Bio-pesticides Research at ICRISAT: A Consortium Model

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Abstract: There is an increasing pressure on agriculture to produce more food to meet the demand from the growing populations all around the world. As the demand for food production increased the need for intensive plant protection also stepped up with increased use of toxic pesticides resulted in complex environmental, economical and operational implications. Several national, International agencies and non-Governmental organizations are presently engaged in supporting research, and application of eco-friendly approaches that sustain plat protection and environment.

ICRISAT initiated a consortium called "Bioproducts Research Consortium (BRC)" in Jan 2005. BRC is a public and private sector partnership initiative, focused on delivering research outputs, capacity building and technologies leading to mass-scale production of quality bio-products. The microbial collection at ICRISAT has over 2000 accessions that includes promising entomopathogens (*Bacillus subtilis* BCB19, *B. thurengiensis* HiB67, in addition to 37 potential isolates) and antagonists of phytopathogens (*B. subtilis* BCB19, *Pseudomonas* sp CDB35, in addition to 154 isolates).

In recent years, bio-pesticide research at ICRISAT has made significant progress in identification, production and field evaluation through participatory approach. The virulence of various bio-agents such as NPV, bacteria and plant products were tested under controlled conditions and the selected ones were evaluated under hot spots. Strategic research related to feasible production technologies, efficient storage to enhance the shelf life, field application, genetic variability, and efficient monitoring has made substantial progress. To strengthen these eco-friendly approaches ICRISAT in collaboration with National Agricultural Research Systems (NARS) assisted in improved the capacity of several researchers and farmers on bio-pesticide production and established 96 village level NPV production units in India and Nepal. On-farm studies with bio-pesticide front indicated substantial reduction in pesticide application from11 to 4 sprays in cotton, 2.1 to 1.6 in rice 2.9 to 2.2 in pigeonpea and 2.9 to2.3 in chickpea. Thus this manuscript discussed various aspects of bio- pesticide research at ICRISAT covering the status, constraints, prospects, their role in integrated pest management and the future strategies for their effective utilization for the benefit of human kind.

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Introduction

The advent of modern agricultural practices comprising of the introduction of high yielding crop varieties, use of chemical fertilizers, assured irrigation facilities and improved agronomic practice during seventies have enabled the farmers in increasing the crop production. The intensive cropping systems have necessitated increased use of pesticides for plant protection. The reduction of losses caused by pests and diseases is the obvious strategy for sustaining the agricultural productivity. Approximately 2.5 million tons of pesticides are used in agriculture annually throughout the World (Meena *et. al*., 2008). In India usage has steadily increased from 2.2 g ha^{-1} active ingredient (a.i) in 1950 (David, 1995) to the level of 381 g ha^{-1} by 2007 i.e about 270 fold (Anon, 2009). Though chemicals gained importance and proved their positive impact in targeting the food security but their continuous and injudicious use in developing countries has resulted in several implications such as development of insecticidal resistance in key pest species (Kranthi, *et al*., 2002), pesticide residues in food chain (Mukherjee Irani, 2003), degradation in the quality of eco-system and human health with eroded profits (Pratap Birthal, 2003).

Increasing cost and negative effects of pesticides and fertilizers necessitates the idea of biological options of crop protection and production. Various biological options such as entomopathogens, antagonistic microbes, endophytes, animal wastes, botanicals and crop residues serves as an alternative to chemical pesticides and fertilizers. Microbial collection at ICRISAT has isolated and identified a large collection of bacteria, fungi and actinomycetes (over 2000) with agriculturally beneficial traits from various herbal composts and rhizosphere soil samples of sorghum, pigeonpea and rice. These accessions possess at least one of seven agriculturally beneficial traits studied viz. phosphate solublization, siderophore production, cellulose degradation, nitrogen fixation, antagonistic to disease causing fungi (viz. *Fusarium oxysporum* f. sp. *ciceri*, *F. udum*, *F. solani*, *Sclerotium rolfsi*
entomopathogens (against *Helicoverpa armigera* and *Spodoptera litura*, the two devastating insect pests of various crops) and *Pseudomonas fluorescens*. Potential cultures were further characterized, evaluated under green house condition for their usefulness.

Eco-friendly approach:

Farmers knew eco-friendly approaches from pre-historic period, however their use has never attained significant level to meet the requirement. Integrated pest management (IPM) approach for managing pest problems emphasize the adoption of available methods and techniques of pest management such as cultural, mechanical, biological and judicious use of chemical pesticides in order to contain the pest populations below economic threshold levels (ETLs).

Biorationals

The term covers a range of alternatives to synthetic chemicals. Their main feature is specificity to avoid non-target mortality and associated problems. The use of bio-pesticides is an important component of IPM strategy for major crops including vegetables. The best-known examples are the neem-based products which have shown to be effective against a number of pests, NPV being used for control of important pests like *Helicoverpa armigera* and *Spodoptera* spp. And Bacillus thuringiensis (Bt) has gained importance in suppressing pest populations in crops like cotton and vegetables (Raheja, 1998).

There are several bio-pesticides that are commercially available to farmers. According to the recent information there were approximately 175 registered bio-pesticide active ingredients and 700 products globally. In India so far only 12 bio-pesticides were registered of which 5 were bacteria (four *Bacillus* species and one *Pseudomonas fluoresens*) three fungal (two *Trichoderma* species and one *Beaveria* species) two viruses (*Helicoverpa* and *Spodoptera*) and two plant products (Neem and *Cymbopogan*). Among various bio-products, *Bacillus thuringiensis* (Bt), *Trichiderma viridae*, *Metarhizium*, *Beauveria bassiana*, Nuclear Polyhedrosis Virus (NPV) and neem are popularly used in plant protection (US Environmental Protection Agency, 2007). Field evaluation of several bio-pesticides either alone or in combination signifies their impact and compatibility with other plant protection options. Several studies indicated their economic feasibility and environmental compatibility to facilitate sustainability in agriculture. A number of neem-based formulations are being produced by small-scale formulators and marketed as insecticides. Most of them are made from neem oil and contain varying amounts of *azadirachtin*. However, there have been problems with inconsistent quality. To overcome this, we encourage farmers to procure neem seed and prepare their own formulation of neem fruit powder (NFP) using the five-step procedure: Collection, drying, pulverizing, storage and application.

Bio-pesticides research at ICRISAT

Viral pathogen

Among microbial insecticides, the insect pathogenic viruses such as baculoviruses are attractive alternatives for biological control under IPM and have been used for more than 20 years with great success (Zhang, 1989). There are several advantages of using insect viruses for pest control: these are highly host specific and are known to be completely safe to humans, animals and non-target beneficial insects such as bees, predatory insects and parasitoids (Monobrullah & Nagata 1999, Nakai *et al.,* 2003, Ashour *et al.,* 2007). In addition, these are highly compatible with other methods of pest control and are well suited for use in integrated pest management (IPM) programs. Another important reason for the interest in baculoviruses as potential insect control agents is that they are relatively easy to visualize and monitor using a light microscope.

Strains of *Helicoverpa* NPV (HaNPV) collected from five locations inIndia i.e. Akola, Coimbatore, Dharwad, Junagadh and Ludhiana were compared with ICRISAT strain for their virulence. The bioassays on third instar larvae indicated that ICRISAT strain had lowest LC_{50} value of $0.54X10^8$ followed by Dharwad ($1.11X10^8$), Coimbatore ($2.63X10^8$), Akola ($14.56X10^8$), Junagadh ($43.97X10^8$) after 5[th] day of infection and Ludhiana strain had not shown any mortality at 5[th] day. However all the strains showed 100% mortality by 8[th] day after infection. Based on the lowest LC_{50} values of 5[th] day ICRISAT strain was found superior to others.

Purified samples of HaNPV from ICRISAT, Dharwad, Tamilnadu, Akola, Punjab and Gujrat were analyzed in 12% SDS-PAGE gels for proteins. This has revealed that all the isolates have 4 major polypeptides of 30.66 - 42.32 kDa, and several minor peptides. Three major proteins were present in most of the isolates. The molecular weights of the major proteins were nearly similar, but not identical 42 and ca. 34 kDa protein.

Studies conducted in evaluating different preservatives for efficient long term storage of HaNPV indicated HaNPV + 10% Acetone with 73% mortality of larvae followed by 70%, 63%, 57%, 53%, 47% with 10% ethyl alcohol, 10% phenol, 10% dettol, 10% methanol and 10% ethyl acetate respectively after 10 months of storage. Most of these preservatives showed good response to HaNPV storage up to six months. However, prolongation of storage until to 10[th] month showed 10% acetone and ethyl alcohol as the most efficient.

Under scanning electron microscope (SEM) the Poly ocular bodies (POBs) of NPVs appeared as crystalline structures of variable shapes (irregular) of size 0.5 to 2.5μm (HaNPV), 0.9 to 2.92μm (SlNPV) and 1.0 to 2.0μm (AmalNPV) in diameter. Under transmission electron microscope (TEM) the cross-sectioned POB revealed multiple nucleocapsids in each envelop, which were of bacilliform shaped structures of 277.7 × 41.6nm (HaNPV), 285.7 × 34.2nm (SlNPV) and 228.5 ×

22.8nm (AmalNPV) in size. The POBs of HaNPV and AmalNPV contained 2 to 6 and SlNPV contained 5 to7 nucleocapsids per envelope.

Among monitoring tools, the DAC-ELISA is a rapid and highly sensitive tool, which can detect low levels of NPV at early stages of infection in larvae as well as latent infection in pupae. While competitive-ELISA, western immunoblotting and indirect immunofluorescence tools were highly specific but not much sensitive than DAC-ELISA to detect low levels of NPV infection. Both DAC-ELISA and IC-ELISA tools were sensitive to the analysis of alkali dissolved protein extracts of POBs or infected larval extracts than direct POBs or larval extracts whereas, western immunoblotting and indirect immunofluorescene tools were specific to both. As part of the quality control during mass production of NPVs used for commercial viral insecticide preparations at ICRISAT, Patancheru, India, the present study developed some sensitive immunochemical methods such as DAC and IC-ELISA and evaluated their performance in quantification of POBs in commercial NPV preparations.

The recovery of the virus needed to be quantitatively optimized to enhance its efficiency and economy as a microbial bio-pesticide. An attempt has been made in this regard to quantify the viral recovery at different post inoculation (PI) days to obtain the maximum poly inclusion bodies (PIBs) and to regulate the malodor through several techniques. Maximum larval mortality was found to be 88% on 7^{th} day of PI followed by 50% on 6^{th} day of PI. The NPV yield was maximum, 0.70 LE/larva at 7^{th} day followed by 0.64 LE/larva at 6^{th} day of PI. The ideal period of viral harvest can be suggested to be 6^{th} day of PI when the mortality percent and NPV yield were in accord for optimal viral recovery to avoid the constraint of malodor associated with the *H. armigera* NPV production.

Evaluation of ELISA tools at field level efficacy study of NPV: The ELISA tools developed in the present study was applied to monitor the NPV infection status in field population of *H. armigera* on pigeonpea crop after field application of NPV. The DAC-ELISA results showed that the concentration of NPV used for field spray (250 LE/ha) successfully infected the field population. The details of total number of larvae sampled per dpa, number of NPV positive larvae observed by DAC-ELISA, percent of infection among sampled larvae per dpa and gross virus concentration (POBs) in infected larvae per dpa estimated by ELISA (DAC and IC) were represented in Table 1.

The data revealed that 10 ± 1.7% of the field collected larvae were NPV positive on 3rd dpa, 15 ± 2.2% on 4th dpa, 32 ± 2.6% on 5th dpa, 50 ± 3.2% on 6th dpa , 65 ± 2.5% on 7th dpa, 71 ± 2.5%, on 8th dpa and 70 ± 5.9 on 9th dpa. But, on 10th dpa the percent infection was decreased to 27 ± 5.7%. In parallel, the DAC-ELISA results of the individual larvae collected from control (untreated) plot showed that most of the larvae were free of NPV and very few larvae were found to be NPV positive.

Phylogenetic relation at nucleotide level of HaNPV-P polyhedrin gene with known polyhedrin and granulin genes: The polyhedrin gene sequence of HaNPV-P was more close to group-II NPVs. Among which, it was showing maximum homology of 98.2% with McNPV, 98% with MbNPV, 96.1% with LsNPV and 90.6% with PfNPV.

HaNPV production and utilization: During 2005-2006, the emphasis was on the establishing bio-pesticide units and imparting training on HaNPV production to farmers, extension officers for their effective utilization. In this process, 76 HaNPV production units in India and 20 in Nepal have been established after detailed training of two farmers and one extension staff from each village. Through these interactions (on site training, and village wide interactions), this influenced the farmers in judicious use of pesticides in plant protection and the importance of protective clothing, which was well adopted in all the villages. The village level bio-pesticide units commissioned production of HaNPV (500-20000 LE) and utilized on several crops including cotton, vegetables, chickpea and pigeonpea with satisfactory results.

Botanicals as pesticides

Insecticidal properties of several plant species have been known since ages. In recent years botanical insecticides played critical role in the management of several insect pests. However, their exploitation on a commercial scale is limited. Amongst these, neem (*Azadirachta indica*) has been the focus of a large number of studies over the past two decades. Today, neem products are used as pesticides against >250 insect species all over the world. In India alone, neem extracts have been evaluated against 106 species of insects.

Neem is a source of eco-friendly pesticides and fertilizer. Several workers have reported the repellent, anti-feedant, growth inhibition and oviposition suppression effects of neem against a large number of insects. Neem products are believed to be relatively harmless to natural enemies, pollinators and other non-target organisms. Thus several IPM programs had adopted neem as one the prime options for greater stability and sustainability in crop production. However, the cost of neem products are on par with chemicals. In order to over come the constraints ICRISAT encouraged the self help groups (SHGs) to undertake collections of neem fruits and making powder as a micro-enterprise following the set procedure: Collect enough quantity of neem fruits, dry the fruits under shade, make the powder from dried fruits, pack in water proof bags before use. Strategic research at ICRISAT with several potential indigenous plants also revealed satisfactory clues to take up further in depth studies in this area.

Evaluation of botanicals with insecticidal properties: Ten indigenous plant materials (*Cleistanthus collinus*, *Calotropis gigantea*, *Pongamia glabra*, *Sphaeranthus indicus*, *Cassia occidentalis*, *Chloroxylon swietensia*, *Vitex negundo*, *Madhuca indica*, *Strychnos nuxvomica*, and *Strychnos pototorum*) known for insecticidal properties collected from Andhra Pradesh and Chhattisgarh, India were evaluated against *H. armigera* larvae. The water extracts of these products against second instar larvae clearly indicated the superiority of *Cleistanthus collinus*, and *Sphaeranthus indicus* with 57% larval mortality one week after exposure. Though the other plant products were inferior in their insecticidal properties to the above, they also caused 48% larval mortality in *Chloroxylon swietensia*, 37% in *Calotropis gigantean* and *Strychnos nuxvomica*, 30% in *Pongamia glabra* and *Madhuca indica*, 23% in *Strychnos pototorum*, 13% in *Vitex negundo* and 10% in *Cassia occidentalis*. Further observations on larval mortality two weeks after exposure revealed similar trend with a range of 17-63%. The maximum mortality was observed in both *Cleistanthus collinus* and *Sphaeranthus indicus* with 63% and were found superior to other plant extracts. Since these botanical extracts have shown encouraging results hence their potential need to be further evaluated under laboratory and field conditions to utilize them as one of the options in integrated pest management programs.

Larvicidal activity of 18 different botanical extracts viz. foliage powder of *Anona*, *Parthinium*, *Datura*, neem fruit powder (NFP), rain tree pod powder, foliage powder of *Pongamia*, *Tridax*, Neem, *Chrysanthemum*, *Calotropis*, *Jatropha*, Rain tree, *Prosopis*, *Vitex*, *Anona* rind and seed powder and tobacco wastes was studied against the neonates of *Spodopetera litura*. Commercially available neem oil (1%) was used as reference. Of the 18 evaluated, *Pongamia* foliage powder showed maximum mortality (86%) followed by NFP (80%), Tobacco waste (72%), neem foliage powder (70%), *Anona* rind (68%), foliage powder of Rain tree, *Prosopis*, *Vitex* (66%), neem oil (64%), pod powder of rain tree (58%) and *Datura* (52%). Neem oil, used as a reference killed only 50% neonates. Some botanicals such as *Tridax, Vitex* and *Anona* shell had antifeedent activity indicated by reduced growth of larvae by 56 to 60%.

Further studies on larval mortality and oviposition deterrence of various botanicals against *Helicoverpa* brought out highest larval mortality in neem extracts followed by *Datura*, Rain tree pod and *Chrysanthemum.* In case of *Spodoptera* maximum mortality was recorded with neem fruit extract followed by *Pongamia*, Rain tree pod, *Datura* and *Anona*. Oviposition of the two species was severely affected by the plant extract sprays reflecting the potential of the botanicals in the suppression of key pests (Table 2).

In order to assess the compatibility of botanical extract and some selected entomopathogenic microorganism such as *Bacillus subtilis* and *Metarhizium anisopliae* and four botanical powders viz., *Anona, Datura*, neem fruit and *Parthinium* were selected. Neem fruit and *Datura* were found to be compatible with *Bacillus subtilis* (BCB19). When carrier based formulation of BCB 19 mixed with extraction of neem fruit powder and *Datura* powder, separately, there were no signs of suppressing BCB19 for up to 8 days. None of the four botanical powder extractions suppressed *Metarhizium anisopliae* (Ma) up to eight days. Count of *M. anisopliae* on 8th day ranged between 5.90 ($\log_{10}$ mL^{-1} of suspension) in Ma+*Anona* and 6.41 ($\log_{10}$ mL^{-1} of suspension) in Ma+neem Fruit. In another study, three botanicals (*Anona, Datura*, neem fruit powder) and three entomopathogens viz. *Bacillus megaterium* (SB9), *B. pumilus* (SB21) and *Serratia marcesens* (HIB28) were checked for their compatibility. There were no definite signs of suppression by any of the botanicals of the bacteria. But there were some signs of improved growth in case of SB9 + neem fruit, SB9 + *Anona* and HIB28 + *Datura* (Table 3).

Bacterial and fungal pathogens

The microbial collection at ICRISAT has over 2000 accessions that includes promising entomopathogens (*Bacillus subtilis* BCB19, *B. thurengiensis* HiB67, in addition to 37 potential isolates) and antagonists of phytopathogens (*B. subtilis* BCB19, *Pseudomonas* sp CDB35, in addition to 154 isolates) (Table 4). Compatibility studies among microbials also showed encouraging results (Table 5). Though several bacterial strains have shown promise, the only strain used extensively in field studies is BCB19 of *Bacillus* spp. BCB19 is an aerobic spore-forming bacterium that is easy to maintain under laboratory conditions. It survives under high

temperatures, and therefore remains viable on leaf surfaces long after it has been sprayed. Its use, combined with other non-chemical methods has helped protect pigeonpea and cotton (Fig.1 & 2). The yields of these two crops in bio-intensive and farmers practice were on par. In view of eco-friendliness, cost effectiveness
of the potential options in on-going IPM programs. Characterization studies suggest that BCB19 is safe for humans.

Bio-efficacy of three different fractions (crude, adsorbed and non- adsorbed) of 7 bio-washes namely *Anona, Datura, Pongamia, Parthinium, Gliricedia*, Neem and *Jatropha* were studied. In case of crude bio-washes, maximum mortality was observed in the *Anona* (38.2%) followed by *Pongamia* (37.8%), *Gliricedia* (37.4%) and neem (36.5%). The mortality in the control was 15.6%. All the seven crude bio-washes showed the reduction in the weight as compared to control. The range of weight reduction over control was 24.7% (*Parthinium*) to 60.3% (*Anona*). Partially purified fraction exhibited a mortality ranging between 42.4% (*Datura*) to 82.3% (*Jatropha*) and in the control it was 22.2%. Except *Datura*, all other bio-washes had done exceptionally well and the difference was statistically significant at 0.001%. Partially purified fraction of all the seven bio-washes showed the reduction in the weight as compared to control. The maximum weight was recorded in *Jatropha* (97%) followed by *Parthinium* (89%), Neem (84.3%), *Pongamia* (73%), *Datura* (71.3%), *Gliricedia* (70%) and *Anona* (65%).

Of the bio-washes studied, *Jatropha, Parthenium* and *Anona*, inhibited all the three tested pathogenic fungi viz. *Fusarium oxysporum f.* sp. *ciceri* (FOC), *Macrophomina phaseolina* and *Sclerotium rolfsii*. The crude biowash (at 75%) suppressed the growth of FOC and *M. phaseolina* but did not show any effect on *S. rolfsii*. The partially purified and concentrated fraction (at 12 times concentrated = 20% of the medium) showed complete inhibition on *S. rolfsii* and *M. phaseolina* where as no inhibition was found in FOC.

Vermicompost: Bio wash

In recent years, production of vermicompost not only enhanced the quantity of available organic manure but created an alternative to overcome the prevailing deficit of chemical fertilizers. The encouragement and importance given from various organizations made an impact in initiating vermicompost production in several regions. However their use as plant protection option has not been exploited. Vermicompost can be made from several bio- materials, but some have proven better impact when applied as wash on plants in terms of plant nutrition as well as insect protection.

Research at ICRISAT has shown that neem foliage (leaves and tender twigs) can be vermi-composted using earthworms. The wash of this compost, when sprayed on the third instar larvae of the pod borer *Helicoverpa,* killed at least 50% of the larvae. A 100-liter capacity drum to provide 30 to 50 L compost wash per week, can be constructed for an initial cost of Rs 500/- (US$ 12). The wash collected week^{-1} is enough to spray crops on one ha after dilution. The process is used repeatedly, and the cost eventually breaks even on the initial investment.

On-farm evaluation of bio-pesticides

Results from the field investigations carried on the efficacy of various IPM (IPM involve intensive monitoring, following thresholds , use of bio-products, encouraging natural enemies, and need based application of chemical) options in chickpea crop revealed neem as an effective oviposition deterrent of *H. armigera*. HaNPV proved effective in reducing larval population and was on par with plots treated with endosunfan (1.25, 1.33 larvae plant^{-1} respectively) (Tables 6&7). Among the various IPM components (neem, HaNPV, bird perches and need based chemical application), plots treated with HaNPV did not show any significant effect on natural enemies (267.1 trap^{-1}) present on the chickpea crop and was on par with control plots (302.4 trap^{-1}) (Vishalakshmi *et al.*, 2005).

In pigeonpea IPM interventions at farm level during 2001-02 resulted in substantial decrease in borer damage to pods and seeds. IPM plots had 34% pod damage compared to 61% in non-IPM plots. The seed damage was also low in IPM plots (21%) compared to non-IPM plots (39%). This lower pod borer damage in IPM plots also reflected in higher yield of 0.77 t ha^{-1} compared to 0.53 t ha^{-1} in farmer practice (Fig 3).

During 2003-04, twelve out of 17 cotton IPM farmers obtained 20-80% higher yields, while four farmers realized 0-20% better yields and in only one farmer's field the yield was lower (4%) in IPM treatment compared to farmers practice. When all the farmers' yields were considered the IPM fields yielded 30% better than non-IPM fields (Table 8). In the next season (2004-05) 4 out of 9 farmers obtained > 20% yield (range 20-45%), two out of nine received 5-6% higher yield and three farmers realized less yield in IPM plots. In the third year three out of six farmers realized 33-74% higher yield and two out of six farmers got 9-12% better yields, while one farmer obtained 3% lower yield in IPM plots. In general, majority of farmers harvested higher yields through IPM compared to complete chemical based farmers practice.

After realizing good results from IPM in cotton, six farmers from the same village adopted the technology in protecting tomato from insect pests. During 2005, IPM farmers realized 2-322% yield gain over the plots covered with conventional chemical pest management (Table 9). The average biopesticide investment over the six farmers was around Rs 2057 ha^{-1} compared to Rs 2637 in farmer practice. This clearly showed the economic feasibility of bio-intensive options over conventional chemicals.

The IPM plots were noted to have a higher population of coccinelids (0.2 $plant^{-1}$) and spiders (0.1 $plant^{-1}$) as indicators of healthy environment than the FP plots, where activity was nil. Crops in IPM lots generally remained productive for about three weeks longer than the FP plots that generally senesced suddenly.

Further scale up studies in addressing eco-friendly approaches clearly brought out substantial reduction in pesticide use with out scarifying yields in wider areas covering several crops (Table 10). Though the awareness and adoption of bio-products increased, the further spread of concept is not up to the expectations.

Bio-product research consortium (BRC)

In order to overcome the constraints induced by injudicious use of chemical pesticides, ICRISAT in collaboration with private sector initiated the bio-products research consortium (BRC) during 2005 with the following objectives:

- Develop promising entomopathogenic microbial strains and improve their delivery to farmers.
- Isolate promising strains of actinomycetes from new niches and evaluate them for their efficacy as entomopathogens.
- Evaluate the compatibility of potential microbial entomopathogens and botanicals etc. for enhancing their efficacy.
- Develop eco-friendly modules for managing pests of important crops and evaluate them on-farm.

Outputs achieved

- New formulations of *B. subtillis* strain BCB 19 developed
- Formulations of *M. anisopliae* produced
- Field bio-efficacy data for the above two strains generated
- Identification of actinomycetes with ability to manage *Helicoverpa*
- Powder formulations of HaNPV developed
- Microbial germplasm made available to members
- NPV quality control and monitoring technique using ELISA developed
- Level of farmers confidence in bio-pesticide based plant protection improved

Discussion

The studies conducted by ICRISAT revealed that most of the farmers (52%) in India get their plant protection advice from pesticide dealers, 22% from extension officials, 15% from neighbors, and 11% make their own judgment in initiating plant protection. Among Nepal farmers, majority of them (69%) make their plant protection decisions through advice from agricultural officers, 10% from senior farmers and 17% based on their own experience. Farmers receiving advice from the pesticide dealers' was negligible (3%) in Nepal. Thus, the decision making in pesticide use was significantly influenced by dealers in India, and agricultural extension in Nepal. This indicated the farmers' dependence on dealers in India, which was primarily due to the credit facility provided by the dealers for major inputs such as pesticides and fertilizers (Anon, 2008). It clearly shows the need for strengthening the on-going extension and the farmers knowledge on plant protection in order to prevent the injudicious use of pesticides and to promote eco-friendly options.

At present though farmers in Asia are aware of importance of IPM and its impact on health and environment the adoption level was up to the expected levels. However, latest estimates are quite encouraging with reduction in chemical use to $25.3 billion in 2010 compared to $26.7 billion in 2005. On the other hand interestingly the biopesticides market is growing rapidly from $672 million in 2005 to over $1 billion in 2010. Biopesticides currently has 2.5% of the overall pesticides market, but its share of the market will increase to over 4.2% by 2010 (Anon, 2009).

The efficacy of the product is crucial in ensuring acceptance and sustained use by the farmers. The issue of erratic performance of viral biocontrol agents has been recognized as a significant factor in the limited successful commercialisation (Lisansky, 1997). It has been widely perceived that viral agents have not achieved a level of efficacy comparable with that of chemicals or other biopesticides such as *Bacillus thuringiensis* (Berliner). Many of the viral products available in the markets in developing countries were found weak, with poor efficacy, questionable quality (Harris, 1997) and are failing to meet acceptable standards (Kern and Vaagt, 1996). Unless this matter is addressed effectively, there is serious danger that poor quality products with their inevitable failures will erode the farmers' confidence in bio-products and significantly retard the promotion of this potential technology.

While NPV insecticide production methods have been well established in many developing countries, the microscopic counting procedure used to screen the larvae for NPV infection and quality control of the viral insecticide lots has low-detection efficiency, unknown specificity and is laborious and requires considerable skill (Wigley, 1976). Because of this, many NPV products produced have poor efficacy and found to be ineffective under field conditions. To over come this problem and for effective production of viral insecticides, it is necessary to have an efficient strategy for virus production, combined with rapid and specific diagnostic and quality control tools (Shieh, 1989). Strategic research organized at ICRISAT in collaboration with National Agricultural Research and Extension Systems (NARES) in India have made significant progress in the identification, production and field evaluation of bio-pesticides. There was significant progress in developing feasible production technologies, efficient storage to enhance the shelf life and field applications. In this process, ICRISAT trained several NARES scientists and farmers on bio-pesticides production to encourage their use.

For village level NPV production, the live larvae are obtained from the fields through shaking pigeonpea crop and manual collection in other crops. This simple technology includes six step procedure i.e., insect collection, inoculation with virus, rearing infected larvae, virus harvest, processing virus, and monitoring quality. The village level NPV production units can make product that of one sixth of market price, hence the virus producing farmers can have plant protection at six times lower cost with no risk to environment.

At present the occurrence of toxic residues of chemical pesticides in the produce hinder the export from developing countries and there is a growing demand for residue free products to suit the exports. In order to achieve this situation the basic problem in the present day agriculture is the negligence of safety intervals after the sprays and also lack of periodic residue monitoring mechanism in the products and environment. There are many reports on the presence of pesticide residues in the environment, food as well as in human beings. Reports from developing world revealed 87% contamination of mothers' milk from 8 districts of Tamil Nadu with HCH and 100% with DDT (Handa, 1995). This is a classic example in developing countries where poor people are vulnerable to the toxic effects (Mancini *et. al.*, 2005) and are unable to feed newly borne babies with healthy mother's milk. Very high levels of pesticide residues were also found in blood samples from four villages in Punjab showed 15 to 605 times higher residues, as compared to samples of people in the USA of certain persistent organo-chlorine pesticides (OCs) through food chain resulted in blood cancers and other abnormalities (Anon, 2005b).

While discussing the status of bio-pesticides and their utilization it was always perplexing for plant protectionist on the out come of the strategies developed. The news in media were also not consistent by providing some negative and positive statements, which confuses the whole group (researchers, producers and consumers) about the facts. The details of plant protection perception at various levels (Policy makers, farmers and consumers) were furnished in the Table 11 and the reasons for low uptake are discussed as follows:

- Bio-pesticide science is knowledge intensive, needs more time to understand the effectiveness.
- Intensive pest monitoring which is a pre-requisite for decision-making at farm level is a specialized job hence farmers considered it as impractical.
- Some farmers also felt that they did not have time to keep a close watch on their fields to monitor pests and their natural enemies to calculate and follow economic thresholds.
- Farmers have several miss concepts about bio-pesticides such as they are less effective, costly difficult to produce, not compatible with other options
- In general the extension programs have very little knowledge and experience of bio-pesticides.
- In the absence of active promotion of bio-pesticides the demand for these products has not developed, for this reason that most private shops and dealers do not stock and sell bio-pesticides.

Lessons learnt

- Need for authenticated data on injudicious use of chemicals on food quality and farm health to justify the alternative options is of high priority to encourage bio-pesticide use and its greater adoption.
- Chemical control was the most commonly adopted (> 90% of the farmers) in the developing world. Though farmers are aware of cultural, biological and other non- chemical plant protection means, the proportion of their adoption was low (<10%).
- Though IPM has been advocated for two decades, only 38% of the farmers in India and Nepal were aware of bio-pesticides.

- Among the various bio-pesticides, majority of the farmers have adopted only neem in their pest management programs.
- Existing pesticide dealer influence on decision making in plant protection
- Bio- products require specialized skills and effective in specific situations
- Complicated policies towards registration discouraged several promoting agencies

Conclusions

- Over the past 25 year, the approach to bio-pesticide research has evolved toward being more ecologically holistic with industry's concerns.
- Although bio-pesticides still represents a very small portion of plant protection at present, their role was considered significant.
- Bio-pesticides though gained prominence as environmentally friendly alternatives to chemical insecticides but still face a number of hurdles in their production, marketing and utilization.
- Importance of effective multidisciplinary research, public, private, people partnerships
- Need for in-depth knowledge among farmers, extension and policy makers about bio-pesticides.
- Lack of effective regulations can lead to poor product quality, performance and loss of user confidence.
- Bio-pesticides that can perform effectively in wider environments have immense potential.
- Prioritize research for better integration of bio-agents into production systems, such as in rotating these with chemical pesticides and developing these into effective bio-models
- Thus ICRISAT successfully addressed millennium development goal on ensuing environmental sustainability by strengthening eco-friendly pest management research

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Table 1: Evaluation of ELISA tools at field level efficacy study of NPV against *H. armigera* on pigeonpea crop.

Days post application (DPA)	Treatment (250 LE ha^{-1})				Control		
	No. of larvae sampled at DPA	No. of larvae with NPV + ve	Infection (%)	Virus conc. (LE ml^{-1})	No.of larvae sampled at DPA	No. of larvae with NPV + ve	Infection (%)
0	30 ± 0.0	0 ± 0.0	0 ± 0.0	0.00 ± 0.0	30 ± 0.0	0 ± 0.0	0 ± 0.0
1	34 ± 4.0	0 ± 0.0	0 ± 0.0	0.00± 0.0	32± 2.0	0 ± 0.0	0 ± 0.0
2	38 ± 2.1	0 ± 0.0	0 ± 0.0	0.00 ± 0.0	34 ± 1.0	0 ± 0.0	0 ± 0.0
3	34 ± 2.0	3 ± 0.6	10 ± 1.7	0.00 ± 0.0	34 ± 2.5	0 ± 0.0	0 ± 0.0
4	33 ± 3.1	5 ± 1.0	15 ± 2.2	0.02 ± 0.0	32 ± 1.5	0 ± 0.6	1 ± 1.9
5	36 ± 2.5	12 ± 2.5	32 ± 2.6	0.07 ± 0.01	31 ± 1.2	0 ± 0.6	1 ± 1.9
6	35 ± 5.0	17 ± 3.1	50 ± 3.2	0.10 ± 0.02	31 ± 1.0	0 ± 0.0	0 ± 0.0
7	35 ± 4.6	23 ± 3.8	65 ± 2.5	0.29 ± 0.06	31 ± 1.2	2 ± 0.6	6 ± 1.9
8	36 ± 5.3	25 ± 3.1	71 ± 2.5	0.33 ± 0.07	31 ± 1.0	2 ± 1.2	4 ± 5.1
9	35 ± 1.2	24 ± 2.1	70 ± 5.9	0.74± 0.07	30 ± 0.0	0 ± 0.6	1 ± 1.9
10	30 ± 0.0	8 ± 1.5	27 ± 5.7	0.41 ± 0.07	30 ± 0.0	0 ± 0.0	0 ± 0.0

Note: 1 LE = 6×10^9 POBs; ± Standard deviation

Table 2: Evaluation of one percent botanical extract against neonates of *H. armigera* and *S. litura*.

Botanical	Scientific names	% Mortality		% Repellency (% reduction in egg laying over control)	
		HA	SL	HA	SL
Anona	*Anona squamosa*	20	40	47	96
Anona rind	*Anona squamosa*	8	46	79	90
Anona seed	*Anona squamosa*	25	34	45	93
Calotropis	*Calotropis gagantea*	18	12	33	66
Chrysanthemum	*Chrysanthemum domestica*	38	32	65	55
Datura	*Datura metal*	40	46	32	46
Jatropha cake	*Jatropha curcas*	24	26	8	95
Marigold	*Tagetus erecta*	35	34	66	96
Melia	*Melia azedarach*	22	30	93	87
Neem	*Azadirachta indica*	42	48	9	73
Neem fruit	*Azadirachta indica*	21	74	84	100
Parthenium	*Parthenium hysterophorus*	10	26	13	75
Pongamia	*Pongamia pinnata*	30	64	92	56
Pongamia seed	*Pongamia pinnata*	29	32	4	77
Prosopis	*Prosopis juliflora*	16	44	34	37
Rain tree	*Samanea saman*	31	44	43	ND
Rain tree pod	*Samanea saman*	39	52	21	73
Tridax	*Tridax procumbens*	32	34	32	85
Vitex	*Vitex negundo*	10	44	1	93

ND= Not done; HA- *Helicoverpa armigera*; SL- *Spodoptera litura*

Table 3: Study of compatibility of *B. subtilis* BCB19, *M. anisopliae* (Ma) *B. megaterium* (SB9), *B. pumilus* (SB 21) and *S. marcesens* (HIB 28) with selected botanicals

Treatment	Desired organism population ($\log_{10}$ ml^{-1})			
	Unfiltered botanical Extract		Filtered botanical extract	
	Day 1	Day 8	Day 1	Day 8
BCB19 +neem fruit powder	6.64	6.88	5.26	4.48
BCB19+*Anona*	6.61	C	6.01	5.42
BCB19+*Parthinium*	3.78	3.95	5.0	4.57
BCB19+*Datura*	5.30	6.15	5.42	3.00
Ma + neem fruit powder	5.30	6.41	4.55	4.27
Ma +*Anona*	<5.00	5.90	4.64	4.93
Ma + *Parthenium*	<5.00	6.15	4.38	4.46
Ma +*Datura*	5.60	6.38	4.56	4.71
SB9+neem fruit powder	6.94	7.19	5.15	6.45
SB9+*Anona*	7.03	C	4.57	6.46
SB9+*Datura*	6.92	6.08	5.08	5.76
SB9+*Parthinium*	5.09	5.09	5.05	5.59
SB21+neem fruit powder	7.13	6.7	6.65	7.05
SB21+*Anona*	6.74	7.55	5.75	6.65
SB21+*Datura*	6.51	7.3	4.82	5.11
SB21+*Parthinium*	7.58	6.24	6.69	6.08
HIB28+neem fruit powder	5.6	6.18	5.87	6.39
HIB28+*Anona*	6.38	C	6.05	5.93
HIB28+*Datura*	6.3	C	5.4	5.29
HIB28+*Parthinium*	4.18	4.92	4.98	5.16

<5.00= No distinct growth of target organism (BCB19, HIB28, SB9, SB21 & Ma) was observed in the dilution (10^{-5}), C= contaminants in large numbers did not allow counting

Table 4: Characterization of microbes on the basis of agriculturally important beneficial traits

SNo	Category	No of potential strains *
1	Cellulose degrading bacteria and fungi	119
2	Antagonistic bacateria and actinomycetes	405
3	Phosphate solubilizing bacteria	143
4	Nitrogen fixing bacteria	590
5	Siderophore producing bacteria and actinomycetes	580
6	Fluorescent *Pseudonomonads*	260
7	Entomopathogens	38
	Total	2135

* On the basis of *in vitro* lab test results

Table 5: Study of compatibility among the bacterial entomopathogens

S.No		BCB19	SB9	SB21	HiB28
1	*B. subtilis* BCB19	+	+	+	+
2	*B. megaterium* SB9	+	+	+	+
3	*B. pumilus* SB21	+	+	+	+
4	*S. marcesens* SB28	+	+	+	+
5	*M. anisopliae*	-	-	-	-

+ indicate that both are compatible
- indicate that both are not compatible

Table 6: Cost benefit ratio of IPM components in chickpea during post-rainy 1998-2000 seasons (cumulative of two years)

Treatment	Pod damage (%)	Grain yield (kg/ha)		Gross Income (Rs)	Cost of Insecticidal application (Rs)f	Net income (Rs)	Cost benefit ratio (C:B)
		Gross yield	Additional Yield over control				
Neem	11.98 (20.23)[b]	1129.9	332.0	14,544	2023.5	12,520.5	1:2.05
HNPV 250 LE/ha	12.55 (20.72)[b]	1140.45	342.55	14.697	2322.5	12,374.5	1:1.84
Bird perches one/plot	14.45 (22.32)[c]	977.00	179.1	12,510	350.0	12,160.0	1:6.40
Endosulfan 0.07%	11.21 (19.56)[ab]	1223.05	425.15	15717.5	1,942.0	13,775	1:2.74
IPM	10.38 (18.77)[a]	1264.35	466.45	16,048	1935.0	13,907.5	1:3.01
Control	19.76 (26.41)[d]	797.9	-	10,114	-	10,114.0	-
S.Ed.	0.550	34.26	-	-	-	-	-
CD	1.180	72.02	-	-	-	-	-

Cost of each spray/ha: Neem = Rs 405/-; HNPV = Rs 465/-; Bird perches = Rs 350/-; Endosulfan = Rs 388/-; Cost of chickpea = Rs 12.5/kg

Table 7: Effect of various IPM treatments on larval parasitization by *Campoletis chloridiae* in chickpea during 2003-04

Treatments	Parasitization (%)		Mean reduction over control (%)
	Vegetative (3 DAT)	Reproductive (5 DAT)	
Neem fruit extract @ 15kgha^{-1}	4.7 *(12.4)^{c}	5.0 (12.8)^{bc}	17.09
Neem oil @ 1 ml of 1500 ppm lit^{-1}	5.0 (12.9)^{c}	5.3 (13.3)^{c}	11.96
HNPV @ 250LE ha^{-1}	5.7 (13.7)^{c}	5.7 (13.7)^{c}	2.56
Endosulfan 35% EC @ 0.07%	2.0 (8.1)^{a}	2.7 (8.3)^{a}	59.82
Novaluron 10% EC @ 0.01%	3.0 (9.8)^{b}	3.7 (10.9)^{b}	42.73
Flufenoxuron 10% DC @ 0.01%	3.3 (10.4)^{b}	4.0 (11.4)^{b}	37.60
Control	5.7 (13.7)^{c}	6.0 (14.1)^{c}	-
CD (P=0.05)	1.41	2.04	-

*Figures in parenthesis are arc sign transformed values;
DAT = Days after treatment.
Values followed by same letters in each column are statistically not significant.

Table 8: Cotton yields in IPM and FP plots during three seasons in Adarsha Watershed, Kothapally, India 2003-06.

Season (No. of farmers)	Mean yield (t ha^{-1})		
	IPM	FP	SE±
2003/04 (17)	2.43	1.87	0.080
2004/05 (9)	0.74	0.68	0.058
2005/06 (6)	1.74	1.38	0.096

Table 9. Tomato yields in IPM and FP treatments in six farmers fields in Adarsha Watershed, Kothapally during 2005.

Name of farmer	Yield (t ha^{-1})		Yield increase over control (%)	Cost of plant protection (Rs ha^{-1})	
	IPM	FP		IPM	Non-IPM
T. Pochaiah	5.53	1.31	322	2870	2929
B. Narayan Reddy	7.93	5.34	49	2154	2344
Md. Yousuf	3.21	2.35	37	1848	2344
T. Kishtayya	2.12	1.85	15	3144	2929
K. Laxminarayana	2.42	2.22	9	1764	2344
K. Permaiah	1.68	1.65	2	561	2929
Mean	3.82	2.45	55.9	2057	2637
SE ±	0.488				

Table 10: Details of cost of plant protection in IPM and non-IPM fields at different locations during 1997-2000.

Village & NGO	Cost of plant protection (Rs ha^{-1})		Cost reduction (%)
	IPM	Non-IPM	
Hamsanpalli (REEDS)	898	1144	21.5
Bollibaithanda (REEDS)	1194	1870	36.1
Chincholi (CEAD)	859	1618	46.9
Kanjar (CEAD)	649	1467	55.8
Maddur (CHRD)	388	1177	67.0
Panyala (ROAD)	584	1492	60.9
Marlabeed (SEVA)	318	1994	84.1
Punukula (SECURE)	458	1017	55.0
Deverajugattu (CAF●RD)	431	2061	79.1
Itagi (PRERANA)	846	1448	41.6
Jeedigaddathanda (VIKASAM)	789	3404	76.8
Pastapur (DDS)	406	569	28.6
Bhavanandapur (TREES)	353	759	53.5
Pothinenipalli (PILUPU)	375	821	54.3
Ashta (NCIPM/MAU)	800*	-	-
Nellipaka (FRSF)	800	2000	60.0
Sategaon (CARD)	2490**	2380	-4.6

* All farmers followed IPM

** High cost was due to higher HNPV procurement price.

Mean of three season's data

Table 11: Present status of chemical and biological plant protection options in Asia.

Aspect	Chemicals	Biologicals
Market situation	Dominant	At infancy
Concept promotion	Well established- Easy to adopt	Needs strategic extension and capacity building
Government attitude	Unchanged policies	Encouraging through IPM programs
Farmers point of view	Continuing the existing practice is easier	As a supplement
Consumer point of view	Apparently unfavorable	Favorable

Figure 1: On-station Evaluation of bio-pesticides in cotton during 2002-03.

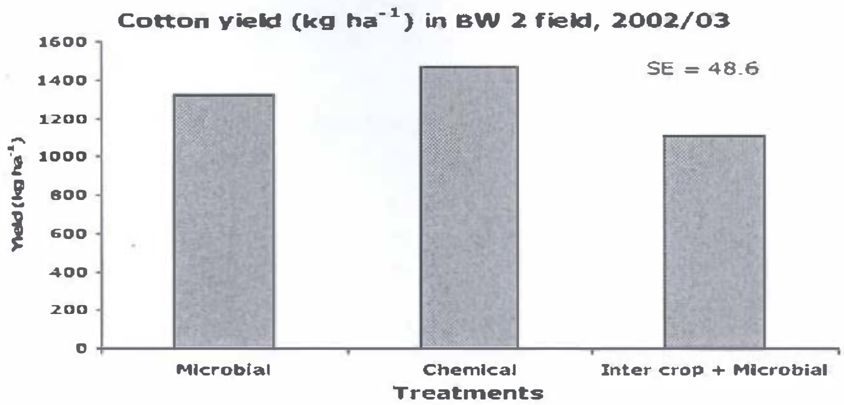

Figure 2: On-station Evaluation of bio-pesticides in pigeonpea during 2002-03.

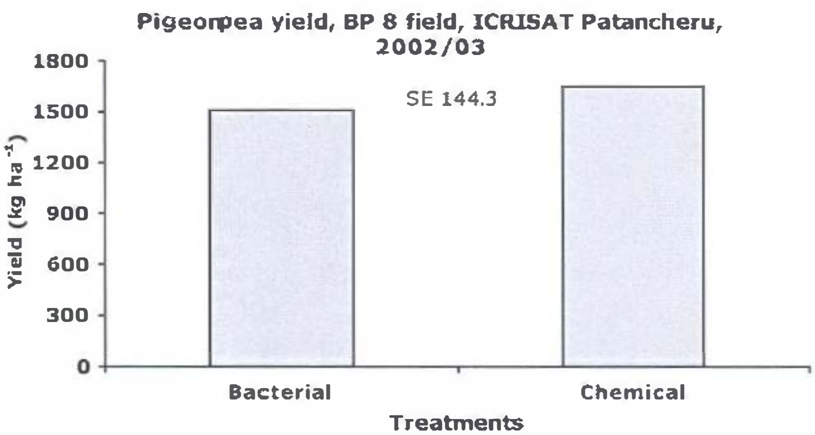

Figure 3: Pod damage, seed damage and grain yield in pigeonpea IPM and farmer practice plots in Adarsha Watershed, Kothapally rainy season, 2001-02.