alternative methods of crop protection. It is particularly important that efforts are made to substitute chemical pesticides with biopesticides, which are environmentally friendly.

Biopesticides are living organisms which can destroy agricultural pests. The two most important advantages of biopesticides are that *a)* they are target specific and do not destroy beneficial organisms and *b)* do not leave harmful residues. Some of the important biopesticides include:

1. *Trichogramma* (egg parasitoid) for control of Lepidopteran pests such as sugar cane inter-node borer;

2. Fungi (*Trichoderma* and *Gliocladium*) for control of root rot and wilt disease in pulse crops;

3. Baculovirues. These include:

   a) Nuclear polyhedrosis virus (NPV) of *Heliothis armigera* for cotton, oil seeds, pulses, vegetables and millets.
   b) NPV of tobacco caterpillar (*Spodoptera litura*) for tobacco and cotton.
   c) Granulosis virus (GV) for sugar cane inter-node borer

4. *Bacillus thuringiensis*

5. Neem

Although the importance of biological control of pests has been known for many years, it is only during the last decades that organised efforts to popularise the use of biopesticides has begun in India. Their use is being encouraged by the Indian government as part of an integrated pest management programme (IPM). The Ministry of Agriculture and the Department of Biotechnology are largely responsible for supporting the production and application of biopesticides. The agencies engaged in these activities include the Directorate of Plant Protection and Quarantine (DPPQ), Directorate of Biological Centre and the National Centre of Integrated Pest Management (NCIPM).[57] In addition to promotional activities, these agencies are responsible for the surveillance and forecasting of pest problems in different parts of the country. These agencies also produce biopesticides, but the facilities in most of these centres are basic, and the quantity of biopesticides produced is very small. Many of these agencies suffer from resource constraints and their overall contribution to the popularisation of biopesticides is small.

The Department of Biotechnology (DBT) has recently set up a comparatively ambitious project to promote the use of biopesticides. The main emphasis of the project is to demonstrate the technical viability of various biopesticide production technologies developed in India. The project will also concentrate on training farmers, NGOs and extension workers in the production and use of biopesticides. The objectives of the programme include:

a) the setting up of biocontrol production units in different states.

b) the setting up of Repository Centres for the collection, maintenance and supply of nucleus cultures of biocontrol agents and host insects to production units.

The programme will run for 5 years, during which 50 demonstration units in different agro-climatic regions will be set up.[58] The cost of setting up the 50 units is estimated to be about Rs. 106 million ($3.39 million).

In spite of these programmes, at present the use of biopesticides in India is limited. Serious problems concerning the availability and acceptance of these products have restricted their use to government-sponsored promotion programmes, and sales to individual farmers are small. Furthermore, the situation is unlikely to change in the near future; the demand is expected to grow very slowly. According to most plausible estimates, biopesticides can be expected to take only 3 per cent of the pesticide market in India by 2000 A.D.

As the performance of the currently available biopesticides is poor and inconsistent, they are not considered by farmers to be economically attractive alternatives to chemicals. Biopesticides have to break into a market which is completely dominated by chemical insecticide companies. The information available to farmers, both from public and private channels, is still largely confined to the use of chemical insecticides. In the face of the aggressive marketing practices adopted by the chemical firms, and general unawareness of farmers about biopesticides, the demand for the latter will continue to be small.

The experience of developed countries shows that, when faced with competition from the existing chemical pesticides, the adoption of these agents has been slow and limited. For example, in spite of their obvious advantage as environment friendly products, microbial insecticides account for less than 1 per cent of the total yearly insecticide sales in the United States. The poor acceptance of these products is also shown by the fact that more companies have given up the production of biopesticides in the last 20 years than are producing today.[59]

When compared with chemical pesticides, the present day biopesticides suffer from certain limitations. These are related to:

Mode of Action

The mode of action of microbial biopesticides creates difficulties. Most chemical insecticides introduced since World War II are nerve poisons and can enter the insect in a number of ways. Also, most of them affect all stages of insect development. As a result, chemical insecticides are effective even when the spray coverage is not thorough. On the other hand, all microbial insecticides (excepts fungi), enter the insect primarily by ingestion. Also, in most cases only one stage (usually larva/nymph) of the pest is susceptible. Therefore, in order to be effective, biopesticides must be placed exactly where the susceptible target stage of the pest will consume it

Effective Life

The other important difference is in the effective residual life on the plant surface. Most chemical insecticides have an effective life ranging between several days and 4 weeks. The effective life of most microbial pesticides, on the other hand, is much smaller; commercial preparations of *Heliothis* NPV, for example, are reported to have a half life of only 12 hours.[60]

These, and other, advantages (as perceived by the farmers) put the chemicals in a preferred position. In most cases farmers are interested in using biopesticides only when the use of chemical pesticides is not feasible (as with the use of *Trichogramma* on sugar cane) or when the pest has developed resistance to chemicals (as in the case of *Heliothis*). In other instances, farmers are reluctant to try biopesticides. In fact, a recent study based on an international survey of researchers shows that there is little likelihood of biopesticides making a major breakthrough in the near future. According to experts, unless the effectiveness of biopesticides improves drastically, the growth in their popularity is likely to be only gradual.[61]

Biopesticide Research

Research on biopesticides is funded mainly by the DBT. Out of 14 studies concerning biopesticides listed in the DBT directory of research, seven were supported by the Department. (see Table 3.1)

Table 3.1. **Research Projects on Biopesticides According to Funding Agency**

Biopesticides	DBT	ICAR	Others	Total
Parasites/predators	2	1	-	3
Fungi	2	-	3	5
Baculoviruses	2	-	2	4
Bacillus thuringiensis	1	-	1	2
Total	7	1	6	14

Note: The funding agencies are public research organisations.
Source: Compiled from "Research profile of Biotechnology Activities in India", Publications and Information Directorate, New Delhi, 1993

Research activities on various biopesticides are described in the following paragraphs.

Trichogramma

Trichogramma belongs to a group of minute wasps, which parasitise eggs of more than 200 insects species, many of which are common pests of important crops such as sugar cane and cotton. The use of *Trichogramma* is particularly beneficial as they destroy pests before they have a chance to do any damage to crops.

Trichogramma is one of the most popular biocontrol agents and is widely used against Lepidopteran insects in a number of countries. For example, in the former Soviet Union more than 10 biological factories were reported to produce about 50 billion *Trichogramma* and other parasites per season. Similarly, more than 50 commercial insectaries are reported to be producing *Trichogramma* and other parasites in the United States and Canada. A number of communes in China are also known to produce *Trichogramma* on a large scale.[62]

Although the rearing and application of *Trichogramma* in India began more than 50 years ago, the early trials were not very successful, and the performance of the parasites was found to be less than satisfactory in most parts of the country.[63] These early failures, which are now believed to be largely a result of faulty experimentation techniques and wrong dosage and release timings, led to a severe decline in research on *Trichogramma*.

With the increased interest in the use of biocontrol agents, research on *Trichogramma* has been revived in recent years. It has been shown that if appropriate strains of the parasite are chosen and released at the right time in correct dosages, *Trichogramma* can be highly effective in many agro-climates.[64]

The mass production of *Trichogramma* is seriously constrained by the difficulty in rearing *Corcyra* moths, whose eggs are used as host. The technology for large scale production of *Corcyra* is not available in India and most rearing facilities are very small. Recent developments in the United States, Russia and China have led to the development of synthetic diet, which can be used in place of *Corcyra* eggs. This technology has allowed the setting up of *Trichogramma* production on a scale which was not possible in the past. However, this technology has not been developed in India.

The difficulties of rearing *Corcyra* on a large scale are compounded by the difficulty in storing the eggs of *Corcyra* and *Trichogramma*. As the demand for *Trichogramma* is seasonal (it is highest in summer), the volume and economics of production would improve if the *Corcyra* eggs could be stored to be used for parasitisation in the summer. Research has failed to increase the life of eggs for more than 15 days, without affecting their suitability for parasitisation.[65]

Parasitised *Trichogramma* eggs have a comparatively short shelf life. They can be stored at low temperature (at about 5 degrees centigrade) for about 3 weeks only. This also acts as a serious restraint on large volume production.

The demand for *Trichogramma* is also limited by a number of factors. Some of these are:

1. sensitivity to chemical pesticides, which limits its use to fields where no chemicals are used.

2. inability to survive harsh environmental conditions. This causes high seasonal mortality during adverse conditions, necessitating repeated releases every season.

3. the need to coincide the release with the egg-laying period of the pest.

As a result of these difficulties, the production and use of *Trichogramma* (and other parasites) in India is still very limited. There are fewer than ten commercial insectaries, most of which are very small. The largest of these, Biocontrol Research Laboratories (BCRL), was set up in 1981 and produces about 2 million parasites per day.

Trichogramma is also being produced in small numbers by some sugar co-operatives and government agricultural departments.

The research on *Trichogramma* has two main focuses: *a)* development of technology for the mass production (rearing) of *Trichogramma* eggs and *b)* study of the effectiveness of various strains of *Trichogramma* in different agro-climatic conditions;

Most efforts to improve production technology are aimed at *a)* the improvement of production efficiency; *b)* reduction in the possibility of contamination; *c)* limited degree of mechanisation and *d)* reduction in health hazards. As a result of work done during the last three years, some improvements in the production technology have been made. These include the mechanisation of certain processes which either pose serious health hazards to workers and/or are considered excessively labour intensive. The two most important examples of these developments are:

a) Moth scale separator for *Corcyra*;
b) Moth collection device for *Corcyra*.

These simple devices have increased the safety and productivity of *Trichogramma* production processes. In the conventional process, *Corcyra* moths are collected manually, exposing the workers to serious discomfort and health risk due to the presence of *Corcyra* scales in a closed atmosphere. The labour productivity of this process is also very low. In the past, these limitations have prevented large scale production of *Corcyra* moths (and, therefore, *Trichogramma*). The use of these devices is likely to make the production of *Trichogramma* a more attractive proposition than in the past.

The production of *Trichogramma* (and other parasites) can be undertaken at two levels: *i)* small scale, decentralised production facilities using labour-intensive technology. *ii)* large scale, centralised production facilities using a high degree of mechanisation and automation. The technology currently available in India is suitable for small to medium sized production. Discussions to import technology from Russia for the setting up of large scale production have also been held.

No research is being undertaken on the genetic improvement of *Trichogramma* and (other parasites) to expand their shelf life, and chances of survival in hostile agro-climatic conditions. Research using conventional breeding techniques to produce *Trichogramma* with temperature and humidity tolerance was started in the early 1970s. The results, however, were not promising and research was abandoned.

Trichoderma

The use of *Trichoderma*, which is a fungus, to control plant pests has been common for a number of years. *Trichoderma* is effective against root pathogens and is used for seed treatment. First used in 1930, it is one of the oldest and most widely used fungi-based pesticides in the world.

Trichoderma (and other fungi-based biopesticides) is particularly effective as it does not have to be ingested by pests but acts through physical contact. It is particularly effective in the case of groundnut, sunflower, sesamum, blackgram, green gram and chickpea crops, which are particularly susceptible to root rot. It can also be used for greenhouse crops and commercial nurseries, where environmental factors can be controlled.[66]

The large-scale production of *Trichoderma* is carried out in fermentors, and the quality and quantity of yield depends largely on the operative conditions (aeration, pH, temperature), media constituent and the rate of biomass production. Aeration and agitation to maintain the required oxygen tension in the medium are particularly critical for the achievement of optimum yield. At present, the average time needed for the production of an optimum quantity of *Trichoderma* is between 6 and 7 days. This is considered to be too long, and efforts are being made to reduce it.[67]

The shelf life and effectiveness of *Trichoderma* (and other fungal and microbial agents) depends largely on appropriate formulation. In fact, product formulation is one of the most complex R&D problems and is considered to be one of the most secret assets of the production technology.[68]

The effectiveness of *Trichoderma* (and other fungal pesticides used for seed treatment) can be improved by:

- the use of genetically superior strains;
- the development of seed treatment techniques which will provide a conducive environment for the growth of biopesticides and will minimise competition from soil born micro-flora.

In India, research and production of *Trichoderma* is of relatively recent origin and is largely limited to the Tamil Nadu Agricultural University (TNAU), which has set up a pilot plant for this purpose. The only other research — testing of *Trichoderma* on cardamom — is being done by the Cardamom Board.

TNAU produced and sold 830 kilograms of *Trichoderma* between November 1991 and November 1993, which was sufficient to treat enough seeds for more than 3 500 hectares. Compared to the total requirement, this is a very small amount. According to estimates prepared by TNAU, India's annual requirement for *Trichoderma* is about 6 000 tones.

The technology developed by the TNAU researchers is suitable for only small scale production. They use a small fermentor, which can produce batches of only 100 Kgs. Further research to update this technology to large scale production is necessary before commercial production facilities can be set up.

TNAU research, however, has successfully shown the effectiveness of *Trichoderma* in controlling root rot. The results of some of their experiments are shown in Table 3.2.

Table 3.2. **Effectiveness of *Trichoderma***

Crop	Root Rot per cent		Yield (Kg/ha)	
	Trichoderma	Control	*Trichoderma*	Control
Ground nut	3.7	16.5	2500	1900
Sesame	4.3	15.5	983	841
Sun flower	18.1	37.1	940	740
Urud Beans	3.0	10.2	910	775
Mung Beans	7.3	17.1	800	688
Chick peas	2.5	14.4	400	350

Source: Information provided by the Tamil Nadu Agricultural University.

TNAU's current research activities include the identification of more suitable strains of *Trichoderma* to suit different soils. The strains already identified work well on neutral and acidic soils but not on alkaline soils. Research is being carried out to isolate strains which can work on soils with pH of more than 8.

Research is also being carried out to improve the strains by genetic manipulation. Two of their scientists have received training in the United States and the work had already started by 1994, but it would be some time before new strains would be available.

Baculoviruses

Baculoviruses are target specific viruses which can infect and destroy a number of important plant pests. A number of these viruses have been registered in the United States, Europe and the former USSR. At least seven baculoviruses are registered in the United States and Canada, four in Europe and eight in the former USSR. They have also been developed in China. The most commonly used baculoviruses are the NPV of *Heliothis* and *Spodoptera.*

For a list of important baculoviruse products, see Table 3.3.

Table 3.3. **Baculoviruses Registered For Commercial Application**

Virus	Trade Name	To be used Against	Registrant	Year
NPV	ELCAR	Bollworm (*Heliothis*) and Tobacco bud worm (*H.virescens*) on cotton.	Sandoz	1973
NPV	BIOCONTROL	Douahals fir (*Oraya pseudot-suqata*) trussock moth	USDA Forest Service	1973
NPV	GYPCHECK	Gypsy moth (*Lymantriadispar*) on forest, shade and ornamental trees.	USDA Forest Service	1976
NPV	NEOCHECK	Pine saw fly (*Neodiprion certifer*)	USAD Forest Service	1978
CPV	MATSUKEMIN	*Dendrolimus spectabilis*	- -	

Source: Reproduced from Rabindra, R.J. and S.Jayaraj, "Genetic Improvement and Development of Baculoviruses as Microbial Pesticides.", in C.Sen and S.Dutta (eds.), Biotechnology in Crop Protection.

For commercial production of baculoviruses host larvae are inoculated with the virus under optimum conditions which promote the growth of the virus. Once the infected larvae dies, the viruses are separated through differential centrifugation.

As the process requires a very large number of larvae, the mass production of baculoviruses has faced serious problems. The maintaining of these larvae on a large scale poses serious technical problems due to the possibility of contamination. The difficulty in maintaining optimum conditions on a large scale and the cost of automation have limited the popularity of baculoviruses in the past.

The acceptance of baculoviruses has also suffered from competition from chemical and other biopesticides (mainly *B.t*). In fact, even large multinationals have faced serious difficulties in setting up commercial production facilities. An example is the case of "*Elcar*", a NPV of *Heliothis* introduced in the United Stated by Sandoz. The production facilities, set up in 1974, had a production target of 50 000 larvae/day. Even though, in order to reduce production costs and achieve high and consistent quality, the production process was partially automated, the level of contamination was found to be very high (40 per cent). After initial success (160,000 acres of cotton were covered during 1976 - 1977) the production was found to be commercially unattractive, and the facility was closed down.[69]

The situation has not changed a great deal since then and, although a number of firms have registered various baculoviruses, their production continues to be beset by production and marketing problems.

Recent increased concern for the environment has, however, led to a renewal in research interest in baculoviruses, and new approaches to production and application are being tried. The most promising of these is the possibility of using insect cell lines for the mass production of baculoviruses, without the use of host larvae.

Considerable success in this direction has already been made, although, at present, the cost of production is too high to be economical. Efforts to increase the production efficiency of cell lines is expected to bring down the costs and to make these products competitive with other biopesticides such as *B.t.* In one of these developments, Boyce Thompson Institute, USA is reported to have developed insect cell cultures which are up to 25 times more efficient for the production of insect viruses than the current standard lines of baculoviruses.[70]

In another important development, the scientists at the USDA Agricultural Research Services have identified an insect virus which is highly effective against a wide range of Lepidopteran pests. The broad target range of the virus is expected to improve the economy of application of baculoviruses.

Sandoz has signed an exclusive license with the USDA Agricultural Research Services to develop the virus further. Research is now being carried out to produce this virus through cell lines. For this purpose, Sandoz will take the help of Biosys of the United States, which will provide its patented liquid fermentation process and formulation technology. The commercial production, however, is not expected before 1998. Other major pesticides firms, such as Dupont and American Cynamide are also working on these lines.[71]

New application methods are also being studied. USDA, for example, have used new application techniques on 100 sq. miles of cotton fields. The techniques involved the spray of weeds and not cotton, as scientists have found that larvae develop on weeds during spring and then attack cotton plants in the season. The experiments were reported to be very successful; the number of pests was reported to be reduced by 88 per cent-95 per cent.[72]

Baculoviruses have attracted attention in India also, and a number of agricultural universities, research institutes and government agricultural departments are engaged in research/production. The scope and size of this work, however, is extremely limited. In most cases the research work is confined to testing the effectiveness of NPV and other baculoviruses on local crops. The production volumes are also very small.

The progress in the development of production technology for baculoviruses has been very slow. Barring the efforts of the TNAU, which has standardised technology for commercial production of NPV and granulosis virus (GV), no systemic work has been done. TNAU research is based on two pilot plants (set up jointly with the Biotech Consortium India Ltd.), in which about 0.38 million LE of NPV of *Heliothis* was produced between November 1991 and November 1993. In addition to this, 0.17 million LE of NPV of *Spodeptera* and 11,150 LE of Chilo GV were produced during the same period.

In order to improve the efficiency of baculoviruse production technology, TNAU has developed the following equipment:

i) Artificial diet production plant;
ii) Multi cellular rearing trays;
iii) Egg separator.

The use of multicellular trays has been particularly effective. TNAU is reported to have achieved a cell occupancy rate ranging from 75-80 per cent, which is comparable with the levels achieved by Sandoz in the United States. However, the yield of virus per larvae achieved by TNAU is low; it is reported to be one third the yield obtained by some of the United States researchers in the 1960s.[73]

The current research at TNAU is aimed at

1. Low-cost sterilisation and sanitation systems to minimise contamination.

2. Productivity improvement. It is hoped that the current level of virus recovery will be doubled, which will bring down the production cost to about 1/3 of its present level.

3. Improved formulation. TNAU has made an arrangement with the National Chemical Laboratories (NCL) to collaborate in the development of formulations. They have also entered into an arrangement with NRI of the U.K. for this purpose.

4. Screening of baculoviruses from different geographical locations in India for the selection of the most virulent strains.[74]

Although TNAU has introduced a number of improvements, the process continues to be basically labour-intensive and is still not suitable for large-scale production. The university has recently entered into a collaboration with the British NRI in order to obtain technology with a high degree of automation. This technology, which is reported to be suitable for setting up large scale production facilities, will also reduce the risk of contamination and will produce NPV of high and consistent quality.[75]

Research on the possibility of using cell lines for the production of NPV is being considered only now. A number of research centres have plans to initiate preliminary studies in the near future.

Research aimed at improving the effectiveness of NPV is also being planned. The acceptance of NPV suffers due to the slow pace of its action. It is known that this is caused by a gene (ET gene). Researchers at the Indian Agricultural Research Institute (IARI) are planning to start work on the removal of this gene from NPV to increase the pace of action. The project is being funded by the DBT and is to start very soon. The immediate goal of the project is to identify and clone the gene. The Institute has received an EGT probe from a British university.[76]

Bacillus thuringiensis

With a world market of about $140 million a year, *Bacillus thuringiensis* is the world's largest selling biopesticide. In fact, 80-90 per cent of biopesticides produced in the world are *Bt* based.[77] It is primarily a pathogen of Lepidopteran pests. When ingested by pest larvae, it releases toxins (commonly known as *B.t* toxin) which damage the mid gut, eventually killing the pest.

First produced in the United States in 1957, *B.t* was registered as a biopesticide in 1961. Today, there are more than 400 registered formulations of *B.t*, which are approved for use against insect pests.[78] The current *B.t* market is estimated to be more than $100 million and is expected to increase to $300 million in 1999.[79] The market is dominated by large multinationals. Three of these: Abbot Laboratories (50 per cent), Sandoz (25 per cent) and Novo Dorsik (25 per cent) accounted for 95 per cent of the world's *B.t* production in 1991. The other major producers include Mycogen, Dupont, Ecogen and Monsanto.[80]

Current research on *B.t* is aimed at improving the strains to increase the target range to non Lepidopteran insects and non-insect pests. Researchers at the University of Maryland, for example, are developing genetically improved strains of *B.t* which will attack more than one order of insects. Using a technique called conjugal transfer, they have crossed a Lepidoptera active strain with a Cleoptera active strain to produce a hybrid which kills both beetle and moth larvae.[81]

In another effort, Mycogen has screened *B.t* strains from more than 50 countries, has already found strains which are effective against nematodes and is developing a biopesticide based on it.

New techniques for devising more effective *B.t* formulation and application are also being developed. Mycogen, for example, replaced its M-One biopesticide based on *B.t.* against Colorado potato beetle with an improved version called *M-One plus.* It incorporates the *MCap* delivery system, which involves genetically engineering a *B.t.* toxin gene into a *pseudomonas* bacterium which is then killed to provide an encapsulated biopesticide.[82]

The use of *B.t* in India was delayed as there were fears about its possible damaging effect on silk worm.[83] It is only in the last few years that government permission to use *B.t* has been given. Estimates of the total market are not available, but a large demand, especially in cotton cultivation areas is said to exist. A number of firms — Sandoz, Lupin, Rallis and the Gujrat State Fertilisers Corporation (GSFC) — are importing *B.t* to sell in the Indian market. The GSFC has entered into an arrangement with Ecogen, according to which it will be the exclusive distributor of Ecogen's bio-rational agricultural products in India and neighbouring areas. It will first introduce *Cutlass* for vegetable crops and then *Condor* for cotton. Both are based on *B.t.* Later, *NoMate PBW Spiral*, a pheromone based insect attractant to control pink bollworm on cotton, will also be introduced.[84]

The early 1990s have also seen the beginning of research on various aspects of *B.t* toxin. Researchers at Anna University have developed fermentation technology for the production of *B.t.* An agreement for the transfer of this technology has been made with a private company (Tuticorin Alkali Chemicals and Fertilisers Ltd.), which belongs to a large producer of agro-chemical inputs. The production is planned to begin some time during 1994.

Researchers at the IARI are working on the possibility of using *E.coli* with *B.t* toxin gene for mass production of the toxin. They have transferred *B.t* gene to *E.coli* and report a high production of *B.t* toxin. While in a *B.t* cell only 0.5 per cent of the cell protein is toxin, in the case of *E.coli* with *B.t* gene, 20 per cent of the cell protein is found to be toxin.[85]

Research aimed at reducing the possibility of accidental damage from *B.t* toxin to silk worms is also being carried out. Researchers at the IARI are working on the transfer of *B.t* gene to non pathogenic bacteria (such as *Pseudomonas*) which are found naturally on plants. Unlike *B.t*, these bacteria do not form spores and can not be transported by wind to neighbouring fields where silk worms are cultivated.[86]

Neem

Interest in the use of neem as a biopesticide has also increased in recent years. Neem contains several chemicals, including "*Azadirachtin*", which affects the reproductive and digestive process of a number of important pests. Neem also acts as a repellent and anti feedant, and its oil is effective against leaf folders (rice), *Heliothis* (chickpea) and aphids and bollworms (cotton). In addition to being environmentally safe, neem is effective against a wide range of pests. In fact, 200 species of insects are known to be controlled by neem.[87]

As neem is non toxic to birds and mammals and is non-carcinogenic, its demand is likely to increase sharply all over the world. Large international biotechnology firms are already engaged in research and commercial production of neem-based pesticides. W.R.Grace, for example, has developed a neem-based pesticide called *Margosan-O*, which contains 0.3 per cent *Azadirachtin*. It is licensed by the US authorities for use in greenhouses, nurseries and forestries.[88] Another firm, Agri Dyna Technologies Inc. has filed registration application for neem-based pesticides for 4 European and 14 Latin American Markets. These pesticides cover both food and non food crops. They will be sold as *Azatin* and *Turplex*.[89]

The commercial production of neem-based pesticides is being undertaken in India also; ten Indian firms are already registered with the Central Insecticide Board for the production of neem-based pesticides and 37 neem-based pesticides are already being manufactured. Research on improving the effectiveness of neem has, however, begun only recently. Some private firms (such as SPIC) and government-funded research laboratories (such as the NCL) are now engaged in isolating the active ingredient of neem. Despite the fact that the neem tree is indigenous to India, the size and scope of these research activities is very small.

Summary

The effect of indiscriminate and excessive use of chemical pesticides on the environment is a cause of serious concern. The use of biopesticides provides a safe and environment-friendly alternative for crop protection. Recent years have seen a considerable increase in the interest in biopesticides.

Although the intensity of pesticide application in India is less than that in developed countries, their ill effects on health and environment are already visible. This has led the government to set up policies and institutions to promote the substitution of chemicals with biopesticides. However, in spite of these steps, the production and use of biopesticides in India is still extremely small. Most of the production is undertaken in laboratory sized facilities by research centres and government agricultural departments. By and large, the production technology used in these facilities is inappropriate for large scale production. Limited success in up-scaling the production technologies of some of the biopesticides has been achieved. These, however, have not been transferred to industry.

The demand for biopesticides is seriously constrained by their mode of action. In particular, their target specificity and slow pace of action put them at a disadvantage vis-à-vis chemical pesticides. Further research to improve the effectiveness and broaden the range of action are necessary before biopesticides can find wider acceptance among farmers. Limited research aimed at some of these objectives is being carried out in India. However, the research is at an elementary stage and its results will not be available for many years.

IV. BIOFERTILISERS

Introduction

The potential of certain micro-organisms to improve the availability of nutrient to crop plants has long been known. In view of the rise in the cost of chemical fertilisers and their adverse effect on the environment, these organisms (collectively called biofertilisers) have become increasingly important. They are considered to be particularly important in tropical countries like India, whose soils are deficient in organic matter and essential plant nutrients, due to high temperature and intense microbial activity.[90]

Most biofertilisers are nitrogen-fixing in nature; they fix atmospheric nitrogen to ammonia by a complex metabolic process. Broadly speaking, these are of two types: symbiotic and free living. The former, which require symbiotic association with plants, are represented by *Rhizobium*. The latter, which can fix nitrogen independently, include Azatobacter, Azospirillium, blue green algae (BGA) and *Azolla*.[91]

Rhizobium is the most researched and well known biofertiliser, and its role in nitrogen fixation in legumes is well established. It infects the lateral roots of these crops to form nodules, where nitrogen fixation is carried out. Although *Rhizobium* forms symbiotic association with legume crops naturally, in many cases its numbers are too small to fix sufficient nitrogen. Furthermore, not all strains of *Rhizobium* are efficient fixers of nitrogen. For example, In a survey of 87 groundnut rhizobial strains isolated from different parts of India, only five were found to be effective.[92] Artificial inoculation of soil with suitable *Rhizobium* strains to augment their nitrogen-fixing capability can contribute to crop yield in such situations. It is reported that, in favourable conditions, *Rhizobium* can fix 40-60 kilograms of nitrogen/hectare, and that 200 grams of *Rhizobium* is enough to meet one third of nitrogen crop requirements.[93]

Azotobacter and *Azospirillum*, which are commonly found in the rhizosphere of cereals, grasses and vegetables, are also bacteria. In addition to fixing nitrogen, they are known to produce growth-promoting substances and antibiotics. As in the case of *Rhizobium*, they can either be applied as seed inoculants, or the roots can be dipped in a suspension before planting.[94] Reports suggest that in favourable conditions their use can reduce the nitrogen requirement by 25 to 50 per cent.[95]

The other important, free-living, nitrogen-fixing agents are blue green algae (BGA) and *Azolla*. While, as the name suggests, BGA is an algae, *Azolla* is a water fern. Both prefer standing water for growth and are suitable for use as a source of nitrogen for rice. Of the two, BGA has attracted greater attention in India. In fact, Indian researchers were amongst the first in world to notice and study its nitrogen fixing properties.[96] The early interest in BGA, however, was not followed up by systematic research. It was only in the 1970s, that efforts were initiated to examine the potential of using BGA as a biofertiliser on a large scale. The initiative was taken by the Department of Science and Technology, which supported an All India Coordinated

Programme on algae during 1976-84. The programme is reported to have shown that in favourable conditions, the use of BGA can reduce the nitrogen consumption by 20-30 kg/hectare without affecting rice yield.[97] Similar increase in production has been reported in the case of *Azolla*.[98]

In addition to the nitrogen fixing micro-organisms, there are biofertilisers which can improve the availability and uptake of other nutrients. Two of these are considered to be most important. The first group consists of certain fungi mycelium which form a symbiotic relationship with plant roots. The symbiotic relationship, called vesicular-arbuscular *Mycorrhiza* (*VAM*), is considered to be associated with increased plant growth and enhanced accumulation of plant nutrients such as phosphate, zinc and copper.[99]

The second group of non-nitrogen-fixing biofertilisers are phosphate solubilising microorganisms. These micro-organisms, which include bacteria, fungi and yeast, excrete organic acids which solubilise rock phosphate and tricalcium phosphate by decreasing the size of particles to near amorphous forms.[100] Indian soils are characterised by poor to medium phosphorus availability; only about 25-30 per cent of the phosphorus applied to the soils is available for the crops. The presence of these microorganisms is reported to increase the availability of phosphorus considerably.[101]

Role of Government

Much of the production of biofertilisers is directly or indirectly supported by the government. Firstly, the Ministry of Agriculture of the central government has a national project on the development and use of biofertilisers, which was set up in 1983. A number of zonal production facilities have been set up under the National project, which produce biofertilisers suitable for various regions. Secondly, most of the state agricultural departments and state agricultural universities produce biofertilisers. Thirdly, a number of public sector firms and cooperatives have been encouraged by the government to set up production facilities. Fourthly, the government provides substantial subsidies to cover the cost of plant and equipment required by private industry for setting up production facilities.[102] Fifth, the state governments purchase a large proportion of the yield for distribution to farmers, thus providing the producers a guaranteed market.

In addition to its support to production, the government is also closely involved with programmes to popularise the use of biofertilisers. The National Centre of Biofertilisers, the state departments of agriculture and some of the agricultural universities are engaged in these programmes.

Production

More than 60 units are engaged in the production of biofertilisers; the total output in 1992-93 was 2 211.8 tons.[103] Most of the producers are small and only a handful of them produce more than 100 tons of biofertilisers per year. These include the production facilities set up under the National Biofertiliser Project, which produced about 350 tons of biofertilisers in 1992-93. In addition to these and various state agricultural departments, some industries in the public and private sectors and cooperatives are also engaged in the production.

The two largest producers are NAFED and Gujrat State Fertilisers Corporation (GSFC). NAFED, a government sponsored co-operative, was one of the first agencies to undertake large scale manufacturing of biopesticides in India. This began in the early 1970s when facilities for the production of 150 tons of *Rhizobium* for soybean, ground nut and pulses were set up. NAFED was also the first Indian producer to use fermentation technology and, at present, uses a 2000 litre fermentor.[104]

GSFC, the other large producer, is a public sector fertiliser firm, which began the production of biofertilisers in 1984. It produced a total of 266 tons of biofertilisers during 1992-93. This included 55 tons of azatobacter, 56 tons of *Azospirillum*, 58 tons of *Rhizobium* and 95 tons of phosphate solublising microorganisms.[105]

The other major producers are Madras Fertilisers (a public sector fertiliser firm), Zuari Agro-chemicals (a private sector agro-chemical firm), SPIC (private sector petrochemicals firm), Stanes and Co. (a private sector firm).

Rhizobium is the most commonly produced biofertiliser in India. About 1 000 tons of *Rhizobium* is being produced currently. A very large proportion of this is produced by government departments and public-sector firms. The private-sector firms account for only 16 per cent of *Rhizobium* production. Government agencies dominate the production of other biopesticides also.[106]

Demand Constraints

In spite of the government's policy of encouraging their use, the demand for biofertilisers continues to be very small. Even the comparatively small amount of biofertiliser produced can not be sold on commercial basis and, in most cases, is procured by the government for free distribution among farmers. Efforts to sell them on a commercial basis have, by and large, failed and, according to one of the producers, the production cannot be continued without substantial government support.[107]

The situation is unlikely to improve in the near future. According to official projections, the production of various biofertilisers at the end of this decade will be sufficient to meet less than 2 per cent of the perceived requirement. (See Table 4.1).

Table 4.1. **Projection for the production of Biofertilisers in the Year 2000 A.D.**

Biofertiliser	Production (tons)	Percentage of Potential Demand
Azospirillum	7 230	0.8
Rhizobium	14 460	1.7
Azotobacter	7 230	0.8
PSM	7 230	0.8
BGA	3 015	1.1

Source: Motsara M.R., "National Project On Bio-fertlisers-Status Position-VIIIth Plan Proposal", National Conference on Biofertilisers and Organic Farming, Organised by the Ministry of Agriculture, Madras, 1993 pp. 14-20

The acceptance of biofertilisers among farmers is very low. This, in turn is due to *a)* the poor and uneven quality of biofertilisers produced and *b)* the relatively small and uncertain contribution to crop yield.

Quality

The problem of quality has been serious since the 1980s, when the production of biofertilisers on a large scale was first started. Tests conducted in recent years show that the situation has not seen much change since then. For example, according to tests (which are based on the number of inoculi found in one gram) carried out by the ICRISAT on *Rhizobium*, a majority of the samples failed to pass.[108] Similarly, in tests carried out by the National Biofertiliser Project, more than one third of the samples were found to be of unacceptable quality. (See Table 4.2).

Table 4.2. **Quality of Biofertiliser Samples Tested Under National Biofertiliser Project**

Number of Samples Tested	Number of Samples Found Below Standard	per cent of Samples Below Standard
430	160	38

Source: Motsara M.R., "National Project on Biofertiliser- Status Position-VIII Plan Proposals", Paper presented in National Conference on Bio-Fertilisers and Organic Farming", Organised by the Ministry of Agriculture, Madras, 1993.pp 14-20

Unlike chemical fertilisers, the quality of biofertilisers is not regulated by the government. Also, except in the case of *Rhizobium*, there are no official quality standards for biofertilisers. Even in the case of *Rhizobium* the standards are not followed.

A number of factors contribute to the poor quality. The quality of strains and carriers, production processes and methods of packaging, storing and transport are largely responsible for this. In the case of bacterial biofertilisers (*Rhizobium*, *Azospirillum* and *Azotobacter*) carriers, which are known to be particularly important for increasing the shelf life, have been the focus of much recent debate and research.

Research has shown that peat, which has a high moisture-retaining ability, is the most suitable carrier for *Rhizobium*. Its use is particularly common in some of the developed countries such as Australia and the United States, where it is commonly available. In India, the availability of peat is limited and a number of alternative carriers have been tried, unsterilised lignite being the most common.[109] Although producers claim that the lignite impurities are within the limit, the performance of these and other materials used as carriers has been found to be less than satisfactory, as their moisture-retaining ability is inadequate, and they are prone to contamination. Consequently, the shelf life of biofertilisers using these materials as carriers is short. While many producers claim that their products have a shelf life of more than six months, in most cases it is found to be less than three months. For carriers used by various *Rhizobium* producers, see Table 4.3.

Table 4.3. **Carriers Used By Various Rhizobium Producers.**

Organisation	Carrier
TNAU	Peat
Madras Fertiliser	Lignite
GSFC	Lignite
NAFED	Lignite
Biofertiliser Centres	Charcoal

Source: Firm interviews

The situation is worse in the case of BGA as most of it is produced in open tanks, which are easily contaminated. Also, soil and straw, which are commonly used as the carriers, are also highly prone to contamination. The contamination not only reduces its effectiveness, but also sometimes damages the crop.

Performance

Most of the field trials highlight the fact that the contribution of biofertilisers to crop yield varies greatly, and that the farmers are justified in being sceptical of their contribution. The results of some these trials are listed in the following paragraphs.

— *Rhizobium*: An increase of only 3 to 12 per cent in yield has been achieved in chickpea.[110] Similar results, showing poor and inconsistent effects on crop yield have been reported by other researchers.[111]

— *Azospirillum*: An increase in yield was achieved in only 6 out of 9 tests on pearl millet. The change in yield varied between -10 to 17 per cent. In case of sorghum, an increase in yield was achieved in only 4 out of 9 tests.[112] In case of rice, the application of *Azospirillum* was found to make an important difference in only 48 out of 108 trials.[113]

— *Mycorrhiza*: Only in 50 per cent of the cases was a significant improvement in yield achieved. The plant response to *VAM* varied with soil type, soil fertility and *VAM* cultures.[114]

— *Azotobacter*: significant response to inoculation was observed in 342 out of 411 trials in wheat.[115]

Phosphate-solublising micro organisms: Out of 37 field trails conducted, only 10 showed significant increase in yield in the case of rice, wheat, chickpea, pigeonpea, soybean and groundnut.[116]

The crop response to biofertilisers depends on a number of factors. The most important of these are:

1. Number of living cells in the inoculum;
2. Suitability of the strain to soil and crop;
3. Competitiveness of the inoculant against microorganism already present in the soil;
4. Tolerance of inoculants to abiotic (salinity, pH, moisture and heat shock) stresses;
5. Application techniques.

Research Activities

Systematic research on biofertilisers began only in late 1980s. The two important objectives of research, most of which have been concentrated on *Rhizobium* and BGA, are: *a)* optimisation of the production process to improve the quality and the quantity of the yield and *b)* strain selection and improvement.

Improvement of Production Process

Rhizobium

Until the beginning in the 1980s *Rhizobium* (and other bacteria based biofertilisers) were produced in flasks. As a result, the quantity and quality of yield was extremely poor. The first major effort towards improving the production process was undertaken jointly by the Ministry of Agriculture and the FAO in the late 1980s. The programme involved the transfer of production and formulation technology and protocols developed by NIFTAL (an international research institute specialising in nitrogen fixation) to India and the training of Indian technical personnel. Some modification of protocols to suit Indian strains and agro-climatic conditions was also undertaken.

The other major research efforts — concentrating on *Rhizobium* and blue green algae — were initiated by the Department of Biotechnology in the early 1990s. This research has more or less the same goals as the Ministry of Agriculture initiative, namely optimisation of the process for the production of *Rhizobium* of suitable strains. According to DBT, a process for large scale production has been optimised. Some of the large producers (Madras Fertilisers, GSFC and NAFED) are also undertaking research aimed at the optimisation of large scale fermentor based production technology. NAFED, for example, is currently using a 2000 litre fermentor.

While the efforts to improve production technology have been successful to some extent, the shelf life of the products continue to be short and varying. This is because the efforts to develop suitable carriers which have desirable moisture retaining and other qualities, and are not prone to contamination, have not been very successful. Experiments with synthetic materials were planned, but it would be some time before results will be available.[117]

Blue Green Algae

Progress in the improvement of BGA production technology has been even less marked. As mentioned earlier, until recently BGA was being produced in open tanks. As these tanks are extremely prone to contamination, the quality of production was very poor. The problem of contamination was made worse by the use of soil and straw, as carriers.

Current research efforts are focused on the production of BGA in plastic bags and containers, which are used as simple bio-reactors. As a result of these efforts, which are funded and co-ordinated by the DBT, trials to produce BGA in liquid cultures using breathing bags are being undertaken. The technology, which is reported to have been optimised, is yet to be used for large scale production.

Studies have also been initiated recently to produce near pure forms of BGA in open ponds without soil. It is planned to mix pure BGA with suitable carriers (Soft stone, kaolin and clay are being considered) for transportation, storage and application.[118]

Strain Improvement

The effectiveness of microorganisms as biofertilisers depends very largely on the selection of strains which are suitable for a particular crop, soil and other factors. This is particularly true in the case of *Rhizobium* which is highly specific in its action. Collection of strains from Indian soils and their screening for survival and nitrogen fixing abilities is being carried out by a number of agriculture universities and research institutes. The Department of Biotechnology has supported the setting up of a germplasm bank at the Indian Agricultural Research Institute (IARI) for maintaining a variety of *Rhizobium* strains. The bank has about 200 strains of *Rhizobium* specific for chickpea, arhar, soybean, french bean and groundnut. It also has about 2 000 soil samples, which are used for studying the effectiveness of *Rhizobium* strains.[119]

Except for a handful of large producers such as NAFED and GSFC, who have their own strain collection, most producers depend on universities and research institutes for strains. NAFED has established a large culture bank which consists of suitable strains of Rhizobium, *Azotobacter*, azospirllum, *Pseudomonas*, *Aspergillus*, *VAM* and phosphate-solublising micro-organisms. Some of the institutes which provide these strains are IARI, ICRISAT, BNFRC (Bangkok) and NIFTAL (Hawaii). GSFC are working on isolating strains which can survive high-temperature and drought conditions. This will increase the life and effectiveness of the strain both on shelf and in soil.

Strains of *Rhizobium* are being screened with the help of genetic techniques. Protein finger printing techniques to study rhizobial variants are also being developed. Also, methods of strain identification using intrinsic antibiotic resistance have also been developed, and are being standardised.

Studies on the development and testing of mutants of microbial biofertilisers are also being undertaken. For example, a number of mutants of *Rhizobium* are being studied for a) the variation in symbiotic effectivity, b) the ability to grow at low pH and c) the competitiveness with native *Rhizobium*.[120]

More ambitious research involving genetic manipulation of micro-organisms to improve their effectiveness as biofertilisers (by improving survivability and competitiveness) has also begun. For example, researchers at the IARI have isolated genes responsible for stress-tolerance (for heat shock, high temp, low moisture) from alfa *Rhizobium* and have cloned these to chickpea *Rhizobium*. This was done during 1990-93. The strains with stress tolerance genes have already been tested in the laboratory and are now undergoing national trials. The new strain is expected to have a longer shelf life as it is likely to withstand stress during transportation and storage better.[121]

Researchers at the Tamil Nadu Agriculture University are also engaged in genetic manipulation of bacterial biofertilisers. They have transferred genes responsible for abiotic stresses in *Rhizobium* and *Azospirillum*. They are now exploring the possibility of transferring genes responsible for the production of indole acetic acid, which is a plant growth hormone, to bacterial biofertilisers.

In another study, researchers at IARI are hoping to increase the effectiveness of *Rhizobium* inoculi by introducing genes which can increase their competitiveness vis-à-vis *Rhizobium* already present in soil. They have isolated certain *Rhizobium* strains which are resistant to toxins (produced by competing *Rhizobium*) but are poor fixers of nitrogen. The genes responsible for toxin resistance from these strains will be transferred to those which are high nitrogen fixers. The research is in its preliminary stages and is currently focused on isolation of genes responsible for toxin resistance. The gene transfer is expected to take about one year.

Compared to *Rhizobium*, the research efforts aimed at improving the strains of other fertilisers are much smaller. A National Facility for Blue-Green Algae, which holds 550 strains of BGA has been set up with the support of the DBT. The facility acts as a source of strains to researchers throughout the country.

Some work on the selection and improvement of BGA strains suitable for various soil conditions is also being done. For example, the DBT has recently funded research on isolating strains of BGA suitable to local conditions. Attempts are also being made to introduce genetic markers in BGA to be used for quality control.[122]

Research to combine the beneficial properties of more than one microbial fertiliser through genetic manipulations is also underway. The IARI researchers, for example, are working on isolating the genes responsible for the production of phosphate solublizing acids in bacteria. They plan to transfer these to nitrogen-fixing *Azotobacter* and *Azospirillum*, so as to combine nitrogen fixation with increased availability of phosphorous. They have finished the screening of bacteria and will soon start working on the isolation of genes responsible for the trait. The completion of work will take at least 3 years. Once isolated, these genes can also be transferred to bacteria for use as bio-reactors which can produce phosphate fertilisers from rock phosphates on an industrial scale, without using energy and causing pollution.[123]

Summary

Biofertilisers are micro-organisms which can improve the availability of nutrients to plants. They are an important component of sustainable agriculture, as they can reduce the use of chemical fertilisers. *Rhizobium*, blue green algae, *Azospirillum* and *Azotobacter* are some of the important nitrogen-fixing biofertilisers. Others, such as *Pseudomonas* and VA-*Mycorrhiza* are phosphate-mobilising biofertilisers.

The Indian government has taken a number of steps to promote the use of biofertilisers. These include technical support and financial incentives to encourage the production and use of biopesticides. However, in spite of these steps, the acceptance of biofertilisers among farmers continues to be very low. Consequently, almost all the biofertiliser produced in India is procured by the agricultural departments for free or heavily subsidised distribution.

The demand for biofertilisers suffers from three factors: poor and uneven quality, short shelf life and small contribution to crop yield. Research aimed at the optimisation of production process and improvement of quality is being undertaken at a number of centres. The progress in this direction, however, has been small. Work to increase the survival and effectiveness of biofertilisers through genetic manipulation of strains has begun very recently. A significant improvement in the performance and increase in acceptance of biofertilisers is possible only if these efforts are successful. Otherwise, the contribution of biofertilisers to sustainable agricultural development will continue to be small.