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# *Tailoring production technology: Bacillus thuringiensis (Bt) for localized production*

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**Abstract**

*Castor cultivation in the dryland districts of Andhra Pradesh is constrained by the insect pest castor semilooper Achoea janata. The insect pest is affected by several natural enemies that include the egg and larval parasitoids. Use of chemical pesticides for management of the pest results in death of the natural enemies thereby necessitating repeated sprays of the insecticide leading to development of pest resistance and resurgence. An insect pathogenic bacterium Bacillus thuringiensis (Bt) holds great promise as an eco-friendly option for management of the pest with safety to the natural enemies. However, prohibitive cost of the commercial Bt formulations traditionally produced through submerged fermentation with high capital investment by the multinational companies will not favor their recommendation for castor semilooper management owing to the resource poor nature of the farmers in the region. This led to development of a novel production methodology for the cost-effective mass production of Bt based on the principle of solid state fermentation with less capital investment and low skill requirement with twin advantages of enabling localized production for bringing Bt to the dryland farmer at an affordable price and generating local employment as well.*

## 1. Introduction

Andhra Pradesh (AP) is India's fifth largest state, with about 70% of the population engaged in agriculture (some 10 million households). Over 80 per cent of those are small and marginal farmers and landless laborers who own a mere 35% (3.5 million hectares) of the total 10 million hectares of cultivated land. Mahabubnagar and Nalgonda districts of Andhra Pradesh are dryland tracts where the farmers are resource pair and agriculture is totally rainfed (Pimbert and Wakeford, 2002).

The crops cultivated predominantly in these two districts are castor, red gram, sorghum and groundnut. Statistics from the Commissionerate of Agriculture, Government of Andhra Pradesh during the year 2004 indicate that the area under cultivation in Mahaboobnagar and Nalgonda districts is 700,538 hectares and 425,642 hectares respectively. Of this, castor occupies the highest acreage of 27.30% and 19.84% in Mahaboobnagar and Nalgonda districts respectively while pigeon pea and groundnut occupy 8.39 and 7.53% in Mahaboobnagar district, 7.96 and 5.29% in Nalgonda district respectively (Vimala Devi and Rao, 2005). Cultivation of these crops in the rain fed areas is constrained by the vulnerability of the improved cultivars to several insect pests of which castor semilooper *Achaea janata*, gram pod borer *Helicoverpa armigera* and tobacco caterpillar *Spodoptera litura* are economically important.

*Bacillus thuringiensis* (Bt) is a naturally occurring soil bacterium. The potential of this bacterium in the management of several caterpillars causing serious damage to the cultivated crops world over has been documented over the last fifty years. There are currently more than a hundred products of Bt registered for the management of important lepidopteran insect pests such as *Helicoverpa armigera* (Cotton bollworm), *Ostrinia nubilalis* (Europeon corn borer), *Plutella xylostella* (diamond back moth), *Trichoplusia ni* etc. Insecticides of microbial origin occupy only 1-2% of the global market for insecticides. However, Bt occupies 95% share of the microbial pesticide market. The production of Bt worldwide is dominated by the multinational companies and amounts to several thousand tons annually. Bt production by these companies involves high capital investment and is carried out traditionally through submerged fermentation in large fermenters.

Over the past three decades, interest in microbial insecticides has been revived leading to development of protocols for large-scale production of Bt and its registration under several trade names world over. This paper presents the initiative towards tailoring the Bt production technology in order to enable cost-effective localized production of Bt in Mahabubnagar and Nalgonda districts under the Andhra Pradesh-Netherlands Biotechnology program.

## 2.0 Technicalities of Bt production

### **2.1** Specific growth requirements of Bt

Bt is a bacterium requiring oxygen, readily utilizable sources of nitrogen and carbon for its growth. The standard method of production of microorganisms is the process of fermentation (Taborsky, 1992). There are many types of fermentation. The two most common are submerged and solid state.





**2.1.1. Submerged or deep tank fermentation** is as the name implies, a growth of microorganism in a fully liquid system. There are a number of advantages to these systems, which include the ability to hold temperature and pH constant, the ability to pump large quantities of air into the system and disperse it by means of stirring impellers, and the ability to generate reasonably homogenous conditions to maximize the growth of microorganism. Small-scale production of Bt through submerged fermentation involves seed fermenters of 20-40 l capacity, which contain 10-25 l of medium. Culture from the shaker or laboratory fermenter (1-3 l) serves for inoculation at 1-3% of the volume of the medium. The culture from the seed fermenters is used for inoculation into industrial fermentation tanks of 100 l capacity and above. The seed tanks can be made of stainless steel or glass but the fermentation tanks are always made of stainless steel. An aeration ring is used to aerate the culture, together with an agitator or an air outlet under a propeller. The air volume used for aeration should correspond to between ½ and full volume of the cultivation medium. Unless foam suppressing agents are used, the liquid in the fermentation tanks will be subject to intensive foaming thereby lowering the efficiency of air uptake by the organism thus affecting its growth. This whole period works out to 72 h.

Production of Bt is done in large capacity industrial fermenters employing the method of submerged or deep tank fermentation, which is as the name implies growth of microorganism in a fully liquid system. For optimal growth of Bt, pH and temperature are important factors. In addition number of essential services namely air, water and electricity should be available 24 h a day throughout the fermentation period, which is 72 h in general for Bt.

**Sterility criteria:** A basic requirement for microbial technology is the stringent requirement of absence of contamination by a foreign organism during the production process right from stock culture to termination of growth. Large fermenters offer a greater possibility of culture contamination. The fermenters, their pipelines and fittings are sterilized prior to medium sterilization, sometimes together with air filters. Contamination may often be caused by minute leakages in cooling coils or jackets, faulty packing or air filter failure. Hence Bt production in fermenters has to undertaken with utmost care, therefore requires great skill and training.

The scale of production and media cost make Bt the most inexpensive of all the microbial insecticides produced. However, it is still expensive compared to many synthetic insecticides. Fermentation optimization has the potential to greatly influence cost of production and ultimately commercial competitiveness.

**2.1.2. Solid-state fermentations** are relatively easy to develop on a small scale. Scaling them up to the sizes necessary for commercial production presents numerous problems, aeration becomes a major difficulty as the volume of the solid mass increases more rapidly than the available surface area. Although multiplication by solid-state fermentation demands less capital investment as well as technical skill nevertheless is not considered suitable for multiplication of aerobic organisms. However optimization of Bt multiplication on the principle of solid-state fermentation can effectively contribute towards promoting Bt usage in insect pest management programs. Manpower requirement is relatively higher for undertaking Bt production through solid-state fermentation, which therefore finds promise in the developing countries where labor is cheap.

**2.2.** Microbial toxins

A microbial toxin can be defined as a biological poison derived from a microorganism such as a bacterium or fungus. Toxins of Bt is the most widely investigated example. The basic active agent, called delta endotoxin, is produced in the form of crystalline parasporal inclusions during sporulation and liberated in the medium after its completion. Maximum toxin production can be achieved only by careful attention to the interaction of fermentation conditions, media and the isolates involved.

Before one can sell a live microorganism as a pest control agent, a reliable method of production must be developed which yields large quantities of the product for which a specification can be drawn up.

**2.3.** Issues that constrain local production of Bt through the traditional method of submerged fermentation**:**

In spite of the convenience of Bt production employing fermentors, the production till date is in the reach of multinational companies only with no scope for encouraging its production at the village level, which can be primarily attributed to the following:

1.High capital investment
2.High level of automation
3.High technical skill requirement
4.Continuous power supply requirement

Promotion of Bt production locally entails a need for development of alternate strategies to overcome these constraints. One important option is the production through solid-state fermentation that is relatively easy to develop on a small scale. Till date, this option has been effectively employed for mass production of entomopathogenic fungi wherein aerial conidia are produced from the surface of the substrate while it has not been exploited for mass production of Bt.





**2.4.** Medium development

Whichever type of fermentation is chosen, nutrient must be provided so that the microorganism can grow efficiently. The nutrients chosen will markedly affect how fast the organism grows, how much is produced and often, how infective the final product is. Nutrients to be provided include a carbon source e.g.. Glucose or molasses, nitrogen source e.g.. Soya bean meal or yeast extract. Some ions which supplement nutrition are indispensable for the growth and sporulation of Bt. They are supplied in the form of salts of magnesium, manganese, iron, zinc and calcium.

A successful microbial process on a small or large scale requires the research and development (R&D) of methods satisfying the following demands:

. Maintenance of stability of the production culture
. Maintenance of suitable conditions ensuring reproducible yields
. Application of the technology for maintaining strictly aseptic conditions

Microbial insecticides do not directly depend on the effect of a poisonous chemical but exploit the activity of living (or self replicating) entities. An exception is the enterotoxinosis caused by Bt where a preformed toxic glycoprotein is essential for infection to occur.

**3.0 Need for tailoring Bt production technology for enabling localized production**

**3.1.** Case background

Castor crop is cultivated predominantly in the dryland tracts of Andhra Pradesh, India. The crop yields are greatly affected by the attack of the insect pest *Achaea janata* known popularly as castor semilooper. Castor semilooper is a voracious feeder causing extensive defoliation, also feeds on tender shoots and developing capsules leading to considerable reduction in yields (Vimala Devi *et al*., 2000). Yield loss to the tune of 20 % has been reported while defoliation during early stages of the crop can lead to resowing. Outbreaks of this pest are common during July to September (Gaikwad. and Bilapate, 1992). In nature, the pest is known to be attacked by several natural enemies. Bt holds promise in the management of castor semilooper and is reported to cause feeding cessation within hours after feeding followed by death of the larvae within 2-5 days of exposure to Bt treated foliage Cost of the commercial Bt formulations is prohibitive. Hence large-scale exploitation of Bt for castor semilooper management can gain momentum only if low cost Bt formulations are made available through localized production. With this objective, efforts were initiated to exploit the principle of solid-state fermentation for the mass production of Bt. The issues addressed were

- Enabling Bt production with low capital investment without a fermentor
- Aeration for meeting oxygen requirement for optimal growth of Bt,
- Medium standardization employing local agricultural wastes
- Minimal recurring expenditure

**3.2.** Technology development For Mass Production of Bt by addressing the identified issues

In tune with the growth requirements of Bt, we developed a novel protocol based on the principle of solid-state fermentation for enabling mass production employing agricultural wastes/by-products such as wheat bran and molasses. Magnesium, manganese, iron, zinc and calcium ions are present abundantly in the wheat bran, hence the need to supplement them in the medium has been overcome. The media used however represents only a small share of the costs of equipment, servicing and utilities required for operation. The mass production of Bt is being carried out successfully in a micro-enterprise established by us at Wanaparthy, Mahbubnagar district, Andhra Pradesh, India, under the AP-Netherlands Biotechnology Programme in association with a non-governmental organization called SDDPA (Society for Development of Drought Prone Area).

The multiplication is done on a medium based on wheat bran supplemented with carbon and nitrogen sources to a minimal level so as to permit the growth of Bt immediately after inoculation. The components are added to the wheat bran, mixed well. This medium is packed in polythene covers and sterilized in an autoclave at 15 pounds pressure and 121$^{o}$C for 20 minutes. After cooling, the medium from the covers is transferred aseptically to plastic tubs sterilized with rectified spirit (a by-product in sugar production) and exposed to the UV light in a laminar airflow. The seed culture is added to this medium and mixed well. The tub is then covered with a polythene sheet kept in place with a rubber band.





For scaling up the production, a clean room with a suitable capacity hepa filter has been established. A wooden cabinet with shelves of 80 cm height and 2 feet depth with sliding glass doors has been fixed to the wall adjacent to the laminar airflow on the left. UV lights have been provided in each shelf of the cabinet on the upper surface. The plastic tubs are wiped with the rectified spirit using cotton and placed under UV in the cabinet for one hour. After this, the tubs are taken into the laminar airflow, medium is transferred to these tubs and inoculated with the seed culture. The tubs are incubated in a room maintained at 30$^{o}$C for 65 h. Aeration is provided at 8-10 h intervals from the second day by exposing the medium in the tubs to the sterile air from laminar flow. In total four aerations are given. After 65 h of incubation, sterile water is added to the medium in the tubs, mixed well and filtered through a double-layered muslin cloth and pressed well for complete extraction. The filtrate is centrifuged at 10,000 rpm. The supernatant is discarded and the pellet is mixed with either the medium residue obtained after filtration or a suitable inert carrier. This mixture is shade dried, powdered and formulated by mixing with stickers/wetting agents. This formulation is suspended in water and sprayed on foliage for management of the insect pests.

In the scale-up production of Bt at the micro-enterprise, one tub yields 100 g of Bt product on an average. 100-150 l of spray suspension is required per acre for the management of castor semilooper depending on the stage of the crop. At the effective dose of 1g/l of spray suspension, the amount of Bt required would work out to 100-150 g per acre with material cost ranging from 3.2 to 4.8 rupees. The material cost for production of one kg of Bt would be Rs.32/-. Multiplication in plastic tubs brings down the cost of production because of the low cost of plastic tubs as well as their re-usable nature when compared to use of glassware that are costly as well as easily subjected to breakage (Vimala Devi *et al.*, 2005).

The whole process is very simple and can be carried out efficiently with some amount of practice. It is not demanding in skill and thus enables localized production at village/district level. Undertaking fresh batches inoculation everyday makes the production cost-effective. In addition the tubs used for inoculation are cleaned and reused. The technology therefore has the potential to generate employment for the local youth. These advantages will encourage the localized production of Bt by the above protocol thereby contributing to promotion of its extensive use in pest management.

**Infrastructure requirement:** Inoculation chamber provided with a hepa filter, UV cabinet and a laminar airflow; media preparation room with an autoclave, weighing balance, water distillation unit; downstream processing room with a centrifuge; drying room with steel racks and a fan and a wash area.

**Medium development:** The nutritional requirements of carbon, nitrogen and trace elements were met by using locally available agricultural by-products/wastes as organic sources of carbon and nitrogen however supplemented with inorganic carbon and nitrogen to a minimal required level.

**3.3** Comparison of Steps in Bt Production in Submerged and Solid-state fermentation:

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| Preparation of seed culture | Seed culture requirement is ranges 3-5% of the total medium. Bt multiplication employing fermentors of 100 l or more capacity would require a minimum of 5-10 l seed culture for which small fermentors are required. | Seed culture multiplication can be taken up in conical flasks on a laboratory shaker. Flasks containing the broth are inoculated and maintained overnight at 300C at 200 rpm on a shaker overnight. |
| Preparation of medium | Sources of carbon and nitrogen in a fine, readily usable form supplemented with trace elements have to be used since the baffles which supply air should not be clogged. | The agricultural by-products/wastes can be used directly with little need for processing. The trace elements requirement is generally met from the organic agricultural by-products/wastes used for multiplication. |





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| Autoclaving of medium | The sterilization of the medium is done either in situ if the provision is available or else outside and then transferred to the fermentor aseptically. This involves high skill requirement and understanding of the process. | The medium can be conveniently packed into polythene covers and sterilized in a fermentor. The procedure does not require skill and understanding and can be carried out routinely |
| Inoculation of medium with seed culture | Inoculation of the medium in large fermentors is fully automated while in small fermentors it is done employing peristaltic pumps and this involves skill. A small fault in inoculation could lead to loss of the whole batch through contamination. | noculations here are being carried out in a laminar air-flow or a chamber wherein conditions in a laminar air-flow are simulated. Since the inoculations are carried out in tubs, mistakes in inoculation could lead to loss of only small amount of the medium. These inoculations are carried out aseptically but do not require much skill and can be carried out routinely. |
| Air supply for growth of the organism | Sterile air is supplied to the medium through air filters using air compressors. The air filters are to be sterilized before they are connected to the fermentor and have to be cleaned from time to time.. | Air is supplied by exposing the medium in the tubs to the sterile air from laminar flow at 8-10 h intervals. Although the step is a bit laborious nevertheless it does not require high skill and can be carried out routinely with perfection through a little practice.. |

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| Efficient growth of the organism | Apart from maintenance of controlled growth conditions, anti-foam agents are required to be added essentially to enable the uptake of the air by the organism effectively. This requires monitoring. Leakages in the fermentation vessel can also result in contamination leading to discarding the whole batch of the medium. | There is no need to use any anti-foam agents. The tubs containing the medium inoculated with the seed culture are only incubated at the required temperature. This does not require monitoring. Contamination problem is not an issue to reckon with. |
| Maintenance of the optimum temperature for growth of the organism | Maintenance of temperature is made possible through use of heater and chillers in fully automated conditions while alternately placing the fermentor under controlled ambient conditions. | Since inoculation is carried out in tubs, they can be maintained in room with the required temperature. This has no skill involvement and can cater to incubation of a large quantity of medium at a given point of time. |
| Time duration for growth and lysis of the organism | Time required for growth and complete lysis of Bt is around 60 h. | Time required for growth and complete lysis of Bt is around 65 h. |





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| Downstream processing for obtaining the final product | This is carried out through spray drying preceded by thickening of the fermentation medium with biomass either through centrifugation or evaporation. This again is automated where large volumes are processed and requires trained personnel. | This is carried out trough centrifugation at 10,000 rpm for 10 minutes. The supernatant containing the beta exotoxin is discarded and the pellet containing the spores and crystals of Bt is mixed with the carrier, shade-dried and powdered. This process is carried out mechanically to a large extent and does not involve skill. |
| A number of essential services are required to run an efficient fermentation laboratory, namely air, steam, water and electricity. | All the services should be available 24 h a day throughout the fermentation period, which is 72 h in general. | All the services are not essential throughout the fermentation period which is 65 h in general. |

**3.4** Advantages that accrue from adoption of the new methodology:

. Enabling localized Bt production by way of micro-enterprise establishment

. Micro-enterprise can be established with less capital investment employing simple equipment

. Simple production methodology that does not require high education and training and can be undertaken with local unemployed youth with school education

. Assured availability of quality product to the farmers at an affordable price

. Generation of employment for local youth

. Lowers dependence on local pesticide dealers

. Eco-friendly technology contributing to lowering the environmental pollution including the toxic effect of the chemical insecticides on the farmers who spray them.

**3.5.** Educational and skill requirements: Since the production methodology is simple, it can be undertaken employing local youth who have the high school education. Skill requirement is not demanding. Hence working with care can avoid problems of contamination.

**4.0. Our experience in establishing Bt production units at village level:**

The project was identified through an Interactive Bottom Up (IBU) approach involving the various stakeholders namely end users i.e., farmers in Mahaboobnagar and Nalgonda districts of Andhra Pradesh, researchers, policy makers, government and non-governmental organizations during a pre-project formulation workshop on "Biofertilizers and Biopesticides" organized in the year 1996, by the Biotechnology unit of the Institute of Public Enterprise (AP-Netherlands Biotechnology Programme - APNLBP). In order to encourage and enable the large-scale field use of Bt for castor semilooper management in the dryland areas of Mahaboobnagar and Nalgonda districts of Andhra Pradesh, the APNLBP approved the project to the Directorate of Oilseeds Research, Hyderabad, with the major objectives of identifying potent local isolates of Bt, development of a protocol for cost-effective mass multiplication of Bt followed by the establishment of a production facility, employ the local youth in the production thereby generating employment as well as make Bt available to the farmers of Mahaboobnagar and Nalgonda districts at an affordable price for castor semilooper management.

**4.1.** Farmers involvement in technology development and transfer

Farmers involvement in the technology development was there right from identification of the problem followed by the phases of technology development and technology transfer by way of large scale demonstrations and trainings, culminating in the establishment of a micro-enterprise for localized mass production of Bt. Collection of soil samples for carrying out Bt isolations was carried out in the farmers fields after holding discussions with them for creation of awareness about Bt as a living organism naturally occurring in their soils and its potential in insect pest management. Technology development included identification of potent local isolates of Bt and development of a mass production protocol. This protocol was developed after discussing with the farmers and NGOs about their affordability and also the feasibility for taking up Bt mass production locally. This led to the development of the novel production methodology based on the principle of solid substrate fermentation. Apart from the formal trainings, informal discussions with farmers were held about the mode of action of Bt which includes feeding cessation in the larvae within few hours after feeding on Bt sprayed foliage followed by death within 2-5 days depending on the stage of the larva. These discussions, held during the initial field trials for generating data on the efficacy of DOR Bt-1 formulation as well as the demonstrations in their fields with the Bt formulation for management of castor semilooper, also laid emphasis on the eco-friendly nature of Bt and its safety to the naturally occurring parasitoids of castor semilooper. This awareness creation coupled with involvement of the farmers at every step of technology development suited to their needs and transfer was crucial to success of the program and acceptance of the technology leading to the establishment of the mass production unit locally for making Bt readily available to them at an affordable price.

In the fields where farmers adopted the regular practice of semilooper management through use of pesticides like quinalphos, 4-5 sprays of the insecticide had to undertaken while the pest could be managed in Bt sprayed fields with only 2 sprays of Bt since the natural enemies like *M. maculipennis, Euplectrus maternae* and several others were conserved and resulted in their build-up thereby leading to natural pest suppression in the later stages. The participating farmers could clearly perceive the difference between pesticide sprayed fields and Bt sprayed fields with lack of natural enemies in the former while there was abundance in the latter thereby depicting the importance of the natural enemies in pest suppression. The cost benefit ratio for DOR Bt-1 treatment (2 sprays) was 3.10 while it was 1.62-1.80 in quinalphos - 5 sprays (Ekalux EC 25) (Vimala Devi and Rao, 2005).

**5.0. Conclusion**

Bt has been the most widely used and successful microbial insecticide ever registered. However, their prohibitive cost coupled with lack of ready availability does not encourage the use of Bt in pest management in the dryland crops like castor and red gram. Bt availability at an affordable price to the farmers of the district is all set to become a reality through the micro-enterprise established in the region.

Large-scale field-testing of the DOR Bt-1 formulation in Mahaboobnagar and Nalgonda districts has shown that the pest can be effectively managed with 2 sprays at 15 days interval. The cost of pest management has been lowered three fold when compared to the chemical insecticide. The yield increase per hectare was 132.5 kg approximately which accounted for an increased income of Rs.2840/-. Even if 10% of the existing area under castor cultivation can be protected from castor semilooper attack employing DOR Bt-1 formulation, the income would increase by Rs.78.10 million rupees annually.

The focus of the program was product development and enabling its adoption. The success of the programme can be attributed to development and transfer of technology in tune with the requirement of the end user namely the resource poor, dryland farmer with major emphasis on the participatory approach.

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