

SAARC Regional Training
on
Integrated Pest Management
(28-31 May 2018)



SN Alam | MA Sarkar | PR Pandey | S M Bokhtiar



SAARC Agriculture Centre
Bangladesh Agricultural Research Institute
Asia-Pacific Association of Agricultural Research Institutions

SAARC Regional Training on Integrated Pest Management

Edited by

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May 2018



**SAARC Agriculture Centre
Bangladesh Agricultural Research Institute
Asia-Pacific Association of Agricultural Research Institutions**

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Minister
Ministry of Agriculture
Government of the People's
Republic of Bangladesh

Message

It is indeed a gracious initiative by the SAARC Agriculture Centre (SAC) in collaboration with Bangladesh Agricultural Research Institute (BARI) and Asia-Pacific Association of Agricultural Research Institutions (APAARI) to organize the SAARC Regional Training Program on **“SAARC Regional Training on Integrated Pest Management”** to be held during 29-31 May 2018 at BARI, Joydebpur, Gazipur.

Agriculture in SAARC region continues to be the priority sector, where almost 60% of the population engaged in farming for their livelihood. It contributes on an average of 18.2% to regional GDP (3.5% in Maldives to 33% in Nepal). Agriculture is the main source of food energy, vigor, labor force, and intellectual power which are indivisible and interrelated. Under the efficient and far-sighted leadership of Hon'ble Prime Minister Sheikh Hasina, the present agriculture and farmer-friendly government, giving highest priority on agriculture, has strengthened the innovation and use of modern, advanced and sustainable technologies for food production. With the active support of the present government, science and technology based agricultural production system is being developed in the country in place of the traditional agricultural production methodology. The agricultural production of the country has increased and as a profession, agriculture is going to be promoted from livelihood to the commercial level. With the adoption of various timely steps taken by the government of Sheikh Hasina, Bangladesh has become a country of food surplus though in small scale. The government is committed to sustainable and nutritious food security. Bangladesh has become self-sufficiency in food production and furthermore, it has already started exporting rice. In 2014, Cornell University of USA awarded Hon'ble Prime Minister Sheikh Hasina for her great contribution in attaining self-sufficiency in food production and for taking initiatives in using modern technology in agriculture. Earlier in 1999, in recognition of her contribution to the fight against hunger, the United Nations World Food Programme awarded 'Ceres Medal' to the Hon'ble Prime Minister Sheikh Hasina. It certainly indicates that whenever Hon'ble Prime Minister Sheikh Hasina is in the driving seat, the country comes out of the clutches of hunger and

poverty. The present government is working sincerely to achieve this goal. Bangladesh under the leadership of Sheikh Hasina has been included in the developing world by passing the lower middle income level. Bangladesh towards overwhelming progress.

In this standpoint, pest management system in agriculture was characterized by indiscriminate use of different types of toxic synthetic chemical pesticides in Bangladesh till 2008. This practice was not only polluting our environment and causing serious health hazards but also increase the overall production costs. Abuse of chemical pesticides caused destruction of beneficial organisms, flora and fauna. Since 2009, the present Government has taken several pragmatic steps to develop and promote cost-effective and environment friendly, health hazard free Integrated Pest Management technologies to reduce the sole dependency on toxic chemical pesticides for pest management. Different National Agricultural Research Institutes, especially BARI has already developed different bio-rational based pest management tactics to control destructive pests of different crops. Due to farm level acceptance of the developed IPM technologies, use of chemical pesticides is declining. On an average 29% less chemical pesticides were imported during 2017 than 2009.

I am personally encouraged by the timely initiatives of SAC and BARI to organize a SAARC Regional Training Program on “Integrated Pest Management (IPM) in SAARC Member States” at BARI, Gazipur, where the participants can share the success stories of the recent IPM technologies for this region.

We hope, the outcome of this training program will help to implement a sustainable bio-rational based pest management system among the farming community of all SAARC countries, which will help us to ensure higher yield for the farmers and safe food for the consumers.

I firmly believe that maintaining the continuity of development in various sectors under the efficient and far-sighted leadership of Hon'ble Prime minister Sheikh Hasina, the present people-friendly government, keeping pace with the Vision-2041, will be able to make our country a happy, prosperous and developed one dreamt by the Father of the Nation Bangabandhu Sheikh Mujibur Rahman.

I wish a grand success of this regional training program.

Joy Bangla, Joy Bangabandhu
Long live Bangladesh.


(Matia Chowdhury, MP)



Senior Secretary
Ministry of Agriculture
Government of the People's
Republic of Bangladesh

Message

It gives me immeasurable pleasure to note that SAARC Regional Training Program on “SAARC Regional Training on Integrated Pest Management” is going to be held during 28-31 May 2018 at Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur jointly organized by SAARC Agriculture Centre (SAC), Dhaka in collaboration with BARI and Asia-Pacific Association of Agricultural Research Institutions (APAARI), where scientists and extension officers from SAARC Member States are going to participate.

Currently, pest management of different crops relies heavily on toxic synthetic chemical pesticides in SAARC Member States. Indiscriminate application of toxic chemical pesticides over the years in crop fields has resulted in many undesirable effects on human health, environment and overall sustainability of the farming system. The increased public concerns about the adverse environmental effects and food safety associated with the use of synthetic agro-chemicals prompted search for the development of bio-pesticides and other environment friendly approaches to manage the destructive pests. The present government of Bangladesh has given top most priority in reducing the adverse impacts of toxic chemical pesticides through popularizing the developed bio-rational based pest management approaches at farm level. Accordingly, the scientists of BARI along with other National Agricultural Research Institute have developed different bio-rational based management technologies as alternates of toxic chemical pesticides against the destructive pests of different crops, which significantly contributed to reduce the pesticide use in Bangladesh.

Therefore, I firmly believe that organizing of this training program jointly by SAARC Agriculture Centre (SAC), Dhaka, Bangladesh Agricultural Research Institute (BARI) and Asia-Pacific Association of Agricultural Research Institutions (APAARI) will assist to

implement a sustainable pest management system among the farmers of all SAARC countries.

I wish the success of the training program as well as the expansion of bio-rational based pest management practices in this region to ensure higher yield and safe food for the consumers.

A handwritten signature in black ink, consisting of a series of loops and a long horizontal stroke ending in an arrowhead.

(Mohammad Moinuddin Abdullah)



Director General

Bangladesh Agricultural Research Institute
Government of the People's Republic of Bangladesh

Message

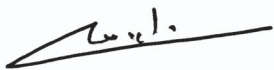
It is my great pleasure on behalf of Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur to arrange a regional training program on **“SAARC Regional Training on Integrated Pest Management”** which is going to be held on 28-31 May, 2018 at BARI, Joydebpur, Gazipur in collaboration with the SAARC Agriculture Centre (SAC), Dhaka and Asia-Pacific Association of Agricultural Research Institutions (APAARI). In this training program 20 scientist/ extension workers from SAARC member countries will participate.

In SAARC Member States, agriculture has had to face the destructive activities of numerous insect pests from time immemorial, leading to radical decrease in yields. With the advent of chemical pesticides, this crisis was resolved to a great extent. but the over dependence on chemical pesticides has given rise to manifold problems such as insecticide resistance, resurgence of insect pests, secondary pest outbreak, environmental pollution, human health hazards etc. So, we need to develop and implement sustainable bio-rational based pest management approaches at farm level. It is a matter of great pleasure that scientists of Entomology division, BARI has developed different bio-rational based management practices to control the insect-pests of different crops and many of them already became very much popular as a toll for pest management in the farm community. The present government of Bangladesh has given highest priority in reducing the adverse impacts of toxic chemical pesticides through popularizing the developed bio-rational based pest management approaches at farm level.

I do hope and believe that the participants from the SAARC Member States will take efforts to develop and promote sound and sustainable bio-rational based management practices to manage different destructive pests and disease of different crops by implementing the acquired knowledge from this training program.

I thank the SAARC Agriculture Centre (SAC), Dhaka and Asia-Pacific Association of Agricultural Research Institutions (APAARI) for cooperating and funding to arrange this training program at BARI, Gazipur.

I wish a grand success of this training program.

A handwritten signature in black ink, appearing to read 'A. K. Azad', written over a horizontal line.

(Dr. Abul Kalam Azad)



Director

SAARC Agriculture Centre

Foreword

Agricultural impacts of climate change will manifest in terms of changes in land and water resources, insect pest populations, diseases, etc. which ultimately translate into a change in productivity and profitability of agriculture. In this connection, IPM is an ecosystem approach to crop protection and production that combines different management strategies and practices to grow healthy crops and minimize the use of pesticides. IPM is not a single pest control method but encompasses a series of pest management evaluations, decisions and control methods. It is based on the concept that it is not necessary to eliminate all pests but to reduce pest populations to levels where pests cannot cause significant loss. An IPM strategy includes the use of pest-resistant crop varieties, modification of agronomic practices to reduce pest incidence, biological control, other innovative pest suppression approaches and need-based, judicious use of chemical pesticides. IPM can make a key contribution to not only addressing the challenge of food insecurity but also meeting the growing consumer demand in the Asia-Pacific region for safe food.

Considering the importance of safe food productions to review the concepts and principles of bio-control and bio-rational based IPM is very important and make urgent action in collaboration of regional institutions and development partners. As a result this training program will be yielded the best experiences, opportunities and challenges for quality production and sustainable utilization of bio-control and bio-rational IPM technologies for the farmers of the SAARC Member countries. Therefore, current regional level training program would be catered the different issues of bio-rational based IPM and open the opportunity of the investment in practical exercises in laboratory and field visit for the scientists and extension officers of the SAARC Member States. Thus, SAARC Agriculture Centre in collaboration with Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh and Asia-Pacific Association of Agricultural Research Institutions (APAARI) is organizing a **“SAARC Regional Training on Integrated Pest Management”** in BARI, Gazipur, Bangladesh during 28-31 May 2018. This training manual is a collection of papers contributed by the expert resource persons for the training program.

Meanwhile, I would like to take this opportunity to express my sincere appreciation to Dr. Syed Nurul Alam and M. A. Sarker from Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh and Dr. Pradyumna Raj Pandey, Senior Program Specialist (Crops), SAARC Agriculture Centre, Dhaka for their hard work to put together the training materials and bring in this form. I am confident that this compilation will facilitate further research and development in IPM technologies and produce safer food in SAARC Region.

A handwritten signature in black ink, appearing to read 'S.M. Bokhtiar', is written above a horizontal line.

Dr. S.M. Bokhtiar

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Integrated pest management – An overview

Dr. Syed Nurul Alam

Entomology Division, Bangladesh Agricultural Research Institute
Joydebpur, Gazipur, Bangladesh

Different types of pest attack are most important limiting factor to different crop production. A world scenario indicates that due to pest infestation more or less about 30-52% of crop loss happens every year, which means the crop losses in the world are anywhere between one-third and one half of attainable crop production. The crop losses are a higher percentage of output in the developing countries than in the developed countries - with a substantial absolute value of crop losses in Asia. Therefore, effective measures must be ensured for protecting the crops against this colossal loss caused by pests. Till today crop protection of South Asia including Bangladesh is mostly dependent on chemical pesticide. Pesticide use in Bangladesh started from mid-fifties and gained momentum in early 1970's with the introduction of green revolution through the use of HYV rice. Before this period, the farmers were not aware of the use of chemical pesticides. Through the importation of 3 metric tons (MT) of insecticides in 1956, Bangladesh entered into the era of the synthetic chemical pesticides for pest control. During that time pesticides were imported by the Government and supplied free of cost. Subsidies were halved in 1973 and withdrawn entirely in 1979, although after that pesticide use went down. However, the consumption began to rise again as agriculture activities expanded. Sales of pesticides doubled in the period 1982-89 and tripled in the last decade.

Till today farmers of South Asia are mostly depending on the toxic synthetic pesticides to combat with different pests attack, in spite of the developments in organic agriculture and pesticide-restricted crop cultivation. Indiscriminate and excessive uses of toxic synthetic pesticides are common scenario in many areas to combat with destructive pests and diseases. However, the frequency and amount of pesticide applications per unit area is highest in the high valued crops. Due to development of resistance by different insect pests to different chemical pesticides, it was reported that for vegetables and fruits in general, an increasing trend was observed in use of pesticides by farmers in combating the pests in this region. Since majority of the farmers do not maintain any waiting period before crop harvest, the consumers become inevitably exposed to high levels of pesticide residues in their diets. The situation is compounded by the fact that, unlike cereals, vegetables or fruits are often consumed soon after harvest and there is little time for the chemicals to degrade. Different crops especially vegetables and fruits are harvested and marketed without knowing any residue status. This is most likely to cause serious health hazard to the consumers. Moreover, the repeated applications have induced multiple resistances of different pests against various pesticides. It is also suspected that the fish populations in the open water bodies as well as in the rice fields have been reduced due to the adverse effects of the chemical pesticide use. Not only that, frequent use of toxic pesticides have boost up the population of many minor pests like whitefly, jassid, fruit

borers, prodenia caterpillar, red pumpkin beetle, cut worm, red mite, aphids, mango twig galls, mango fruit weevil, mango fruit borer, jack fruit borer, litchi mite, litchi borer, guava spiraling whitefly, guava fruit borer, different viruses etc. Just before one decade none of those pests were considered as the major pests of vegetables or fruits.

One of the ways to avoid this measurable pest management system may be to develop eco-friendly, sustainable, socio-economic acceptable integrated pest management (IPM) strategies. IPM is one of a number of integrated approaches that are gaining credence for use in sustainable agriculture development. It involves the integration by farmer of the most appropriate management strategies for pest control where sole dependency on pesticides can be avoided.

Over four and a half decades have elapsed since V. F. Stern and his colleagues introduced the concept of "Integrated Control" (presently called Integrated Pest Management) in 1959 as a method of reducing pesticide use without affecting crop yields. It was a time when the hidden effects of pesticides were just beginning to come to light. The world came to know about the ill effects of DDT in 1962, three years after the IPM concept was introduced, through the classic work of Rachael Carson published in the book "Silent Spring". She proved through numerous tests that DDT, although controlled the crop pests, also killed birds and fish and this chemical was not safe. Obviously, the concept of IPM was rooted in the recognition of the deleterious consequences of harmful pesticides. On the contrary, Dwight Isley successfully established in the early 1940s a sound program for the management of the boll weevil, boll worm and spider mite of cotton in Arkansas, USA using many of the IPM components based on applied ecological principles that are used at present. This indicates that IPM as a concept is old, but the term was not known. The need for an integrated approach for pest control was evidently the result of the recognition that no single approach to pest control, such as the use of pesticides only, offers a universal solution considering the dynamic characteristics of the pest species and that the best protection can be provided by fusion of various tactics and practices based on sound ecological principles.

The different components of IPM are:

1. Cultural control: Purposeful manipulation of environment to make it less favorable for the pests. Cultural control may be employed by using the following techniques: a) Destruction of crop stubble and affected plant parts, b) Tillage/Ploughing, c) Clean cultivation, d) Use of resistant crop variety, e) Changing of planting date, f) User of crop rotation technique, g) Use of inter-cropping, mixed cropping, h) Optimizing density of plants, i) Optimizing fertilizers, j) Use of trap cropping, k) Use of stripe cropping for enhancing natural enemies, l) Water management, m) Optimizing depth of planting.

2. Physical and mechanical control: Methods employing manual devices and mechanisms are included in physical and mechanical control. Physical and mechanical control will be employed as described below:

Physical control: a) Superheating of empty godown to a temperature above 50°C for 10-12 hours, b) Exposing of infested grain to the sun and c) Exposing thickly coated seeds to 52°C.

Mechanical control: a) Hand picking for caterpillars, beetles, bugs, b) Shaking, beating, killing and collecting insect pests, c) Use of sweeping net/hand net, d) Use of light trap, bait trap, sticky trap, window pan trap, suction trap, pheromone trap, yellow pan trap, e) Sieving and winnowing, f) Hooking, g) Banding/use of sticky traps, h) Screening window, door and ventilator, i) Wrapping fruits with cloths, j) Trenching field and erecting barrier, k) Smoking by flame thrower.

3. Application of pheromones: Pheromones are naturally produced chemicals or blends of chemicals that elicit a behavioral response from a member of the same species. Similarly, chemicals that elicit a behavioral response from members of another species are known as allelochemicals. These can be subdivided according to the species that benefits. Thus, kairomones benefit the receiver while allomones benefit the emitter. Other allelochemicals benefit both receiver and emitter and are known as synomones. The various classes of "behavior-modifying chemicals" are collectively known as semiochemicals. Semiochemicals are of enormous economic importance to agriculture. The pheromones are used in pest management in the following purposes: a) Population monitoring e.g., African armyworm, *Spodoptera exempta*, b) Mating disruption e.g., Pink bollworm, *Pectinophora gossypiella*, c) Mass trapping e.g., Yellow rice stem borer, *Scirpophaga incertulas*, Brinjal shoot and fruit borer (BSFB), *Leucinodes orbonalis*.

4. Biological control: Total or partial destruction of an insect population by other organisms or insects is generally known as biological control. Out of the total identified population of insects in the world, only 10% considered as pests and the rest 90% are beneficial and harmless. So, there are huge sources of biological agents that can be used in biological control of insect pests.

5. Microbial control: The use of microbial organisms to control the insect pests of crops is regarded as microbial control. Microbes are highly host specific, environmentally sound, effective in low dose and generally inexpensive. The examples of microbial agents are given below. **Fungus:** *Verticillium lecani* and *Beauveria bassiana*; **Virus:** Nuclear Polyhedrosis Virus (NPV), Granulosis Virus (GV) and Cytoplasmic Polyhedrosis Virus (CPV); **Bacteria:** *Bacillus thuringiensis* as BT powder.

Bio-rationales including botanicals (plant extracts), bio-pesticides (microbial – fungi, bacteria, viruses) and pheromones for management of insect pests are considered by crop protection scientists as environmentally sound and with great potential to replace chemical pesticides if production protocols can be standardized and the private sector becomes involved. Pheromones have proved effective for mass trapping and mating disruption of specific insect pests. Even in Bangladesh, studies showed that several devastating pests like brinjal fruit and shoot borer, cucurbits fruit fly etc. can be economically managed with the sex pheromone based IPM packages.

Need for IPM in the South Asia: Considering the facts that (a) South Asia needs to increase its food production on a sustainable basis, (b) pests and diseases continue to cause serious crop losses and (c) the use of toxic pesticides is the main method of pest management and that such continued reliance on chemicals would lead to serious environmental and human health problems, pest resurgence, new pest problems etc. there is a real need for an alternative strategy to sole reliance on pesticides for crop pest management in South Asia. IPM is considered to be the most appropriate strategy.

IPM packages should mainly be dependent on 'Bio-rationales/Bio-pesticides'. It refers to products from natural sources such as animals, plants, and micro-organisms, including "natural ingredient pesticide," "microorganism pesticides" and "biochemical pesticides." 'Bio-rationales/ Bio-pesticides' offer several advantages over traditional synthetic chemical pesticides. They are safer and less toxic to human and animals than chemical ones. Moreover, bio-rationales/ bio-pesticides will not endanger birds or other non-target animals, which makes them safer to the habitat and environment. 'Bio-rationales/ Bio-pesticides' can offer much more targeted activity against a desired pest, as opposed to conventional pesticides, which can affect a broad spectrum of pests and non-target arthropods including beneficials. 'Bio-rationales/ Bio-pesticides' often are effective in very small quantities, thereby offering lower exposure. Furthermore, they decompose more quickly than the conventional synthetic pesticides. Due to those reasons, bio-pesticides are supplementing the synthetic pesticides in the IPM programs, which offer potentially higher crop yields and can dramatically reduce the use of chemical pesticides.

The potentiality of 'bio-rationales/ bio-pesticides' has increased substantially throughout the world including South Asia and Bangladesh. Extensive and systematic research and development works on mass production, storage, transport and application of 'bio-rationales/ bio-pesticides', augmentation and application of bio-control agents have improved both in public and private sector in recent years with the ultimate objective of improving its commercial production and use. With the increased environmental awareness, increase activities of IPM, widening of organic farming, more R&D of bio-pesticides etc., use of synthetic pesticides especially in case of insecticides are showing a declining trend, whereas the bio-pesticide market is growing.

Best practices of IPM application in Bangladesh

It has been observed that it is difficult to control a pest only by the application of a single tactic. In order to achieve sustainable result, it is necessary to develop complete and coherent packages of technologies that meet farmers' needs and completely replace the need for application of toxic chemical pesticides. It has been observed that effective management of *Spodoptera litura* can be ensured with the mass trapping of *Spodoptera litura* by pheromone traps along with 2-3 application of SNPV, inundative release of two parasitoids, *Tricogramma chilonis* and *Bracon habetor* can reduce the brinjal fruit damage to less than 10% along with the pheromone mass trapping and weekly removal of pest infested shoots and fruits from the field. In Bangladesh, scientists of different public research institutes and universities, viz. Bangladesh Agricultural Research Institute (BARI), Bangladesh Agricultural University etc. have already developed 20 bio-pesticide based pest management packages against several destructive insect pests and diseases of different crops.

Conclusion and recommendation

Pest management is a dynamic approach and pesticide is still essential for pest management. However, the pest management tools should be safe, cost-effective and have minimum risk or hazard to human and desirable components of environment. In Bangladesh and also most of the countries of South Asia, till now research efforts have

been unexpectedly slow and limited for the development of bio-pesticide based IPM technologies for different crops. As a result, the availability of bio-pesticide based IPM technologies for different crops lagged behind seriously for years, compelling the farmers to have no other option than to rely solely on pesticide use for pest management. So, extensive research work especially in the public sector should be undertaken for the development of effective and cheap bio-pesticide based IPM technologies against major insect pests & diseases. At the same time extensive promotional works all over the country should be undertaken for the quick dissemination of the developed bio-pesticide based IPM technologies. Steps should be undertaken to give legal permission to the authorities for easy availability of different bio-pesticides.

Strengthening biocontrol by transboundary exchange of tools, techniques and expertise amongst SAARC countries

Malvika Chaudhary

Regional Coordinator for Asia Plantwise, CABI-South Asia
New Delhi-INDIA

Biocontrol technology and its significance

The indiscriminate use of pesticide in agriculture has resulted into innumerable adversaries which has imperatively led the mankind to search an alternate which is safe to use both for environment and human and ensures food security through sustainable agriculture. Biocontrol agents provide solution as they are naturally occurring organisms predating, parasiting on pest of crops and offering a natural control. But due to many factors including excess use of chemicals the balance between these natural enemies of pest and pest themselves has been disturbed. Hence there is a dire effort required to restore this imbalance by conservation and augmentation of biocontrol agents in the environment. Classical and augmentative biological control of insect pests and weeds has enjoyed a long history of successes. However, **biocontrol** practices have not been as universally accepted or optimally utilised as they could be (Barratt, et. al., 2018).

This demand and search is only amplifying with time as we are experiencing an impact of climate change on the pest dynamics and realisation that farmers cannot handle such unpredictable conditions with chemicals. Including this various other reasons emerge that adds to the significance of biocontrol in agriculture. Few are mentioned to help the readers rationalise the need of biocontrol in agriculture.

Climate change and invasive: The rise in temperature and other resulting changes in the climate is leading to an altered pest dynamics, is affecting their metabolism hence various unpredictability in the field. This has also led to transboundary migration of certain pest which becomes a new entity to manage. Although the variety of insect and plant responses to **climate change** reported in the literature do not currently allow for strong generalizations to be made as to the best biological control agents or strategies in novel **climates**, pertinent considerations for selecting effective biological control agents in the future is a strong option for addressing this challenge (Reeves, J. L. 2017).

Pesticide poisoning: The havoc created by confusion of new pest in the area and incapability of handling these with existing plethora of chemicals leads to the inappropriate use of these inputs resulting into use of some very harmful chemicals that directly affects the health of the users. About 80% of farmers do not have adequate materials for handling and application of **pesticides** and are thus exposed to **pesticide poisoning** (Agboyi et. al., 2015). The inadequacy of information reaching farmer regarding such chemical molecules and the carelessness through which they are handled certainly carves the need of safer alternates.

Exports Rejects: Growing awareness of the harmful properties of certain chemicals are leading to their restricted use and even unacceptability of application to be complied as global standards. This leads to the need of intensive research of either new chemicals or promotion of biocontrol as alternatives.

Above given are just few examples which are thought provoking enough to realise the need. These are equally balanced by the various numbers of opportunities that exist to adopt biocontrol technology at large scale and make it a sustainable practice. Amongst these are:

Utilisation of natural resources : The tropical natural conditions of SAARC countries provides rich biodiversity and availability of natural resources in form of plants and organisms that can act as natural pesticides and enemies to the pest of agricultural importance. With the indigenous traditional knowledge combined with novel global findings, these options can be explored to work out a biocontrol optioneg. In India, there are many locally available plants like beshram, neem, garlic, triphala, pinuskesiaetc which can be easily processed and increase the biopesticide consumption in India (Srijita Dutta, 2015).

Growing organic market: The awareness amongst the consumer regarding ill effects of chemicals is leading to the rise in demand of safe food. More and more retail outlets are mushrooming up to cater these demands. To supply to both domestic and international market the need to grow the food cautiously with less or no chemicals defines the opportunity of using biocontrol technology as viable alternative.

Most recent status of biocontrol in SAARC countries

The status of biocontrol in SAARC countries has been recently discussed in a regional consultation wherein seven SAARC countries have expressed the status and the limitation they are facing to promote and adopt the technology (Wickramaarchichi et. al., 2018). However the status through these interactions is clear that except for India, Bangladesh and Nepal the use of BCA is not utilised to the expected level.

Afghanistan: Efforts are underway to standardise and produce biopesticides like *Trichoderma*, NPV, *Trichogramma* but due to lack of infrastructure and more importantly absence of skilled manpower and proper guidance, the progress is impeded.

Bangladesh: The presence of national research institutes dedicated towards research of biocontrol technology and some associate private industry reflects a better status of the technology in the country. However the need of structured monitoring system to assess the quality of the products in the market will further enhance the use of natural enemies in the country.

Bhutan: This country provides ample of opportunities for a biocontrol to be a success owing to its status of completely organic agriculture. Biopesticides are still imported from the neighbouring countries. Lack of manpower, resource capacity, laboratory facilities, awareness and entrepreneurial aptitude are some of the main challenges faced in the country to adopt the technology at large scale.

India: From the period of initiating research of biocontrol way back in 1950 till date, India has made progress in popularising and adoption of this technology through more

than 400 private industries commercialising the concept with 990 registered products. These products are both utilised in the domestic market and are also exportable. Though the main challenge lies in getting these products across the vast nation, increased number of production units for uniform availability and to enable so public private partnerships are sought to make it a reality. Further research on efficient storage of biocontrol products, packaging and their transport and simplification of registration process will encourage more investment in the industry.

Maldives: Though microbial pesticides are being used at large commercial scale their production and availability are a challenge in the country. Insufficient financial resources, and poor coordination amongst the stakeholders restricts implementation of technology. Moreover the illegal trade of counterfeit chemicals are the major motivation for the government to promote biocontrol technology, lack of technical staff and awareness amongst farmer have to be overcome as serious considerations.

Nepal: Bio pesticide have been imported in Nepal since 2004, seventy eight microbial products have been registered for use. With no discrimination between the registration process of chemicals and bio pesticides, the process of registration becomes tedious and a impediment for the availability. The regional plant protection laboratories are putting in immense efforts in research and production of bio pesticides.

Sri Lanka: Botanicals are most exploited category of bio pesticides in the country owing to its utilisation through indigenous traditional knowledge but lack of donors to fund development of this technology are the major constraint. Huge investments are required from the entrepreneur which may not much profit in short term is a demotivation and knowing that the registration of these products can take a long time in the country.

Way forward forging the adoption and utilisation of biocontrol technology in SAARC countries.

Policy and Regulation: SAARC countries should look into their Nation Agricultural Policies and bring interventions for amendment of existing legislations to accommodate promotion and utilisation of biocontrol products and technologies on priority. They should include these components their National agricultural plans so as to have dedicated funds to manage resources for implementation of plans especially focussing on the actions to bring the technology at user's level. Adoption of standard procedure like ISPM8 and separating legislation for chemical pesticide and microbial pesticide should be worked out for speedier process. Uniform and harmonised registration across the SAARC countries without any disparity will ease import and export of the products to support the agriculture of those SAARC countries where the manufacturing has to been initiated at the industrial scale.

Research: Research and Experts from the countries where technology has already been developed for scale up stage should be adopted by other countries with proper intellectual property consider derations and should be utilised instead of wasting resources in reinventing the wheel. Focus should be on isolation of indigenous isolates which should be explored for standardisation of production of the microbial pesticide in the country. These isolates can be most effective under the field conditions and can result into good products. Repository of such strains at national level can be effective for strengthening

the research and utilisation of these in most appropriate manner. Their identity at molecular level and validating the same from time to time will ensure proper use. Research should also be focussed on better formulations, storage and transport of biocontrol product to retain their integrity and deliver desired results in the field. Regular exchange visits of scientists amongst SAARC region is recommended for getting exposure to novel research techniques and considering these objectives for their own respective countries subjected on the suitability and feasibility of adoption.

Production: Technical audit and need assessment in SAARC countries should take place in identifying various gaps. Public private partnerships should be encouraged with national and donor funding to support a program of scaling up of lab technologies into commercialisation of mass production of bio control agents. Experts from SAARC countries should be identified and deputed to go and help standardise the scale Skilling the manpower by production techniques should be taken up on priority. SAARC countries should also look into international resource pool where technologies are at much advanced stages and methods have been already developed for production of these agents at much higher scale.

The private companies should be given motivational seminars and exchange visit in more successful countries to understand the potential investment required for this potential technology. Economics of production and utilisation of microbial pesticides should be computed and communicated. Linkages of stakeholders ensuring utilisation of the end products, relaxed policies and risk coverage will encourage investments from the private sector.

Quality Assurance: To ensure consistency of the quality of the produced biocontrol product standard specifications for these products should be framed with proper guidelines. The referral labs should be instituted with mechanism to draw samples from the market from time to time for testing the products. Capacity building to enhance the skills of the officials as quality inspectors should be in place to develop proper human infrastructure to backstop the quality of the products in the market. The testing of these products can either be linked to existing laboratories in research institutes and universities or dedicated laboratory for the specific purpose should be strengthened with basic equipment to support the testing. Development of referral material like protocols and manuals will further provide technical guidance to the technicians working in these laboratories. Proper action should take place in case the quality of the products is not conformed in order to avoid discontinuation of the use of technology at the user level.

Marketing: Proper research to forecast the demand of the biocontrol products is the backbone of the continuous production and utilisation of the products. Since these products have a shorter shelf life and have to be handled with much care than the chemical pesticide proper marketing channel and capacity building of handling the same at distribution and dealer level should be done. This includes enhancing of these stakeholders in terms of knowledge and encouraging them to invest in certain resources which can support storage and distributions of the BCA in proper way. For strengthening the Import and Export procedures careful monitoring should be instituted to address utilisation of counterfeit products. Such technical can be tested by sending them in international referral laboratories if national facilities are not available.

Extension: Enough printed material and other resources should be developed, adopted customised to increase the awareness of the use of biocontrol agent at the user level. Programmes with proper funding should be operationalised to encourage the use. In the era of devolution and decentralisation, private extension should be encouraged as another source of reaching out farmers in SAARC countries. PPP mode should be highly encouraged so that the onus to extend this technology is not only left on government sector but also is responsibility of IGOS/NGOs and other private companies. Universities should work in collaboration to ensure that the proper standardised recommendations are channelled through these parallel extensions. Motivational training should be provided by NGOs demonstrating the benefit of bio pesticides. Microbial pesticides should be utilised in all sectors of agriculture including plantations. Appropriate documentation of success stories will motivate use of this technology amongst the users. Bio pesticide knowledge bank with open access on information available should be promoted for better learning and utilisation of knowledge across the SAARC countries. This technology can be best explained by use of approach that can offer proper diagnosis and recommendation of the products as early intervention for timely management of pest and diseases. ICT, Mass media and social network can provide maximum outreach and should be thoroughly utilised for the extension purpose of biocontrol agent thus promoting the awareness and utilising the technology in most appropriate manner.

Utilisation at farmer level: Farmers should be given demonstration at field level for the better understanding. Village models utilising the biocontrol technology as prime method of plant protection should be developed to give the farmers encouragement for the use of this technology at farm level. Promotion of this technology through organised farmer association and cooperatives will lead to adoption. Farmer Producer organisation should be linked to proper stake holders for procurement and increasing the availability of products at farm level. Farmers should also be linked with proper banking and risk covering agencies to strengthen the adoption of novel technologies.

Conclusion: International donor agencies and SAARC should make efforts to facilitate implement the recommendations of the various regional consultations that are held on the subject to ensure the utilisation of technology. In South Asia the current status is that the biocontrol technology is established to an extent wherein we should look into the possibilities of channelize it to the user level. An effort for extending biocontrol with use of advanced technology and proper cooperation is the need of the hour and can be established through proper cooperation and under guidance of experts amongst the global community and SAARC countries .

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Sampling procedures of different arthropods emphasize on biological control agents

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Sampling

Insect sampling is usually conducted to collect population and diversity information about insect fauna of a particular area. Sampling involves collecting repeated systematic data of an insect in its environment over a specified time. A group of sampling unit is referred to as the sample, which is used to estimate the population.

Why taking insect samples?

- Sampling is a fundamental component of an IPM program.
- It increases awareness of insect activity in orchards, fields and storages.
- Provides reliable indications of presence, abundance and distribution of pests and beneficial insects.
- Give information on injury and damage caused by the pests.
- Make cost effective and environmentally sound insect management decisions by ensuring-
 - When (if) to apply control measures
 - Apply the right control
 - Avoid pest outbreaks/ yield loss
 - Avoid unnecessary treatments
 - Resistance management
 - Determine population trends
 - Determine effect of treatments

Components of an insect sampling program

- **Knowledge of pest and beneficial insects**
 - **Identification:** Generally need to determine the species level. Through identification we could understand: Is it pest or beneficial? Which pest is it? What stage is it?
 - **Life cycle and biology:** The life cycle and biology tells us when and where and how often to sample. It gives information on overwintering (when, where and what stage), host (the plants attacked, used), plant parts attacked, damage stage, when it's present, number of generation per year and generation time.

- **Injury caused:** Injury- the effect that the pest has on the crop or commodity. Damage – the effect that injury has on man's assessment of the crop's economic value.

For crops, "Injury" is biological and "Damage" is economic. For non-crops, "Injury" = "Damage".

Direct damage: Pests attack harvestable commodity (fruit, fruit buds etc).

Indirect damage: Pests attack non-harvested plant parts (roots, shoots, leaves etc).

- **Action/ economic thresholds**

- **Economic (action) threshold:** Level at which pest should be treated to prevent exceeding the EIL.
- **Economic injury level:** Pest density that causes economically significant crop loss, or when: Cost of yield loss = cost of control efforts.

- **Pest distribution**

- **Uniform distribution:** When a pest is distributed evenly throughout the sampling universe, e.g., every leaf on a plant has 3 adult whiteflies.
- **Random distribution:** It occurs when a pest is distributed haphazardly in the field.
- **Clumped distribution:** It occurs when a pest is aggregated within certain areas of the field such as field borders, or centrally within the orchard or planting.

- **Sampling program**

- **Sampling equipment supplies**

- **Sampling unit**

A sampling unit is a proportion of the habitable space from which insect counts are taken. The sampling unit concept is most easily explained when total counts are taken from a unit-area of land surface. For example, direct counts of all the caterpillars in 1 m² of alfalfa could be considered a sampling unit. If the caterpillar population occupies 100 m², the habitable space is composed of 100 sampling units. Sampling unit depends on- pest species and size of the population, mobility and distribution of pest, cost of the sampling unit and accuracy of the sampling unit.

- **Sampling accuracy**

Sampling accuracy depends on field size and shape, variation between individuals and differences in perception, consistency of sampling methods and the numbers of samples taken.

Types of sampling

- **Random sampling:** Random sampling involves selection a number of samples from more or less homogeneous population such that every individual has an equal chance of being selected as part of sample. Random sample is the simplest method for field to estimate insect infestation. It can be easily done by using a quadrat.

- **Stratified random sampling:** In stratified random sampling the population is divided into strata and each stratum is randomly sampled and the samples from different strata are pooled. The strata are subdivisions of the sample based on distribution of the population.
- **Systematic sampling:** Systematic sampling involving taking samples and fixed intervals. The first sample is taken at the reference point and subsequent samples are taken at successive intervals. For example- collection of sample for the number of plant of every third row of every plot or collection of sample of every fifth plant.

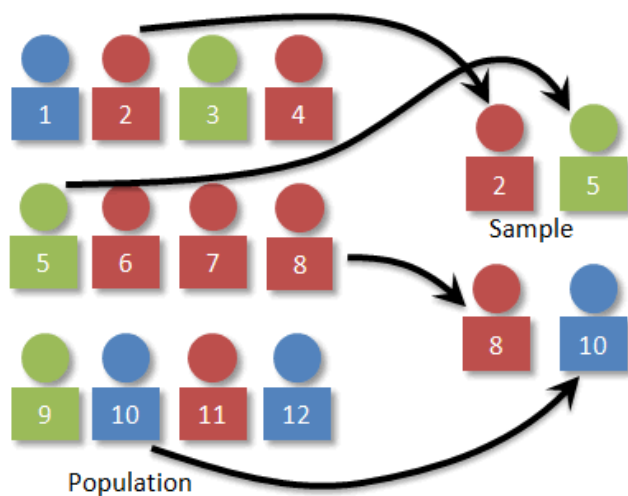


Fig. Random sampling.

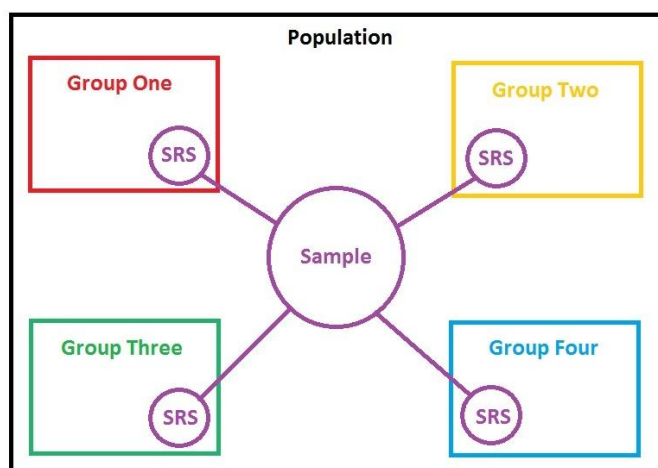


Fig. Stratified sampling.

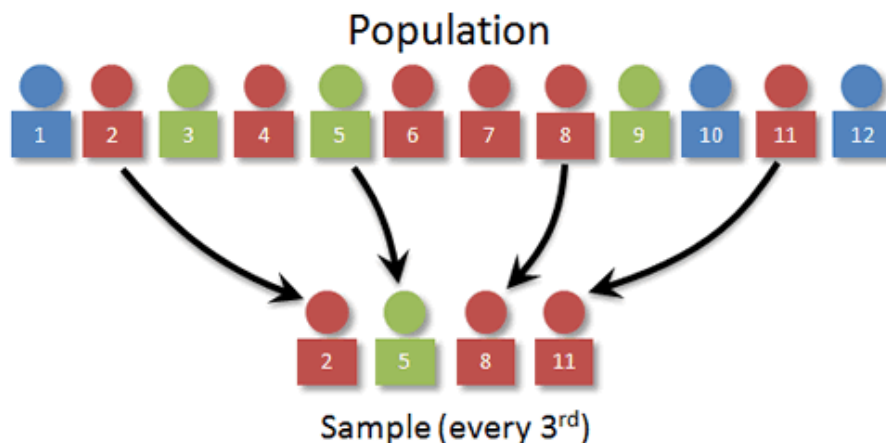


Fig. Systematic sampling.

Common sampling techniques in insect pest management

Most common techniques are (i) In situ counts (ii) Knockdown (iii) Netting (iv) Trapping (v) Extraction from soil.

In Situ counts

In situ (Latin in place) counts, also known as direct counts and direct observation, often required no special equipment but rely on good eye.

What kind of insects and locations are feasible for in situ method?

With in situ method, large and mostly conspicuous insects are viewed in the habitat, and counts are recorded. Usually the habitat viewed with this method is the plant canopy, a specific plant part. If numbers of insects are relatively low and the plant is small and isolated from adjacent plants (as with seedlings), all insects on the plant may be counted. Therefore, the procedure is frequently used with early-season pests of agronomic crops, or in later season with crops where herbage is removed. When plants are large, such as mature trees or shrubs, only certain numbers of leaves, stems, flowers, buds, or pods may be counted.

How the samples are taken?

- **Line-transect method:** Walk through the field/ orchard and count pests and beneficial insects. If one walks in a straight line at a constant speed through a habitat, the number of individuals can be counted. This technique is used for quantitative comparisons both between different species, and between different occupiers of habitats.
- **Visual sampling:** Counts of insect (aphids, scale insects, mites, leaf miners, small caterpillars, leaf hoppers, immature psylla) or damage directly on leaves, stems, fruit, roots etc.
- **Insect aspirator:** Small insects are captured (aphid, jassid, mealy bug etc).

- **Quadrat method:** Small areas or quadrates are chosen at random from a large area. From a quadrate, the insects may be counted or collected directly as in the case of fairly immobile but relatively large insects such as cutworms, caterpillars and grasshoppers. In case of tissue borers such as sugarcane borers, maize borer, etc., the estimate is done by insect removing the infested plants from the quadrates and then counting them after splitting open the plants.
- **Knockdown:** Knockdown is closely related to in situ counting but in this instance, the insects are removed from the habitat by jarring, chemicals, or heating, and then counted. Jarring is probably the most common method of knockdown from plants.
- **Where and how knockdown method is applied?**

It has been used particularly when sampling insects occurred on the lower branches of trees and shrubs. Here a cloth, tray, or other receptacle is placed on the ground, a branch is pulled down over the receptacle, and the branch is struck a prescribed number of times with a stick. Insects knocked off fall into the receptacle and are counted.
- **Netting:** Netting insects is one of the most widely used techniques in population sampling. Netting can usually be accomplished rather inexpensively and is adaptable to sampling a vast array of agricultural pests and their natural enemies. In this instance, a muslin 'net' is swung into the plant canopy, jarring the plants and causing insects on them to fall off into the net. After a prescribed number of such swings, called sweeps, plant debris is removed, and the insects are counted.
- **Sweep net sampling:** Collects many insects quickly. Not as useful for tree and small fruits. Useful for sampling field crops, ground cover and field edges.



Fig. Sweep net.

- **D-frame and O-frame aquatic nets:** These nets are much sturdier than the sweep nets to accommodate the resistance of the water. D-frame net is best for sampling in flowing water.



Fig. D-frame net



Fig. O-frame net

- **Trapping:** Various types of traps are flight traps, aquatic traps, pitfall, light and other visual traps are used.
- **Attractant traps:**
 - **Visual traps:** Colors and/or shapes used to attract insects. Yellow sticky cards are used to capture aphids, fruit flies, thrips etc.
 - **Food attractants:** Food source scents. Often an ammonia source (ammonium acetate, or ammonium carbonate), may have a protein source (casein). Often combined with visual/sticky traps.
 - **Pheromone traps:** Most commercial pheromones are synthetic versions of natural scents produced by insects to attract mates. Most are female-produced scents that attract males. Usually species specific.



Fig. Pheromone trap.

- **Bait traps:** Many insects, such as ants, nitidulidae beetles, dung beetles, carrion beetles, bark beetles, moths, and others are attracted to various baits. Baits can be used to the ground, on trees, ropes, or elsewhere, and insects can be collected directly from them. Commonly used baits are- brown sugar yeast bait, turpentine bait, beer/molasses bait, wine/fermenting fruit bait etc.



Fig. Bait trap.

- **Light traps:** Light traps make use of a light source to attract night-flying insects. They are especially useful to monitor certain species of moths. While the light source attracts the insects, the traps need another device to kill or capture them. Often this is a container with water that is placed under the light source.



Fig. Light trap.

- **Malise trap:** Malaise traps are tent-like traps made of fine mesh material and used primarily for the collection of flies (Diptera) and wasps (Hymenoptera), although they also catch a great many other flying insects. Malaise traps are generally set out for long periods of time and checked at least weekly, or occasionally every other week.



Fig. Malise trap

- **Pan traps:** Pan traps are primarily used to capture micro Hymenoptera, but also trap many other insects. Yellow, blue, white, and red colored bowls are placed on the ground and partially fill with water and a drop of dish liquid or other soap. Pan traps need to be checked at least daily.



Fig. Pan traps.

- **Berlese funnel:** Berlese funnels are used for extracting arthropods from soil and litter samples. They work on the principle that the insects and other arthropods that normally live in soil and litter will respond negatively to light. Therefore, a light source is used to force the arthropods to move downward, where they will fall into a funnel and then into a container of ethanol.



Fig. Berlese funnels.

- **Pitfall traps:** A pitfall trap consists of some type of cup or other container (gallon bucket, for example) that is submerged in the soil and partially filled with a preservative. Insects crawling about on the ground simply walk into the container and then cannot get out.

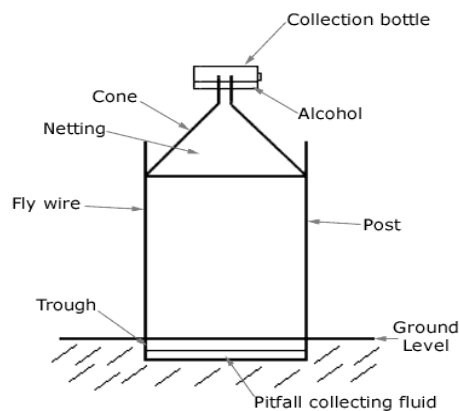


Fig. Pitfall trap

Use of different biopesticides for pest management and their quality control

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About Biopesticide

As defined by the United States Environmental Protection Agency (EPA), biopesticides are certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals. Plant growth regulators (PGRs), which exhibit no pesticidal activity but instead can promote, inhibit or modify the physiology of plants, are also regulated by the EPA as biopesticides. In commercial terms, biopesticides include microorganisms that control pests (microbial pesticides), naturally occurring substances that control pests (biochemical pesticides), pesticidal substances produced by plants containing added genetic material (plant-incorporated protectants) and biochemical plant growth regulators. Biopesticides are employed in agricultural use for the purposes of insect control, disease control, weed control, nematode control, and plant physiology and productivity.

Benefits of Biopesticide

Environmental Safety with Biopesticides: The general public's concern for environmental safety is one of the leading drivers of biopesticide usage. As growers focus on this aspect of production and market these practices to their customers, dealers may recommend biopesticides as an environmentally sound and efficacious product. Non-toxic to non-target organisms, including beneficial insects and wildlife, many biopesticides also are biodegradable. They decompose quickly and do not negatively impact surface water and groundwater. Biopesticides typically are effective in small quantities, which eliminates pollution concerns sometimes associated with traditional chemicals. In addition, biopesticides are manufactured from naturally occurring raw materials in an environmentally responsible and sustainable manner.

Add Value and Yield: Crop quality and yield largely determine a grower's income. Biopesticides provide dealers with products that can markedly improve crop quality and yield by preventing pest damage and promoting physiological benefits in plants, including increased fruit size and enhanced color. Dealers who supply biopesticides and encourage their innovative use are on the forefront of yield-and-profit enhancement practices.

Partner with Traditional Chemicals in IPM Programs: Sustainable agriculture relies on integrated pest management (IPM) techniques which combine proven cultural, biological, and chemical methods to control pests as an environmentally sound approach to crop production. Dealers supply products that support these methods and many biopesticides are specifically designed for use in conjunction with stable traditional chemistries in an IPM program.

Biopesticides Allow Labor and Harvest Flexibility: Biopesticides provide dealers with a solution to grower requests for products with labor and harvest flexibility. In terms of application, biopesticides generally have short worker restricted entry intervals. Workers can safely return to a field shortly after treatment, allowing them to assess crop conditions and follow up with applications of other crop inputs when needed. In addition, biopesticides generally have short pre-harvest intervals. This allows growers to better manage their labor, harvest and shipping schedules. In terms of plant growth, Plant Growth Regulators (PGRs) may be used to control and optimize harvest times. PGRs that inhibit the biosynthesis of ethylene in plants, for example, can slow down fruit maturation, ripening, and abscission.

Help with Resistance Management: Long-term continuous use of a single class of pesticides may result in resistance to that class by the targeted pest. Biopesticides with novel modes of action provide dealers with products that manage pest resistance and extend the life of valuable traditional pesticides. Biopesticides optimized for mixtures, tank mixtures and combinations have been used in combination with traditional chemistries to effectively manage resistance, while maintaining high pest control standards.

Make Residue Management Easier: Consumers are increasingly aware of chemical use in food production and the potential for chemical residue on food. Dealers may promote biopesticides, which leave little to no residue on food, to growers as residue management products. Many biopesticides are exempt from residue limits on fresh and processed foods. In addition, biopesticides may be applied in environmentally sensitive areas without residue concerns and may be used to manage residue levels for exported produce.

Types of Biopesticides

The EPA recognizes three major classes of biopesticides: microbial pesticides, biochemical pesticides, and plant incorporated protectants.

1. Microbial Biopesticides: Microbial pesticides are products derived from various microorganisms (e.g., bacterium, fungus, virus or protozoan) that are used as an active ingredient to control pests. Microbial products may consist of the organisms themselves and/or the metabolites they produce. The microorganism may occur naturally, be dead or alive, or be genetically engineered. Microbial biopesticides are generally divided into six subcategories:

Bacteria ~ Many spore forming and non-spore forming bacteria are known to be effective against a wide spectrum of insects and diseases. For example, more than 90 species of naturally occurring, insect-specific bacteria have been isolated from insects, plants, and the soil. To date, *Bacillus thuringiensis*, or Bt, is the species that has been most successfully developed as a microbial insecticide.

Viruses ~ Baculoviruses are a family of naturally occurring viruses known to infect only insects and some related arthropods. Most are so specific in their action that they infect and kill only one or a few species of Lepidoptera larvae.

Fungi ~ With complex lifecycles, some fungi are parasitic to various eukaryotes, including plants and insects. Fungi require specific environmental conditions to proliferate and their means of affecting the target pest are diverse.

Protozoa ~ Protozoa are single-celled eukaryotic organisms that exist in both water and soil. While most protozoa feed on bacteria and decaying organic matter, a wide range of protozoan species are insect parasites.

Yeast ~ A variety of non-pathogenic, naturally occurring yeasts have been investigated for their usefulness in controlling plant diseases.

Nematodes ~ Nematodes act as insecticides by invading insect larvae through bodily openings and releasing toxic bacteria that kills the host.

2. Biochemical Biopesticides: Biochemical biopesticides are naturally occurring compounds or synthetically derived compounds that are structurally similar (and functionally identical) to their naturally occurring counterparts. In general, biochemical biopesticides are characterized by a non-toxic mode of action that may affect the growth and development of a pest, its ability to reproduce, or pest ecology. They also may have an impact on the growth and development of treated plants including their post-harvest physiology. Biochemical biopesticides are generally divided into six subcategories:

Plant Growth Regulators (PGRs) ~ Including both natural and manufactured versions of natural substances that affect major physiological functions of plants, PGRs can promote, inhibit, or modify the physiological traits of a range of fruit, vegetable, ornamental and agronomic crops. PGRs are used to maximize productivity and quality, improve consistency in production, and overcome genetic and abiotic limitations to plant productivity. There are five major classes of these natural plant hormones: gibberellins, cytokinins, abscisic acid, ethylene, and auxins.

Insect Growth Regulators (IGRs) ~ With a unique mode of action, these products prevent insects from reaching a reproductive stage, thereby reducing the expansion of pest populations. The direct impact of IGRs on target pests combined with the preservation of beneficial insects and pollinators aids growers in maximizing yield and product quality.

Organic Acids ~ Acids such as peracids are effective sanitizing agents used for control of pathogens and algae. Peracid products can be used for sanitation of greenhouse surfaces, shock applications for tanks and piping, continuous application at a low concentration, and also as a bactericidal or fungicidal application to plant foliage or roots.

Plant Extracts ~ Many plants have developed natural, biochemical mechanisms to defend themselves from weed, insect and fungal attacks. These products act as insect growth regulators, feeding deterrents, repellents, and confusants. Some plant growth extracts provide relief for abiotic stress such as heat, drought, salinity and even frost.

Pheromones ~ Chemical signals that trigger a natural response in another member of the same species, pheromones are used to disrupt pest ecology and reduce crop damage. Synthetic female pheromonea are used as lures to attract males into traps and are also used for mating disruption.

Minerals ~ Minerals play a key role in a wide range of biopesticide applications that can be divided into three categories: those that create barriers that keep pests from plant tissues and/or impact water supply; those that deliver physical impacts such as smothering or abrasion; and those that act as an inert carrier for companion biopesticides.

3. Plant-Incorporated Protectants: Plant-incorporated-protectants, also known as genetically modified crops, are pesticidal substances that plants produce from genetic material that has been added to the plant, such as brinjal, corn and cotton.

Table. Different Biopesticides for pest management available in Bangladesh

Type of Biopesticides	Active ingredients	Use
Microbial Biopesticides	<i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i>	Control of various lepidopteran pests of 1) a wide variety of agricultural, ornamental and home garden crops 2) trees in forests, woodlots and residential areas
	<i>Spodoptera</i> Nucleo polyhedrosis Virus (SNPV)	<i>Spodoptera</i> spp.
	<i>Helicoverpa</i> Nucleo polyhedrosis Virus (HNPV)	<i>Helicoverpa</i> spp.
	<i>Metarhizium anisopliae</i>	Various lepidopteran pests, spittlebugs of sugarcane, locust and grasshopper pests
	<i>Beauveria bassiana</i>	Whitefly, aphids, thrips, psyllids, weevils, mealybugs, leathoppers, weevils, and leaf-feeding insects on Vegetables, Fruits and Berries, Spices, Ornamentals, Herbs and Orchard Crops.
	<i>Trichoderma harzianum</i>	Control of root diseases caused by <i>Pythium</i> , <i>Rhizoctonia</i> and <i>Fusarium</i> in field and greenhouse crops
Soil recharge	<i>Metarhizium anisopliae</i> , - 2-3% <i>Trichoderma harzianum</i> , - 2-3% <i>Beauveria bassiana</i> – 2-3% <i>Trichoderma viride</i> , - 0.5-1.0%	Weevils, aphids, thrips, whitefly, scarabs, mites, gnats and fruit fly. Vegetables, brassicas, soft fruits, and ornamental plants on field and protect crops.
Pheromone		
Fruit fly (<i>Bactrocera cucurbitae</i>)	Cuelure (4-[4-acetyloxyphenyl]-2-butanone)	
Fruit fly (<i>Bactrocera dorsalis</i>)	Methyl Eugenol (1,2-dimethoxy-4-(2-propenyl) benzene)	

Type of Biopesticides	Active ingredients	Use
Brinjal Shoot & Fruit Borer, <i>Leucinodes orbonalis</i>	(E)11-hexadecenyl acetate (E11-16:Ac) and (E)11-hexadecenyl-1-ol (E11-16:OH) in the ratio 100:1	
Tomato leaf miner, <i>Tuta absoluta</i>	0.8 mg of (3E, 8Z, 11Z)-3,8-tetradecantrien-1-y-1 acetate	
Pod borer, <i>Helicoverpa armigera</i> :	(Z)-9-Hexadecenal, Z-11-Hexadecenal	
Common cut worm, <i>Spodoptera litura</i>	Z9,E11–Tetradecadienyl acetate,Z9, E12–Tetradecadienyl acetate	
Legume pod borer, <i>Maruca vitrata</i>	(E,E)-10,12-hexadecadienal, (E,E)-10,12-hexadecadienol,and (E)-10-hexadecenal in a 100:5:5 ratio	
Attract & Kill for Cucurbit crops (<i>Bactrocera cucurbitae</i>)	Cuelure 30%, abamectin 0.5%.	
Attract & Kill for fruit crops (<i>Bactrocera dorsalis</i>)	Methyl Eugenol 30%, abamectin 0.5%.	
Biochemical	Azadirachtin	Aphid, Jassid & Whitefly
	Spinosad	Borer pest
	Abamectin	Aphid, Red spider mite, Jassid & Whitefly
	Potasium Salt of Fatty Acids	Soft bodied insect
	Lantadenes & Vasine	Red rust

Quality Control

Biopesticides are often criticized for their variable performance and lack of reliability. Quality control (QC) is therefore of paramount importance in order to ensure that products are delivered that comply with pre-determined specifications and deliver the efficacy within the prescribed conditions for use. Quality control does not only refer to the final end-use product, but also to the production and the production processes.

Quality control can be divided in production control, process control, and product control. The first two refer to internal quality control of the production of a microbial pest control product, and ensure a stable production process with a minimum of failures. Product control refers to the quality of the final product that leaves the factory and which needs to perform according to registration and customer satisfaction requirements. Products must meet product specifications, which are set by the manufacturer, until the end of the claimed shelf-life. Registration requirements in terms of quality control are reviewed. Complete quality control procedures and data for validation must be established, although there are no officially recognized criteria. Practical challenges in quality control procedures are reviewed per type of pathogen. Natural variation makes efficacy testing via bio-assays difficult, and setting an internal standard is required. Recommendations for standardization and criteria will be provided. Research needs are identified that may facilitate quality control in the future. Quality control must ensure that end-users receive high quality products. Total quality control covers all aspects of quality, including the field use of a biopesticides.

Production Quality Control and Process Quality Control: Quality control of production concerns internal procedures for checking whether inputs (purchased raw materials: medium ingredients, formulation compounds and packaging materials) comply with their specifications, whether standard operating procedures (SOPs) are well set-up and followed, and whether production and downstream equipment is maintained properly and is operating as expected.

Product Quality Control of Microbial Pest Control Products: Quality control generally refers to product control, i.e. to the final formulated product. In manufacturing, however, there may be several in-between products, like the "technical grade active ingredient" (TGAI), that need to be checked. Material from succeeding production batches may be stored to be formulated together. Checks on propagule numbers, microbial contaminants are needed and some batches may be discarded or handled differently, stored longer or shorter. During formulation, propagule numbers may be set to the specification of the final product, thus propagule counts are needed before final formulation. The final formulated product is subject to quality control and the product needs to conform to previously determined product specifications. These specifications are usually determined with regard to product performance in the field in a broad sense and to registration requirements. Product performance relates to efficacy, obviously, but also to applications characteristics (particle size, emulsion separation, sedimentation in the package) and to shelf-life parameters (moisture content, microbial purity).

Identity of the Microbial Pest Control Agent: The organism needs to be properly identified at strain level using the most appropriate scientific techniques. Molecular

techniques are needed to do this. The area of taxonomic identification is still developing and species and strain concepts are changing with the development of new technologies. Identification is a difficult topic, but very important with regard to a proper risk assessment.

Number of Infective Propagules: A product contains a certain number of infective or virulent propagules. This needs to be specified on the label. This number of infective propagules, when applied according to the recommended methods of application and under the right conditions of use, should achieve the claimed level of control of the pest. Counting of propagules can be done, but is often difficult in technical grade products as well as in formulated products in which remnants of the production medium and/or carrier and particles of formulation ingredients may be difficult to distinguish from spores or virus particles.

Microbial Purity: The total number or percentage of microbial contaminants in a MPCP should be limited. These contaminants may negatively influence product quality in terms of shelf-life, efficacy and even physical characteristics. Furthermore, contaminants may pose a risk to the applicator and the consumer. Registration criteria therefore only accept low numbers of contaminants and require absence or near-absence of human pathogens.

Presence of Toxins: Presence of toxic metabolites is a concern of regulatory bodies and information needs to be presented to the authorities for each specific MPCA where this is considered relevant. The issue of toxins is not well regulated and a discussion between the industry and regulators is ongoing. Clear and appropriate requirements still need to be defined.

Physical, Chemical and Technical Characteristics: Registration requirements demand product stability with regard to physical and chemical stability, and information on technical properties. The formulation needs to be physically stable during the shelf-life period. This means no clump-forming in wettable powders should occur, nor should irreversible sedimentation of propagules in suspensions or separation of carriers in the case of emulsions take place. Chemical stability (like pH) also needs to be considered and checked according to registration requirements, but in reality this is of lesser importance. Each type of formulation has its particular characteristics that need to be checked.

Efficacy: Efficacy is the most significant parameter in quality control. The product's field performance is the most valuable aspect; not only for registration, but also for the company's revenues from the product. If batches of the product do not perform up to expectations, repeated sales will decline. Therefore, every batch should be tested prior to release. This can only be done quickly and in a cost-effective way by using bio-assays. These should reflect the product's performance in the field as much as possible. The relationship between bio-assay results and field results needs to be established from the outset.

Quality Control and Shelf-Life: A product has to comply with its set QC criteria up to the end of its shelf-life period. Usage of a product at the end of its shelf-life should give similar results as does usage of a fresh product. As a consequence, the length of the shelf-life period is determined by the QC criteria and product specifications which it still has to meet at the end of the shelf-life period. For example, if spore germination rate in a fungal

product has a minimum set criterion of 80% and after 6 months the germination rate drops under this level, the shelf-life period is determined at 6 months, even when other criteria are still above their minimum criteria.

Quality Control Parameters for End-Use Products: Product quality control needs to be carried out on the formulated product. It may also be done on bulk material just before packaging if this process is known not to influence quality. Testing should require as little time as possible so that no valuable storage time is lost. Parameters subject to testing at this point in time are:

- (1) number of infective propagules;
- (2) physical-chemical properties;
- (3) microbial contaminants;

Critical Aspects of Product Quality Control with the Various Types of Pathogens Product quality control is an integral part of manufacturing biopesticides to ensure sale of high quality products.

Entomopathogenic Bacteria: In bacterial insecticides, the essential parameters subject to quality control are the same as for fungal products: the number of active propagules, the biological activity, and the microbial purity. Most of the literature discussing QC in bacterial products refers to Bt products. The insecticidal activity of Bt is based on spores and toxins. There are many strains and types of toxins available, each with its specific biological activity. Pathogenicity of Bt's is often primarily due to S-endotoxins (crystallized proteins). Accordingly, spore counts do not correlate well with the biological activity, and hence a bio-assay is a prerequisite for quality product control.

Conclusion

Biopesticides have long been attracting global attention as a safer strategy than chemical pest control, with potentially less risk to humans and the environment. In Bangladesh context, it is not far away to popularize for the poor farmers. Governments are likely to continue imposing strict safety criteria on conventional chemical pesticides, and this will result in fewer products on the market. This will create a real opportunity for biopesticide companies to help fill the gap, although there will also be major challenges for biopesticide companies, most of which are small and medium enterprises with limited resources for R&D, product registration and promotion. To this end, co-operation between the public and private sectors is required to facilitate the development, manufacturing, and sale of this environmentally friendly alternative. In this context, discovery of new substances and research on formulation and delivery would boost commercialization and use of biopesticides. Additional research on integrating biological agents into common production systems is necessary. Maintaining low cost to farmers for a given product quality and availability is also important. Moreover, regulations that promote registration of low-risk compounds with provision of incentives could also facilitate commercialization and availability of biopesticides in the market.

Insect pests of vegetable crops and their integrated management

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Indiscriminate and excessive uses of toxic synthetic pesticides are common scenario in many areas to combat with destructive pests and diseases. However, in Bangladesh, the frequency and amount of pesticide applications per unit area is highest in the high valued crops especially vegetables and fruits. Among the various vegetables crops, profitable crops like brinjal, country bean, cabbage, cauliflower, cucurbits, summer tomatoes, okra, string beans, chilli etc. receive excessive amounts of pesticides as they suffer serious pest damage. On brinjal alone, commercial producers apply toxic chemical pesticides to produce marketable fruits at a cost of more than Tk. 50,000/00) per ha (40% of total production costs). According to Bangladesh Crop Protection Association (2006), pesticide use for growing vegetables was six times higher than the rice (1.12 kg/ha for vegetables, while it was only 0.20 kg/ha in rice). Due to development of resistance by different insect pests to different chemical pesticides, it was reported that for vegetables in general, an increasing trend was observed in use of pesticides by farmers in combating the pests throughout the country. Since majority of the farmers do not maintain any waiting period before crop harvest, the consumers become inevitably exposed to high levels of pesticide residues in their diets. The situation is compounded by the fact that, unlike cereals, vegetables or fruits are often consumed soon after harvest and there is little time for the chemicals to degrade. Different crops especially vegetables and fruits are harvested and marketed without knowing any residue status. This is most likely to cause serious health hazard to the consumers.

To get rid from this measurable pest management system, some new avenues or some alternatives of synthetic pesticides are very much needed. One of the ways is to develop and expansion of eco-friendly, sustainable, socio-economic acceptable integrated pest management or IPM packages. IPM is one of a number of integrated approaches that are gaining credence for use in sustainable agriculture development. It involves the integration by farmer of the most appropriate management strategies for pest control where sole dependency on pesticides can be avoided. Several IPM technologies have been developed by BARI scientists and some of them became very much popular in the farm community.

Brinjal

Brinjal (*Solanum melongena*), also called aubergine or eggplant, is one of the top ten vegetables in the world and the top vegetable crop of the South Asia. However, it's production is severely constrained by several insect, mite pests and diseases. The major pests include brinjal fruit and shoot borer, leafhopper, whitefly, thrips, aphid, red spider mite etc.

Brinjal fruit and shoot borer (BFSB), *Leucinodes orbonalis* Guenee (Lepidoptera: Pyralidae)

Distribution: South and Southeast Asia, Africa and Saudi Arabia

Hosts: Eggplant, Potato

Characteristics

The adult females lay eggs singly or in groups of two to five on the under surfaces of leaves (Plate 1), tender shoots, flower buds, or the base of developing fruits. Each female lays about 250 eggs, which are creamy white soon after laying, but turn red before hatching. The egg period is three to five days. The larva is creamy white to pink in color in the early stages. The grown-up larva is pink with sparse hairs on the warts on the body and a dark brown or blackish head. The full-grown larva measures about 16-23 mm in length. The larva usually has five instars, sometimes six. The larval period is about two weeks in summer and three weeks in winter. The larva pupates on the plant parts or plant debris on the soil surface, or rarely, under the soil. The pupation occurs in tough silken cocoons, and the pupa is dark brown in color. The pupa measures about 13 mm. The pupal period varies from one to two weeks. The moth is white or dirty white with pale brown or black spots on the dorsum of thorax and abdomen. Wings are white with a pink or blue tinge, and have pink or brown and red spots on the forewings. The female is bigger than male, with a bulged abdomen. The female moth tends to curl its abdomen upwards. The adult life span is about a week; the females live longer than males.



Adult



Eggs



Larvae



Pupae

Damage symptoms

BFSB is mostly monophagous, but sometimes also feeds on tomato, potato, *Solanum indicum*, *S. xanthocarpum*, *S. torvum*, and *S. nigrum*. Upon hatching, the larva starts boring near the growing point or into the flower buds or fruits. During the early vegetative phase of the crop growth, it feeds on the tender shoots. Soon after boring into the shoots and fruits, the larva seals the entry hole with excreta. The larva tunnels inside the shoot and feeds on the inner contents. It also fills the feeding tunnels with excreta.

This results in wilting of young shoots, followed by drying and drop-off, which slow plant growth. In addition, it produces new shoots, delaying crop maturity. During the early reproductive phase, the larva occasionally may feed on flower buds and flowers. However, it prefers to feed on the fruit rather than other plant parts during the fruiting stage of the crop. Damaged fruit exhibits boreholes on the surface, which often are sealed with excreta. The larva feeding inside the fruit creates tunnels filled with frass and fecal pellets. Hence, the fruit becomes unfit for marketing and consumption. Under heavy infestation, more than one larva will feed inside the same fruit.



Wilted shoot



Immature fruit infestation



Mature fruit infestation

Management strategy:

- Sanitation : Weekly removal and destruction of pest-damaged shoots and fruits that harbor brinjal shoot and fruit borer (BSFB) larvae from the field.
- Use of sex pheromone: Sex pheromone for BSFB (a combination of two chemicals) has been identified, synthesized, and is now commercially available to trap the male moths before they mate. BSFB pheromone lures (in a plastic tube) baited in a suitable trap, 'BARI trap'. Male moths are attracted to the trap, captured and killed. The pheromone lure hung through the center of the lid inside the trap in such a way that the lure is 2-3 cm above the water level of trap. The trap should be set just above the plant canopy. Baiting should be started from 4-5 weeks after transplanting and continued till last harvest. A distance of 10 m² should be maintained between the traps. The pheromone plastic tubes (lures) should be changed at every 45-60 days.
- Application of bio-pesticide Spinosad: During hot and humid period the population of BSFB increase very quickly. During that period Spinosad (Tracer 45 SC, @ 0.4 ml/liter of water) should be sprayed 3-4 times at 10-12 days interval.
- Artificial release of bio-control agents: To proliferate the use of different bio-control agents, weekly release of two parasitoids should be done. The parasitoids are i) egg parasitoid, *Trichogramma chilonis* (@ 1gm parasitized eggs/ha/week) and ii) larval parasitoid, *Bracon habetor* (@ 1 bunker /ha/week).
- Community approach: Community approach of the IPM package should be followed.
- Cultivation of Bt brinjal: Four varieties of Bt brinjal, resistant to brinjal shoot and fruit borer has been released in Bangladesh. They can be cultivated especially during winter season.

Leafhopper, *Amrasca bigutata bigutata* Ishida (= *Amrasca devastans* Distant), (Hemiptera: Cicadellidae)**Distribution:** South and Southeast Asia**Hosts:** Eggplant, okra, cotton, potato**Characteristics**

The adult females lay eggs along the midrib and lateral veins of the leaves. The egg period is 4 to 11 days. The nymphs resemble the adults, but lack wings. Instead, they have slightly extended wing pads. They are pale green in color. They tend to move sideways when disturbed. The nymphal period varies from one to four weeks depending

on the temperature. The adults are wedge-shaped, pale green insects. They have fully developed wings with a prominent black spot on each forewing. The adults may live for one to two months.

Damage symptoms

Both nymphs and adults suck the sap from the lower leaf surfaces through their piercing and sucking mouthparts. While sucking the plant sap, they also inject toxic saliva into the plant tissues, which leads to yellowing. When several insects suck the sap from the same leaf, yellow spots appear on the leaves, followed by crinkling, curling, bronzing, and drying, or “hopper burn”. Leafhoppers also cause similar damage in okra, cotton, and potato.



Leafhopper Nymphs



Leafhopper Adults



Hopper burn caused by leafhopper

Management strategies:

- Application of neem seed kernel extract (500 gm crushed neem seed kernel should be soaked in 10 liters of water. The filtered water then ready for application) or neem oil (@5 ml/ liter of water + 5 gm detergent or soap powder).
- Application of commercial formulation of Azadirachtrin (neem based formulation) at the stipulated dose at the lower surface of the leaves.
- Spraying (2-3 times) of soap powder solution (@ 5 gm per liter of water) at the lower surface of the leaves.

Whitefly, *Bemisia tabaci* Gennadius, (Hemiptera: Aleyrodidae)

Distribution: Tropical and sub-tropical areas of the world

Hosts: These whiteflies have a wide range of over 500 herbaceous annuals including cotton, okra, tomato, brinjal, peppers, beans, cassava, cucurbits, potato, sweet potato, crucifers etc.

Characteristics

The females mostly lay eggs near the veins on the underside of leaves. They prefer hairy leaf surfaces to lay more eggs. Each female can produce as many as 300 eggs in its lifetime. Eggs are small (about 0.25 mm), pear-shaped, and vertically attached to the leaf surface through a pedicel. Newly laid eggs are white and later turn brown. The eggs are not visible to the naked eye, and must be observed under a magnifying lens or microscope. Egg period is about three to five days during summer and 5 to 33 days in winter. Upon hatching, the first instar larva (nymph) moves on the leaf surface to locate a suitable feeding site. Hence, it is commonly known as a “crawler.” It then inserts its piercing and sucking mouthpart and begins sucking the plant sap from the phloem. The first instar nymph has antennae, eyes, and three pairs of well-developed legs. The nymphs are flattened, oval-shaped, and greenish-yellow in color. The legs and antennae are atrophied during the next three instars and they are immobile during the remaining nymphal stages. The last nymphal stage has red eyes. This stage is sometimes referred to puparium, although insects of this order (Hemiptera) do not have a perfect pupal stage (incomplete metamorphosis). Nymphal period is about 9 to 14 days during summer and 17 to 73 days in winter. Adults emerge from puparia through a T-shaped slit, leaving behind empty pupal cases or exuviae. The whitefly adult is a soft-bodied, moth-like fly. The wings are covered with powdery wax and the body is light yellow in color. The wings are held over the body like a tent. The adult males are slightly smaller in size than the females. Adults live from one to three weeks.



All stages

Adult whitefly

Damage symptoms

B. tabaci is highly polyphagous and is known to feed on several vegetables such as tomato, eggplant, okra, etc. Both the adults and nymphs suck the plant sap and reduce the vigor of the plant. In severe infestations, the leaves turn yellow and drop off. When the populations are high they secrete large quantities of honeydew, which favors the growth of sooty mould) on leaf surfaces and reduces the photosynthetic efficiency of the plants.



Crowding of *B. tabaci*



Sooty mould on *B. tabaci* infested leaves

Management Strategies

- Unnecessary use of insecticides should be avoided.
- Sticky yellow traps can be used for population level evaluation and control.
- Application of neem seed kernel extract (500 gm crushed neem seed kernel should be soaked in 10 liters of water. The filtered water then ready for application) or neem oil (@5 ml/ liter of water + 5 gm detergent or soap powder).
- Application of commercial formulation of Azadirachtrin (neem based formulation) at the stipulated dose at the lower surface of the leaves.
- Spraying (2-3 times) of soap powder solution (@ 5 gm per liter of water) at the lower surface of the leaves.

Red spider mite, *Tetranychus urticae* Koch, (Aranae: Tetranychidae)

Distribution: Cosmopolitan

Hosts: Polyphagous

Characteristics

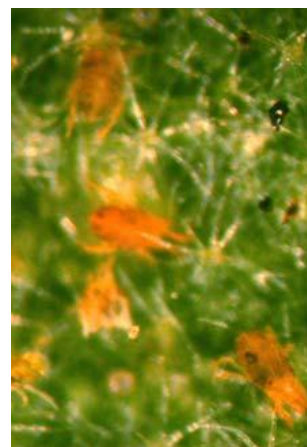
T. urticae is commonly known as red spider mite or two-spotted spider mite. They are minute in size, and vary in color (green, greenish yellow, brown, or orange red) with two dark spots on the body. Eggs are round, white, or cream-colored; egg period is two to four days. Upon hatching, it will pass through a larval stage and two nymphal stages (protonymph and deutonymph) before becoming adult. The lifecycle is completed in one to two weeks. There are several overlapping generations in a year. The adult will live up to three or four weeks.

Damage symptoms

Spider mites usually extract the cell contents from the leaves using their long, needle-like mouthparts. This results in reduced chlorophyll content in the leaves, leading to the formation of white or yellow speckles on the leaves. In severe infestations, leaves will completely desiccate and drop off. The mites also produce webbing on the leaf surfaces in severe conditions. Under high population densities, the mites move to the tip of the leaf or top of the plant and congregate using strands of silk to form a ball-like mass, which will be blown by winds to new leaves or plants, in a process known as “ballooning.”



White and yellow speckles caused by spider mites



Adult spider mite

Management Strategies

- Regular syringing (water) can keep spider mites under control. This technique also helps conserve natural predators.
- Foliar spray of Neem oil 5 ml with Trix 2 ml /L at 10 days intervals.
- Alternate application of miticides with different mode of action should be sprayed, such as Bio-miticide, Abamectin @1.5 ml/ L of water (Vertimec 18EC, Ecomac 1.8EC) along with chemical miticide, such as Propargite @ 2ml/L of water (Omite 57EC), Dicofol @ 1.2ml/L of water (Dicofol 18.5EC) and Sulphur fungicide @ 1.2ml/L of water (Kumulus DF) at 10 days interval.
- Indiscriminate use of pyrethroid insecticide should be avoided, because the population of natural enemy is reduced.

Cucurbit Crops

The family Cucurbitaceae, also known as the gourd family, includes many crops that are predominately grown in the sub-tropics and tropics, such as pumpkin, bitter melon, ribbed melon, snake melon, watermelon, cucumber, squash etc. Cucurbits crops are often attacked by different insect pests but cucurbit fruit fly, epilachna beetle, whitefly, fruit borer like *Spodoptera sp.* or pumpkin caterpillar are considered as the major pest.

Melon fly, *Bactrocera cucurbitae* (Coquillett), (Diptera: Tephritidae)

Distribution: Asia and Africa

Hosts: Cucurbits, tomato, capsicum, beans, passion fruits, eggplant (over 125 hosts have been recorded).

Characteristics

Adults require protein for sexual maturity and in order to develop eggs. Mating is necessary for the production of fertile eggs. Each female is capable of laying over 1,000 eggs. The female inserts her ovipositor directly into the host fruit or vegetable and lays

her eggs. There are three larval instars and a prepupal stage. Pupation takes place in the soil beneath the host plant.



Egg



Larvae



Pupae



Adult

Nature of damage

The melon fly is the most important fruit fly pest of vegetable crops, especially cucurbits. Damaged fruit becomes deformed and later turns yellowish with rotten flesh as a result of the feeding activities of the maggots. In addition to causing direct damage to fruit, its occurrence in a country leads to quarantine restrictions.



Bitter gourd



Pumpkin



Cucumber



Ridge gourd



Teasel gourd



Sponge gourd



Sweet gourd



Water melon

Infestation on different cucurbit crops

Management Strategies

- Sanitation: Collection and destruction of infested fruits along with larvae.
- Pheromone bait trap: The sex pheromone, 'cuelure', which mimics the scent of female flies, attracts the male flies and traps them in large numbers resulting in mating disruption. Simple plastic containers known as 'BARI trap' or 'Magic trap' were used for deployment of the pheromones. The rectangular plastic container had around 3-liter capacity and 20-22 cm tall. A triangular hole measuring 10-12 cm height and 10-12 cm base was cut in any two opposite sides. The base of the hole would be 3 cm above the bottom. Water containing two-three drops of detergent would be maintained inside the trap throughout the season. The pheromone soaked cotton was tied inside the trap with thin wire. Fruit fly adults enter the trap and fall

into the water and die. Water inside the trap was replenished often to make sure the trap was not dry. The pheromone dispensers were continued throughout the cropping season. The pheromone bait traps should be in the cucurbit field at a distance of 12-15m² starting from first flower initiation and be continued till last harvest.

- Community approach: Community approach of the IPM package was practiced.
- Attract and kill method: Setting of cuelure pheromone lures at the border plant rows at 10 m distance and female attractant in the inner rows at 15 m distance within one month of plant age. Both male and female attractant was used to trap and kill fruit flies in the cucurbit crops.

Pumpkin beetle, *Aulacophora* spp. (*Aulacophora similis*), (Coleoptera: Chrysomelidae)

Distribution: Cosmopolitan.

Host: All cucurbits and also reported from Leguminosae and Malvaceae.

Characteristics

Adults are known to live 100-200 days. Eggs are yellow and laid singly or in masses of two to five in the soil near the base of the plant. The incubation period ranges from 8-15 days. Newly hatched grubs are pale yellow and turn orange-yellow when full-grown. Grubs bore into roots, and the larval stage lasts for 18-35 days. Pupation takes place in the soil within a cell. Pupae are elongate and pale yellow, and the pupal stage ranges from 4014 days. The duration of life cycles varies among different species and also depends on host plants and the environment.



Adult



Larvae



Pupa

Nature of damage

Adults cause serious damage by feeding on leaves and flowers. Seedlings may be heavily attacked to the point that they are totally destroyed. Grubs bore into the roots, causing them to swell, be discolored, misshapen, and in some cases, plant growth is retarded or plant are killed.



Damage on leaf



Damage on roots



Grubs in the soil

Management Strategies

- Cover with mosquito net from emergence to 35 days age of the seedling.
- Collection and destruction of adult beetles by sweep net.
- Seed treatment with seed treating chemicals before sowing.
- Application of Carbofuran 5G (5 gm/pit) in the pit during seed sowing/transplanting.
- Foliar spray of Neem oil 5 ml with Trix 2 ml /L or commercial neem formulation at 10 days intervals for 2-3 times.

Spotted beetles, *Epilachna dodecastigma* (Wiedemann) and *E. vigintioctopunctata* Fabricius, (Coleoptera: Coccinellidae)

Distribution: South and Southeast Asia

Hosts: Eggplant, tomato, potato

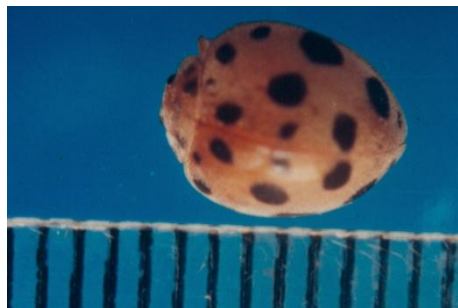
Characteristics

The females lay eggs mostly on the lower leaf surfaces. Each female lays about 100-400 eggs. The egg is spindle-shaped and yellowish in color. Eggs are laid in clusters of 10-40. The egg period varies from two to five days. The grub is creamy white or yellowish in color with black spiny hairs on the body. The grub period is two to five weeks depending on the temperature. Grubs pupate on the leaves and stem. The pupa resembles the grub but is mostly darker in color, although it sometimes is yellowish in color. The pupa bears spiny hairs on the posterior, but not the anterior, part of the body. The pupal period is one to two weeks. The subfamily Epilachninae contains plant-feeding ladybird beetles because most other ladybird beetles are predators, not plant pests. These brownish or orange-colored, hemispherical beetles are larger than other ladybird species. *E. vigintioctopunctata* (in Latin, *viginti* means 20 and *octo* means 8) has 28 black spots on the forewing (elytra). *E. dodecastigma* (*dodeca* means 12 in Greek) has 12 black spots on the elytra. However, beetles with 14, 16, 18, 20, 22, 24 or 26 spots have been observed

under field conditions, due to mating between females of *E. dodecastigma* and males of *E. vigintioctopunctata*.



Adult of *E. dodecastigma*



Adult of *E. vigintioctopunctata*



Eggs



Neonate larvae



Mature grub

Damage symptoms

Epilachna beetles are polyphagous, and feed on cucurbits, tomato, potato, and kidney bean as well as eggplant. The grub and adult have chewing mouthparts. Hence, they scrape the chlorophyll from the epidermal layers of the leaves. The feeding results in a typical ladder-like window. The windows will dry and drop off, leaving holes in the leaves. In severe infestations, several windows coalesce together and lead to skeletonization—the formation of a papery structure on the leaf.



Ladder-like windows caused by *Epilachna* beetle feeding

Management Strategies

- Collection and destruction of larvae and adult.
- Foliar spray of Neem oil 5 ml with Trix 2 ml /L or commercial neem formulation at 10 days intervals for 2-3 times.
- At severe infestation contact insecticide may be sprayed.

Cabbage & Cauliflower

Leaf eating lepidopterous pests like cabbage worm and diamond back moths are the main constraints for cabbage production. However, several integrated pest management packages to control those pests have been developed.

Diamondback moth, *Plutella xylostella* (L.), (Lepidoptera, Plutellidae)

Distribution: Cosmopolitan

Hosts: Crucifers

Characteristics

Eggs are oval, flat, and laid singly or in groups up to eight, mostly on the upper side of the leaves. The incubation period is 4-8 days. Upon hatching, the first instar larvae crawls to the lower surface of the leaf, bores into the epidermis, and mines into the tissue. Subsequent instars feed on the lower surface of the leaf, leaving the upper epidermal layer intact, and resulting in a characteristics window effect. Larvae are green, and the larval duration ranges from 9 to 30 days. Pupation takes place inside a finely woven silken cocoon, and the pupal duration ranges from 5-15 days.



Larva



Pupa



Adult

Nature of damage

Fourth instar larvae cause most of the feeding damage. Unless proper control measures taken, they are capable of total destruction of the crop.



Initial infestation



Severe infestation

Management Strategies

- Clean cultivation, burning of stables after crop harvest.
- Collection and destruction of the larvae by hand picking.
- Conservation of different predators and parasitoids in the field by limiting the chemical pesticide use.
- Application of microbial pesticide *Bacillus thuringiensis* (Bt strain EG 7841) from the initial infestation.

Common Cutworm, *Spodoptera litura*, (Lepidoptera: Noctuidae)

Distribution: South and Southeast Asia and South Pacific islands.

Hosts: Polyphagous pest of crops belonging to the families Solanaceae, Malvaceae, Cruciferae, Fabaceae, Musaceae and others.

Characteristics

Adult of *S. litura* is stout-bodied moth with a wing span of about 40 mm. The adults are usually brown in color. Eggs are laid in groups on leaves and are covered with hair scales for protection. Each egg cluster contains 100-300 eggs which hatch in 3-6 days. Larvae feed together as a cluster after hatching and disperses as they mature. The neonate larvae are translucent green with dark thorax, while the grown-up larvae are green, pale greenish brown, or black in color, with stout cylindrical bodies with prominent black spiracles. There are six larval instars and the larval period is about 15-30 days. Pupation takes place in soil. Pupae are shiny reddish-brown. The pupal period varies from one to three weeks.



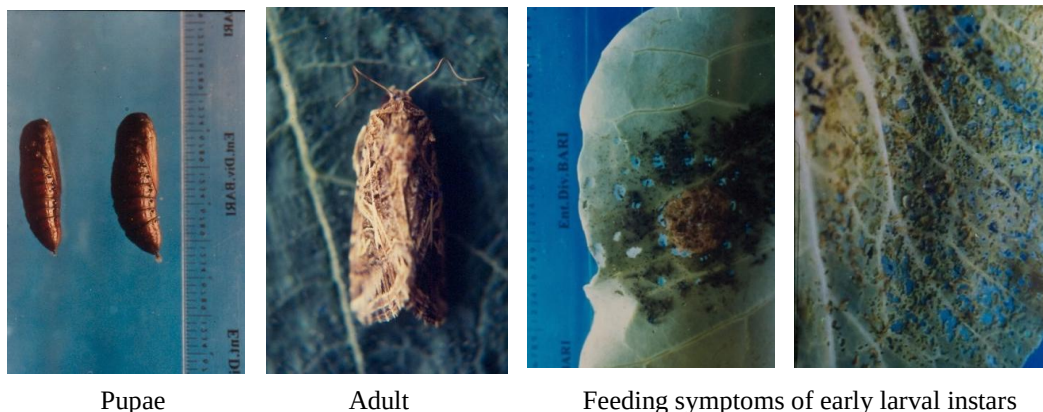
Egg deposition



Hatching of neonate larvae



Mature larvae



Pupae

Adult

Feeding symptoms of early larval instars

Nature of damage

The most conspicuous damage is caused by early larval instars as hundreds of caterpillars feed in clusters and quickly skeletonize the leaves (windowing). Feeding by older larvae causes numerous round holes between the main veins of the leaves. At high density, the larvae also feed on the flowers and pods.

Management Strategies

- Mechanical control: Hand picking and destruction of *Spodoptera litura* or DBM egg/larvae during initial stage should be done in the cabbage and cauliflower fields.
- Use of pheromone bait trap and application of SNPV & Bt: Sex pheromone for *Spodoptera litura* is now commercially available to trap the male moths before they mate. *Spodoptera litura* pheromone lures (in a plastic tube) baited in a suitable trap, 'BARI trap'. Male moths are attracted to the trap, captured and killed. The pheromone lure hung through the center of the lid inside the trap in such a way that the lure is 2-3 cm above the water level of trap. Trapping should be started from 2-3 weeks after transplanting and continued till last harvest. A distance of 30 m² should be maintained between the traps. If infestation starts even after pheromone trapping then 2-3 application of SNPV (@ 0.2 gm/liter of water) and Bt (@ 0.4 gm/liter of water) should be done.
- Artificial release of bio-control agents: To proliferate the use of different bio-control agents, weekly release of two parasitoids should be done in the field. The parasitoids are i) egg parasitoid, *Tricogramma chilonis* (@ 1gm parasitized eggs/ha/week) and ii) larval parasitoid, *Bracon habetor* (@ 1 bunker /ha/week). Those bio-control agents would manage the fruit borer problem.

Tomato

The key constraints to tomato production relate to tomato leaf curl virus, particularly in summer production when total crop loss is possible because of the efficiency of the vector, *Besimia tabaci*, transmission and susceptibility of currently available varieties. Other key constraints of tomato production is the attack of fruit borer, *Spodoptera litura* & *Helicoverpa armigera*.

Tomato fruit worm, *Helicoverpa armigera* Hubner, (Lepidoptera: Noctuidae)

Distribution: Europe, Africa, Australia, Asia

Hosts: Tomato, cotton, corn, peppers, brinjal, melons, crucifers, beans, others.

Characteristics

The tomato fruit worm is a polyphagous and highly mobile insect and pest of economic importance on many agricultural and horticultural crops. The adult is a stout-bodied moth with a wingspan of about 35-40 mm. The adult males are usually pale yellow with olive greens or grey color while the adult females are reddish-brown. The adults feed on nectar and lays singly and scattered, usually on or near the leaflets, floral buds, or young fruit. On an average a single female can lay about 730 to 1702 eggs in an oviposition period of 10-23 days. The eggs are spherical shaped, about 0.5 mm in diameter, creamy white when laid but change through greenish yellow to dark brown to black before hatching. Egg hatch in about 4-5 days and young larvae feed on tender leaves, flowers and terminal shoots. Larvae pass through six instars and the larval period ranges from 17-25 days. Mature larvae vary in color and generally prefer to feed by boring directly into the fruits. Often only the front portion of the body is inside the hole. Larvae are cannibalistic and pupate in soil. The pupal stage lasts for 13-14 days.



Larva on fruits



Larva

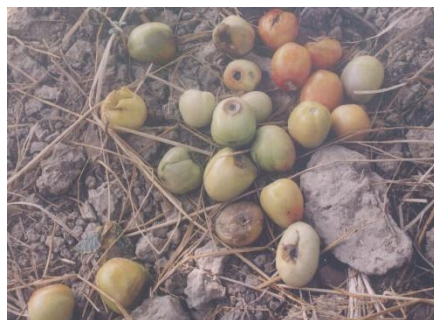


Pupae



Adult

Nature of damage



The neonate larvae feed on the surfaces of leaves or floral buds. However, the grown-up larvae prefer to feed on the contents of reproductive parts such as floral buds, flowers, and young fruits. The larvae make holes in these parts and feed by thrusting their heads inside. Later, the larva feeds on most of the inner contents of the fruits, hollowing it out. Severely damaged fruit rots and fall down; partially damaged fruits may become deformed.

Management Strategies

- Setting up of sex pheromone traps @ 20 traps/ha before the initiation of flowering and collection and destruction of moths caught in the traps.
- Sequential release of two parasitoids should be done in the field. The parasitoids are i) egg parasitoid, *Tricogramma chilonis* (@ 1gm parasitized

eggs/ha/week) and ii) larval parasitoid, *Bracon habetor* (@ 1 bunker /ha/week). Those bio-control agents would manage the fruit borer problem.

- Spray application of Spinosad (Tracer 45SC) @ 0.4ml/l of water combination with *Bacillus thuringiensis* kurstaki three times at fortnightly interval commencing from flowering affords protection.
- Spray application of HaNPV at dusk @ 0.1g/l of water.

Common cutworm or cluster caterpillar, *Spodoptera litura* (F.), Lepidoptera: Noctuidae

Distribution: South and Southeast Asia and South Pacific islands.

Hosts: Polyphagous pest of crops belonging to the families Solanaceae, Malvaceae, Cruciferae, Fabaceae, Musaceae and others.

Characteristics

Adult of *S. litura* is stout-bodied moth with a wing span of about 40 mm. The adults are usually brown in color. Eggs are laid in groups on leaves and are covered with hair scales for protection. Each egg cluster contains 100-300 eggs which hatch in 3-6 days. Larvae feed together as a cluster after hatching and disperses as they mature. The neonate larvae are translucent green with dark thorax, while the grown-up larvae are green, pale greenish brown, or black in color, with stout cylindrical bodies with prominent black spiracles. There are six larval instars and the larval period is about 15-30 days. Pupation takes place in soil. Pupae are shiny reddish-brown. The pupal period varies from one to three weeks.

Nature of damage

The most conspicuous damage is caused by early larval instars as hundreds of caterpillars feed in clusters and quickly skeletonize the leaves. The larvae also feed on the fruit, boring single or multiple holes in the flesh.



Egg deposition



Hatching of neonate larvae



Mature larvae



S. litura larva infesting tomato fruits

Management Strategies

Given in cabbage pest management

Whitefly, *Bemisia tabaci* Gennadius, (Hemiptera: Aleyrodidae)

Distribution: Tropical and sub-tropical areas of the world

Hosts: These whiteflies have a wide range of over 500 herbaceous annuals including cotton, okra, tomato, eggplant, peppers, beans, cassava, cucurbits, potato, sweet potato, crucifers etc.

Characteristics

The females mostly lay eggs near the veins on the underside of leaves. They prefer hairy leaf surfaces to lay more eggs. Each female can produce as many as 300 eggs in its lifetime. Eggs are small (about 0.25 mm), pear-shaped, and vertically attached to the leaf surface through a pedicel. Newly laid eggs are white and later turn brown. The eggs are not visible to the naked eye, and must be observed under a magnifying lens or microscope. Egg period is about three to five days during summer and 5 to 33 days in winter. Upon hatching, the first instar larva (nymph) moves on the leaf surface to locate a suitable feeding site. Hence, it is commonly known as a “crawler.” It then inserts its piercing and sucking mouthpart and begins sucking the plant sap from the phloem. The first instar nymph has antennae, eyes, and three pairs of well-developed legs. The nymphs are flattened, oval-shaped, and greenish-yellow in color. The legs and antennae are atrophied during the next three instars and they are immobile during the remaining

nymphal stages. The last nymphal stage has red eyes. This stage is sometimes referred to puparium, although insects of this order (Hemiptera) do not have a perfect pupal stage (incomplete metamorphosis). Nymphal period is about 9 to 14 days during summer and 17 to 73 days in winter. Adults emerge from puparia through a T-shaped slit, leaving behind empty pupal cases or exuviae. The whitefly adult is a soft-bodied, moth-like fly. The wings are covered with powdery wax and the body is light yellow in color. The wings are held over the body like a tent. The adult males are slightly smaller in size than the females. Adults live from one to three weeks.



All stages

Adult whitefly

Damage symptoms

B. tabaci is highly polyphagous and is known to feed on several vegetables such as tomato, eggplant, okra, etc. Both the adults and nymphs suck the plant sap and reduce the vigor of the plant. In severe infestations, the leaves turn yellow and drop off. When the populations are high they secrete large quantities of honeydew, which favors the growth of sooty mould on leaf surfaces and reduces the photosynthetic efficiency of the plants.



Crowding of *B. tabaci*



Sooty mould on *B. tabaci* infested leaves

Management Strategies

- Use of virus resistant germplasm: Resistant/tolerant line against whitefly and leaf curl virus (containing the Ty-2 resistance gene) were developed. Those line (TLB 182) and BARI released variety BARI Tomato – 16 were promoted among the farmers to resist whitefly and whitefly transmitted leaf curl virus disease.

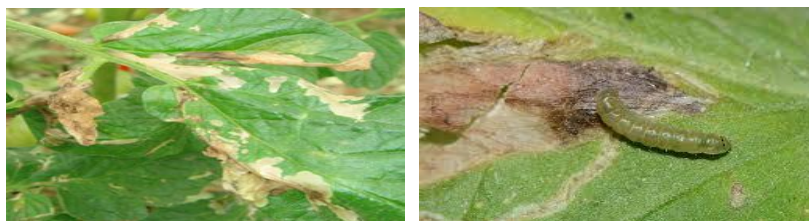
- Use of pheromone bait trap and application of SNPV & HNPV: Sex pheromone for *Spodoptera litura* is now commercially available to trap the male moths before they mate. *Spodoptera litura* pheromone lures (in a plastic tube) baited in a suitable trap, 'BARI trap'. Male moths are attracted to the trap, captured and killed. The pheromone lure hung through the center of the lid inside the trap in such a way that the lure is 2-3 cm above the water level of trap. Baiting should be started from 2-3 weeks after transplanting and continued till last harvest. A distance of 30 m² should be maintained between the traps. If infestation starts even after pheromone trapping then 2-3 application of SNPV (@ 0.2 gm/liter of water) and HNPV (@ 0.1 gm/liter of water) should be done.
- Artificial release of bio-control agents: To proliferate the use of different bio-control agents, weekly release of two parasitoids were done in the trial areas. The parasitoids are i) egg parasitoid, *Tricogramma chilonis* (@ 1gm parasitized eggs/ha/week) and ii) larval parasitoid, *Bracon habetor* (@ 1 bunker /ha/week). Those bio-control agents would manage the fruit borer problem.

Tomato leaf miner, *Tuta absoluta*, (Lepidoptera: Gelechiidae)

The larva feeds voraciously upon tomato plants, producing large galleries in leaves, burrowing in stalks, and consuming apical buds and green and ripe fruits. It is capable of causing a yield loss of 100%. Adult moths are grey-brown, approximately 6 mm in size and have a wing-span of 10 mm. Males are somewhat darker than females. Newly hatched caterpillars are small (0.5 mm) in size and yellowish. When maturing, caterpillars turn yellow-green and a black band develops behind the head. Fully-grown caterpillars measure approximately 9 mm with a pinkish color on their back. Pupae are light brown and approximately 6 mm. In favorable weather conditions eight to ten generations can occur in a single year.

Host range: Tomato is the main host plant, but *T. absoluta* also attacks other crop plants, viz. potato, eggplant, pepino, pepper and tobacco, many solanaceous weeds, including *Datura stramonium*, *Lycium chilense* and *Solanum nigrum*.

Distribution: This moth was first known as a tomato pest in many South American countries, then it invades Europe, Africa, Middle East, South Asia and forwarding towards Southeast Asia.



Tuta absoluta infestation on tomato leaf



Tuta absoluta infestation on tomato fruit



Alternate hosts of *T. absoluta*

Okra

Okra (*Abelmoschus esculentus*) is of Ethiopian origin, but it is widely cultivated throughout the tropical and subtropical parts of the world. It grown mostly for its semi-mature pod. In some parts of the world, it leaves and dried pods are used in cooking. It is one of the more nutritious vegetable grown in the tropics. Major pests and diseases of okra are the whitefly and its biotypes, cotton stainer (red cotton bug) and leafhoppers, while yellow vein mosaic virus, fusarium wilt, white mold fungus are the major diseases of okra.

Okra fruit and shoot borer (OFSB), *Earias vittella* (F.), (Lepidoptera: Noctuidae)

Distribution: Asia, Australia, Pacific Islands, and Africa

Hosts: Okra and other members of the family Malvaceae

Characteristics

Eggs are laid singly on shoots, flower buds, and young fruits. Eggs hatch in about 3 days, and the larvae are dirty brown with white spots. Larval duration is about 20 days, and pupation occurs in a lathery brown cocoon attached to the shoots, fruits, or leaves. The moth emerges from the pupal case in about 10 days.



Larva

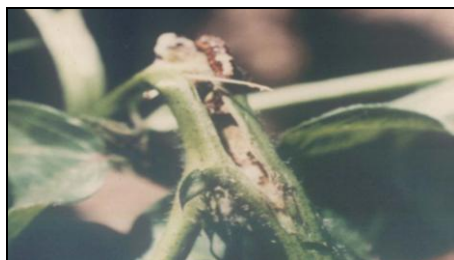


Adult



Nature of damage

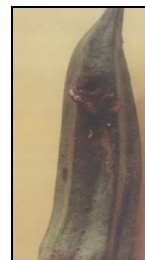
This borer is one of the most serious pests of okra, as a single larval bore hole renders the fruit unmarketable. Also larval boring into stems kills shoot tops and retards plant growth. The larva feeds and completes its larval period inside the stems and pods. Damaged plant part can be easily detected through the fresh frass and holes on the pods. Damaged plant tissue serve as entry sites for disease-causing microorganisms such as fungi. Early damage on pods also results in pod deformation.



Shoot infestation with OFSB



Fruit infestation with OFSB



Management Strategies

- Setting up of sex pheromone traps @ 40 traps/ha before the initiation of flowering and collection and destruction of moths caught in the traps.
- Sequential release of release of two parasitoids should be done in the field. The parasitoids are i) egg parasitoid, *Tricogramma chilonis* (@ 1gm parasitized eggs/ha/week) and ii) larval parasitoid, *Bracon habetor* (@ 1 bunker /ha/week). Those bio-control agents would manage the fruit borer problem.
- Spray application of Spinosad (Tracer 45SC) @ 0.4ml/l of water combination with *Bacillus thuringiensis* kurstaki three times at fortnightly interval commencing from flowering affords protection.

Chille pepper

Prodenia caterpillar, *Spodoptera litura* and fruit borer, *Helicoverpa armigera* are two devastating pest of chilli and key constraints to its production. However, the following management approaches can effectively control those insect pests:

Common armyworm or cluster caterpillar, *Spodoptera litura* (F.), (Lepidoptera: Noctuidae)

Adult of *S. litura* is stout-bodied moth with a wing span of about 40 mm. The adults are usually brown in color. Eggs are laid in groups on leaves and are covered with hair scales for protection. Each egg cluster contains 100-300 eggs which hatch in 3-6 days. Larvae feed together as a cluster after hatching and Chili fruit infestation by *S. litura* disperses as they mature. Pupation takes place in soil. Pupae are shiny reddish-brown. The most conspicuous damage is cause by early larval instars as hundreds of caterpillars feed in clusters and quickly skeletonize the leaves.



S. litura infesting chilli fruits

Fruitworm, *Helicoverpa arimigera* (Hubner), (Lepidoptera: Noctuidae)

Distribution: Europe, Africa, Australia, Asia

Hosts: Tomato, cotton, corn, peppers, eggplant, melons, crucifers, beans, others

Characteristics

Cosmopolitan pest. The young, small caterpillars have prominent rows of dark bumps (tubercles) on their backs. The older, larger ones vary in color from dark gray to light brown and have lengthwise stripes on their bodies. Adult moth are usually active at night. In warm weather, several generations may occur. Caterpillars often move from one fruit to the next destroying only small portions of each fruit. Pupation occurs in soil near the base of the plant. These insects are pests of other solanaceous family, viz. tomato. They are also commonly feed on maize.

Damage symptoms

A small darkened partially healed hole at the base of the fruit pedicle is evident. The inside of the fruit has a cavity that contains frass and decay. Damaged fruits ripen early, but those are not usually marketable.



Fruits infested by fruitworm

Management Strategies

- Use of pheromone bait trap and application of SNPV & HNPV: Sex pheromone for *Spodoptera litura* is now commercially available to trap the male moths before they mate. *Spodoptera litura* pheromone lures (in a plastic tube) baited in a suitable trap, 'BARI trap'. Male moths are attracted to the trap, captured and killed. The pheromone lure hung through the center of the lid inside the trap in such a way that

the lure is 2-3 cm above the water level of trap. Bating should be started from 2-3 weeks after transplanting and continued till last harvest. A distance of 30 m² should be maintained between the traps. If infestation starts even after pheromone trapping then 2-3 application of SNPV (@ 0.2 gm/liter of water) and HNPV (@ 0.1 gm/liter of water) should be done.

- Artificial release of bio-control agents: To proliferate the use of different bio-control agents, weekly release of two parasitoids were done in the trial areas. The parasitoids are i) egg parasitoid, *Tricogramma chilonis* (@ 1gm parasitized eggs/ha/week) and ii) larval parasitoid, *Bracon habetor* (@ 1 bunker /ha/week). Those bio-control agents would manage the fruit borer problem.

Thrips, *Scirtothrips dorsalis* Hood and *Thrips palmi* Karny, (Thysanoptera: Thripidae)

Distribution: Cosmopolitan.

Host: Polyphagous, some common hosts are eggplant, tomato, chili pepper, okra, coffee, cocoa etc.

Characteristics

Cosmopolitan pest. They have modified piercing sucking mouthparts. These insects are very small and are just visible to naked eyes. The young are yellow to white. Adults are darker and brownish with or without stripes on their back. Adults have narrow wings that are fringed with hairs, which are only visible under microscope. Thrips are difficult to find by those not familiar with them. They often congregate along the leaf veins. They can develop several generations in each cropping season and hot and dry weather favors population build up. Adults are excitable and fly off if disturbed. Pupation occurs in the soil usually at the base of the plant.

Damage symptoms

Areas near the mid-vein are brown and dried up. The major damage occurs on the undersides of new or old leaves. Leaves tend to curl upward like the shell of a boat. They appear distorted or misshapen.



Adult thrips on leaves around the leaf veins



Favorable weather:
hot and dry weather conditions

Leaves tend to curl upward
like the shell of a boat

Management Strategies

- Irrigation and flooding can be used to destroy thrips as they spend pre-pupal and pupal stages in the soil. By watering plants once or twice daily, the population of thrips can be reduced.
- Aluminium soil mulches may deter aphids and thrips from landing on plants. This is perhaps associated with a disturbance in the orientation of the insects prior to landing by the presence of a reflective surface near the host plants. The use of black plastic mulch is also effective in suppressing the population of thrips on chilies.
- Chili seedlings are highly susceptible to thrips attack, and such seedlings should be grown in isolation from other host plant species.
- Use of blue sticky traps can reduce the populations.
- Application of soil recharge can destroy the pre-pupa and pupal stage in the soil.
- Spray application of Spinosad (Tracer 45SC) @ 0.4ml/l of water

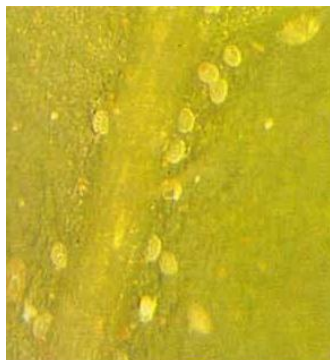
Broad mite, *Polyphagotarsonemus latus* (Banks), (Acarina: Tarsonemidae)

Distribution: Considered cosmopolitan.

Hosts: Polyphagous, many vegetable and fruit crops are attacked.

Characteristics

Extremely small in size, about the size of a particle of sand. They are not visible in naked eye. They are hidden and grouped around the mid vein undersides of the leaves. They appear crablike and are yellow or white. Female lays eggs singly on the surface of the leaves. These eggs are white, oval and extremely large compared to adults. Populations are continual but appear to be limited at high temperatures. Broad mites also feed on tomato, potato, beans and pepper.



Mites on the under surface
of the leaf



Leaf damage

Damage symptoms

Damage is usually confined to undersides of leaves, where areas between veins are brownish and dried out and brittle in severe cases. Young leaves are cupped downward and narrower than normal.



Infested leaves and fruits by broad mite

Management Strategies

- Foliar spray of Neem oil 5 ml with Trix 2 ml /L or commercial formulation of neem at 10 days intervals.
- Alternate application of miticides with different mode of action should be sprayed, such as Bio-miticide, Abamectin @1.5 ml/ L of water (Vertimec 18EC, Ecomac 1.8EC) along with chemical miticide, such as Propargite @ 2ml/L of water (Omite 57EC), Dicofol @ 1.2ml/L of water (Dicofol 18.5EC) and Sulphur fungicide @ 1.2ml/L of water (Kumulus DF) at 10 days interval.
- Indiscriminate use of pyrethroid insecticide should be avoided, because the population of natural enemy is reduced.

Country Bean

Country bean is one of the most popular vegetables in Bangladesh. Now- a- days country bean became a year round crop instead of only growing during winter. So, due to year round availability infestation of different pod borers were also increased. Previously, pod borer, *Maruca vitrata* was considered as the single borer pest of country bean in Bangladesh. But recently it was observed that *Helicoverpa* sp. infesting pods of country bean. On the other hand aphids infestation is also considered as the limiting factor for country bean cultivation especially during cool and dry period.

Farmers are spraying different types of insecticides at a very high frequency and dose on country bean to save the crop from the borer complex and aphids. Even after that proper management of those insect pests become difficult.

Bean pod borer , *Maruca vitrata* (F.), (Lepidoptera: Crambidae)

Distribution: Tropical Asia, Africa, Australia, North and South America, Pacific Islands.

Host: Legumes

Characteristics

Adult females lay eggs singly or in groups of two to ten on flowers, flower buds, or pods. Eggs are oval in shape and greenish white when laid, later turning to pale yellow. The incubation period ranges from 2 to 4 days. Newly hatched larvae feed on flowers and later bore into the bean pods. The immature is dull to yellow white and often reaches a length of 18 mm. Each segment has dark spots which form a distinct series along the length of the body. This is especially obvious on the upper surface. The head is dark brown to black. Larval duration is about 10 days. It then descends to the soil and pupates beneath leaf debris.



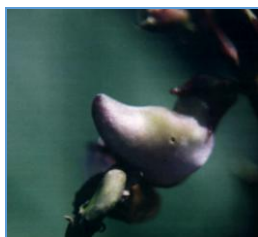
M. vitrata larva



M. vitrata adult

Nature of damage

Flowers may be damaged and discolored. The reproductive parts of the flower are damaged and missing. There is flower bud shedding and pod production is reduced. Pods have small darkened entry holes on the surface and bores inside. Leaves and pods are stuck together by webbing and show signs of surface feeding.



Infestation on flower



Infestation on pod



Infestation on seed

Bean lycaenid / Blue butterfly, *Euchrysops cnejus* (F.), (Lepidoptera: Lycaenidae)

Distribution: Asia, Australia

Hosts: Bean



Characteristics

Eggs are spherical in shape and are laid singly on young beans or flower buds. The larval stages last from 11 to 14 days. Larvae grew to 18 mm in length. The pupal stage lasts 6-7 days.

Nature of damage

Larvae feed on pods of long bean and other beans. Damage is characterized by round holes in pods and feeding on pod contents.



Infestation on pods



Larva



Pupa

Management Strategies

- Mechanical control: Hand picking and destruction of *M. vitrata* and *H. armigera* infested flowers and pods should be done at alternate days in the country bean fields.
- Application of bio-pesticide Spinosad: During hot and humid period the population of the borers increase very quickly. During that period Spinosad (Tracer 45 SC, @ 04 ml/liter of water) should be sprayed 3-4 times at 10-12 days interval especially during the flower initiation period till harvesting.
- Spraying of soap water: Spot application of powder soap (5 gm/liter of water) during initial aphid infestation can manage aphid population in a sustainable manner or spot application of Azadirachtin (neem based formulation) also control aphid population.

Conclusion and recommendations

The availability of IPM technologies for different crops especially in vegetables lagged behind seriously for years, compelling the farmers to have no other option than to rely solely on pesticide use for pest control. So, extensive research work should be undertaken for the development of effective and cheap IPM technologies against major insect pests & diseases. At the same time extensive promotional works all over the country should be undertaken for the quick dissemination of the developed IPM technologies. Private sector should assist in different aspect to develop the commercial venture of the IPM inputs, so that those products can be available at the farm level. Massive awareness campaign among the producers and consumers should be done jointly by public – private institutes on the deleterious effects of chemical pesticides. In that way a holistic and sustainable way of integrated pest management system for different crops especially vegetable crops should be undertaken thereby reducing the pest management cost with minimum risk or hazard to human and desirable components of their environment.

Insect pests of fruit crops and their integrated management

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About 70 different fruits are grown in Bangladesh. However, at present, fruits occupy only 1.2% of the total cultivated land area of the country. The consumption of fruit per head per day is about 67g as against the minimum requirement of 115g per head per day which indicates that the present production can meet about 58% of the requirement. There is an ample opportunity to increase the area and production of fruit crops in the country to meet the local as well as export demand. Pest attacks are one of the most important limiting factors for satisfactory production of different crops. Annual yield loss due to insect pest alone is 30 percent for fruits and that is one of the major cause for low production of those crops in Bangladesh. Therefore, effective measures must be ensured for protecting the crops against this colossal loss caused by pests. Till today crop protection of Bangladesh is mostly dependent on toxic synthetic chemical pesticides. Due to indiscriminate application of toxic chemical pesticides, its consumption increased several folds in the last decade.

Development of eco-friendly, sustainable, socio-economic acceptable integrated pest management or IPM packages is one of the best way to get rid from this measurable pest management system. IPM involves the integration of the most appropriate management strategies for pest control where sole dependency on chemical pesticides can be avoided. Scientists of Bangladesh Agricultural Research Institute have already been developed some effective IPM technologies, and some are in pipeline to control the devastating pests of fruits.

Mango

Mango (*Mangifera indica*) originated in the South Asian region. Over the years mango cultivation has spread throughout tropical and sub-tropical regions of the world. It is rich source of Vitamins A and C, beta-carotene, and fiber. India is one of the largest producers of mango. However, Thailand, Pakistan, the Philippines and Bangladesh are major exporters of mango in Asia. Quarantine restrictions on fruit flies and stone weevil are the major impediments to international trade of this fruit. Several species of other pests and diseases are also constraints for mango production.

Mango Hoppers, *Idioscopus clypealis* Lethiery, *Idioscopus niveosparsus*, *Idioscopus nitidulus* Walker, *Idioscopus incertus* Baker, (Hemiptera: Cicadellidae)

Distribution: *Idioscopus nitidulus* in Malaysia; *Idioscopus clypealis* in the Philippines, Indonesia and in South Asia; *Idioscopus niveosparsus* in South Asia, Indonesia and northern part of Queensland, Australia; *Idioscopus incertus* in Guangxi Province, China.

Host: Mango

Characteristics

The mango hoppers are one of the most destructive pests of mango. The adult mango hoppers are small (3.4 to 5.3 mm long), wedge-shaped, greenish-brown, and winged insects that remain hidden in shady places. Adult female lay 100-200 eggs in their lifetime. Eggs are laid in rows of 1-12 and are inserted into the tender stems, midribs, and panicles. Egg hatches in 4-7 days and there are 4-5 instars. The nymphs are also wedge-shaped, whitish, yellow, yellowish-green or greenish-brown in color with two red eyes and developing wing pads. Enormous number of nymphs are found clustering on inflorescence and new leaves during flowering seasons. Nymphal stages last about 8-10 days, but the adult leaves up to 300 days. Mango hopper populations reach their peak during the flowering season.



Adult hopper

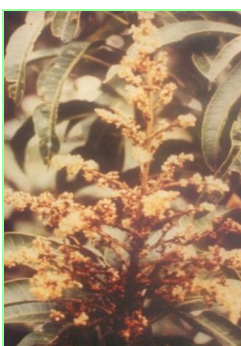


Hopper nymph on leaves and inflorescence



Damage symptoms

Damage is mostly caused by the enormous number of hopper nymphs which feed on inflorescence and new leaves by sucking plant sap. The voracious feeding of the nymphs causes the inflorescence to wither and turn brown. Even fertilized flowers may not give fruits and very young fruits may drop due to withering of florets due to physical injury and sap-sucking by the nymphs. Mango hoppers produces honeydew which results in the growth of sooty mold that retards plant growth. The mango hoppers may cause a loss of 20-100 percent of the inflorescence and thus affecting fruit yield in the infested mango trees.



Sooty mould in the inflorescence



Puncture in midrib
For egg laying



Sooty mould
in the fruit

Management Strategies

- Annual pruning and thinning of densely planted mango orchard after harvesting especially at the end of rainy season and the cut surfaces must be sprayed with copper fungicide to protect from pathogenic infection.
- Spraying of microbial pesticide, *Beauveria bassiana* @ 5.0g/litre of water within 10 days of flowering and spraying of Bioneem plus (Azadiractin) @ 1.0ml/litre of water + Indofil M 45 @ 2.0g/litre of water at pea stage of fruit growth can effectively control both nymph and adult population of mango leaf hopper as well as anthracnose.
- Two full cover spray applications (covering leaves, branches and trunk) with imidacloprid (Confidor) 70WG @ 0.2g per litre of water or cypermethrin (Ripcord/Cymbush/others) 10EC @ 1.0 ml per litre of water with the help of a high pressure and high volume power sprayer or a foot-pump sprayer, first within 10 days of flowering when the flower-buds are not opened and the second after one month of the first application, are very effective in controlling mango hoppers.

Mango pulp weevil, *Sternuchus frigidus* (F.), (Coleoptera: Curculionidae)

Distribution: South Asia, Malaysia, Palawan Island in the Philippines and part of Indonesia.

Host: Mango

Characteristics

Adult insects are 5-7 mm long, stoutly build, ovoid and dark brown colored weevil with lengthwise parallel and ridge-like lines on the hard elytra (front wings). The snouts of the weevil remains bent and concealed under the head when. Adults insect laid eggs in the silt-wounds made by the ovipositor on young (pea size) fruits and the larvae burrow into the flesh. Wound for egg-laying is healed up afterwards with no visible external injury on the skin of the infested mangoes. The legless grubs, pupae and adults are found inside the infested fruits. Adult weevils emerges from the fruits by making small exit holes. After the fruiting season the adult weevils hibernate under the bark of the mango trees, in the orchard soil, or in the debris. The life cycle completed in 40-50 days with only one generation of the pest in a year.



Mango pulp weevil adult



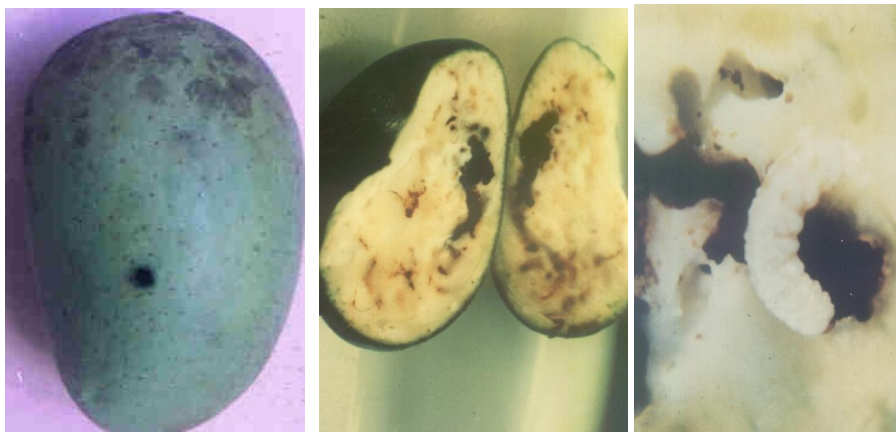
Larva



Pupa

Nature of damage

The legless grubs hatch from eggs inside the skin of infested fruits and they made tunnel through the pulp while feeding. Tunnels with black excreta, grubs, pupae and adults are found in the pulp of the infested fruits. Infested mangoes are not in a edible condition and rot easily when stored. The insect is monophagous and is a pest of quarantine importance.



Scars of adult secretion

Larval feeding in side mango fruit

Management Strategies

- Destroying of insects together with the infested fruits.
- Destruction of all parasitic plants and debris from the tree and keeping the trees clean.
- Destruction of the weeds and spading the ground around 4 meter of a tree to kill the hidden weevil in the soil during January-February
- Sparying with Lebaycid 50 EC or Sumithion 50 EC @ 2.0 ml/l of water during marble size of the fruits and at least two more time at 15 days interval.

Mango fruit fly, *Bactrocera dorsalis* (Hendel) [*Bactrocera zonata* (Saunders), *Bactrocera frauenfeldi* (Heardy), (Diptera, Tephritidae) (+52 more species)

Distribution: Asia

Hosts: Mango and many other fruits and vegetables

Characteristics

The mango fruit fly is a major pest of the matured fruits. The adult *B. dorsalis* are about 7 mm long and 13-15 mm wide when measured across the expended wings. Hyaline wings, ferrugineous thorax without any yellow middle stripe, yellow legs and dark brown triangular abdomen are the identifying characters for the adult flies. Female lays up to 200 eggs in batches of 2-15 directly inside the host fruits with their ovipositor. Maggots hatch out in about 6 days from the eggs and bore further into the fruits to feed on the soft pulp. There are three larval instars, and a prepupal stage followed by pupation within a

cocoon usually found in the soil below the host plant. The complete life cycle varies from 18-29 days.



Bactrocera dorsalis adult



B. zonata adult



B. correcta adult

Nature of damage

Infested fruits show depressions with dark-greenish puncture. Maggots are found inside the infested ripe fruits when they are cut open. The affected fruits get malformed and rot. Such fruits cannot be stored or transported. Female fruit flies also often sting immature fruits without laying eggs. This causes a sap exudate that discolours the skin of the fruits. Fruit flies are a serious constraints to the export of mangoes.



Maggots of fruit flies are found inside the infested ripe fruits when they are cut open.



Male attractant
Attract and kill method



Female attractant



Methyl eugenol pheromone trap

Management Strategies

- Clean cultivation. All infested fruits should be destroyed by burning or burying deep into the soil.

- Harvesting of mature mango before proper ripening.
- Use of methyl euginol pheromone traps starting from pea stage of mango.
- Wrapping of individual fruits with waxy paper before one month of harvesting.
- Attract and kill method: Setting of methyl euginol pheromone lures at the border plant rows at 10 m distance and female attractant in the inner rows at 15 m distance within one month of plant age. Both male and female attractant was used to trap and kill fruit flies in mango.

Red banded mango caterpillar, *Deanolis sublimbalis* Snellen (*Noorda albizonalis* Hampson, (Lepidoptera: Pyralidae)

Distribution: India, Myanmar, Thailand, China, the Philippines, Bangladesh, Brunei

Host: Mango

Characteristics

Eggs are white to crimson and are laid in groups of 1-4 on or near the peduncle. Eggs hatch in 3-4 days, and larvae have 5 instars in 14-20 days. Larvae have a brown or black head and white body with red rings in each abdominal segment. They first feed on the pulp of the fruits and later bore into the seed. Pupation takes place in an earthen cocoon in the soil. The pupal period lasts 9-14 days, and adult longevity is 8-9 days.



Adult



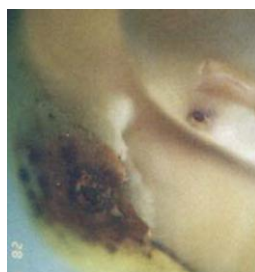
Larva



Pupae

Nature of damage

Mango fruit is attacked at all developmental stages. The caterpillar feeds on fruits as small as 2 cm and destroys the fruit from the inside out. Fruits do not reach maturity when attacked by this caterpillar. It is becoming a serious pest of mangoes in the South and Southeast Asia.



Infestation by red banded mango caterpillar

Management Strategies

- Destruction of infested fruits along with the insect larva.
- Spraying of Neem oil @ 10ml/L + Trix 5 ml/L of water or commercial neem formulation at fortnightly interval from marble size stage to fruit maturity were the best management option for the effective management of the mango fruit borer.
- Application of chemical insecticide twice, once during first observation of the infestation and second after 15 days of the first application. The insecticides like Fenitrothion (Emithion 50 EC / Sumithion 50 EC) or Shobicron 425 EC @ 2.0 ml/liter of water or Mipcin (isoprocab) 75 WP or Sevin (carbaryl) 85 SP @ 2.0 g/liter of water can reduce the infestation significantly

Mango trunk or branch borer, *Rhytidodera simulans* White, (Coleoptera: Cerambycidae)

Distribution: Southeast Asia, South Asia

Hosts: Mango, cashew, rose apple, star fruit

Characteristics

Eggs are deposited on the bark of braches, and, upon hatching larvae bore into twigs. As they develop, they move into larger twigs, branches, and trunk. Before pupating, mature larvae prepare an exit hole which is plugged with wood fiber. Adults are nocturnal and live for 50-100 days. Each female lays about 160 eggs. The life cycle takes 7-8 months.



Mango trunk borer larva



Mango trunk borer pupa



Adult

Nature of damage

This pest causes drying of branches and, in severe infestation, kills total branch and sometime the total plants.



Infestation by mango trunk borer

Management Strategies

- Insertion of petrol soaked cotton in the trunk hole made by the trunk borer and then close the hole with mud.

Mango mealy bugs, *Drosicha mangiferae*, (Hemiptera: Pseudococcidae)

Distribution: South and Southeast Asia

Hosts: Mango, Rambutan, Cocoa

Characteristics

There are three nymphal stages, and adult males have one pair of wings, well-developed eyes, antennae, and legs. Females are wingless and covered with long waxy filaments. Mealy bugs lay eggs in soil near tree trunk. It feeds on wide range of plant species.

Nature of damage

Both nymphs and female insects suck sap from all parts of the mango tree (i.e., tender leaves, shoots and inflorescence). The infected inflorescences may dry up affecting the fruit set and may cause fruit drop. Severely infected plants may show wilting and thereby affect fruit setting. The mealy bugs secrete the honey dew which causes sooty mold. That affects the growth of the trees.



Mealybug on leave



Mealybug on mango fruits

Management Strategies

- Stem banding with wrapping tape and application of pesticide carbaryl (@ 2 gm/liter of water) can effectively control adult mealy bug during March-April and also effectively control the neonate larvae during November.
- Application of bio-pesticide potassium salt of fatty acid (Phytoclean) @ 10 ml/liter of water on the adult mealy bug can effectively control it.

Guava

Guava *Psidium guajava*, is an evergreen shrub or small tree in the family Myrtaceae grown for its edible fruits. It is native to tropical America, covering the region from

Mexico to Peru eastern Brazil. It is well adapted to hot climates and is grown throughout the subtropical and tropical regions. It is rich in vitamin C, iron, calcium, and phosphorus. There are several pests and diseases, which are considered as the serious constraints in guava production in the tropics.

Spiraling whitefly, *Aleurodicus disperses* Russell, (Hemiptera: Aleyrodidae)

Distribution: Central and South America, Caribbean, the Pacific Islands, Asia and Africa

Hosts: Polyphagous: it is also a pest of vegetables, fruit trees, ornamental and shade tree

Characteristics

Adults (1.5 to 2 mm) are white and lay eggs in spirals covered with waxy secretions, usually on the undersurface of the leaves. The eggs stage takes about 7 days. There are four larval instars which last about 30 days. Only the first instar is mobile. Adults and larvae feed on plant sap.

Nature of damage

Feeding by large populations can cause desiccation of plants. Whiteflies also produce honeydew which encourages the growth of sooty mold. This hamper the normal photosynthesis process of the plant.



Waxy filaments produced by the spiraling whitefly on guava leaf

Management Strategies

- During initial attack spraying of soap water (5 g soap powder/L) can control the pest
- Spraying of neem oil (+ detergent) @ 5 ml/L or neem seed extract (50 g/10 L) or neem formulation commercial products can manage the pest.
- Application of Imidachlorpid (Admire 200 SL @ 0.5 ml/L) during severe infestations can control the pest. However care should be taken about the repeated spraying of same insecticides as the pest grows resistance very quickly.

Oriental fruit fly, *Bactrocera dorsalis* (Hendel) [*Bactrocera zonata* (Saunders), *Bactrocera frauenfeldi* (Heardy), (Diptera, Tephritidae) (+52 more species)

Distribution: Asia

Hosts: Guava, Mango and any other fruits and vegetables

Characteristics

The fruit fly is a major pest of the matured fruits. The adult *B. dorsalis* are about 7 mm long and 13-15 mm wide when measured across the expended wings. Hyaline wings, ferrugineous thorax without any yellow middle stripe, yellow legs and dark brown triangular abdomen are the identifying characters for the adult flies. Female lays up to 200 eggs in batches of 2-15 directly inside the host fruits with their ovipositor. Maggots hatch out in about 6 days from the eggs and bore further into the fruits to feed on the soft pulp. There are three larval instars, and a prepupal stage followed by pupation within a cocoon usually found in the soil below the host plant. The complete life cycle varies from 18-29 days.



Adult (Female & Male)



Maggots



Pupae

Nature of damage

Infested fruits show depressions with dark-greenish puncture. Maggots are found inside the infested ripe fruits when they are cut open. The affected fruits get malformed and rot. Such fruits cannot be stored or transported. Female fruit flies also often sting immature fruits without laying eggs. This causes a sap exudate that discolours the skin of the fruits. Fruit flies are a serious constraints to the export of mangoes.



Infested fruit



Bagging



Attract and kill method



Management Strategies

- Clean cultivation. All infested fruits should be destroyed by burning or burying deep into the soil.
- Harvesting of mature guava before proper ripening.
- Use of methyl euginol pheromone traps starting from pea stage of guava.
- Wrapping of individual fruits with waxy paper before one month of harvesting.
- Attract and kill method: Setting of methyl euginol pheromone lures at the border

plant rows at 10 m distance and female attractant in the inner rows at 15 m distance within one month of plant age. Both male and female attractant was used to trap and kill fruit flies in guava.

Papaya

Papaya, *Carica papaya*, is an herbaceous perennial in the family Caricaceae grown for its edible fruit as well as for green vegetable. Papaya is of tropical American Origin and is grown throughout the tropics. Among the pests and diseases fruit flies are constraints in export trade. Aphids, *Aphis gossypii* and *Myzus persicae* are very much important as vectors of ring spot virus, a major disease of papaya. Papaya mealy bug originated in Mexico causing devastation of papaya cultivation in many countries Caribbean, tropical South America, the Pacific, Asia, and recently Africa.

Asian Papaya fruit fly, *Bactrocera papaya* Drew and Hancock, (Diptera: Tephritidae)

Distribution: South and Southeast Asia

Hosts: Papaya, citrus, banana, guava, apple, mango, carambola, cashew and other flashy vegetables

Characteristics

This is a polyphagous pest that can lay eggs in green papaya, young banana, and citrus. Female have a long ovipositor that allows them to penetrate beyond the sap layer of green fruit. The egg stage lasts for 2-3 days, and maggots develop through 3 instars inside the fruit, feeding on the pulp. Mature larvae exit the fruit and pupate in the soil within a puparium. The pupal stage lasts for about 10 days.

Nature of damage

Fruit that is attacked by this fruit fly will rot. It is a serious quarantine pest.



Adult fruit fly

Management Strategies

- Clean cultivation. All infested fruits should be destroyed by burning or burying deep into the soil.
- Harvesting of mature guava before proper ripening.

- Use of methyl euginol pheromone traps starting from pea stage of guava.
- Wrapping of individual fruits with waxy paper before one month of harvesting.
- Attract and kill method: Setting of methyl euginol pheromone lures at the border plant rows at 10 m distance and female attractant in the inner rows at 15 m distance within one month of plant age. Both male and female attractant was used to trap and kill fruit flies in guava.

Papaya mealy bug, *Paracoccus marginatus* Williams and Granara de Willink, (Hemiptera: Pseudococcidae)

Distribution: Carribbean, Central and South America, South and Southeast Asia, Middle east and Africa

Hosts: The pest has wide host range of over 60 species, viz. papaya, eggplant, bullock's heart, china rose, sweet potato etc.

Characteristics

Adult females are yellow and covered by a white waxy secretion. They lay 100-600 eggs in an ovisac. The eggs hatch in 10 days, and the crawlers actively seek new sites for feeding. Females have four instars while the males have five. Males are winged, and female are sessile. A generation takes approximately 1 month. In general, the population of mealy bugs build up in the dry season and decline in the rainy season.

Nature of damage

Heavy infestations occur along the midrib and veins in older leaves and in all areas of tender leaves and fruits. While feeding mealybug injects a toxin into the plant. This along with the feeding activities results in chlorosis, stunting, deformity, and early leaf and fruit drop. Honeydew produced by them causes sooty mold infestation on leaves and fruits, thereby reducing photosynthetic activities. Heavy infestation kill papaya plantations.



Infested with PMB



Infested twigs



Heavily infested plants

Management Strategies

- Biological control with parasitoids, *Acerophagus papayae* is the best management option of papaya mealy bug.

- Application of bio-pesticide potassium salt of fatty acid (Phytoclean) @ 10 ml/liter of water on the adult mealy bug can effectively control it.

Red spider mite, *Tetranychus urticae* Koch, (Aranae: Tetranychidae)

Distribution: Considered cosmopolitan.

Hosts: Polyphagous.

Characteristics

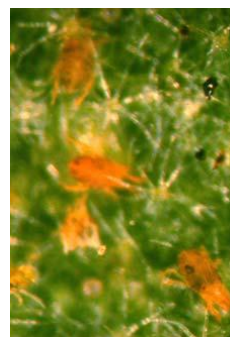
T. urticae is commonly known as red spider mite or two-spotted spider mite. They are minute in size, and vary in color (green, greenish yellow, brown, or orange red) with two dark spots on the body. Eggs are round, white, or cream-colored; egg period is two to four days. Upon hatching, it will pass through a larval stage and two nymphal stages (protonymph and deutonymph) before becoming adult. The lifecycle is completed in one to two weeks. There are several overlapping generations in a year. The adult will live up to three or four weeks.

Damage symptoms

Spider mites usually extract the cell contents from the leaves using their long, needle-like mouthparts. This results in reduced chlorophyll content in the leaves, leading to the formation of white or yellow speckles on the leaves. In severe infestations, leaves will completely desiccate and drop off. The mites also produce webbing on the leaf surfaces in severe conditions. Under high population densities, the mites move to the tip of the leaf or top of the plant and congregate using strands of silk to form a ball-like mass, which will be blown by winds to new leaves or plants, in a process known as “ballooning.” Favorable weather: warm and dry weather conditions



Spider mites in the lower surface of papaya leaf



Adult spider mite

Management Strategies

- Foliar spray of Neem oil 5 ml with Trix 2 ml /L at 10 days intervals.
- Alternate application of miticides with different mode of action should be sprayed, such as Bio-miticide, Abamectin @1.5 ml/ L of water (Vertimec 18EC, Ecomac 1.8EC) along with chemical miticide, such as Propargite @ 2ml/L of water (Omite 57EC), Dicofol @ 1.2ml/L of water (Dicofol 18.5EC) and Sulphur fungicide @ 1.2ml/L of water (Kumulus DF) at 10 days interval.

- Indiscriminate use of pyrethroid insecticide should be avoided, because the population of natural enemy is reduced.

Banana

Banana and plantain, which evolved in Southeast Asia and India, respectively, the most important fruit crops in the tropics. The banana plant, *Musa paradisiaca*, is the world's largest herbaceous perennial plant and belongs to the family Musaceae. Bananas are grown in low input backyard gardens and in subsistence agriculture, as well as in high input export plantations. The banana weevil is one of the most serious pests of bananas throughout Asia, but the banana scrapping beetle, banana aphids etc. are common pests and diseases in Southeast Asia.

Banana weevil, *Cosmopolites sordidus* (Germar), (Coleoptera: Curculionidae)

Distribution: The weevil is present in all banana growing areas of the world

Hosts: Banana, plantain

Characteristics

The adults are nocturnal and live up to 2 years and lay up to 100 eggs per year. The eggs are white, ovoid, 2mm long and are laid singly on the banana corm or pseudostem in small cavities gnawed out by the female near the ground surface. The egg stage lasts about 8 days, and larvae reach maturity in about 20 days. Larvae tunnel deep into the corm, return near the corm surface, and pupate in an oval chamber. The life cycle varies from 1 to 6 months depending on the temperature. Dispersal is mostly through infested plant material.



Banana weevil larvae



Banana weevil adult

Adult insect is a hard-shelled beetle which is almost black in color; adult is commonly found between leaf sheaths; larvae are creamy-white, legless grubs with a red-brown head.

Nature of damage

Most damage is caused by larval tunneling in the corm. Severe damage results in premature death of leaves, development of poor root systems, and toppling and breaking

of the plants. Larval tunneling also may provide entry points for fungi and bacteria that cause rotting.



Tunneling in the corm by banana weevil

Management Strategies

- Clean cultivation is an important measure for its control. Infested plants should be removed from the field and destroyed immediately.
- Regular de-suckering may reduce incidence of the pest and by keeping the bases of plants free from dead leaves, removal of other decaying plant material which harbor the insect.
- Good husbandry practices, such as the prevention of soil erosion, clean weeding, manuring and mulching produce vigorous banana plants which are more resistant to weevil damage.
- Suckers should be used from fields known to be free of weevil.
- Pieces of old rhizomes or pseudo stems 1 to 2 feet in length are cut down, split in half and placed cut face downwards on the plantation floor between the plants. Adult weevils migrate into these stems and can be controlled by hand.

Banana aphid, *Pentalonia nigronervosa* Coquerel, (Hemiptera: Aphididae)

Distribution: The aphid occur in all banana growing countries of the world

Hosts: Banana, plantain

Characteristics

This aphid occurs in almost all countries where banana is grown. Apterous and alate form coexist. This aphid reproduces parthenogenetically throughout the year. Males have been observed and reproduction is viviparous. There are four instars. Adults are about 1.5mm in length and vary in color from reddish to dark brown. This pest completes 21-26 generations per year.

Nature of damage

The preferred sites for feeding are within the whorl of the growing shoot, base of the leaves of young plants and immature fruits. This aphid produces honeydew which attracts ants. This aphid transmit bunchy top virus which makes it a serious pest. Direct damage

by the pest is minimal. Bunchy top virus disease is established in Asia, some parts of Africa, Hawaii etc.



Banana aphids

Management Strategies

- Spot application of powder soap (5 gm/liter of water) during initial aphid infestation can manage aphid population in a sustainable manner
- Spot application of Azadirachtin (neem based formulation) also control aphid population

Fruit-scarring beetle, *Nodostoma viridipennis* Motschulsky, (Coleoptera: Chrysomelidae)

Distribution: South and Southeast Asia

Hosts: Banana, plantain

Characteristics

Larvae is grayish white and found in the soil between 6 and 12 cm deep, feeding on grass root. Adults measure between 5 and 7 mm in length, and feed on leaves and fruit skin. Females deposit the eggs on the ground, in moist conditions. Adults fly directly to the fruit bunches or on leaves after emerging from the soil. Occurrence of this pest is usually high during the rainy season.

Nature of damage

The beetle feeds on young unfurled leaves and stems of the banana and also eats the skin of young fruits making scars. Severe scarring of fruit skin leads to under-developed fruits which fetch lower price in the market. Peak occurrence of this pest is usually during the rainy season. Generally old gardens are more infested than new ones.



Adult fruit-scarring beetle



Fruit-scarring beetle infestation on banana



Management Strategies

- Crop rotation to be followed. Banana cultivation to be allowed up to three years in the same land. More than two years ratoon crop should not be encouraged in the same land.
- Clean cultivation, particularly the removal of grass weeds from plantations where the population of this pest is high can often reduce population levels enough to avoid the use of insecticides.
- Bagging of fruits by transparent polythene is effective for the management of banana scarring beetle.



Bagging with polythene or mosquito net is the effective way to control this pest.

Rice insect pests and their integrated management

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Rice is the staple food in Bangladesh. A total of 232 rice pests have been recorded in the rice field in Bangladesh. One ninety two rice predator and 183 parasitoids have also been recorded so far by Bangladesh Rice Research Institute (BRRI). Among the pests, 20-32 insect pests species are important. The infestation and damage of these pests vary with crop seasons, years, crop growth stages, variety, management practices and the overall agro-ecological conditions. Results of several crop loss assessment trials showed that an average of 18% yield loss due to the infestations of major insect pests (13% in Boro, 24% in Aus and 18% in T. Aman). Another trial also conducted with insecticides showed an average of 13% yield increase in the insecticide-treated plots over the untreated ones. Rice insect pests may be divided into following categories depending upon the nature of their feeding and damage.

Internal Stem Feeder

Stem borer: Yellow stem borer – *Scirpophaga incertulas*

Dark headed borer – *Chilo polychrysus*

Pink borer – *Sesamia inferens*

Gall midge: *Orseolia oryzae*

Leaf Feeders

Whorl maggot: *Hydrellia philippina*

Rice hispa: *Dicladispa armigera*

Leaf roller: *Cnaphalocrocis medinalis*, *Marasmia*(=*Susumia*) *exigua*

Caseworm: *Nymphula depunctalis*, *N. fluctuosalis*, *N. foedalis*

Swarming caterpillar: *Spodoptera mauritia*, *S. litura*

Grasshopper: *Oxya velox*, *O. hyla hyla*, *Euconocephalus incertus*, *Conocephalus longipennis*

Long horned cricket: *Euscyrtus concinnus*

Leaf and Plant Suckers

Green leafhopper: *Nephotettix virescens*, *N. nigropictus*, *N. modulatus*, *N. malayanus*

Orange headed leafhopper: *Thaia oryzivora*

Zigzag leafhopper: *Recilia dorsalis*

Thrips: Complex of six species

Brown planthopper: *Nilaparvata lugens*

White-backed planthopper: *Sogatella furcifera*

Mealy bug: *Brevinnia rehi*

Grain Sucker

Rice bug: *Leptocorisa oratorius*, *L. acuta*

Ear-Cutter

Ear-cutting caterpillar: *Mythimna separate*

Internal Stem Feeder

Stem borers: Among the three species, yellow stem borer (YSB) is the most predominant species on all the rice crops. The dark-headed borer (DHB) is prevalent in Boro and Aus seasons, while the pink stem borer (PSB) in the Boro season. YSB larvae are pale-yellow in colour, sometimes with greenish tinge. DHB larvae are light brown in colour having 5 longitudinal dark-brown stripe on the body and a dark-brown head capsule. PSB larvae are pink coloured with light coloured head capsule. The adult of YSB is straw coloured. The females have a conspicuous black spot on each forewing. DHB moth is light yellow to dark brown in colour with 6-7 black spots in the centre and 7 black dots at the margin of the forewing. PSB moth is broad shouldered and has light brown forewings with dark brown streaks. YSB and DHB lay eggs in masses on the leaf surface, while PSB lays eggs in rows on the inner side of the loosened leaf sheaths. The damage caused by these three stem borer species is identical. The larvae after feeding in the leaf sheath for a few days enter into the stem and cut off the growing part of the plant from the base during their feeding which causes the growing shoot to die. This damage is known as 'dead heart' in the vegetative stage of the plant and as 'white head' in the flowering stage. The following measures can be taken for the control of stem borers:

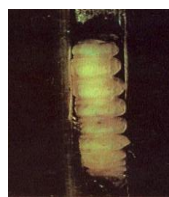
- In case of small holdings, destruction of egg masses by hand picking.
- During high incidence, destruction of moths by collecting in light traps placed 200-300 meters away from the rice field.
- Unharvested stubble particularly of the T. Aman crops, harbour 43-100% borer larvae and pupae. Stubble destruction in large areas can greatly reduce this source of population.
- Egg parasitoids destroy 60-100% YSB egg masses particularly in Aus and T. Aman season. Disruptive insecticide applications should be avoided to boost up parasitoid activity and to maximize and stabilize the natural control systems.
- Perching for insectivorous birds help to reduce YSB moths population.
- Recommended insecticides should be applied only at Economic threshold level (ETL) of 3 female moths or egg masses/m² or more than 5% dead hearts after maximum tillering.



Adult borer



Egg



Larva



Dead heart



White head

Gall midge: It is an important pest mainly of the T. Aman crop. The adult fly is about the size of a mosquito. Females have bright red abdomen; males are darker. It lays eggs singly in groups of 3-4 near the base of the plant. The freshly hatched larvae move into the growing point of the plant and feed inside. Feeding stimulates the leaf sheath to transform into gall known as 'onion shoot' or 'silver shoot'. Tillers with galls bear no panicles. Pupation occurs within the galls.

- Destruction of gall midge by light trapping.
- At present, the use of insecticides is the only method of control.



Gall midge



Onion shoot

Leaf Feeders

Whorl maggot: The maggot feeds on the whorl leaf while it is still within the plant. As the leaves emerge yellow damaged areas of degenerated tissues become visible. Damage occurs mainly at the early stage of the plant. Infestation reduces the tiller number and may delay the crop maturity.

- Use of insecticides is the only control measure available at present



Whorl maggot



Grub



Damage symptom

Rice hispa: At present it is the most menacing pest in Bangladesh. It attacks all rice crops. The adult beetles are bluish black with spine on the body. Both the adult and grub injure the plants. Adults scrape the upper leaf surface down to the lower epidermis. The feeding appears as narrow longitudinal streaks. The grub mines between the epidermal layers creating irregular tunnels. The damaged portions become white and then brown. Severely damaged leaves look burned. The adults lay eggs usually on the lower surface of the leaf, partly inserted into the epidermis. The following control measures may be taken:

- In the primary infestation areas of the southern districts, appropriate control measures should be taken on the T. Aman ratoons and Boro crop early in the season to arrest migration of the adults to larger areas.
- Collection of hispa adults by repeated sweeping.
- Destruction of the grub by clipping the grub infested leaves 2.5-3 cm above the leaf base. This method can remove 75-92% grubs.
- Application of appropriate insecticides at ETL of 4 adults/hill or 35% leaf damage.



Rice hispa



Adult hispa



Damage symptom

Leaf roller: It is common in the Aus season, also occurs in Boro and T. Aman seasons. The adults moth is small with light brown wings having dark brown stripes and spots. The larvae are yellow or yellowish green. Adults lay eggs singly on the leaf surface. After hatching the larvae feed on the leaf tip and then roll the leaf longitudinally. They feed on the green tissues living within the leaf roll. The damage appears as longitudinal white streaks. Severely damaged leaves look scorched. The insect may be controlled by:

- Destruction of moths by light trapping.
- Perching for predatory birds can greatly reduce the population.
- Insecticide should be applied when 25% leaves are damaged at maximum tillering stage.



Larva



Adult



Damage symptom

Caseworm: It is a pest mainly of the T. Aman crop having stagnant water. The adult is a snow-white moth with pale brown markings on the margins of the forewings. They are nocturnal and are strongly attracted to light. The larvae are semi-aquatic. They scrape the

leaf tissues leaving the upper epidermis. Characteristic damage shows longitudinal white streaks. The larvae cut off the leaf tip to make about 2 cm long cases in which they live. The leaf cases float on the paddy water and serve as transport for the larvae to move from one plant to another. It can be controlled through the following methods:

- Destruction of moths through light trapping during peak incidence periods.
- Draining out of stagnant water from the infested fields and destruction of the leaf cases.
- Perching for the predatory bird.
- Use of appropriate insecticides at ETL of 25% damaged leaves.



Caseworm



Case



Damage symptom

Grasshopper: Five species are prevalent; *Oxya* spp. is more common. Their damage is identical. Both adults and nymphs injure the plant by eating the leaves from the margin. In severe infestation, plants are skeletonized leaving only the stem and midribs of the leaves.

The insect may be managed by:

- Perching for predatory bird.
- Light trapping.
- Collection of adults and nymphs by sweeping.
- Insecticides should be used only when 25% leaves are damaged.



Grasshopper



Damage symptom

Long-horned cricket: The adult is golden coloured and has long antennae. Both adult and nymph damage the rice plants. Characteristic damage appears as window like oblong perforations on the leaf blade. Heavily damaged leaves may be skeletonized leaving only the ribs. The following method may be used to control the insect:

- Perching for predatory bird.
- Light trapping.
- Collection of adults and nymphs by sweeping.
- Insecticides should be used only when 25% leaves are damaged.



Adult cricket & Damage symptom

Leaf and Plant Suckers

Green leafhopper: Among the GLH species, *Nephotettix virescens* is predominant and more important. The adult hopper is green coloured with or without black spots on the forewing and about 4.5 mm long. Both adults and nymphs damage the rice plant directly by sucking the leaf sap and indirectly by transmitting the tungro virus disease. Direct feeding cause the plant to become yellow and stunt. GLH is more prevalent as tungro disease transmitter than as a direct feeder. Control measure for GLH includes:

- Destruction of hoppers by light trapping & sweeping.
- Application of insecticides when tungro disease plants and GLH are prevalent.



Green leafhopper

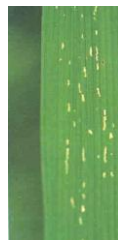


Tungro affected rice plant

Orange-headed leafhopper: The adult is small and slender with orange coloured head. Both adults and nymphs damage the rice plants scraping the leaf surface in a zig zag manner and sucking the sap. 'Hopper burn' may occur in severe infestations. It is more prevalent in Boro season. Presently, it is controlled only by insecticide.



Orange-headed leafhopper



Damage symptom

Zigzag leafhopper: The adult has dark zigzag markings on the wings. The nymphs are yellowish brown. Both nymph and adult damage the plant by sucking sap from the leaves and leaf sheaths. The zigzag leafhopper transmits tungro, yellow dwarf and orange leaf virus diseases. It plays a minor role as a pest because its population is generally low.

- Keep the fields free of vegetation between rice crops.
- Destruction of hoppers by light trapping & sweeping.
- Application of insecticides when tungro disease plants are prevalent.



Zigzag leafhopper



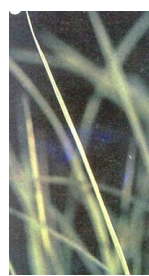
Damage symptom

Thrips: Six species have been identified in Bangladesh but their relative pest status is not known. They cause identical damage. Adult thrips are about 1mm long and dark brown in colour. Both adults and nymphs injure the young rice plants by sucking the leaf sap through laceration. Damaged leaves show fine yellowing streaks which join together. Later, the leaf rolls longitudinally taking a needle-like shape. Severely attacked plants may wither. Higher infestations usually occur in seedbeds or on young plants in Aus season.

- Top dressing with nitrogenous fertilizer is effective for the plants to recover from thrips damage.
- Insecticide application is necessary in case of heavy infestations.



Thrips



Damage symptom

Brown planthopper: It is a serious pest of Boro, but also attacks the T. Aman crop. The adult hopper is brown coloured and about 4mm long. It may be long-winged (macropterous) or short winged (brachypterous). Females insert eggs in rows in the thick tissues of the leaf sheath. About 400-600 eggs are laid by a female. The nymphs are

whitish, gradually turning brown. Both adults and nymph live in a crowded form at the base of the plant and suck sap from the stem. Direct feeding by a large number of hoppers cause the plants to dry up and the damage is known as 'hopper-burn'. BPH transmit grassy stunt and ragged stunt virus diseases which have not yet been reported in our country. BPH can be controlled by adopting the following measures:

- Cultivation of early maturing varieties.
- Draining out of standing water and drying out of the infested fields for about a week when BPH tends to increase sharply.
- Using a wider plant spacing and avoidance of top dressing of nitrogenous fertilizer in the endemic areas.
- Avoidance of disruptive insecticide application to boost up the activities of predators. Insecticide application should be skipped if at least one predatory spider is present/hill in 50% plants of a field.
- Application of appropriate insecticide only at ETL of 4 gravid females/hill or 10-15 nymphs/hill



Nymph



Brachypterous adult



Macropterous adult



Damage symptom

White-backed planthopper: It is slightly smaller than BPH, light brown in colour with a white streak on its back. Only females are short winged. It occurs concomitantly with BPH. Like BPH, it lives in a crowded form at base of the plant, suck sap from the stem and cause 'hopper burn'. The life history of the WBPH is similar to that of BPH. The control measures used for BPH are applicable to WBPH.

- Cultivation of early maturing varieties.
- Draining out of standing water and drying out of the infested fields for about a week when BPH tends to increase sharply.
- Using a wider plant spacing and avoidance of top dressing of nitrogenous fertilizer in the endemic areas.
- Avoidance of disruptive insecticide application to boost up the activities of predators. Insecticide application should be skipped if at least one predatory spider is present/hill in 50% plants of a field.
- Application of appropriate insecticide only at ETL of 4 gravid females/hill or 10-15 nymphs/hill



Nymphh

Adult

Damage symptom

Mealy bug: It is a soft-bodied, pink coloured insect covered with white waxy material. Adult females are wingless and 3-4 mm long. The males are uncommon and have a single pair of wings and a stylet like process at the end of the abdomen but lack mouthparts. Females are sometimes parthenogenetic. They live in a crowded form between the leaf sheath and the stem and lay eggs more or less in a chain inside the waxy materials. The infested plants become stunt and yellow due to sucking of plant sap by a large number of mealy bugs. Severely infested plants have exerted panicles or none. Mealy bug is more prevalent in drought situations in the Aus season, but also occurs in Boro and T. Aman crops. To control the insect:

- Destroying the infested plants
- Insecticide application are needed when more than 5% hill are damaged



Mealy bug



Damage symptom

Grain Sucker

Rice bug: Rice bug is the main grain sucking pest in Bangladesh. Two species are important. The adult bug is 15-20 mm long, slender and brownish green in colour. It emits pungent, repelling smell. Females lay eggs in rows on the leaf surface. Both adults and nymphs cause damage by sucking sap from developing grains. Damaged grains become partially filled or empty. The whole panicle may be damaged during heavy infestations. To control the insect:

- Light trapping can effectively reduce the population.
- Insecticides should be applied when 3 bug are found/panicle.



Rice bug



Damage symptom

Ear-Cutter

Ear-cutting caterpillar: The adult of ear-cutting caterpillar is medium sized and pale brick red to pale brownish in colour. The forewing has a pale central spot and a series of black specks at the margin. Larval colour varies from green pale-grey to greenish grey or pinkish. Larvae have four longitudinal black stripes running laterally on the back. The full grown larvae are 30-35 mm long with 6 ocelli on the brown coloured head. The young larvae eat rice leaves or grasses from the edges. They are active at night and hide at the base of the plants or under the foliage at day time. The 5-6 instar larvae require large amount of food and they become gregarious. They move from one field to another in search of food and cut ripe or half ripen rice panicles. Higher incidence is observed mainly during the T.Aman season. The following control measures are effective:

- Ploughing down the stubble soon after harvest destroys the hibernating population.
- Flooding the infested fields and perching for predatory birds are effective for reducing the caterpillar population.
- Use of insecticides at ETL of one caterpillar/m².



Larva



Adult



Damage symptom

Management of Rice Insect Pests

1. Cultural control

Draining out of standing water –

Whorl maggot

Caseworm

Brown planthopper

White-backed planthopper

* Flooding of field –

Swarming caterpillar

Soil insects

- * Destruction of infested plant – Mealy bug
 - * Early maturing variety – Brown planthopper
White-backed planthopper
 - * Wider plant spacing – Brown planthopper
White-backed planthopper
 - * Application of nitrogenous fertilizer – Thrips
 - * Light trap – All insects attracted to light
 - * Collection and destruction of egg Stem borer masses –
 - * By sweep net – Rice hispa & all other insect
 - * Burning of stubbles – Stem borer
Ear-cutting caterpillar
Swarming caterpillar
- 2. Mechanical control**
- * Leaf clipping – Rice hispa (about 75-92% grubs can be removed)
- 3. Biological control**
- * Perching – Placing of branches in the field to provide perching site for predatory bird

4. Insecticidal control

Economic threshold level (ETL) for different rice insect pest

- ❑ Rice hispa – 4 adult/hill
35% damaged leaves
- ❑ Brown planthopper – 2-4 gravid female/hill
10 nymph/hill in 50% hill (at booting stage)
- ❑ Green leafhopper – 1 hopper/sweep and the presence of tungro virus
- ❑ Leaf feeders – 25% damaged leaves
- ❑ Rice bug – 2-3 bug/hill
- ❑ Stem borer – 10-15% dead heart
5% white head
3 moth or egg mass/m²
- ❑ Gall midge – 5% onion shoot

Biological control of insect pests and mass rearing techniques of predators and parasitoids

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In agro-ecosystems, the components of modern intensive agriculture such as high yielding varieties, excessive use of fertilizers, irrigation, etc. alter the crop physiology, morphology and phenology so as to render the crop attractive for insect pests and diseases. To control or manage pests, synthetic pesticides were developed in the early 1940s. With the success of pesticides, biological, cultural, and mechanical controls were often underutilized or disregarded as viable pest management strategies. Further consequences of overreliance on pesticides became apparent over the next few decades. The first and foremost requirement in the pest management is the knowledge of crop and associated organisms. Biological control is one of the most important components of integrated pest management through promoting natural enemies of key pests of crops. It offers a sustainable, viable and ecological solution to the pest problem. It has three approaches viz. importation, conservation by modifying the environment and periodic release by mass multiplying the bioagents.

In nature, different egg and larval parasitoids are useful biological control agents because parasitization invariably kills the hosts. Among them different species of *Trichogramma* are considered as the most important egg parasitoid especially for augmentation. *Trichogramma* and other egg parasitoids are generally part of the local ecosystem and often contribute to the control of lepidopteron pest in absence of disruptive pesticides. The braconid, *Bracon habetor* Say is a gregarious, idiobiont, arrhenotokous, ectoparasitoid wasp, attacking lepidopteran larvae. It is considered as an ideal biological control agent, as it can be able to regulate a pest species population at a level that is economically acceptable. Earwig can destroy considerable number of borer larvae and both adult and larvae of lady bird beetles are known as voracious feeder of small, soft-bodied insects like aphids, whitefly etc.

So, it is a well-established fact that bio-control based management package can control insect pest population effectively and sustainable manner. However, for that reason sufficient number of parasitoids must be present in the management area and for that reason inundative release is needed and that requires efficient mass production system. In fact naturally occurring predators and parasitoids are often not present in sufficient numbers at the right time to keep pest species within an economically sustainable limit. So, artificial release programs can be used to overcome those limitations. Hence, for the development of sustainable management packages, inundative releases of effective biological agents along with conservations of released agents are very much important. For that reason effective, cheap mass rearing protocols for effective bio-control agents must be developed. On the other hand, bio-control based management is not becoming

popular due to their ready availability among the farmers communities, because the private entrepreneurs are not coming forward to make it as a commercial enterprise.

Mass rearing protocol of egg parasitoids, *Trichogramma* spp. on rice meal moth *Sitotroga cerealella* eggs:

Trichogramma are minute wasps parasitic on eggs of lepidopteran insect pests. *Trichogramma* lays its eggs in the host insect eggs, multiply therein, thus preventing hatching of host insect larvae. *Trichogramma* is considered as an effective parasitoid against many deleterious insect pests. Worldwide it is using in the bio-control based pest management. However, for augmentation easy availability of *Trichogramma* is needed. For that reason easy and cost effective mass rearing is very much important. Presently mass rearing of *Trichogramma* generally was done on rice meal moth, *Corcyra cephalonica* eggs. However, it is not so much cost effective. So, attempt was undertaken to develop cost effective mass rearing protocol of *Trichogramma* sp. on *Sitotroga cerealella* eggs.



Materials and methods

Five kg wheat poured into boiled water for 2-3 minutes. Then the treated wheat kept in steel trays (50 cm x 60 cm), each tray containing 2.5 kg and 1 gm of *S. cerealella* egg and kept for 5-6 days in untouched condition. After that requisite amount of water was added and mixed that properly with gentle stirring. After 22-25 days later, the infested wheat with *S. cerealella* larvae, were put in mass rearing chamber for adult emergence.

From the insect mass-rearing chamber thousands of *S. cerealella* adults were collected and kept in a glass cylinder open at one side covered by 32 mesh net. Adults were kept in the cylinder for one day for mating and subsequent egg laying within the glass cylinder. In the consecutive day the eggs laid on the wall of the cylinder were brushed and were sieved to collect fresh eggs. The adults and their body parts and scales were cleaned by holding the cylinder near an exhaust fan to get the fresh eggs. Five gm fresh eggs of *S. cerealella* were then put in a long moist glass cylinder (glass cylinders were moistened by keeping them inside freezer for few minutes) and the eggs were spread over the cylinder. Vial containing one gm parasitized eggs with *Trichogramma* were then kept inside the glass cylinder. The glass cylinders were then kept continuously in florescent light at 25.0 ± 2.0 °C for 9-11 days.

Results

Within 9-12 days parasitism of almost all eggs of *S. cerealella* were happened. The duration of the egg stage of *Trichogramma* spp. within the host egg was 1-2 days, larval stage 5-6 days Pupal stage 3-4 days (total 9- 12 days). The parasitised eggs were collected and kept in desiccators in $3-4^{\circ}\text{C}$ temperature and 75-85% RH for 1-1.5 months.



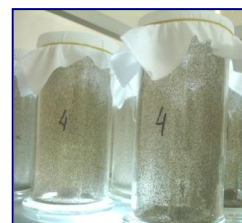
Mass rearing
chamber
of host insects



Egg laying of host
insects in glass
cylinders



Collection of host
eggs



Parsitisation of host
eggs

Field release techniques of *Trichogramma* spp.

Trichogramma spp. are efficient egg parasitoids of many lepidopterous pests. Artificial release of this parasitoid can also control many devastating pests like *Leucinodes orbonalis* or *Helicoverpa armigera* etc. However, the parasitism efficacy of mass reared parasitoids depends upon the field release techniques. One of the most drawbacks of inundative release of *Trichogramma* spp. are its field release through paper strips methods and this method is popular due to its easy handling technique. In the paper strip method *Trichogramma* spp. are released at pupation stage. So natural predation of those parasitised eggs are common phenomena in that method. Even sometimes about 40-50% of the parasitised eggs by *Trichogramma* spp. are destroyed by different predators after their field release. So, this study was undertaken to develop a techniques to release adult parasitoids in the field instead of release during pupation stage in the paper strips. A comparison between the two techniques was also done in the field condition.

Materials and methods

This study was undertaken in the eggplant field in micro plot condition and in open field condition at BARI, Gazipur during February 2012 – May 2012. Parasitism efficacy of three different host eggs, viz. rice meal moth (*Sitotroga cerealella*), meal moth (*Corcyra cephalonica*) and eggplant shoot and fruit borer (*Leucinodes orbonalis*) eggs were evaluated by releasing *Trichogramma chilonis* through paper strip method (release during pupation period) and adult release methods in micro plot condition as well as in open field condition.

In micro plot condition: Five host egg strips (containing 200 host eggs of each insect pests/ strip) were clipped on the eggplant leaves of 04 plants in the field. The plants were then covered with fine siphon net. Vial containing 500 parasitised host eggs of *Trichogramma chilonis* were kept inside a microplot, on the other hand in another microplot paper strips containing 500 parasitised host eggs of *Trichogramma chilonis* were placed. After 03 days the host egg strips were collected from the field and kept in test tubes. The number of parasitised eggs as well as the number of adult parasitoids emergence were recorded. One microplot was considered as one treatment replication and in the same way there were three replications of those two treatments.

In open field condition: Five host egg strips (containing 200 host eggs of each insect pests/ strip) were clipped on the eggplant leaves of 04 plants in the field. Paper strips

containing 500 parasitised host eggs of *Trichogramma chilonis* were placed in that field. Releasing of adult parasitoids was done in another field having same number of host egg strips. When emergence of the adults of *Trichogramma chilonis* in a vial (containing 500 parasitised host eggs) started, then those adults and parasitised eggs were placed in a plastic container containing 300 paper chocklets (1.5"x2.0" size newspaper in a twisted form like a chocklet covering). Within 3-4 hours all *Trichogramma chilonis* adults emerged from the parasitised eggs and took shelter in the paper chocklets. Then those paper chocklets were distributed within the plots having the host egg strips. After 03 days the host egg strips were collected from the field and kept in test tubes. The number of parasitised eggs as well as the number of adult parasitoids emergence were recorded. One field was considered as one treatment replication and in the same way there were three replications of those two treatments.

It is revealed from the results that host egg parasitism was 2.26 times (mean of three host eggs) higher in adult parasitoid release than parasitised eggs on paper strips in micro plot condition. The same trend was also observed in the open field condition, where egg parasitism was 2.42 times (mean of three host eggs) higher in adult parasitoid release than parasitised eggs on paper strips.



Parasitised eggs in paper strips



Steps of adult parasitoids release

Mass rearing protocol of larval parasitoid, *Bracon hebetor*

Bracon hebetor is medium sized wasp parasitic on later instars non-hairy larvae of wide range of insect pests. *Bracon hebetor* is an aggressive ecto-endo parasitoid. Female *Bracon* at first inject venom and thus paralyze insect larvae. As little as one part of venom in 200 million parts of host blood was sufficient to cause permanent paralysis of insect larvae. A female *Bracon* can paralyze 500-1000 larvae. Paralyzed larvae cannot survive. Female *Bracon* then lays its eggs on the host larvae; multiply therein and thus destroying the pests. As it is considered as an effective parasitoid, so it can be used in the augmentation process. For that reason easy and cost effective mass rearing protocol development is very much important.



Materials and methods

Mass rearing of *B. hebetor* parasitoid was done at IPM laboratory, Entomology Division, BARI. Later instar (5-6 instar) wax moth, *Galleria mellonella* larvae was used as the host of *B. hebetor*. At first a parent stock of wax moth was developed in honeycomb in

glass jars. First to second instar larvae of wax moth were released in the artificial diet (made with definite proportion of wheat flour, maize flour, milk, animal fat, sugar and yeast and autoclaved at 125°C and 1.5 PSI for 70 minutes). The larvae when attained full growth length (18 to 20 days later), they were transferred in a plastic bottle (200 larvae /bottle) with a corrugated paper sheet. The full-fed larvae took position on the corrugated paper sheet for pupation. Then 40 adult *B. hebetor* (30 female and 10 male) were released in the plastic bottle with honey cube for their food. The open end of the jar was closed with black cloth. The wax moth larvae and *B. hebetor* were then kept in rack for 8-10 days for parasitism, egg laying, pupation and adult emergence of *B. hebetor*. It has been observed that 100% parasitism of wax moth larvae happened by *B. hebetor*. The duration of the egg stage of *Bracon hebetor* on host larva was 3-4 days, larval stage inside the host larva was 6-8 days, pupal stage outside the host larva was 5-6 days. Total adult longevity in the laboratory was 20-25 days (with honey as food). However, in nature adult *B. hebetor* can survive up to 45 days.



Parent stock of wax moth larvae



Artificial diet for wax moth larvae



Full grown larvae in the diet



Full grown larvae put in plastic jar with a corrugated paper sheet

Parasitisation of *B. hebetor* in darkAdult *B. hebetor* ready to use in field

Mass rearing protocol predator *Lady bird beetle*:

Ladybird beetle is an effective predator against many soft-bodied insect pests, viz. aphids, white fly, jassid etc. as well insect eggs. It is very much helpful for natural control of many deleterious insect species. However, due to indiscriminate use of insecticides the natural population of ladybird beetle is declining. So, in many crop fields augmentation of ladybird beetle is very much necessary for the natural control of its insect pests. Due to that reason development of mass rearing protocols of ladybird beetle is important. Hence, this experiment was undertaken to develop protocol for the rearing of ladybird beetle, *Menochilus sexmaculatus* (Fab.), Coleoptera: Coccinellidae.

Materials and methods

Mass rearing of *M. sexmaculatus* were done in the IPM Laboratory, Entomology Division, BARI, and Ispahani Biotech (IBT) Laboratory, Konabari, Gazipur during December 2010- June 2011. Single pair of newly hatched *M. sexmaculatus* were kept in petridish. The young pairs were supplied with requisite quantity of *Sitotroga cerealella* aged eggs (more than 3 days old), *S. cerealella* eggs + live aphids, only live aphids collected from country bean and mustard and mealy bugs. Data on different life stage parameters, viz. egg laid/female, egg hatching period, larval period, pupal period and adult longevity were recorded.

Results and discussions

Some differences were observed in the LBB's growth parameters in natural conditions than that in the laboratory condition. Egg laid/female were 50-300 in the laboratory condition, while LBB can lay up to 1000 eggs in the natural condition. In the natural condition the larval period and adult longevity is longer than the laboratory condition. Although there are some hindrances happened in the growth of *M. sexmaculatus* on *S. cerealella* eggs, but overall growth was satisfactory.

However, weight loss of LBB population was observed in the successive generations. Weight loss was 26.67% after three generations if they are reared only with *Sitotroga cerealella* eggs. It might be due to the effects of food quality. As all the requisite amount of food sources are not available in those eggs. However, if it can be reared only in live insects (aphids, mealy bugs etc.) then healthy LBB can be reared in the successive generations.

Mass rearing protocol of predatory earwigs, *Euborellia annulata* in the laboratory

Mass rearing of earwigs generally done in a plastic pot having 6.0" diameter and 6.0" height. Netting was done in the middle portion of the top end of the plastic pot for proper aeration. Around 3-4 kg organic matter mixed soil was taken in the pot in such a way that there would be 3.0" soil layer in the pot. In that soil rice or wheat husk, rotten straw etc. were added to increase the organic matter contain as well as those can be used as earwigs alternate diet also. Requisite amount of water must be added in the soil before put it in the plastic pot (it would be enough to make a ball with the soil). However, precaution should be taken to add water, because due to excess water earwigs can be infected with different fungal diseases and due to less water proper growth of earwigs would be stunted. After mixing of water, the soil was autoclaved at 121°C and 15 PSI pressure for 25-30 minutes to kill unwanted insect pests and diseases. After that 10 pairs of earwigs were released in that soil. Proper food of earwigs, viz. live insects (aphids, whiteflies etc.) or insect eggs were supplied everyday. Requisite amount of water were also supplied everyday by putting 2-3 moist cotton pieces (2.0"x2.5") on the soil top inside the plastic pot. The pots were then kept in dry place having temperature range from 25-27°C. The adult earwigs

generally lay eggs in the soil in batches. Within 25-30 days around 100 earwigs nymphs can be reared in that pot. When those nymphs molted 2-3 time then the old earwigs were taken out from the pot. For separating the old earwigs from the new ones, honey solution (60%) in a cotton piece kept on a petridish and put on the soil of the pot. The adult earwigs gathered near the petridish with honey and in that way they were removed from that pot to a new pot for more egg laying. In this way continuously earwig's mass production can be done.

Commercial formulation and use of *Trichoderma* in Bangladesh

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A. *Trichoderma* and *Trichoderma* based products

Trichoderma is a beneficial antagonistic fungus that is present in all types of soils in Bangladesh. The fungi are opportunistic, avirulent plant symbionts, as well as parasites of other fungi. *Trichoderma harzianum*, *T. viride* and *T. virens* are the three common species used as biological control agents. This biological control promotes seedling establishment, enhanced plant growth and elicited plant defense reaction in different crops as reported by many scientists. The potential *Trichoderma* isolates are formulated using different organic and inorganic carriers either through solid or liquid fermentation and prepared called *Trichoderma* based products. These products are delivered either through seed treatment, bio-priming, seedling dip, soil application, and foliar spray.

B. History of *Trichoderma* research in Bangladesh

Research on *Trichoderma* spp. was started at Bangladesh Agricultural Research Institute (BARI) in 1998. Dr. M A Bari, PSO 1st worked on *Trichoderma* in the laboratory of Plant Pathology Division, BARI, Gazipur. Most of his research on isolates collection and evaluation against soil borne diseases in vitro. Later on Dr. Sachin Barman, SSO; Dr. Nazrul Islam, SO; Dr. Iqbal Faruk, SSO, Dr. Mynul, SO; Dr M. A. Rahman, PSO and Dr. M S Nahar had research on *Trichoderma*. More than 100 isolates were collected. Among them two isolates of *Trichoderma harzianum* found to be most effective to control various soil borne pathogens. The isolates were maintained and performed a series of experiments at net house in plant pathology Division. Bangladesh Agricultural University (BAU) started work on *Trichoderma* at the same time of BARI.

For large application and formulated were carried out through IPM IL (CRSP) in 2005. In parallel, Plant Pathology Division had work on *Trichoderma* formulation and isolates collections. In 2007-8, different formulations such as compost base, talc base, barley, rice husk, chickpea bran etc were tested in the fields. Among them, compost base formulation namely Tricho-compost is most popular and farmers' are using for management of diseases in the fields.

Bangladesh Agricultural University, Mymensingh and Rural Development Academy (RDA), Bogra are doing research on *Trichoderma*. Natore Development Society (NDA), Natore; Ispahani and some other NGO are producing and selling different formulation of *Trichoderma*. However, one NGO GKSS, Gabtoli, Bogra has commercial production factory and been producing Tricho-compost (60 ton/month) and selling different region of Bangladesh.

C. Different commercial formulations of *Trichoderma*

Solid media base:

Solid media such as barley grain base, rice husk base, grass pea bran base & chickpea bran base *Trichoderma* are producing in the laboratory (Fig. 1.). These organic materials are autoclaved and inoculated by mother culture of *Trichoderma* spp. and incubated for 7-10 days at 25-30°C. Then the air dried materials are ready to use. These products suitable for furrow application in the fields.

Talc base formulation:

Talc base formulation of *Trichoderma* is recently developed (Fig. 2.). Spore suspension of *Trichoderma* mixed with talc power (Calcium magnesium silicate) and air dried to reduce moisture at 8°C. This formulation is suitable for seed treatment and making Tricho-compost at farmers' level.

Compost base formulation:

The most popular formulation of *Trichoderma* is using at farmers' level. Mixing of a definite concentration of spore suspension of a *T. harzianum* strain with measured amounts of processed organic materials (de-composted poultry litter and cow dung, processed water hyacinth, vegetable waste/ kitchen waste, fine sawdust, mushroom waste/ash, maize bran, neem leaf and molasses) is used to develop *Trichoderma*-based compost fertilizer namely Tricho-compost (Fig. 3.). The composting process produces drainage enriched with *Trichoderma*, called Tricho-leachate which can be collected and reused for composting. Tricho-compost was applied in soil for soil borne disease control and Tricho-leachate is used for foliage disease management.



Fig. 1. *Trichoderma* in solid media (barley grain, rice husk, grass pea bran & chickpea bran)



Fig. 2. *Trichoderma* in talc base formulation



Fig. 3. *Trichoderma* in compost base formulation namely Tricho-compost & Tricho-leachate (by-product)

Tricho-compost and Tricho-composting

Composting is the natural process of 'rotting' or decomposition of organic matter by microorganisms under controlled conditions. *Trichoderma* take part in this natural composting process and enhance the process and itself play important role for crop protection. Raw organic materials such as crop residues, animal wastes, food garbage and some municipal wastes, enhance their suitability for application to the soil as a fertilizing resource, after having undergone *Trichoderma* composting.

Organic materials often contain complex chemical compounds such as lignin, cellulose, hemi-cellulose, polysaccharides, proteins, lipids etc. These complex materials cannot be used as such as resource materials unless it is converted into simple inorganic element as available nutrient. The material put into soil without conversion will undergo conversion inside the soil. This conversion process takes away all energy and available nutrients from the soil affecting the crop. Hence conversion period is mandatory before application in soil. Tricho-compost provides a stable organic matter that improves the physical, chemical, and biological properties of soils, thereby enhancing soil quality and crop production. When correctly applied, compost has the following beneficial effects on soil properties, thus creating suitable conditions for root development and consequently promoting higher yield and higher quality of crops.

E. Tricho-compost and Tricho-leachate for soil borne disease manage of vegetable crop

These two products are found to be effective to reduce diseases and increase yield of vegetable and fruit crops. A numbers of adaptive trials were conducted during 2012 to

2016 for validation of Tricho-compost and-leachate in vegetable crops at farmers' level (Picture 1, 2 & 3). Application of Tricho-compost and-leachate reduced bacterial wilt about 45-72.5% in eggplant and 79.5% in summer tomato. *Fusarium* wilt was reduced about 66.6-84.8% in cauliflower. *Phomopsis* blight of brinjal was reduced about 50.1-65.7%. *Phytophthora* fruit rot of pointed gourd was reduced about 33.7-36.8%. Anthracnose disease of country, bottle gourd and chilli was reduced about 37.6-52.8% in summer country bean, 58.7-6.6% in winter country bean and 27.3-48.6% in bottle gourd. Yield of brinjal, tomato, pointed gourd, bottle gourd, summer country bean, winter country bean, cauliflower and cabbage about 20.2-37.0%, 27.5%, 20.7-42.4%, 28.2-31.8%, 29.5-33.5%, 20.0-30.4%, 18.2-38.0% and 18.5%, respectively were increased.

BCR was increased by 19.7 - 32.1% in pointed gourd, 24.3-27.8% in bottle gourd, 28.1-31.9% in summer country bean, 20.0 - 30.4% in winter country bean, 20.2-31.3% in eggplant, 18.2-38.8% in cauliflower, 20.5% in cabbage and 21.4-28.4% in cucumber due to application of Tricho-compost and-leachate. On the other hands, application of Tricho-compost reduced Urea, TSP, DAP and MoP fertilizer by 18.1-41.6%, 16.6%, 16.6-33.3%, 18.7-33.3% depending on soil type and crop variety.

F. Management of soil health using Tricho-compost and Tricho-leachate

Beside disease control, Tricho-compost is a source of organic matter. Tricho-compost contains as much as 20% organic carbon and considerable amounts of as many as 11 elements, such as Nitrogen (N-1.2%), Phosphorus (P_2O_5 -1.41%), Potassium (K_2O -0.93%), Sulphur (S-0.24%), Calcium (Ca-1.71%), Magnesium (Mg-0.4%), Boron (B-0.01%), Copper (Cu-0.01%), Iron (Fe-0.12%), Manganese (Mn-0.026%), and Zinc (Zn-0.02%). Tricho-leachate, on the other hand, contains 2.05% organic carbon, and five kinds of elements, such as Nitrogen (N-0.01%), Phosphorus (P_2O_5 -0.05%), Potassium (K_2O -0.50%), Sulphur (S-0.10%), and Zinc (Zn -0.003%). As Tricho-compost is highly rich in various elements, it can enrich soil fertility as well as provide nutrition to the crops. Application of Tricho-compost also increases the water-holding capacity of the soil directly by binding water to organic matter, and indirectly by improving the soil structure, thus improving the absorption and movement of water into the soil. It helps bind the soil particles into crumbs by the fungi mycelia contained in the compost and stimulated in the soil by its application, generally increasing the stability of the soil against wind and water erosion. Improves soil aeration and thus supplies enough oxygen to the roots and escapes excess carbon dioxide from the root space.

G. Advantages of Tricho-compost over other organic composts:

Tricho-compost increase soil pH that may create an unfavorable condition for bacterial diseases. It contains different volatile substances (H_2S , NO, NO_2 etc) and phenolic compounds that may directly kill or act as repellent for parasitic nematodes. *Trichoderma* acts as phyto-pathogens by competing for i) nutrients and space, ii) modifying the environmental conditions or iii) promoting plant growth and plant defensive mechanisms, iii) as myco parasitism i.e. feed of pathogens hyphae or iv) antibiosis -secrete toxic enzyme which breakdown the cell wall of pathogens. These properties make Tricho-compost superior than other organic compost.



Picture 1. Tricho-compost and Tricho-leachate applied eggplant fields at Jessore



Picture 2. Tricho-compost and Tricho-leachate management bottle gourd field at Bogra



Picture 3. Chemical spray (left) & Tricho-compost and Tricho-leachate management (right) chilli field at Bogra

Production and quality control methods for the development of an effective microbial pesticides as a viable IPM inputs for the management of crop pests and diseases

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Introduction

The feasibility of economic mass production of the selected strain and the development of a stable product are key factors to a successful microbial pest control product. Two phases are distinguished, the development of the production process, and the development of the product, including formulation, quality control , packaging and field testing. The critical technical and economic factors are identified and evaluated for both soil-borne and foliar disease causing pathogens and also various insect pests like cryptic , defoliator , borer and sucking pests . The promising biocontrol agents likes bacteria, fungi and actinomycetes are generally produced by in-vitro method and baculloviruses are produced by in-vivo method whereas entomopathogenic nematodes are produced by both in-vitro and in-vivo methods. Advantages and disadvantages in terms of costs, manageability and versatility are the critical factors for selecting cost-effective production system. The development of a stable microbial product that is able to deliver effective pest control requires a formulation. The four main objectives in formulation are: to stabilize the propagules; to make a user-friendly product; to protect the propagule, once applied; and to minimize risks of exposure to the applicator. Formulation considerations are provided as per formulation function as well as types of pest and pathogens. Field testing is a key phase in the product development which links all steps in the developmental process. It provides information on the efficacy of the selected strain, on the quality of the produced propagules, on the formulation, on the optimal application strategy, on efficacy that is necessary for registration, on compatibility, on the implementation of the product in an IPM system, and on the marketability of the final product. The method of field testing is crucial and should reflect “real world” conditions. A cost price model for biopesticides is provided with cost factors involved from production to product, and from product to market.

Since quality plays a vital role for the success of any microbial formulation a special attention would be provided towards the quality of each BCA including mother culture maintenance , production process maintenance and finally product quality. Finally, the performance of different biocontrol products under real field conditions to be shared for better understanding of a user for their proper handling and usage to make them an effective IPM inputs for the future agriculture for mitigating the hazardous impact of deadly chemical pesticides.

Production of Antagonistic Fungal Bioagent

Trichoderma viride

Trichoderma viride besides other species of the genus, is an omnipresent saprophytic fungus. Its colonization in rhizosphere of crop plants renders long time protection against diseases, improved growth of plants besides imparting resistance. Such species as *T. harzianum*, *T. hamatum*, *T. lignorum*. *Trichoderma* bio fungicides are highly effective against the powdery mildew, the grey and the white rot, the mildew and other diseases. Active components of biopesticides made from this fungus are their spores, mycelium and metabolites. *Trichoderma* is able to suppress more than 60 species of pathogens (*Pythium*, *Botritis*, *Phoma*, *Sclerotinia*, *Fusarium*, *Ascochyta*, *Alternaria*, etc.) on different plants (cucumbers, tomatoes, cabbages, peppers, various ornamentals, cereals and grain legume crops).

Taxonomy, Identification and distribution Kingdom: Fungi

Class: Sordariomycetes

Order: Hypocreales

Family: Hypocreaceae

Genus: *Trichoderma*

Species: *viride*

Trichoderma viride is widely distributed in the world and found in substantial numbers in nearly all agricultural soils and in other environments such as decaying wood, etc.

Mother culture:

Colony of species fulfilling the criterion of mass production which will be used for further propagation by sub culture.



Fig. 1: A microscopic view of *Trichoderma viride*

- **Obtaining pure culture:** Obtain pure culture of *Trichoderma* from reputed institute or do in-house isolation after collecting soil sample from different agro-condition.
- **Bio-efficacy check:** Check the bio-efficacy of the mother culture by dual culture technique and if the culture shows > 80 % mycoparasitization against target pathogen, Select it for mass production.
- **Procedure of dual culture technique (mycoparasitism):**
 - Pour PDA media in 90mm dia. petri plates and divide the petri plates into four quarters.
 - Inoculate the opposite quarters with 1mm dia. of host (pathogen) as well as antagonistic pathogen.

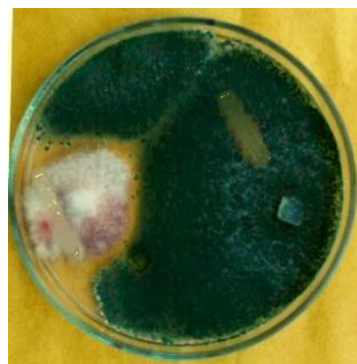


Fig. 2: A view of mycoparasitism of *Trichoderma* on *Fusarium*

- Keep control petriplate for both host pathogen and antagonistic bioagent on separate PDA petri plates.
- Incubate for a week at 28⁰ C.
- Compare the growth of both host and pathogen with control petri plates of both host and pathogen.
- **Isolation-Purification:** From dual culture isolate and purify the starter culture by using TSM media and SS (single spore) isolation technique. Do further sub-culturing on PDA media for.
- **Preservation:** To guard accidental loss of culture follow two methods of storage.
- **Short-term:** Keep fully sporulated PDA slopes at 4-8⁰C conditions and use them for regular production purpose.
- **Long-term:** Store under mineral oil.

Production process: A biphasic production process is being discussed here for the production of *Trichoderma viride*. This could be a cost-effective production method of other fungal bioagents (Ghosh and Jayanth, 2003). The process includes.

- **Plate culture:**
 - Prepare the PDA media from potato or use commercially available PDA (Himedia) @ 39 gm /litre of single distilled water in a 250ml conical flask. Close the mouth of flask with non-absorbent cotton plug and seal it with aluminum foil. Autoclave it for 15 minutes at 15 psi.
 - Bring the media at 40 to 50°C and pour in hot sterilized dry Petri plates (90mm dia.) @ 20ml/ petriplates under Laminar air flow chamber and allow for solidification under UV light for 45 minutes.
 - Under total aseptic condition of Laminar air flow chamber, inoculate a small quantity of culture at the center of the petriplate from pure slant of *Trichoderma* by the help of inoculation loop.
 - Incubate the petriplates at 26±2⁰C in incubator for 4 to 6 days. Properly conidiated petriplates will give a dark green appearance for *T. viride* and pale green for *T. harzianum*.
- **Preparation of liquid broth inoculum**
 - Use 5 litre conical flask to prepare liquid broth inoculum.
 - Prepare liquid medium by using readymade PD broth or by soaking 100 gm potato or tapioca/sago in 2 litre single distilled water for 12 hrs. at room temperature.
 - Add 18 gm Yeast Extract Powder and 8gm Ammonium Nitrate in soaked medium.
 - Check the pH of the medium (6-7).
 - Close the mouth of the conical flask by non-absorbent cotton plug and seal it with aluminum foil.
 - Autoclave the media in commercial autoclave for 30 minutes at 15 psi.

- Bring the media to room temperature and inoculate the petriplate culture under highly aseptic condition @ one petriplate culture /Conical flask
- Incubate the liquid medium by placing the conical flask on heavy-duty mechanical shaker (130 to 140 rpm) for 80 to 100 hrs. (10-12 working days) at room temperature.
- Visually check the growth of culture on the wall of the conical flask and also the change of broth colour from whitish cream to light green.
- **Aerial conidiation on semi-solid substratum fermentation (SSSF)**
 - Use a tray method (50cm L x 30 cm W x 8.5cm H) for aerial conidiation of *Trichoderma* on semi-solid substratum.
 - **Preparation of semi-solid culture medium**
 - Use 5l. Conical flask to soak 120gm tapioca/sago in 4-litre tap water for 12 hrs.
 - Add 40 gm agar shreds/powder to the soaked medium and close the mouth with non-absorbent cotton plug and seal it with aluminium foil.
 - Place the conical flask in autoclave and sterilize it for 30 minutes at 15 psi.
 - Bring the temperature of the media to 40-50°C
 - Pour the media in the plastic tray (@ 700 ml / tray) and allow it to solidify for about 45 minutes under UV light.
 - Inoculate liquid broth culture on semi-solid medium @200 ml/tray. (1 flask covers 10 trays)
 - Incubate the inoculated trays at $28 \pm 2^{\circ}\text{C}$ temperature and 60 to 70 % RH for 4 to 5 days under semi-dark condition.
- **Harvesting of fermented biomass from tray:**
 - Select properly conidiated trays with uniform green look appearance.
 - Collect the total tray material, having pigmented aerial conidia, mycelial mat and nutrient base in a table top mixer by a spatula and make a jelly like biomass by blending it.

Formulation process:

- **Mixing:**
 - Mix fermented biomass along with carrier with the help of a power operated powder-paste mixer (100 -150kg cap.).
 - For making final product directly from fermented biomass, mix fermented biomass with carrier in ratio of 1:3.
- **Drying:**
 - Immediately after mixing the product will have around 20-25% moisture content which should be brought down upto 8% at the time of packing.

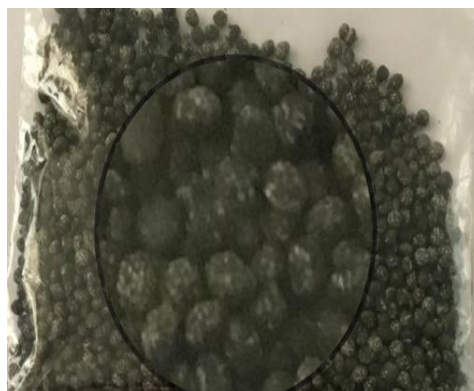


Fig. 3: A granular formulation of *Trichoderma viride*

- The drying process is being carried out on a polythene sheet for about 24hrs under the roof by creating artificial air-flow by the help of fan.
- Adding adhesive agent: After 24 hrs. of drying when moisture level comes down to <8% add adhesive agents @ 0.5% (Carboxymethyl cellulose or Gour gum can be used as adhesive agent).
- Packaging of the product in particular pack. Use milky white polythene pouches for primary pack to prevent the adverse effect of light on the active ingredient of the product.
- **Precautions**
 - Mixed material never be dried under sun or in oven.
 - Adhesive material should be added prior to pack (after checking moisture).
 - Talc powder should have 350-400 mesh size with high magnesium content otherwise mixing will create problem by making hard ball like structures in the powder-paste mixer.

Quality control of final product

Quantitative Analysis: Assess the number of propagules in final product to meet to product specification

Colony forming units (CFU):

Table-1: Composition *Trichoderma* selective media

Sl. No.	Media	Quantity / Litre (gm)
1.	Magnesium Sulphate	0.20
2.	Pot. Di hydrogen orthophosphate	0.90
3.	Pot. Chloride	0.15
4.	Yeast	1
5.	Sucrose	3
6.	Agar	20
7.	Chloramphenicol	0.25
8.	Rose Bengal	0.15
9.	Captan	0.02

Procedure:

- Take 1 gm of sample and mix it in 10 ml of sterilized water in a clean and sterilized vial (1: 10)
- Shake well by vortex and take 1 ml of the suspension to 9 ml of sterile water in a vial (resulting dilution: 1: 100).
- Make seven more serial dilutions to arrive at a dilution of 1: 10⁸.
- Transfer 1 ml of this suspension to a sterile petri dish.
- 10 ml of sterile, cool, molten Trichoderma selective medium is poured into the plate.
- The plate is gently rotated to ensure the even distribution of the sample and allow it to cool.
- Incubate the plate at 25°C for 3-4 days.
- The number of colonies of each kind is counted with a colony counter. For analysis, calculate the number of colonies per ml of the original dilution as follows:

Colonies per gram or ml of sample =

(No. colonies [average of 3 replicates]) / (amount plated out) * dilution factor

- This number is then multiplied by the dilution factor to find the total number of cells per gram or ml of the original sample. A standard, talc-based product (WS or WP) of *Trichoderma viride* should have a count of more than 2 x 10⁶ CFU per gram.

Qualitative Analysis: To understand how to do qualitative analysis of the finished Trichoderma product

1. Colony Morphology:

2. **Product Performance Analysis(PPA):** Having known the product is meeting the requirement in terms of quantity of active molecule present per gram (through quantitative analysis) and the colonies obtained are identified (with help of microbiological test), it is important to know about their capacity to produce the desired result in the field. To assess this, we have to plan the following test.

a) Dual culture (Mycoparasitism):

Procedure:

- Take a 1mm dia. small disc of the freshly culture pathogen and Product culture and placed them just opposite to each other in the PDA Petri plates (for Pathogen and Trichoderma).
- Similarly, inoculate only pathogen in another PDA plate as control.
- Incubate the Petri plates for 4-5 days at room temperature at 25°C.

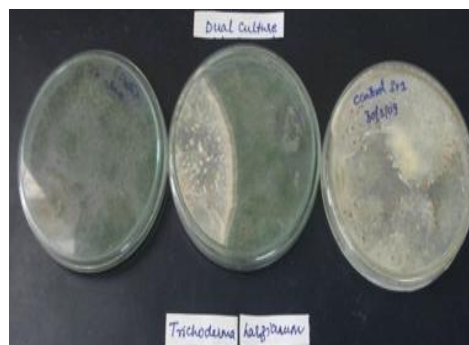


Fig. 4: A dual culture technique to assess mycoparasitism

- Calculate the % inhibition of pathogen growth in the treated plate compared to the control plate of the pathogen.

$$\% \text{ inhibition} = (C-T)/C * 100$$

whereas *C* = Growth of the pathogen in control plate and *T* = growth of the pathogen in the Treated plate.

- b) Germination test
- c) Plant emergence test
- d) Rhizosphere colonization test
- e) Plant vigor test



Fig. 5: Germination test



Fig. 6: Plant emergence test



Fig. 7: Plant vigor test

Production of Entomopathogenic Fungus (EPF)

Entomopathogenic fungi (EPF) such as biological control agents can be used as a component of integrated pest management (IPM) of many insect pests. Under natural conditions, these pathogens are a frequent and often cause natural mortalities of insect populations. There is a high potential for the use of Hyphomycetes such as *Metarhizium* or *Beauveria* for biological control because such fungi can be cost-effectively mass-produced locally, and many strains are already commercially available. Study was conducted to evaluate the mass production of fungal spores using the two-stage technique liquid-solid fermentation developed for the LUBILOS which is particularly well adapted to the production of EPF's *B. bassiana* and *M. anisopliae*. Rice is used as a solid substrate in this experimental system. The fungus also grows equally well on maize or other grains. The general procedures for two stage production of *Beauveria bassiana* is given here as reference.

Beauveria bassiana

Beauveria bassiana is a mitosporitic and aggressive entomopathogenic fungus, attacking a wide host range of insects at larval or adult stages. It was the first recorded entomopathogen in world (Bassi 1835). It attacks all stages of insects of all groups. *B. bassiana* occurs world-wide and has one of the largest host lists among imperfect fungi. It occurs as a ubiquitous saprophyte in soil (Bambawale et al 2005). The pathogen is of cosmopolitan nature. The hosts include mainly Lepidoptera, Coleoptera, Hemiptera, Diptera and Hymenoptera.

Taxonomy, Identification and distribution of common species

Valid scientific name: *Beauveria bassiana* (Balsamo) Vuillemin.

Kingdom: Fungus

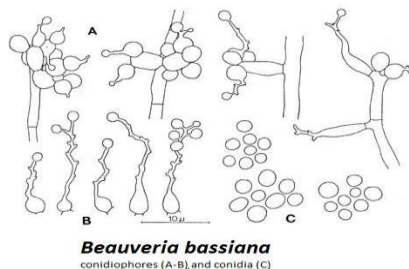
Class: Sordariomycetes

Order: Hypocreales

Family: Clavicipitaceae

Genus: *Beauveria*

Species: *bassiana*



Biology of *Beauveria bassiana*

B. bassiana is an anamorphic fungus with septate hyaline mycelium. Colonies appear white in cultures or tufts of white mycelium bearing masses of powdery spores. Conidiophores bear conidia in acropetal succession. The conidiospores are tiny, measuring only a few microns. A spore is produced at the tip of the mother cell and the growth of the mother cell ceases. A new growing point initiates just below this terminal spore, grows past it and a second spore is produced at a higher level. This uses up the new growing point and a third growing point is then initiated just below the second spore. Every time a spore is produced the hyphal tip is used up and a new growing point is produced. In this way a succession of spores is produced with the youngest spore at the tip (= i.e. acropetal succession) and the spore head gets longer and longer. When all the spores are dislodged the spore-bearing tip of the conidiogenous cell has a zig zag appearance and is referred to as a rachis.

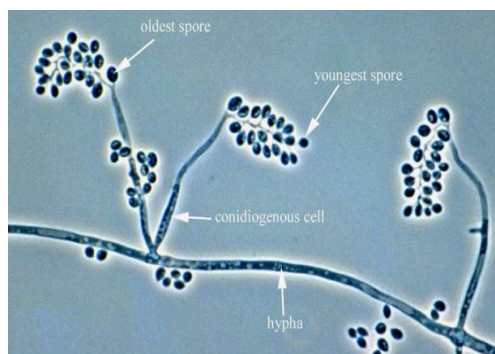


Fig. 8: Microscopic view of *B. bassiana*

Mode of action :

B. bassiana in action as entomopathogen. The action of *B. bassiana* on insects begins from the penetration of spores in a body cavity through dermal coat (cuticle). High humidity or free water is essential for conidial germination and infection establishes between 24 and 48 hours. Having penetrated in a body the spores germinate in hyphae, then a mycelium overgrows from which conidia split off. Having proved in the body the conidia begin to circulate in haemolymph. Mycelium gradually fills up the whole body

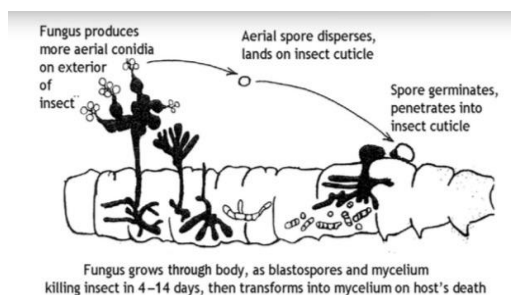


Fig. 9: Diagrammatic representation of infection process of EPF into insect body

of the insect. In the beginning muscular tissue is affected. Fungus growth continues until all the tissues are destroyed. The infected insect may live for three to five days after hyphal penetration and, after death, the conidiophores bearing conidia are produced. The fungus can form conidiophores, which rupture the cuticle and the envelope of a dead larva. The affected insect is covered with white, wadded coating (conidiophores). Then it is observed spore maturation, and mass sporulation begins. The spores are tiny, measuring only a few microns. Colonies appear as tufts of white mycelium bearing masses of powdery spores burst out through the body parts of infected insects. The effectiveness of *B. bassiana* depends very much on climatic conditions, the methods of application and doses. The best hydrothermal conditions for the development of *B. bassiana* are: a temperature of 25–28°C, a relative air humidity of 80–90%.

Production of *Beauveria bassiana*

Mother culture:

- When mass producing a fungal pathogen, it is essential that the strain is well maintained. It must be free from all contamination and carefully conserved so that it not only remains viable in agar culture, but also retains its virulence.
- If fungal strains are sub-cultured too often on artificial media, they can lose their virulence. This can be prevented by maintaining isolates for storage on Potato carrot agar (PCA) slopes, which may be kept in the refrigerator for between six months and one year.
- The spores from these cultures can then be used to inoculate working cultures on Sabouraud dextrose agar (SDA) slopes (the recipes for these media can be found below).
- In the longer term, fungal strains should be passed through the original host or closely related species periodically to maintain virulence.

Production process : Diphasic fermentation

Phase -1 :

Preparation of liquid broth

This is a simple liquid broth which encourages the production of hyphal bodies and mycelium. These can then be used for inoculation into the second (solid) stage of production. Brewers' yeast is added to this medium in a dry, granulated form using the following procedure:

- Mix 20 g dried yeast/yeast extract powder) and 20 g sucrose in 500 ml of tap water.
- Heat the broth in a pan of water for 10-15 minutes, this brings the solution to boiling point.
- Homogenize in a Wareing blender at high speed for 60 seconds or until there are no more lumps of yeast.
- Leave to settle for 2-3 hours or overnight in the refrigerator, so that the foam can settle out, then add a further 500 ml of tap water to make up to one litre.

- Mix the broth gently by hand until the yeast is suspended homogeneously in solution.
- Put 75 ml of the solution into a 250 ml conical flask (This gives optimal aeration during growth of the fungus). If larger conical flasks are used, adjust the amount of liquid medium so that the proportion of liquid to flask capacity is about the same as in the 250 ml flasks above.
- Plug the flasks with non-absorbent cotton wool bungs or polyurethane bungs and cover with aluminum foil.
- Autoclave at 121°C (15 psi) for 20-30 minutes.

Inoculation of liquid broth

- It is essential that each production run has a consistent inoculum.
- Take spores from a stock culture growing on SDA as described above, suspend spores in sterile water containing 0.05% Tween 80. The spore suspension should contain approximately 6×10^6 spores per ml. This can be achieved by adding approximately 10 inoculating loops full of spores to 50 ml of 0.05% Tween water. If you are not sure, take a small sample haemocytometer. When you have done this several times, you will soon be able to recognise the concentration of spores in a suspension by the colour of the suspension and you will no longer need to count the spores to get the right concentration.
- Shake the spore suspension thoroughly and use 1 ml of spore suspension to inoculate each flask (75 ml) liquid medium.
- Check the spore suspension, a sample of uninoculated broth and a sample of inoculated broth for contamination using the contamination check.
- Put the inoculated flasks on the incubated shaker at approximately 150 rpm for three (3) days at approximately 25-30°C (room temperature).



Fig. 10: SS fermentation of *B. bassiana*

Phase- 2:

Preparation of rice substrate bags

- Soak parboiled rice in water for 30 min
- Drain & fill in polythene bags @ 1 kg equally distributed in 5 bags (200 g/ bag)
- Close mouth of bag with cotton plug & tie with thread
- Place about 15 bags (3 kg material) in the pressure cooker and cook with some quantity of water. After 3 whistles reduce the flame and allow heating for 15 min.

- Clean working table with 5% sodium hypochlorite solution & switch on the UV lamp.
- Take out the bags & remove water adhering to the sides with tissue paper and place in a clean tray.
- Switch off UV lamp. Place the bags on working table and allow further cooling.

Inoculation of rice with liquid medium

- Take a small sample of the liquid broth to be used for inoculation for contamination control (CC).
- Take a sample of un-inoculated rice for CC.
- Use one flask of liquid inoculum (75 ml) for each 500 g bag of rice, or if you used 1000 g of rice, dilute the liquid medium with 75 ml of sterile water. Flame the neck of the flask and pour the whole contents of the flask onto the rice in the bag.
- Massage the bag from the outside to evenly distribute the inoculum over all the rice grains.
- Take a small sample of inoculated rice from one bag in every ten that you inoculate for testing the moisture content and for CC.
- Place the inoculated rice bags on the rack in slanting manner for incubation.
- Incubate the bags at room temperature (25-30°C) for about 10 days, during which sporulation will occur. (if temperature goes above 30°C use AC to maintain required temperature for incubation).
- White mycelial growth will be noticed within 72-96 hours of incubation which slowly turn into conidiation.
- Check all the bags for growth of contaminants. Throw away all contents of any bags with any visible contamination. Be careful not to spread contamination and ensure that all the contaminated waste is either autoclaved or burnt.
- Once conidiation over transfer the conidiated substrate into a brown paper bag and allow for drying. Use tray dryer for large scale production.
- Once dried, the conidia can be extracted from the rice.

Extraction of dry conidia from rice

- Carefully pour the dry rice into the top of a metal sieve with a mesh size of 300 µm or less (if the sieve has a receiving tray at the bottom, you should use this to collect the spore powder. If you do not have a receiving tray, the spore powder can be collected directly inside a plastic bag).
- Place the top on the sieve and put the entire sieve assembly into a large plastic bag and tie with a knot to seal it.
- Shake the sieve inside the bag for several minutes, then allow to stand before opening the plastic bag. The spore powder will settle to the bottom of the bag, or if you are using a receiver, the spore powder will have been collected in this.

- Empty the old rice out and add more sporulated rice to the sieve, seal the bag again and shake well.
- Repeat the process until you have sieved all of the rice.
- For larger quantities, use a two-man sieve. This should also be completely contained within plastic during the sieving process. Microreactor/ cone harvester can also be useful for large scale production system which needs some standardization of the process. Carefully transfer the conidia from the bag into plastic boxes or another open container for further drying. The spore powder should not be too deep to ensure that the spores at the bottom will dry as well as those on the top.

Final drying of the conidial powder

- The extracted conidial powder should be dried down to 5% moisture content before being stored in a refrigerator. This can be done on a relatively small scale using a standard glass (or plastic) desiccators by using dried silica gel at the base. The conidial powder can be placed in the desiccator to dry for 5 days.
- Fresh dry silica gel beads (non-indicating if possible) should then be added to the conidial powder (these may be contained in small packages made from muslin or nylon netting) at a rate of 20% w/w.

The whole product may then be stored in a refrigerator or cold room or a deep freeze until use.

Quality control of pure spores

Quality control should be carried out both during the production process and ultimately, on the end product. Both of these aspects of quality control are essential for the successful production of good quality viable conidia which are free of potentially dangerous contaminants.

- Quality control procedures carried out during the production process and monitoring for the entrance of contaminants into the system have been incorporated into the production process in order to help in locating the source of these contaminants when detected (Contamination Control, CC).
- Quality control carried out on the final product (the spore powder) is designed to ensure that the product meets pre-determined specifications. These include viability, virulence, moisture content (for long shelf-life), the number of infective propagules per g product, particle size spectrum (suitable for the application equipment) etc



Fig. 11: Conidiated rice substrate of *B. bassiana*



Fig. 12: Conidiated rice substrate of *M. anisopliae*



Fig. 13: A simple method to harvest dry fungal spores from conidiated rice substrate by using multiple sieves



Fig. 14: Pure and dry spores of *B. bassiana*



Fig. 15: Pure and dry spores of *M. anisopliae*

Formulation

Formulation of entomofungal propagules is the most challenging considering their mode of action which is primarily by adhesion to the outer parts of an insect and penetration through the cuticle. The infection process is complicated compared to bacteria and baculoviruses with which infection takes place after take-up per oral, or compared to the active search and penetration behaviour of EPNs as part of the infection process. Formulation of fungi poses challenges related to targeting, persistence, protection against harmful environmental factors, growth stimulation and enhancing agents. Generally, formulations of fungal products contain spores, either conidiophores or blastospores, sometimes mycelial particles as active ingredient along with different additives. Commonly found different types of entomofungal formulations are:

- **Liquid formulations:** Fungal spores are used as active part along with different additives like wetting agents, dispersing agent thickener, antifreeze agents, stabilizer, thickener and carrier material as oil or water. Different form liquid formulations are
 - Suspension concentrate (SC)
 - Aqueous formulation (AS): Spores as active ingredient, sterilized water and thickener and stabilizer.
 - Oil dispersion (OD): spores, oil, emulsifier

- Ultra-low volume suspension (ULV)
- Dry formulations : Fungal spores are used as active ingredient along with different additives like dispersing /wetting agent , nutrient, starch as binder, inert filler material as carrier material. Different dry formulation are
- Wettable powder (WP)
- Granular (GR)
- Water dispersible granule (WDG)

Oil-based formulations of *M. anisopliae* var. *acridum* for locust control have proved to be a major breakthrough, enabling the usage of this fungus in hot and dry environments with ULV applications (Bateman et al. 1993). This formulation technology has several advantages that made it so successful shelf-life is quite long for a mycoinsecticide (>18 months at 17°C); good persistence in the field; enhanced biological activity; and usage by conventional equipment at ultra-low volumes (Bateman 1997). Oil based formulation enhances the spore persistence and secondary pick-up from plants plays a greater role in developing infection. Myco-jaal is a similar oil-based suspension concentrate (SC) formulation of *B. bassiana* for diamondback moth which is commercially available in India (Ghosh et.al. 2007).



Fig. 16 : Oil based SC formulation of *B. bassiana*



Fig. 17: Oil based SC formulation of *M. anisopliae*



Fig. 18: A granular formulation of *M. anisopliae*

Quality control of entomopathogenic products

1. **Germination test:** Fungal spores are living organism. The viability of the fungal spores diminishes with time depending in the conditions in which they are kept . It is therefore Weigh SDA media (dehydrated media) as recommended.

Procedure :

- Sterilize the media with chloramphenicol antibiotic solution in the pressure cooker for 1 hr at 160°C or use autoclave and sterilize for 20 min at 15 psi.
- Remove from the pressure cooker /autoclave and cool the media till warm.
- Lay out sterile glass petri plates in the laminar air flow chamber and pour the media to the petriplates (approx-15 ml) and allow it to solidify.

- Make the spore suspension and use cyclo mixer to mix thoroughly the product with water.
- Take the spore suspension as low at 104-105 / ml
- spread 0.1-0.2 ml spore suspension on the media and put the lid of the Petri plates.
- Using a microscope check the spore are well distributed.
- Incubate the plates at 25°C+ 2°C for 20-24 hrs, then using a microscope count the number of germinated spores and non-germinated spores.
- Count at least 300 germinating and non-germinating spores by moving the field view of the microscope so as to cover a large area of the petri plate.
- Calculate the percentage of germination as follows:

$$\% \text{ germination} = a / (a + b) \times 100 \text{ where } a = \text{germinating spores and } b = \text{non-germinating spores}$$

- Repeated the observation for 3 times on the same petriplates or 3 petriplates may be set up at the same time.
 - Finally calculate the rate of germination by average of three observation.
2. **Enumeration of colony forming units (CFU'S):** This test gives an estimation of viable spores present per unit quantity (gm or ml) of sample and helps to determine the effective dosage for application.
- Procedure :** Follow same procedure as mentioned in Trichoderma section.
3. **Product efficacy studies :** This study helps to understand the product potency of creating infection to target pests.

Procedure

- Fresh undamaged radish/cabbage leaves free from pesticide application are to be collected and washed thoroughly in sterile distilled water and air dried.
- Dilute the test formulation in different concentrations as recommended.
- Individual leaves are dipped in respective test concentration for 30 sec.
- After complete drying of leaves minimum 10 nos. of early 3rd instar test larvae to be released on the treated leaf.



Fig. 19: *B. bassiana* infected larva of *H. armigera*

- A water dipped radish/cabbage leaf disc is to be maintained simultaneously as control.
- To prevent dessication of leaves the petiole of radish/cabbage leaves are to be moist with cotton swab. If studies are carried out on cabbage leaf disc, each treated leaf to be placed in a plastic container of 50-60 diameter containing moist filter paper, wattman No. 41 to be provided for humidity.
- Each treatment should be replicated in thrice. If necessary, fresh leaves to be provided as feed at 48 hrs interval.
- Total experiments set up has to be maintained at $25 \pm 100^\circ\text{C}$ and 70-80% RH for 7 days.
- Observation on larval mortality to be taken on 1,3,5,7 days after treatments.
- Mortality due to test fungus to be confirmed by keeping the dead insects in sterilized humid camber for 48 hrs.
- Data to be analyzed to assess the virulence of the fungal isolates/product against the target insect.

(Note: Diphasic production process can be followed to produce other entomofaunal bioagents like *Metarhizium anisopliae*, *Verticillium lecanii*, *Paecilomyces fumoroceus*, *Hirsutella thompsonii*, *Nomurea riley*).

Mass production of antagonistic bacterial bioagents as biofungicides

Pseudomonas fluorescens

A Gram-negative bacterium with antifungal properties and used to protect against pathogenic fungal diseases of fruit and other crops. *P. fluorescens* has multiple flagella. It has an extremely versatile metabolism, and can be found in the soil and in water. It is an obligate aerobe, but certain strains are capable of using nitrate instead of oxygen as a final electron acceptor during cellular respiration.

Taxonomy, Identification and distribution of common species

Kingdom : Bacteria

Phylum : Proteobacteria

Class : Gammaproteobacteria

Order : Pseudomonadales

Family : Pseudomonadaceae

Genus : *Pseudomonas*

Species : *fluorescens*

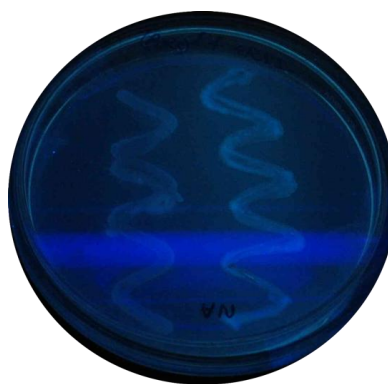


Fig. 20: A fluorescens appearance of growing pseudomonas culture under UV illumination

Mode of action:

- Antibiotic production
- Siderophores production
- Induced systemic resistance
- Competition
- Hydrogen cyanide production
- Plant growth promotion by producing phytohormones.

Production process:

Mother culture Establishment:

1. Collection of soil from different ecosystem:

- Collected soil from the healthy plant of the infected plot/ Area.
- The soil intimately adhering to the roots was collected and mixed to provide a composite soil sample

2. Isolation & Purification method:

- Take 10g of soil from each soil sample and mix thoroughly with 90ml of saline water in a conical flask.
- Agitated the soil suspension for 15 minutes on a vortex and then do serial dilutions up to 10^{-5} .
- Spread 0.1ml of suspension of different dilution (10^{-3} to 10^{-5}) on sterilized petri plates containing specific culture media like for Pseudomonas agar for fluorescein (Pseudomonas).
- Incubated the petriplates at room temperatures ($28^{\circ}\text{C} \pm 2^{\circ}\text{C}$) for 24-72 h. Three replicates to be maintained for each dilution.
- Examine the petriplates daily up to 3 days for the confirmation of bacterial colonies.
- Confirm the culture purity by subjecting different biochemical and qualitative analysis.
- Take a loopful of inoculum from the bacterial colony.
- Inoculate approximately one-third of the agar surface (at the edge) using the infected inoculating loop (without scratching the agar).
- Cross over the streaks of the first inoculation when streaking the second part of the agar surface.
- Flame and cool the loop again before repeating the streaking process on the third part of the agar surface. (Quadrant streaking method.).
- Incubate the culture at 28°C for one week.
- Check the growth for bacteria.

3. **Sub-culturing:**

- Place both test tubes in one hand to form a V-like shape. Take the inoculating loop in the other hand and flame it over a Bunsen burner.
- Remove the cotton plug from the test tubes, and briefly flame the neck of the tubes.
- Pick up a loopful of bacterial cells from the culture using the sterile inoculating loop and inoculate the surface of the sterile agar slant in zigzag streaks.
- Re-flame the necks of the tubes, close with cotton plug and re-flame the inoculating loop.
- Allow to incubate at room temperature.

4. **Culture preservation method:**

- **Short-term preservation:** The simplest way to preserve a culture is to add 15 % glycerol to the culture and then to store it at -20°C or -80°C in a freezer. Cultures can be preserved for a number of months in glycerol, at a temperature of -40°C in a freezer.
- Long-term:
 - (a) Lyophilisation.
 - (b) Freeze drying method.

Production of *Pseudomonas fluorescens* by liquid fermentation process:

1. **Lab Scale:**

- The viable slant culture to be taken out from preservation
- A loopful of mother slant culture to be inoculated in the specific sterilised medium of 30ml in 250ml conical flask. For *Pseudomonas fluorescens* king's both medium used as standard media.
- Allow the Inoculated broth for incubation on a shaker for 48-72 hrs at room temperature.
- Once incubation is completed draw sample to check the purity of the culture through different Quality parameters.
- Select / Stored the only the pure starter culture for next step production process as an inoculum.

2. **Large Scale:**

- Generally, the Up scaling of bacterial production is getting done in fold from one step to next.
- Similarly, to make seed culture the viable starter culture is getting used as inoculum for 300ml of sterilized seed culture and allowed for incubation.
- Based on capacity, this seed culture once again is getting inoculated in 3000litre of sterilized culture media in 5litre conical flask and allowed for incubation.

- Once incubation is completed the culture is being subjected to quality assessment and formulation process or used as inoculum for commercial scale production by using bioreactor.
3. **Formulation process:**
- Aqueous Formulation:** Once the Fermenter sample has been approved from the Quality Department, the formulation process starts by adding protective additives like glycerol, PVP. If the culture is highly concentrated it need to be diluted by addition sterilized water to make as per specification.
 - Wettable Powder Formulation:** For Powder formulation Talc powder (Magnesium silicate) is used as a Carrier material and its specified particle size should be 350-380 mesh. The viable Fermenter broth is used as active ingredient during formulation by using a ribbon blender machine. During formulation the culture is mixed at the ratio of 25-30% of the carrier material. Once the formulation is done, sample has to be drawn for its quality assessment.

Quality control

Quantitative analysis:

Colony forming units : These are the number of live organisms present in the product and are determined by serially diluting the product and plating the specified dilution in a suitable media to quantify.

Procedure:

- Take 1gm sample and mix it in 10ml of sterilized water in a clean and sterilized vial (1:10)
- Shake well in vortex and take 1ml of the suspension to nine ml of sterile water in a vial (1:100).
- Make seven more serial dilutions to get 1:108 dilutions.
- Transfer 1ml of this suspension to a sterile petridish.
- 10ml of sterile, cool, molten KING'S MEDIA BASE AGAR (KMBA) medium is poured in the plate.
- The plate is gently rotated to ensure the evenly distribution of the sample and allowed to cool.
- Incubate the plate at 30oC for 48hours.
- The number of colonies of each kind is counted in COLONY COUNTER.

Pseudomonas selective medium (KMBA)	
Ingredients	Quantity
Peptone	20g
Di-potassium Hydrogen Phosphate	1.5g

Magnesium Sulphate	1.5g
Glycerol	10g
Agar	15g
Distilled water	1lt
Cycloheximid	100ppm
Ampicillin	50ppm
Chloramphenicol	12.5ppm

Analysis: -Calculate the number of colonies per ml of the original dilution as follows
Number of colonies (average of 3 replicates) Colonies /gm or ml of the sample = ----- Amount plated x dilution.
This number is then multiplied by the dilution factor to find the total number of cells per ml of the original sample. A standard product which is 1% W.P. <i>Pseudomonas fluorescens</i> should have 2×10^8 cfu/gm.

Qualitative analysis:**Morphological tests :****1. Colony Morphology**

- Serial dilution of the test sample is done and 1ml of this dilution is then pour plated.
- Incubate the plates for 24-48hrs in the incubator at 30 degrees
- Study the colony morphology of the organism on the media.
- The standard product containing *Pseudomonas fluorescens* contain round & mucoid colonies.

2. Gram staining**Procedure :**

- Apply 1 or 2 loopfuls of broth to a clean slide. DO NOT spread out drops. Air dry sample.
- If Sample is from PLATE: Place a loopful of tap water onto a slide. Touch a colony with a loop and mix the bacteria with the water until there is a uniform, thin film of microorganisms on the slide. DO SPREAD out the liquid as much as possible. Air dry sample.
- Heat fix the slide. Allow the slide to cool.
- Stain with Crystal Violet for 1 minute by flooding the slide with stain. Rinse with water.

- Apply Gram's Iodine solution for 1 minute by flooding the slide with iodine. Rinse with water.
- CAREFULLY Decolorize the slide by flooding with decolorizer(Alcohol). IMMEDIATELY, rinse with water.
- Counterstain with Safranin for 30 seconds by flooding the slide with stain. Rinse with water.
- Dry the slide by putting it between the pages of a book.
- Remove slide and view organisms using the oil immersion objective of your microscope.

The standard product containing *Pseudomonas fluorescens* contain colonies which appear as Gram negative(Pink) Rods.

3. Motility test

Procedure

- Apply Vaseline around the depression of the hanging drop slide.
- Using the inoculation loop, aseptically transfer one drop of the culture to the center of a cover slip.
- Invert the hanging drop slip and center its well over the drop of the culture to the center. Press down on the edge of the cover slip so that the Vaseline makes a firm seal.
- Quickly and carefully turn the slide right side up so as to suspend the hanging drop in the well. Don't let the drop fall or touch the bottom of the well.
- To examine, first locate its edge in center of the microscopic field with low power objective and markedly lower the light. The edge will be visible as a bright wavy line against a dark background. Now the slide can be focused under oil immersion.

The standard product containing *Pseudomonas fluorescens* contain bacteria which appear as fast motile microbe:

Pigment Production

- Inoculate the test organism on the media / in broth in an aseptic condition.
- Incubate at 37°C for 24-48hrs.
- Observe for the colour change in the media under ultra-violet light.
- Colour change is due to the pigment production by the organism.

The standard product containing *Pseudomonas fluorescens* contain colonies that give fluorescence appearance:

Endospore staining:

- Prepare a thin smear on a clean slide.
- Place the slide on staining rack above boiling water.
- Cover the smear with small pieces of paper towel, keep saturated with malachite green (5% aqueous solution) and continue heating for 5 mins.

- wash gently with water.
- Counter stain with safranin for 30 secs.
- Wash with water and blot dry.
- Examine under oil immersion objective

The standard product containing *Pseudomonas fluorescens* contain colonies that does not contain endospore so green colour will not appear at center.

Biochemical test:

1. **Catalase:** Metabolic reactions that occur in the presence of water and oxygen often result in the formation of hydrogen peroxide (H₂O₂). This compound is toxic to cells. Therefore, most organisms that can grow in the presence of oxygen possess catalase, an enzyme that converts hydrogen peroxide to water and oxygen.
2. **Oxidase:** Cytochrome oxidase C is an enzyme that reduces (adds electrons to) oxygen. This enzyme, therefore, is an oxygen reductase. Cytochrome oxidase is the last step in the electron transfer system in most aerobic organisms. It transfers electrons from the electron transport chain to oxygen, forming water in the process. Not all aerobic organism, though, possess cytochrome oxidase. Some species possess alternative electron transport molecules. Because this enzyme is present in some, but not all bacteria.
3. **Gelatinase:** The standard product having *Pseudomonas fluorescens* contain colonies that will hydrolyse gelatine and liquidity it.
4. **Starch (Amylase) Hydrolysis:** The standard product of *Pseudomonas fluorescens* contain colonies that are capable of digesting starch hence the result will be positive.
5. **Caseinase test:** Casein is the principal protein of milk. It exists as a colloidal suspension that gives milk its opaque whiteness. Many bacteria are equipped with caseinase enzyme that hydrolyses this protein. The standard product of *Pseudomonas fluorescens* contain colonies that will undergo a chemical reaction resulting in a clear zone.
6. **Siderophore Test :** *Pseudomonas fluorescens* secretes siderophore which binds to iron in the environment and carries it back to the bacterial cell. The siderophore-iron complex actually interacts with a receptor on the bacterial cell membrane when delivering the iron to the bacterial cell. If bacteria are growing in a medium or environment where iron is scarce, the bacteria secrete large amounts of the fluorescent siderophore to capture and deliver iron to the bacteria. Conversely if bacteria are growing in a medium or environment rich in iron. An abundance of iron represses the formation of siderophore receptors on the bacterial cell membrane. The standard product of *Pseudomonas fluorescens* contain colonies that will not produce fluorescens on fortified nutrient agar media with iron.

Performance analysis:

Having known the product is meeting the requirement in terms of quantity of active molecule present per gram (through quantitative analysis) and the colonies obtained are

identified (with help of microbiological test), it is important to know about their capacity to produce the desired result in the field. To assess this, we have to plan the following test.

1. Germination / Plant emergence
2. Plant vigour
3. Root colonization.

(Note: Same production procedure can be followed for *Bacillus subtilis* which is very promising BCA for different foliar diseases like powdery mildew. Downy mildew)

Mass Production of Entomopathogenic Bacterial Bioagents as Bioinsecticides

Bacillus thuringiensis

B. thuringiensis is a common Gram-positive, spore-forming aerobic bacterium that can be readily isolated on simple media such as nutrient agar from a variety of environmental sources including soil, water, plant surfaces, grain dust, dead insects, and insect feces. Its life cycle is simple. When nutrients and environmental conditions are sufficient for growth, the spore germinates producing a vegetative cell that grows and reproduces by binary fission. Cells continue to multiply until one or more nutrients, such as sugars, amino acids, or oxygen, become insufficient for continued vegetative growth. Under these conditions, the bacterium sporulates producing a spore and parasporal body which is composed primarily of one or more insecticidal proteins in the form of crystalline inclusions, causing mortality of insects.



Fig. 21 : Bt infected larva of *Spodoptera litura*

Taxonomy, Identification and distribution of common species

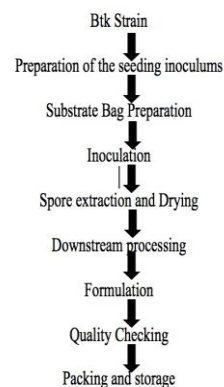
Kingdom : Bacteria
Phylum : Firmicutes
Class : Bacilli
Order : Bacillales
Family : Bacillaceae
Genus : *Bacillus*
Species : *thuringiensis*

Entomopathogenic bacteria can be easily produced in in-vitro systems, although one species, *B. popilliae*, used for the control of the Japanese beetle, can only be produced in its natural host. *Bacillus thuringiensis* is the most successful biopesticide and its three subspecies are produced, and commercial on the basis of these subspecies: Bt kurstaki

against caterpillars, *B.t. israelensis* against mosquito larvae and *Bt tenebrionis* against larvae of the Colorado potato beetle. *Bt* can be produced by (semi-)solid state fermentation, but industrial production is performed by liquid state fermentation. Semi-solid and solid-state fermentation is done on a small scale in developing countries for local use. Downstream processes have become an integral part of *Bt* production and large facilities are needed to accomplish drying of the bacteria sludge by spray-drying and/or fluidized-bed drying. Obviously, this production has become capital-intensive because of its size and technology and it shows that competitive biopesticides can be produced in this way. Here we will highlight an in-house production process of Btk by solid substrate fermentation method.

1. **Virulent isolate of Btk:** Obtain a virulent strain from culture bank of reputed institute and store in refrigerated condition.
2. **Preparation of the seeding inoculums:** 2 loops full of 4-day old Btk culture to be selected to inoculate 500 ml Erlenmeyer flask containing 200 mL nutrient broth with an initial pH of 7.0. and then allow to incubate for 18 hours at $28 \pm 2^\circ\text{C}$ using a rotary shaking incubator shaker 200 rpm
3. **Substrate Bag Preparation:** Based on literature and basic research wheat bran as a substrate for SSF production of Btk.

S.No.	Requirements	Weight/volume (gm/ml)
1	Sucrose/ Maltose	36g
2	K_2HPO_4	3.6g
3	KH_2PO_4	3.6g
4	Yeast	7.2g
5	Soya bean meal	7.2g
6	$\text{D.H}_2\text{O}$	2500ml
7	Wheat Bran	1500g



4. Take 150g of Wheat bran in each polythene bag and then add 250ml of nutritive supplement (as mentioned in the table) into each wheat bran bag. These wheat bran substrates mix thoroughly and seal by sealing machine and insert the 3cm PVC pipe with cotton plug and sterilize at 121°C for 25 min and then allow them to cool down at room temperature.
5. **Inoculation:** Introduce 10% of the seed culture as inoculum in each bag and incubate at $28 \pm 2^\circ\text{C}$ with 50-60% ambient relative humidity (RH). Bacterial load in the inoculum to be assessed after 3-4 days of incubation.
6. **Spore extraction and Drying:**
7. After 72 h of incubation at 30°C , the fermented substrate, having *Bt* spores and crystals are collected into a plastic tub with 800 ml of sterile distilled water.

8. Mixed well the substrate with water and filtered through a muslin cloth. Then the filtrate allow to centrifuge at 10,000 rpm for 10 min. The resulting pellet at the base of centrifuge tubes are collected and then spread in a thin layer on a sterile polythene sheet for air drying for overnight at 30°C under laminar airflow chamber.
9. Once it dry up make fine powder by using kitchen blender or ball mill.
 - Each packet could yield 20 g of Bt technical powder which passed through a sieve (Jayanth test sieve) of 105 µm mesh size to obtain a fine powder of Bt technical.



Fig. 22: Steps involved in production process of Bt through SSF method fermentation

- **Formulation:** Most Bt formulations are wettable powders or water dispersible granules, some are oil-based or water-based or mixtures thereof. Shelf-life is generally 2 years at room temperature. Points of attention here are the concentration of the spores, the drying technology, and the determination of the potency standard. Formulation requirements and formulation types are different depending on the usage of a Bt product, in agriculture, forestry or in aquatic habitats. The large Bt producing companies have developed successful and convenient formulations, but details of commercial formulations are proprietary.

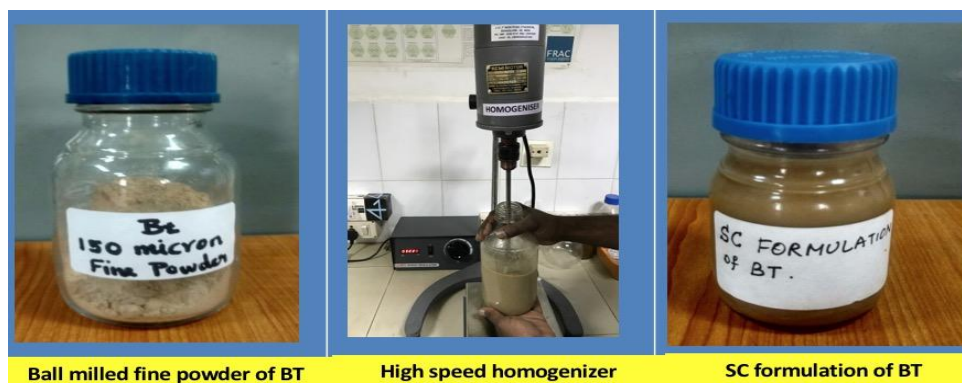


Fig. 23 : preparation of Suspension concentrate of Bt from its pure dry toxin powder

Quality control :

Determination of heat resistant viable spore count of Bt:

- Weigh 1g or 1 ml of the Bt formulation.
- Transfer it in a sterile 250 ml conical flask with 100 ml sterilise water.
- Mix it gently to form a uniform suspension.
- Heat this material in a preheated water bath at 80 °C for 15 Minutes ensure that the vegetative cells for Bt and all other micro-organism killed except the Bt spores.
- Add 9ml of sterile water in each 10 sterile test tubes number 1 to 10.
- Add 1ml of heat treated Bt suspension to test tube no. 1.
- Shake well and transfer 1 ml of the suspension from test tube 1 to labelled 2.
- Repeat the procedure till the last test tube to give the dilution of 1/100 1/1000, 1/10000 i.e., 10⁻¹, 10⁻², 10⁻³ 10⁻¹⁰ and the corresponding dilute factor is 10,100,1000.....respective.
- Dispense 50 ml of diluted sample suspension from 5th to 10th dilute in triplicate in the plates with 15-20ml Nutrient Agar medium. Nutrient agar plates should be prepared the previous day and must observed for any possible contamination.
- Spread the sample suspension on the agar medium thoroughly with glass spreader.
- Incubate the plates at 30°C for 24 hours and count the numbers colonies

Calculation:

No. of Viable spores/gm of ml. = $N \times D \times 2 \times 10^3$

N - No. of colonies in plate (Average of three plates)

D - Dilution factor.

Beta – endotoxin determination by house fly bioassay method:

Procedure:

- 1 g sample thoroughly mixed with 9 ml. of sterile saline.
- This solution is heat treated at 65°C (Water bath) for 45 minutes and incubate at rotary shaker for 2 hrs. at room temp.
- Then centrifuge this sample at 12,000 RPM for 10 minutes.
- This suspension is serially diluted (1:10) to 10⁻⁶ dilutions.
- Liquid diet 200 g for each replicate is placed in trays/beakers.
- 5 ml. of heat treated culture supernatant (10⁻⁶) is poured on diet. Let it solidify at room temp.
- For control set up use 5 ml. of sterile water
- 5 days old House fly Larvae (50) in each replicate i.e. two replicate each for sample and control & cover with wire mesh /clot.

- Incubate the trays at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ till emergence.
- After 24 hrs. just put 5 g wheat bran in each tray on the top (On to 10th day). On adult emergence freeze the trays for 2 hrs. to control the adults and % mortality may be calculated as: -

% Mortality = $(100 - \text{Number of Normal Adults})$.

House fly assay diet:

- Agar – 16gm
- Milk powder -100gm
- Yeast -100gm
- Methyl paraben -2.1gm
- Water -1000ml

Mass Production of Entomopathogenic Nematodes

Steinernema and *Heterorhabditis* are symbiotically associated with bacteria belonging to the genera *Xenorhabdus* and *Photorhabdus*, respectively. The first attempt in the exploitation of EPNs was taken against Japanese beetle (Glaser, 1932). Later, the programme failed due to lack of knowledge about

EPN–bacteria symbiosis (Gaugler et al., 1992). The mystery of the symbiotic

relationship between the nematode *Steinernema*, and the bacterium *Achromobacter nematophilus* (now *Xenorhabdus nematophilus*) was revealed in 1966 by Poinar and Thomas and again one decade later, i.e. in 1976, by Poinar for the nematode *Heterorhabditis* and the bacterium *Xenorhabdus luminescens* (later on transferred to the genus *Photorhabdus*). These discoveries proved to be a major breakthrough in insect nematology that was later exploited by other research workers and which ultimately led to the mass production and commercialization of EPNs.

EPNs are being mass produced in several countries of North America, Europe and Asia, on both a small scale and a large scale, using bioreactors (Shapiro-Ilan and Gaugler, 2002). They can be mass produced in two ways:(i) in vivo and invitro method. In the case



Fig. 24: Nematodes (IJ) is coming out from infected galleria



Fig. 25: Galleria moth

of in vivo, insects serve as the bioreactor, whereas the in vitro process is carried out in artificial media (Sharma et al., 2011). Each approach has certain advantages and disadvantages that may be reflected in the form of technical expertise, production costs, economy of scale and quality of end product.

Stage -1: Production of host insect (*Galleria larva*)

In vitro rearing of wax moths : Most in vitro lab rearing techniques follow a simple series of events:

- Place wax moth eggs on new diet.
- Allow resulting larvae to feed on diet.
- Harvest late instar larvae or pupa and place into a second container.
- Allow late instar larva to pupate or pupa to emerge as adults.
- Allow adults to mate and allow females to lay eggs.
- Place eggs on new diet.

Artificial Diet for rearing :

Diet composition (Jones et al., 2002)

Mix thoroughly

- 300 ml liquid honey
- 400 ml glycerol
- 200 ml milk powder
- 200 g whole-meal coarse flour
- 100 g dried brewer's yeast,
- 100 g wheat germ
- 400 g bran



Fig. 26: Mass production of *Galleria larva*

Stage-2: production of EPN through in-vivo method:

This is a low technology method with low start-up costs (such as a cottage industry) that involves the production of EPNs by using live insects, which are highly susceptible and easily available at a lower cost. The insects used under this method are the larvae of the greater wax moth, *Galleria mellonella*, the rice moth, *Corcyra cephalonica*, or the mealworm, *Tenebrio molitor*, which are reared in the laboratory. Generally, the last instar of *G. mellonella* is preferred, due to its high susceptibility, easy availability and high yield of IJs (Woodring and Kaya, 1988). The method involves four steps: inoculation, harvest, concentration and decontamination.

Inoculation

- Insects are inoculated with IJs on a tray or dish lined with filter paper or another substrate conducive to nematode infection, such as soil or plaster of Paris.
- The nematode dosage and host density should be optimized for maximum yield. Too low a dosage of IJs may result in low host mortality, whereas too high a dosage may result in failed infections due to competition with secondary invaders.

- Approximately 25–200 IJs are sufficient to cause infection on one insect larva of *G. mellonella*.

Harvest

- This step is performed by using a technique based on the White trap (White, 1927), wherein after 2–5 days, the host insects killed by nematodes are placed above a water reservoir.
- The nematode produced by this method is harvested by placing moist filter paper on a concave side up watch glass surrounded with water in a large Petri dish.



Fig. 27: *Galleria* larvae before exposing for EPN infection



Fig. 28: EPN infected *Galleria* larvae



Fig. 29: White trap method to extract IJ

- The progeny IJs migrate from the depleted host cadaver into the water reservoir, where they are trapped and subsequently harvested.
- Precaution should be taken that there should not be too long a delay in the transfer of the infected insect cadaver to the White trap, as it may cause a negative effect on the reproductive stages of the nematodes, as well as a chance for the insect cadaver to rupture.

Concentration

- IJs are decanted, transferred to a beaker and then kept in BOD incubator at 10–15°C. During the process, care should be taken that settling for a prolonged period may prove detrimental to the nematodes, as this often causes a lack in oxygen content.
- Although this may be accomplished by vacuum filtration or centrifugation, for commercial in vivo operations.

Decontamination

- There is a chance of host material or microbial contamination on nematodes while migrating away from the cadaver. Therefore, the nematodes harvested by this method are washed repeatedly. This can be accomplished by gravity settling wherein antimicrobial compounds such as streptomycinsulfate, Hyamine® (methylbenzene thonium chloride), merthiolate, NaOCl and HgCl₂ are used (Lunau et al., 1993).

These compounds have not been found to have any detrimental effect on nematodes during commercial application.

- The yield could be more than 300,000 IJs/insect.

The method described above is simple and cheap. It is appropriate for laboratory use and small-scale applications, such as for those growers who cannot afford large investments in *in vitro* production. However, for largescale nematode production, this method is not suitable as it demands a large number of labourers, because the two different organisms, i.e. host insect and EPN, are cultured simultaneously, which makes the entire process costly. In spite of this, the above method of nematode production is sustainable as a cottage industry (Gaugler and Han, 2002).

Formulation and storage

An important consideration for the success of EPNs against target insect pests is their formulation into a stable product, which in turn is a prerequisite for successful commercialization, and this can be achieved by maximum survival of IJs in a formulation for a longer period before their utilization in the field. Nematode mortality may vary from 70 to 100% if storage life of the formulated product has expired (Grewal, 2000).

- Alginate encapsulation:
- Flowable gel formulations:
- Aqueous suspension
- **Water dispersible granules.** The nematodes formulated on to inert solid carriers like clays are partially dehydrated. The induction of partial anhydrobiosis reduces nematode activity and metabolism. Sandwich formulation method was found to be very effective where nematodes are mixed in clay to remove excess surface moisture and induce partial



Fig. 30: WDP formulation of EPN

anhydrobiosis. Recently, commercial attention is being gained by water dispersible granules (WDGs)

formulation of EPN where granules consisted of a mixture of various types of silica, clays, cellulose, lignin and starches and diameter ranging between 10 and 20 mm. WDGs increases the storage ability of IJs to several months at 15–25°C, besides enhancing the tolerance capacity of the nematodes to extreme temperature, enabling easier and less expensive transport and labour intensive preparation steps. Above all, this method is cost-effective. However, no EPN formulation has been reported to meet the 2-year shelf-life requirement of a standard. Thus, the shelf life of nematodes in a formulated product can be enhanced by reducing nematode activity and metabolism through physical trapping, metabolic inhibition, cold storage, or by the

induction of anhydrobiosis. The shelf life can also be improved by genetic selection approaches, which may also lead to an improved persistence of these beneficial organisms in the soil.

Quality control:

The parameters involved in manufacturing a quality EPN product are

- Correct identity of species.
- Total number of live nematodes.
- Ratio of live and dead nematodes.
- Matching of host finding behaviour to the target pest.
- Age of nematodes used in the formulation.
- Virulence and reproduction ability of nematodes in the target pest.
- Storability.
- Tolerance to temperature extremes and cold or warm temperature activity.
- Other requirements are size and packaging, reliable instructions for the consumers, ease of transportation, free from contaminants, price, availability and efficacy under field conditions.

Nematologists working in different laboratories are now paying attention to molecular approaches to improve the quality of EPNs towards heat tolerance by genetic modifications.

Application Technology

The application of EPNs (IJs) is done mainly for the control of larval or pupal stages of insect pests in the soil, plant surface or cryptic habitats. The prime care during application should be minimum loss of IJs from sprayer tank to target insect. The following are recommendations for applying a commercial product of EPNs in the field:

- The EPN supplier/distributor should be given the order only a few days prior to the application of EPNs. Shipping of EPN products by overnight delivery and their application within 1–2 days after arrival is expected to provide a satisfactory result.
- The expiry date of the commercial product of EPNs needs to be checked before application.
- EPNs should be kept in a cooler temperature until ready for use.
- Selection of nematode species should be precise and matched with target pest.
- Before application, check the colour and odour of the product. In the case of live nematodes, the odour of the product in the container will be mild, while a strong smell like ammonia indicates dead nematodes.
- To check the viability of the nematodes, a little amount product should be first soaked in water and a droplet of this sample should be placed on a slide or small, clear glass bowl. The sample is viewed with 15X hand lens or microscope. The

motile nematodes will appear as an 'S' shape, live but not motile as a 'J' shape (*S. carpocapsae*). The dead nematode will appear as straight and unmoving

- Soil temperature should be checked before application of EPNs. It should be $>55^{\circ}\text{F}$ but $<90^{\circ}\text{F}$.
- Light irrigation before application is recommended as EPNs require a thin film of water around soil particles to move.
- An appropriate method to use EPNs is to apply them in the morning or evening hours so that the nematode may be protected from desiccation and ultraviolet rays.
- The field wherein EPNs have been applied needs to be kept moist for at least 7 days to obtain a satisfactory result.

Mass Production of Nuclear Polyhedrosis Virus

The nuclear polyhedrosis virus infects the insect in the form of a polyhedral inclusion body (PIB). When the polyhedra enters the mid gut of an insect it dissolves under the alkaline conditions (pH 9-11) releasing the virions. These virions enter the cells of the mid gut and proceed to multiply in the nucleus. From this initial infection new virions are produced which proceed to spread the infection to other body tissues such as haematocytes,

tracheal cells, fat body cells and hypodermis. It is in these tissues during the later stages of the infection that polyhedra are produced in which virions become embedded. When the insect dies it ruptures releasing these polyhedral to infect other insects. Insects killed by NPV will commonly contain up to 100 million PIB. In the wild, infection occurs through the ingestion of PIB.

Insects infected with NPV show few symptoms for the first 2-4 days after ingestion of the virus. The larvae slowly stops feeding and become less active. During advanced stages of the infection, as the epidermis is infected, the skin becomes very fragile and ruptures easily. The larvae become wilted and the body contents become a fluidised mass of decomposed tissues and polyhedra. Just prior to

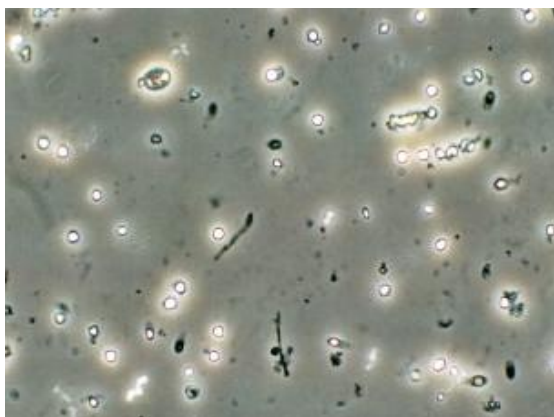


Fig. 31: A view of NPV under phase contrast microscope



Fig. 32: A typical symptom of NPV infection

death infected larvae often climb to the highest parts of the substrate they are located, e.g. tops of plants and attach themselves by their prolegs. On death they hang in a characteristic V-shape.

Insect virus can be produced by in-vivo method where we need to establish an ideal insect culture facility for the production of healthy host insect. A supply of healthy, disease-free insects is the first requirement for the mass production of NPV against the target pest. In small scale set up the field collected target insect also can be used as host to produce NPV but for large scale production an insectary is must. Besides, the production of NPV have also made possible by in-vitro method but found to be very expensive.

Setting up insectary for continuous host supply for NPV production

The insectary for the production of host insects should be designed in such a way as to avoid contamination of the colony with pathogenic diseases. The rearing area should be well isolated with restricted access. *Helicoverpa armigera* is of major pest, damaging a wide variety of food, fibre, oilseed, fodder and horticultural crops. The nuclear polyhedrosis virus of *H. armigera* (HaNPV) is currently used for the management of *H. armigera* on chickpea, cotton, pigeon pea, tomato and sunflower. Mass production of Nuclear Polyhedrosis Virus (NPV) of *H. armigera* has been taken here for discussion which is restricted to *in vivo* procedures in host larvae and obtained by

- Field collection from cotton, pigeon pea and chickpea – *H. armigera*
- Mass culturing in the laboratory in semisynthetic diet – *H. armigera*

Composition of semisynthetic diet

COMPONENT	QUANTITY
Chickpea flour	100 g*
Yeast	30 g
Wesson's salt mix	7 g
Methyl Paraben ²	2 g
Sorbic acid	1 g
Ascorbic acid	3 g
Agar	13 g
Vanderzant vitamin solution	8 ml**
Streptomycin sulphate	40 mg
Carbendazim	675 mg
Formalin	2 ml***
Water	720 ml

* Whole chickpea seeds can also be used (soak in distilled water overnight).

** 28% solution in distilled water.

*** Not included in diets used for inoculation of larvae with virus and post-inoculation rearing.

Some small scale producers use field – collected larvae for mass production of NPV in spite of the following constraints.

- Collection of a large number of larvae in optimum stage (late IV / early V instars) is time-consuming and can be expensive in terms of labour and transportation costs.

- Wild populations of insects may carry disease causing organisms like microsporidians, cytoplasmic polyhedrosis virus, stunt virus and fungal pathogens which will affect both virus production and quality.
- Introduction of wild strains of NPV resulting in quality control problems.
- Transportation of a large number of larvae with cannibalistic behaviour will be a difficult task.
- Parasitized larvae collected from the field will die prematurely yielding little virus.

Rearing of larvae in the natural host plant will involve frequent change of food at least once a day during the incubation period of 5-9 days increasing the handling time and hence the cost. In order to reduce the cost, field collected larvae are released into semi synthetic diet treated with virus inoculum. Mass culturing of insects in semi synthetic diet involves high level of expertise, hygiene and cleanliness.

Production procedure

- The NPV of *H. armigera* is propagated in early fifth instar larvae. The virus is multiplied in a facility away from the host culture laboratory.
- The dose of the inoculum used is 5×10^5 polyhedral occlusion bodies (POB) in 10 ml suspension.
- The virus is applied on to the semisynthetic diet (lacking formaldehyde) dispensed previously in 5 ml glass vials.
- A blunt end polished glass rod (6 mm) is used to distribute the suspension containing the virus uniformly over the diet surface.
- Early fifth instar stage of larvae are released singly into the glass vials after inoculation and plugged with cotton and incubated at a constant temperature of 25°C in a laboratory incubator.
- When the larvae exhausted the feed, fresh untreated diet is provided.
- The larvae are observed for the development of virosis and the cadavers collected carefully from individual bottles starting from fifth day.
- Approximately, 200 cadavers are collected per sterile cheese cup (300 ml) and the contents are frozen immediately.
- Depending upon need, cadavers are removed from the refrigerator and thawed very rapidly by agitation in water.

Processing of NPV

- The method of processing of NPV requires greater care to avoid losses during processing. The cadavers are brought to normal room temperature by repeatedly thawing the container with cadaver under running tap water.
- The cadavers are homogenized in sterile ice cold distilled water at the ratio 1: 2.5 (w/v) in a blender or precooled all glass pestle and mortar.
- The homogenate is filtered through double layered muslin and repeatedly washed with distilled water.

- The ratio of water to be used for this purpose is 1:7.5-12.5 (w/v) for the original weight of the cadaver processed.
- The left over mat on the muslin is discarded and the filtrate can be semi-purified by differential centrifugation.
- The filtrate is centrifuged for 30-60 sec. at 500 rpm to remove debris.
- The supernatant is next centrifuged for 20 min at 5,000 rpm.
- Then the pellet containing the polyhedral occlusion bodies (POB) is suspended in sterile distilled water and washed three times by centrifuging the pellet in distilled water at low rpm followed by centrifugation at high rpm. The pellet finally collected is suspended in distilled water and made up to a known volume, which is necessary to calculate the strength of the POB in the purified suspension. Store in refrigerated condition for further usage.

Formulation: A main issue in virus formulations is microbial contaminants. Purification of the crude technical product is needed for product stability. The formulation ingredients of current baculovirus products are not available as the information is commercial is proprietary. Generally, virus products were often formulated as wettable powders, particularly those used in forestry. Oil formulations have been studied, but oils are often repellent or act as an anti-feedant to the target pests. Nowadays, most formulations are aqueous suspensions along with phagostimulant and optical brightener as UV protectant that are recommended for spray applications in orchards and greenhouses and are user-friendly.

Quality control :

Quantitative analysis : Enumeration of NPV

Use of appropriate dose of NPV is very important for successful biological control of pests. It is therefore, very essential to ascertain the strength of the NPV in the suspension before it is applied on crop.

Materials Required

- Suspension of virus suitably diluted
- Haemocytometer
- Digital micropipettes
- Vortex mixture
- Phase contrast microscope
- Disinfectants (70 % Alcohol)
- Tally counter

Figure 1. Haemocytometer.



Figure 2. Grid on haemocytometer. $\times 100$

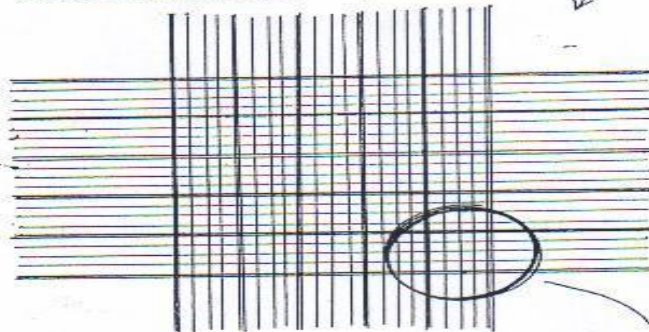
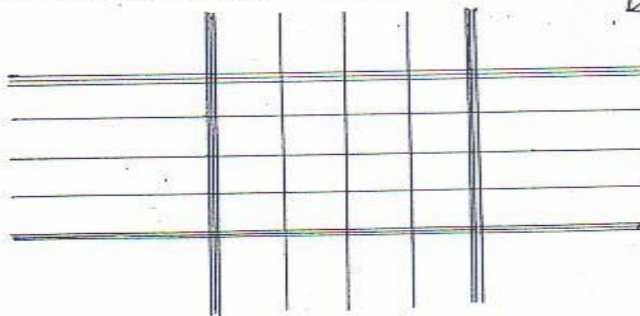


Figure 3. 400x magnification of single large square of grid.



The haemocytometre: It is an essential tool used for estimating the polyhedral occlusion bodies (POB) in a sample. Improved Neubauer Haemocytometer comprises a thick glass slide with a shallow depression in the central section divided in to two halves (Fig.1). On each side, the base of the depression has a fine ruled grid of squares (Fig.2), which is visible under a microscope. The dimensions of this grid are defined. With a thickened cover slip placed over the depression a chamber is created of fixed depth.

Procedure:

Serial dilution

- Shake the container with NPV stock suspension thoroughly before opening the
- Transfer 100 μl of the above suspension to a sterile eppendorf tube using a digital micropipette, make up the volume 1000 μl with distilled water and Label as A.
- Mix the contents using vortex mixture to obtain uniform distribution of POB. From A, transfer 100 μl to another eppendorf tube and make up the volume to 1000 μl with distilled water. Label this as B.

Enumeration.

- Clean the cover slip and the haemocytometer by wiping with tissue paper dipped in 70% ethanol.
- Place the cover slip on top of the slide exactly over the depression in the counting chamber.
- Thoroughly mix the sample with a vortex mixture.
- Using a digital pipette, introduce 10 µl of sample into the chamber directly so that the chamber is filled completely. Avoid overflow of the suspension.
- Firmly but carefully press down on the sides of the cover slip to ensure that the
- Chamber is of the correct depth.
- Leave the haemocytometer undisturbed for 2-3 min. So that the POB in the suspension settles down and Brownian movement is reduced.
- Place the haemocytometer under a 40 x objective and using phase contrast (Phase 2) to focus on the polyhedra.
- The central squares are divided into 5x5 squares equally. Each of these 1/25 squares is further sub divided into 16 smaller squares. Totally there are 400 smaller squares.
- The polyhedral have to be counted systematically in sequence across the grid. By doing so one will be able to count the polyhedra in 160 small squares. The polyhedra within each smaller square and those touching the top and left-hand sides alone are counted.
- The counts have to be taken in three replicates and average has to be worked out.
- Carefully remove the cover slip, thoroughly wash with 70% ethanol and dry the counting chamber and cover slip.

Calculation.

The number of POB/ml is calculated by the formula: $\frac{D \times X}{N \times K}$

Where:

- D = dilution factor
- X = total number of polyhedral bodies counted in 10 big square (Mean of 3 counts) N = number of squares counted (160 smaller square)
- K = volume above one small square in cm³ (i.e., 2.5×10^{-7})

Precautionary measures to be taken while taking POB count:

- Suspension should not dry while counting.
- POB's should be 50-100 per big square.
- Suspension while drawn on first shot and dispensed on second shot.
- Constant fine-tuning of focus is required to count the POB's suspended at different depths of cavity.

- Serial dilution should be done with fresh micro tips at each step.
- If the work area is warm the suspension in the chamber will evaporate rapidly and the counting will become erroneous.
- Therefore, enumerations have to be done preferably in an air-condition room.

Worked example:

Suppose in a sample diluted by a factor of 1000 we count 535 polyhedra in 160 smaller squares then:

$$D = 1000$$

$$X = 535$$

$$N = 160$$

$$K = 2.5 \times 10^{-7}$$

$$\begin{aligned} \text{Thus Number of polyhedral bodies (POB) per ml} &= \frac{1000 \times 535}{160 \times 2.5 \times 10^{-7}} = \frac{5.35 \times 10^5}{4 \times 10^{-5}} \\ &= 1.33 \times 10^{10} \text{ POB / ml} \end{aligned}$$

Assessing for Virulence of NPV

Quantitative enumeration by the use of haemocytometer gives us an indication of the number of polyhedral occluded bodies present in a sample but cannot provide information on the pathogenicity of a sample. Routine assessment of virus pathogenicity is essential in a production system as a quality control procedure to confirm consistent activity over time. The biological activity of a virus can only be assessed by bioassay in which test organisms are exposed to virus samples.

Bioassay Method:

- **Principle:** To provide known doses of the virus samples to test insect and measure mortality induced at regular intervals of time. Method: Diet surface contamination method.
- Diet: The standard chickpea-based diet without formaline.
- Bioassay vials: 5ml glass vials with a diameter of 18mm (255 mm.sq. surface area)
- Doses of NPV:
 - *Helicoverpa armigera*
T1- 5×10^4 POB/ml: T2- 1×10^4 : T3- 2×10^3 : T4- 4×10^2 T5- 8×10^1 : T6- 1.6×10^1
 - *Spodoptera litura*
T1- 1×10^6 POB/ml: T2- 2×10^5 : T3- 4×10^4 : T4- 8×10^3 : T5- 1.6×10^2 : T6- 3.2×10^2
- **Method of dosing:** Dispense 10 micro liter aliquots into each vial and spread uniformly over the entire diet surface using a polished rounded tip of a 4mm glass rod and allow drying off under hood for 10min.

- **Number of larvae/dose:** 35 larvae and maintain 40 larvae without virus inoculation for control.
- **Stage of larvae:** Second instar larvae
- **Observation:** Record mortality in different doses commencing from 24 hours after treatment.
- **Analysis:** A number of methods are available for analysis of dosage-mortality response. However the Finney's method is accepted as the standard method. Mortality data are subjected to probit analysis using a statistical software in a computer. Express LC_{50} in terms of POB/sq.mm of diet surface.

Pre-requisites for bioassay:

Test insect: Test insect used should be genetically pure and not exposed to stress, either physiological or nutritional. The test insect will show heterogeneous response as they grow. This will interfere with the assessment. Therefore, greater control can be exercised if the larvae in early stages are used.

Abiotic factors: Temperature/ Humidity/ Light conditions should be maintained constant during incubation period & bioassays should be carried out in perfectly aseptic conditions.

Toxicant/ Pathogen: For the inoculation, the strain used should be viable, with POB's counted accurately in a haemocytometer with the help of a phase contrast microscope.

Observation: If there is a case of abnormal mortality before 48 hours bioassay has to be repeated, as it can be a case of contamination of the populations used for bioassay.

Important point for producing quality NPV

- Production of NPV can only be carried out in live insect cells, either in whole insects or in cell culture.
- In whole insect's virus production can be highly productive and all commercial production is done this way, as cell culture is still under development as a practical technique.
- Key to production in insects is good inocula, healthy insects, correct choice of NPV dose, harvesting time and stable rearing conditions.
- While harvesting live insects from production takes more care it results in a cleaner more consistent product.
- All processing involves loss of NPV do what you need, no more. Gradient purified NPV is needed for production inocula but not for field use.
- All processing must be carried out at 4-10°C to avoid the development of bad smells and the build-up of bacterial contamination.
- Properly processed centrifuged NPV is better for formulation as it has high activity and low smell.
- It is easier to clean up well-infected, live harvested insects into a low smelling product.
- Drying of virus suspension is the most convenient way of storage.

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Tomato leaf miner, *Tuta absoluta* an invasive pest of South Asia and their bio-rational management

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The tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is one of the most serious pest and invasive species of Tomato originating from South America. It has been spreading in Africa, Asia and Central America. Since its introduction to Spain in 2006, it has invaded most of the countries in Europe, Mediterranean, Middle East, Northern, western and eastern Africa, and India in South Asia (Desneux *et al.*, 2010 and Desneux *et al.*, 2011). It causes 80 to 100% crop loss unless proper management technologies are taken up (Giulianotti, 2010). The pest can breed between 10-12 generations a year and each female can lay 250-300 eggs in her life time (Barrientos, 1998). The pest pupates in the soil and remains of plant materials in the field, infests various plant parts including seedlings, flowers buds and tomato fruits (Desneux *et al.*, 2011). Farmers use various pesticides as a quick and adopted control measure of the pest. Lack of knowledge on pest population monitoring in tomato production has resulted into inappropriate pesticide use which lead into an increase of cost of production as well as build-up of resistant, killing of non-target organisms and pollute water sources (Biondi *et al.*, 2012).

Since invasion of this pest in Tanzania in 2014 no single management approach has been developed but growers were left to use any insecticides available in their vicinity, the outcome being loss of biodiversity of beneficial species (Arnó and Gabarra, 2011), the environment building of insecticides resistant strain (Lietti *et al.*, 2005). It is imperatively necessary to devise sustainable management strategies which can guide tomato growers in the management of this highly appetite pest, *Tuta absoluta* targeting its major life stages.

It is essential to detect and monitor *Tuta absoluta* population early in order develop sustainable management strategy.

First, it is important to differentiate between detection and monitoring. Detection is to establish the arrival of the insect to a location while the monitoring is to estimate the level of the insect population in the field and its life cycle.

Therefore, prior to starting monitoring in the field, the arrival of the pest needs to be established. This can be done by placing pheromone traps in all the potential transfer points. The spread of *Tuta absoluta* so far was mainly carried by human activity more than the insect movement itself.

Key detection steps:

Identify the high risk contact points in which *Tuta absoluta* can spread. This could be at nurseries selling tomato seedlings to farmers, tomato farms, composting plants using plant waste from tomato farms, wholesale vegetable markets and vegetable repacking

and distribution centers, Food processing, salad packing and tomato processing areas and border crossings custom inspection areas.

Trap choice:

Delta traps with non-drying sticky liners are best for this purpose. They can preserve the insect for further examination by a taxonomist.

Pheromone lures: There are three common pheromone dosing levels 0.1mg, 0.3mg and 0.5mg. They release different levels of pheromone. 0.3mg seems to be the optimum dose and seems to be suitable for most applications. 0.1mg is more suitable to detect the insect in enclosed buildings such a packing and sorting houses, Salad packing etc. 0.5mg is more useful for open space, large truck yards and open fields.

Traps placement:

Place 1-2 traps in each area indicated above. Traps should be hung as low as practically possible to be close to the tomato related product. *Tuta absoluta* does not fly very high and therefore trap height should not be higher than 1-2 ft over the tomato or related product. Traps are more effective when placed near open product rather than packaged product. Traps located near farm / tomato processing waste disposal locations has the highest probability of capturing *Tuta absoluta* if present.

Insect Monitoring: If the presence of *T. absoluta* has been established, then fields and greenhouses producing tomato will need to be monitored.

Greenhouses: one trap inside the entrance, 1-2 traps nearest the warmer part of the greenhouse. In Open field: 2-3 Traps per Hectare. 2 traps near the edge of the field form all four directions to help establishing the direction of the infestation. 2-3 traps per hectare for the rest of the field.

Alternative hosts:

It should not be forgotten that *Tuta absoluta* may find other hosts in the absence of Tomato. Other members of the solanaceae family such as potato, Aubergine, peppers, and solanaceous weeds can also host *Tuta absoluta*.

Biology and ecology of *Tuta absoluta*

Tuta absoluta is a micro lepidopteran moth with a high reproductive potential, and although its biological cycle depends on temperature, it is capable of over 10 generations per year under optimal conditions. Its life cycle comprises four development stages: egg, larva, pupa and adult, and there are about 10–12 generations per year. The total life cycle is completed within 24-35 days depending on the temperature. Low temperatures are a limiting factor for its survival, but *T. absoluta* can overwinter as eggs, pupae or adults, depending on environmental conditions (Imenes SDL *et al.*, 1990).



Eggs are small, cylindrical, creamy white to yellow-orange, and 0.35 mm long.

Larvae are whitish soon after hatching, becoming greenish or light pink in the second to fourth instars according to food (leaflet or ripe fruit, respectively). They are characterized by a dark head and a lateral spot that extends from the ocellus to the posterior margin. They lack a typical dorsal plate in the prothorax. Instead, they have a dark oblique band that does not cover the dorsal midline (Imenes SDL *et al.*, 1990).

Pupae are cylindrical in shape and greenish when just formed, becoming darker in color as they near adult emergence. The pupae are often coated with a white silky cocoon (Imenes SDL *et al.*, 1990).



Fig. 1: From top to bottom: young larvae 3mm, fourth instar larvae 4.6 mm, pupae and adult laying eggs of *Tuta absoluta*. © J. Arnó, A. Mussoll and R. Berruezo, IRTA, Spain.

Adults are 5-7 mm long with a wingspan of 8-10 mm. they are nocturnal and hide between leaves during the daytime. The most important identifying characters are the filiform antenna, grey colored scales and black spots present on the anterior wings. Females can live for two weeks, whereas the males live only one week.

Females usually lay eggs on the aerial parts of host plants, on the underside of leaves or stems and to a lesser extent on fruits. A mature female can lay up to 260 eggs. Eggs hatch occurs in 4-6 days.



Fig. 2: in the left, adults of *Tuta absoluta* mating (Ghanem, 2017). In the right female laying eggs on the leaf. © J. Arnó, A. Mussoll and R. Berruezo, IRTA, Spain.



Fig. 3: different larval instars of *Tuta absoluta* (Ghanem, 2017)

After hatching, young larvae penetrate plant tissue (leaves, aerial fruits or stems), begin feeding and create mines. The larval stage is the most damaging to plants and is completed within 12-15 days under optimal conditions. Larvae do not enter dormancy when a food source is available.

Larvae can leave their mines and travel to new locations to mine again. This behavior may result in damage to all stages of plant growth. Fully-grown larvae usually drop to the ground on a silk thread and pupate in the soil, although pupation may also occur on leaves or in the calyx. Pupae have been found in the mines, outside the mines and in the soil, as well as beneath pots and under greenhouse benches (Desneux N *et al.*, 2010).



Host plants

Tuta absoluta is a harmful leafmining moth with a strong preference for tomato (*Solanum lycopersicum*), but *T. absoluta* can also attack potato (*S. tuberosum*), colored peppers (*Capsicum annuum*) and other solanaceous plants such as common thorn apple (*Datura stramonium*), Angel's-trumpets (*D. ferox*), glaucous tobacco (*Nicotiana glauca*), eggplant (*Solanum melongena*), silver-leaf nightshade (*S. elaeagnifolium*), and black nightshade (*S. nigrum*).



Nature of damage and importance

The larvae of *Tuta absoluta* mine the leaves producing large galleries and burrow into the fruit, causing a substantial loss of tomato production in protected and open field cultivations. The larvae feed on mesophyll tissues and make irregular mines on leaf surface.

Tuta absoluta reduced the yield and fruit quality of tomato grown in greenhouse and open field. Severely attacked tomato fruits lose their commercial value. 50–100% losses have been reported on tomato (EPPO, 2008a). On potato, (CIP, 1996) considers it one of the major pests of foliage, occurring in warm zones of low altitudes (below 1000 m). The fact that this pest has 10 to 12 generations per year, damages occur throughout the entire growing cycle of tomato.

As larvae are internal feeders, it is difficult to achieve an effective control. Therefore, it causes an increase in synthetic insecticide applications, disruption of integrated management programs of other tomato pests and an increase in the cost of crop protection and as a result an increase on tomato prices,. In addition, the outbreak of this pest led to an augmentation of bans on the trade of tomato including seedlings and to a significant increase of risks for growers, consumers and the environment with the blind use of chemicals. Considering its high biotic potential, its ability to adapt to various climatic conditions it has rapidly colonized Europe and North Africa (USDA-APHIS, 2012; Zappalà L, 2012; Zlof V, 2012).

Tuta absoluta can rapidly evolve strains with reduced susceptibility to insecticides that have been previously effective. Failure by synthetic insecticides has also been reported in many countries (IRAC, 2012).

Distribution



Fig. 5: Distribution of *Tuta absoluta* according to the (EPPO, 2016).



Fig. 4: (a) damage caused by Tuta's larvae on the leaves (Ghanem, 2017). (b) damage on the fruits (EPPO 2016). (c) Total losses on tomato due to *Tuta absoluta*, Dr Andrea Minuto, CERSAA, Albenga (IT).

Since the first detection in Spain in 2006, this pest is spreading rapidly across Southern Europe and North Africa to engulf the whole of the Mediterranean countries. Until today the presence of *Tuta absoluta* has been reported in Italy, France, Malta, United Kingdom, Greece, Switzerland, Portugal, Morocco, Algeria, Tunisia, Libya and Albania during last two years. *Tuta absoluta* is already a serious pest in South America since the 80's (EPPO, 2008a; 2009a;b; 2011; 2012; 2014). *T. absoluta* has also been detected in South Africa, near the border with Mozambique (IPPC, 2016a;b; 2017).

EPPO Reporting Service (2008/174): first observed in Morocco in April 2008 on outdoor tomato crops in Bouareg (North-East). However, nowadays the pest is now established and widespread in Morocco, Algria, Libya, Egypt, Kenya, Tanzania, Zambia, Southa and Southern Africa, Nigeria, Niger, Middle East and South Asia including Bangladesh, Nepal, India and Pakistan.

Various control technique for *Tuta absoluta*

In many counires *Tuta absoluta* is now well established and complete eradication is impossible. However, it may be controlled by various measures as Integrated Pest Management (IPM) involves various control practices.

Introduction of *Tuta absoluta* may likely be by way of infected plants for planting, since plants can carry all stages of the pest. Exchange of infested tomato, seed and planting materials responsible for rapid dispersal of this devastating pest. An outbreak can occur in a greenhouse or open field where tomatoes and other solanaceous crops (such as potatoes or aubergines) have been grown previously and which support the development of the pest (IPPC, 2009).

Cultural control

Crop rotation with non-solanaceous crops (preferably cruciferous crops), ploughing, adequate irrigation and fertilization, removal of infested plants and complete removal of post-harvest plant debris and fruits. The removal of wild solanaceous host plants in the growing area is also encouraged, as these can host all stages of the pest that can re-infect the growing crop. Keeping a good planting distance between rows is advised, not having crowded plants, as is removal of weeds. Tomato seedlings originating from Nurseries, whether local or foreign, should not show any signs or symptoms of *Tuta absoluta*. Following greenhouses growing season, all plants should be cut from above the soil level and left to dry out inside the greenhouse. Insecticide should continue to be applied in order to completely kill off adult moths before transferring debris outdoors for burning, which should be carried out immediately to avoid spreading of the pest to the outdoor plantations (ONSSA, 2010).

Pheromone based strategy

The use of pheromone-based strategies is recognized as an important control technique for *T. absoluta* (Cocco A *et al.*, 2013; Megido R. *et al.*, 2013)). Enormous advances have been made in the field of semiochemicals to cope with *T. absoluta*, especially sex pheromones which are important male attractants (Desneux *et al.*, 2010). The reproduction biology of *T. absoluta* supports the potential use of male annihilation as an

effective control method as reproduction in this pest has been considered as strictly amphimictic, and males emerge earlier than females and the females mate several times (Garzia GT *et al.*, 2012). Pheromone traps are considered as the first line of defense against this moth in open fields and in greenhouses as they are used for monitoring and male annihilation purposes.

Most of the IPM strategies developed against *T. absoluta* utilize pheromones in combination with other control techniques. This aims to annihilate males using pheromone-baited traps or mating disruption based on atmospheric saturation of the synthetic pheromone to reduce mating chances. Furthermore, lure and kill techniques use a combination of a low amount of the synthetic sex pheromone of *T. absoluta* and an insecticide in order to reduce the male population (Witzgall P *et al.*, 2010; Cocco A *et al.*, 2013).

Monitoring

Monitoring for the pest presence and density is a critical first step for its management. The primary value of sex pheromones, combined with traps is to determine the presence and density of pest populations in the field. The pheromone of *T. absoluta* is highly species-specific. It attracts males to the trap even when the field population is extremely low. Pheromone baited traps are, therefore, remarkable tools for detection of this insect, and should help in determining pest distribution at large geographic scales (Urbaneja A. *et al.*, 2012; Urbaneja A. *et al.*, 2013).

Using pheromone traps as tools for monitoring the pest population in the field requires an appropriate calibration, requiring correlation of certain combinations of trap and lure with the actual presence of the pest in the target area. This is actually done by correlating catches in pheromone traps with the population density of immature forms and level of damages detected in the crop (Urbaneja A. *et al.*, 2013). In addition, pheromone traps give early warning of infestation and exhibit the density of the insect accurately in low population to medium level infestation. In heavy infestation, traps tend to give high level of capture, which makes data collection difficult (Anyanwu, 2009).

Mass trapping

Mass trapping is a technique that helps to remove a sufficiently high proportion of male insects from the pest population. It consists of placing a higher number of traps in various strategic positions of the crop at the base of the plants (Height ≤ 40 cm) with a density of 20 to 25 traps per hectare under greenhouse and 40 in the field. The traps should be distributed evenly along the aisles and rows of the crop while keeping a distance of 25m



Fig. 6: Delta trap with pheromone to attract leafminer's males (Ghanem, 2017)

between two traps and pheromone capsules should be renewed every 4 to 6 weeks with cleaning and maintenance of each trap (ONSSA, 2010).

This method is widely used in conjunction with other control measures to achieve acceptable level of damage and to reduce the reliance on insecticide treatments otherwise it is not sufficient (Cocco A *et al.*, 2013). The release of the eggs parasitoid, *Trichogrammatoideabactrae*+ Mass trapping showed the lowest infestation rate 9.2% comparing to others control combinations (Salem N. M., 2015). Mass trapping is a potential option for open field production. However, and for practical reasons, application in protected agriculture has a higher chance of success (Anynumous, 2009).



Fig. 7: Water trap used for mass trapping of *Tuta absoluta* in Souss Massa region (Ghanem, 2017)

Lure and kill

Lure and kill is a very promising approach to control the male adults of *Tuta absoluta* with minimum amount of insecticide application. This will reduce the mating incidence and therefore reducing the number of viable eggs. Based on sustained release matrix, Lure and kill product can release the pheromone over a long period normally over 6-8 weeks while sustaining the activity of the contact insecticide throughout the same period. Pheromones of other pests many be incorporated to reduce the need to other insecticide applications. It is targeted application in specific locations left over 99% of the plant source insecticide free providing a safe environment for beneficial insect to develop and to participate in the overall control of Strategy (Anynumous, 2009; Russell IPM, 2012).

Attract and kill is a density-dependent phenomenon that inherently requires substantially less pheromone than mating disruption formulations designed to achieve habituation/adaptation sensory imbalance and camouflage. Mating disruption prevents the mating by overwhelming the male with natural and synthetic source of pheromone. Attract and kill requires well balanced pheromone lures that not only elicit upwind flight behavior of the male, but also promote sustained contact with point source (Urbaneja A. *et al.*, 2013).

Biological control

In the area of origin of *T. absoluta*, naturally occurring predators in tomato are important to regulating *T. absoluta* populations, with apparent mortalities of larval instars, reaching values close to 80% (Miranda M. *et al.*, 1998). Naturally, as with all pests, *T. absoluta* is attacked by a set of natural enemies that infect and parasite the different stages of its development. However, their action is limited by the use of insecticides (table below). On the other hand, the larvae lives hidden as miner of leaves, fruits and stems. These enemies include predators and parasitoids.

The following bio-agents have been reported to control *Tuta absoluta*: 1. *Trichogramma pertiosum* 2. *Trichogramma achaeae* 3. *Macrolophus pygmaeus* 4. *Nesidiocoris tenuis* 5. *Nabis pseudoferus*.

The egg parasitoid *Trichogramma achaeae* has been identified as a candidate for biological control of the South American Tomato Pinworm, *Tuta absoluta*. On greenhouse conditions a high efficacy, 91.74 % of damage reduction was obtained when releasing 30 adults/ plant (=75 adults/ m²) every 3-4 days on August and September of 2008 in the southeast of Spain (Cabello T. *et al.*, 2009).

The most common predators of *T.*

absoluta are the mirid bugs *Nesidiocoris tenuis* and *Macrolophus pygmaeus*. These natural enemies are commercially available and widely used in North Africa and Europe. *Macrolophus pygmaeus* Rambur) and *Nesidiocoris tenuis* Reuter (Hem.: Miridae), can adapt to this invasive pest and prey actively on *T. absoluta* eggs and all larval stages, although they preferred first-instar larvae (Urbaneja A. *et al.*, 2008). *N. tenuis* was highly effective in controlling *T. absoluta* under experimental conditions, with reductions of up 97% infestation of leaflets and of 100% of fruits.

Macrolophus pygmaeus was also effective on this new pest, although its efficacy was lower in comparison to *N. tenuis* (76% and 56% reductions of leaflet and fruit infestation) (Mollá *et al.*, 2009). The green Mirid bug. Naturally present in Morocco (Ouardi K. *et al.*, 2012). Both predators have also been observed to contribute to the control of thrips, leafminers, aphids, spider mites and Lepidoptera species (Izquierdo J. *et al.*, 1994; Wei D *et al.*, 1998; Agustí N. *et al.*, 1999; Devi PK *et al.*, 2002; Perdakis DC and Lykouressis DP, 2002; Urbaneja A *et al.*, 2003; Blaeser P. *et al.*, 2004).

The use of biological pest control, the damsel bug *Nabis pseudoferus* has been studied to be applied in Spanish greenhouses. Two semi field bioassays on tomato plants, under controlled conditions, have shown an important reduction in the number of eggs of *Tuta absoluta*, between 92 and 96 %, when releasing 8 or 12 first stage nymphs of *Nabis pseudoferus* per plant (Cabello T. *et al.*, 2009).

Microbial control

Bacillus thuringiensis subspecies *kurstaki* BT

Bacillus thuringiensis sub sp. *kurstaki* have exhibited satisfactory efficacy against *Tuta absoluta* larval infestations. Delayed application of *Bacillus thuringiensis* may cause



Fig. 8: *Nesidiocoris tenuis* adult, Arnó, A. Mussoll and R. Berruezo, IRTA, Spain.



Fig. 9: *Macrolophus pygmaeus* adult, Arnó, A. Mussoll and R. Berruezo, IRTA, Spain.

higher insect mortality if the insects become more susceptible to the pathogen after a longer period of feeding on the resistant crop (Medeiros NA *et al.*, 2006). It produced crystalline inclusion bodies during sporulation, constituted of insecticidal proteins named Cry and classified into 70 groups according to their similarity of amino acid sequences (Schnepf E. *et al.*, 1998; Van Frankenhuyzen K. and Tonon A., 2013). The Cry protoxins were activated by proteolysis into toxins upon ingestion by susceptible larvae. Then bound to specific receptors on the brush border membrane of the midgut epithelial cells, causing pore formation and cell lyses (Jenkins J. and Dean D., 2001; Peyronnet O. *et al.*, 2001; Peng D. *et al.*, 2010).

Natural isolates of *B. thuringiensis* have been used as a biological pesticide since the 1950s for the control of certain insect species among the orders Lepidoptera, Coleoptera and Diptera as an alternative to chemical pesticides. This feature makes *B. thuringiensis* the most important bio-pesticide in the world market (Uribe D. *et al.*, 2003). It is reported that in a combine application of mass release of *Trichogramma pretiosum* and *Bacillus thuringiensis* resulted in fruit damage only 2 % in South America (Medeiros NA *et al.*, 2006). The combination between Azadirachtin and *B. thuringiensis* offered promising results in the fruit damage reduction by 30% (Bue P. L. *et al.*, 2012). *T. absoluta* first larval instar is found to be the most susceptible to *B. thuringiensis* sub sp. *Kurstaki* (Giustolin T. *et al.*, 2001; González-Cabrera *et al.*, 2011; Mollá O. *et al.*, 2014). A significant decrease of 33.36% in the hatching percentage has been observed on treated eggs whereas the control gave 86.74% (Alwan K. *et al.*, 2012).

***Beauveria bassiana* and *Metarhizium anisopliae*:**

Entomopathogenic fungi were successfully worldwide used as a biological control agent since 1880s (Krassiltschik I., 1888). It was used in integrated pest management (IPM) of many insect pests. (Khashaveh A., 2008; Herlinda S. *et al.*, 2010; Barra P. *et al.*, 2013). Several fungal species including *M. anisopliae*, *Verticillium lecani* and *B. bassiana* are being used as bio-control agent for a number of crop pests. *B. bassiana*, *M. anisopliae* and *V. lecani* are facultative pathogens can be mass-produced on various substrates (Prasad C. and Pal R., 2014).

B. bassiana is interesting because it attacks a wide range of insect pests, including whiteflies, moths, aphids and thrips (Saxena S., 2015). It was also known as white muscardine disease. Infections occur once the spores come in contact with an insect body, and then penetrate the cuticle, growing inside the infected insect body thereby causing its death (Rehner S. A. *et al.*, 2011). In whitefly, the second instars are the most susceptible to *B. bassiana* infections (James R. *et al.*, 2003). According to LC50 values, *B. bassiana* and *M. anisopliae* were most effective on larval phase. The effect of pathogen application was dependent on the instar phase at which the larvae were fed on pathogen-treated leaves. The second and the third larval instars give similar larval mortality values when larvae fed with leaves treated with *B. bassiana* and *M. anisopliae* (Shalaby H. H. *et al.*, 2013).

The genus *Metarhizium* includes several valuable entomopathogenic fungi, which are used in biological control (Khashaveh A., 2008). They are widely dispersed in nature and commonly isolated from infected insect carcasses or soil (Razinger J. *et al.*, 2014).

anisopliae has been reported to infect approximately 200 species of insects (Herrera-Estrella A. and Chet I., 1999; De Faria M. and Wraight S. P., 2007). In whitefly nymphs, a mortality of 67–100% has been observed in the laboratory assays and 30–92% in the field trial (Batta Y., 2003). In Morocco, a field trial proved that strategy based on attract and kill Ceranock combined to *M. anisopliae* KN14 soil application reduced the Med-fly population to 41.7%. Whereas In the laboratory bioassay the different life stages of *C. capitata* showed the mortality rate of 70.53%, 60.91% and 15.29% of the 3rd instars larvae, pupae and adults respectively (Oussouque T. *et al.*, 2015).

For *T. absoluta* Studies have revealed up to 54% mortality of *T. absoluta* adults by *M. anisopliae* (Pires L. *et al.*, 2009; Pires L. *et al.*, 2010). *Metarhizium anisopliae* could cause a female mortality up to 37.14% (Gonçalves-Gervásio R. and Vendramim J. D., 2007). Further studies proved that spinosad and indoxacarb were compatible with the two *M. anisopliae* isolates (URPE-6 and URPE-19) (Pires L. *et al.*, 2010). Many authors reported a pathogenic effect of *B. bassiana* and *M. anisopliae* on all the developmental stages of *T. absoluta* (Rodríguez M. *et al.*, 2006; Pires L. *et al.*, 2010).

Botanical extract

Azadirachtin

It is a tetranortriterpenoid extracted from neem trees (*Azadirachta indica* Juss.). It is a strong anti-feedant, repellent and growth-regulating compound for a wide variety of phytophagous insects, including the target insect pests; *B. tabaci* and *T. absoluta*. It delays and prevents molting, reduces growth, development and oviposition; and can cause significant mortality particularly in immature stages (Flint H. and Parks N. J., 1989; Schmutterer H., 1990; Liu T. X. and Stansly P. A., 1995; Mitchell P. L. *et al.*, 2004; Gonçalves-Gervásio R. and Vendramim J. D., 2007).

Azadirachtin acts as contact and systemic insecticide against *Tuta absoluta*. In soil application, 48.9–100% of larval mortality was recorded. Application of neem oil in adaxial surface of the foliage causes 57–100% larval mortality. However, it reported that application directly on larvae caused 52.4–95% mortality (Gonçalves-Gervásio R. and Vendramim J. D., 2007). Furthermore, it have significant inhibitory effects on fertility and fecundity of the adults (Lynn O. M. *et al.*, 2010). Overall, plants that contain such compounds with insecticidal activity such as *A. indica* can make a significant contribution to the development of novel, eco-friendly pest control methods that reduce the use of synthetic pesticides (Brunherotto R. *et al.*, 2010). Such compound is appropriate for control in organic systems of tomato production.

Larvicidal and ovicidal efficiencies of some botanical insecticides on *T. absoluta* in semi-natural conditions were explored. The study stated that spinosad had a 70% impact on the second-stage larvae of *T. absoluta*, and effect of *Azadirachtin* was so low; however, mixture of *Azadirachtin* and neem oil had a significant effect on eggs and larvae (Hafsi A. *et al.*, 2012). Also, (Moussa S. *et al.*, 2013) tested the effects of 17 different insecticides on *T. absoluta*. Results indicate that, out of the tested compounds, spinosad, emamectin benzoate, *Azadirachtin* and *B. thuringiensis* were found the most effective bio-pesticide for controlling *T. absoluta*.

Garlic extract

Garlic (*Allium sativum*) is a close relative of other members of the *Allium* genus including onions, chives and leeks. It is believed to be a native of central Asia but garlic is now cultivated throughout the world.

Garlic has for many years been used as a companion crop for cultivated plants, to help them withstand attack by pests such as aphids and caterpillars. This use exploits the natural biologically active defence compounds produced by plants. The principal biologically active compound produced by garlic is allicin which was discovered in 1944. Allicin is a sulphur containing (thiosulphonate) compound with powerful antioxidant and other properties. Undoubtedly allicin synthesis has evolved in garlic as the plants first line of defence against pest attack (Solufeed, 2015).

In controlling pests, the garlic extract showed good effect in promoting plant growth and suppressing the nematode (Sayeeda F. and Ahmad M.U., 2005). In an in vitro experiment showed that the growth of *Alternaria*, *Botrytis*, *Magnaporthe* and *Fusarium* was inhibited by application of garlic extract (allicin) placed on agar plates seeded with fungal spores (Hannah *et al.*, 2004). Plant extracts including garlic were prepared and tomato plants infested with leaf miner *Tuta absoluta* were sprayed three times at two week intervals starting after 40 days from transplanting. All treatments reduced population density of leaf miner significantly. The highest reduction was recorded by garlic extract followed by lemon grass extract and basil oil. Also, garlic extract increased the yield of tomato significantly (Hussein *et al.*, 2014).

Field study: Use of a biorational control system against *T. absoluta*.

The study was carried out in a greenhouse in Agronomy and Veterinary Institute Hassan II, the Horticultural Complex of Agadir (IAV-CHA) (altitude: 32m; latitude: 30°22' north; longitude: 9°39' west). The experimental station occupies an area of 20ha, the surface of the greenhouse where the work is laid out 1250m².

The substrate used is characterized by a sandy texture consisting of 78% sand, 19% Silt and 3% clay. This substrate is slightly saline (EC values range between 0.3 and 0.4 mmhos/cm). Soil fertility has lightly content of macro- and micronutrients and organic matter. Drip irrigation systems are used to irrigate field crops.

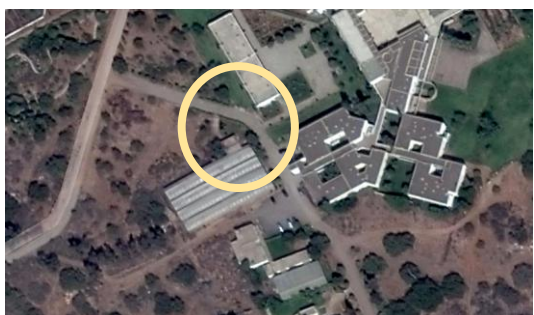


Fig. 10: Satellite view of the experimental greenhouse 1cm -->50m



Fig. 11: The experimental greenhouse

The experiment was carried out in a multi-chapel metallic greenhouse oriented to the East-West, covered by polyethyène plastic and net, with a ridge height of 6 m and over an area of 993 m². In this greenhouse, an open circuit soilless system was installed. The rows are 12 m long, 0.80 m wide and 0.30 m depth, and sealed with black plastic mulch, anti-Ultra-Violet, and 500 µm thick. These planting lines are prepared carefully by tilling the soil. The soil is treated against Nematodes using Metame sodium 3 months before planting. The greenhouse is a simple shelter since no function related to the climate is automated (manipulation of the doors, heating and ventilation).

Climatic conditions

Souss-Massa region is characterized by a semi-arid climate with a warm and dry summer and a relatively humid and slightly mild winter. The average temperature is 20°C with a maximum average of 45°C and a minimum average that can reach 7°C. Precipitation is generally low and irregular rarely exceeding 250 mm (Ouargaga M., 2015). The climatic conditions including: temperature, humidity and dew point inside the greenhouse are recorded using two datalogger (RHT10 extech instrument®) installed on the western and the eastern side of the greenhouse in order to determine the rate of the gradient of the temperature and the illumination.

Conducting the crop

Tomato variety

The variety used is Pristyla. It is a variety with long shelf life; the fruit is round, slightly flat with a caliber of (66-77mm) and a good firmness. The plant is vigorous with an indeterminate growth, short internodes. It is highly resistant to TYLCV, ToMV, *Verticillium albo-atrum*, *Verticillium dahliae* and *Fusarium oxysporum* f. sp. *Lycopersici* (type 0 and 1). This variety has an intermediate resistance to *Meloidogyne* species. It can be used for production under autumn, winter and spring shelters, and in summer fields.

Up keeping the crop.

The removing of the axillary buds and training of the plants through the string are taken place twice every week in order to keep the plants in the appropriate conditions to its growth. Surveillance of diseases and other pests is carried out every day. Other diseases and pests are controlled using the appropriate active ingredients without affecting *Tuta absoluta*'s population see table below.

Table 1: pests and diseases observed on the crop

Diseases and pests
Leaf mold (<i>Fuvi Fulva</i>)
Downy mildew (<i>Phytophthora infestans</i>)
Red mite (<i>Tetranychus urticae</i>)
Whiteflies (<i>Bemisia tabaci</i> and <i>Trialeurodes vaporariorum</i>)

1. Treatments and applications management

Soil treatment: Recharge®

Recharge 2% *Metarhiziumanisopliae*, entomopathogenic fungus for control of Tuta pupae present in soil. Mode of action - sporulation of tuta pupae root zone and areas in between plants should be covered. Dilute recharge in water and apply without spray nozzle.

Nursery application:

- Start with diluting 250g/250 liter of water and apply to all seedling trays. Mix more recharge if needed.

Greenhouse Application:

- First application, 5 kg / ha, 1000 liters of water during planting of the seedling in tomato field.
- Second application of recharge as 1 kg / ha, 1000 liters of water, around 30 days after planting.

Third application should be made at 1 kg / ha, 1000 liters of water around 60 days after transplanting

Foliar treatment 1: Biotrine®

It is a biorational 5% Abamectin, contact and translaminar activity on Tuta larvae larvacide acting on the first and second instars based on a natural fermentation of the soil bacterium *Streptomyces avermitilis* (Abamectin). It triggers the plant defence system against pathogens limiting the secondary infection of the open wounds caused by larvae. It has a broad spectrum. It kills the pest through contact, ingestion, paralyzing the larvae and by suffocation. It acts also as an antifeedant product with residual protection for the crop. It assists the plant in preparing against further attacks from the newly hatched larvae.

- Apply Biotrine when Tuta presence is visible or captured in the trap.
- Application rate 250 ml/ha in 1000 liters of water.
- Total applications : 2 applications and if needed up to 4 applications
- Application of Biotrine has to be rotated with Antario
- Supersede can be mixed with Biotrine for longer translaminar action and affection of the tuta eggs.

Foliar treatment 2: Fytomax® combined with Biotrine®)

Fytomax PM: It is a bio-rational solution based on Azadirachtin 1% (10000 ppm) in combination with neem oil, extracted from *Azadirachta indica* seeds in ULV formulation. Fytomax prevents or interferes with insect's development. It has an ovicidal effect and controls target pests by contact as well as by ingestion. It acts as repellent, antifeedant, and interference with the molting process of insect pest. Treated insects stop feeding and growing.

- Apply Fytomax when *Tuta absoluta* presence is visible or captured in the trap.

- a combined effect, mix 250 ml of Fytomax PM with 50 ml of Biotrine per 1000 L of water, to repel *Tuta absoluta* and whitefly. This mixture acts as a potent insecticide and fungicide, providing protection for 10-12 days. The waiting time is 3-4 days

Monitoring traps

Monitoring traps will be used to observe *Tuta absoluta* emergence, to monitor the pest pressure and to manage the treatments.

- 2 trap per hectare to monitor the population of *T. absoluta* using the pheromone lure Tuta-optima with the suitable delta trap.
- Lure can be changed every 4-6 weeks
- Monitoring of *T. absoluta* has to start from the beginning of the cropping season.

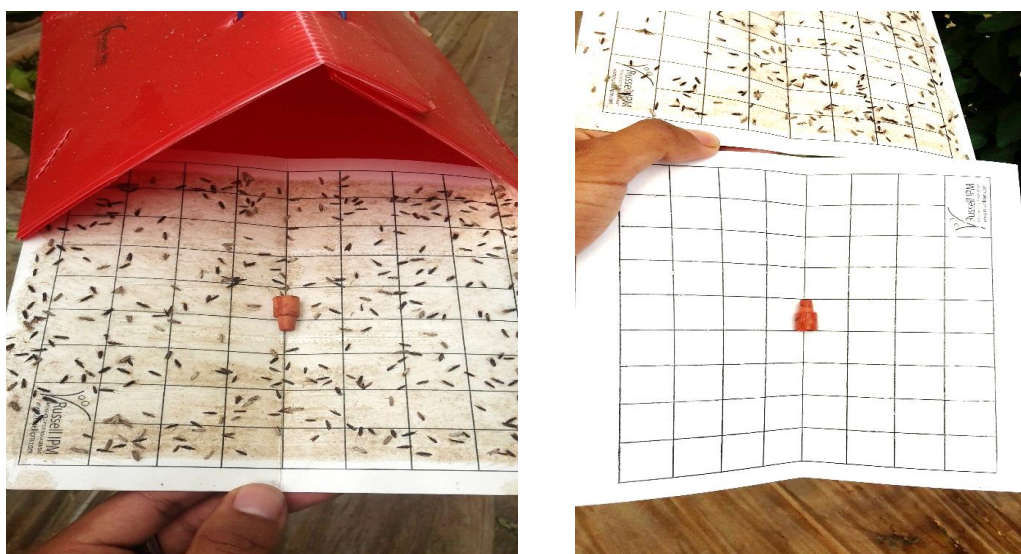
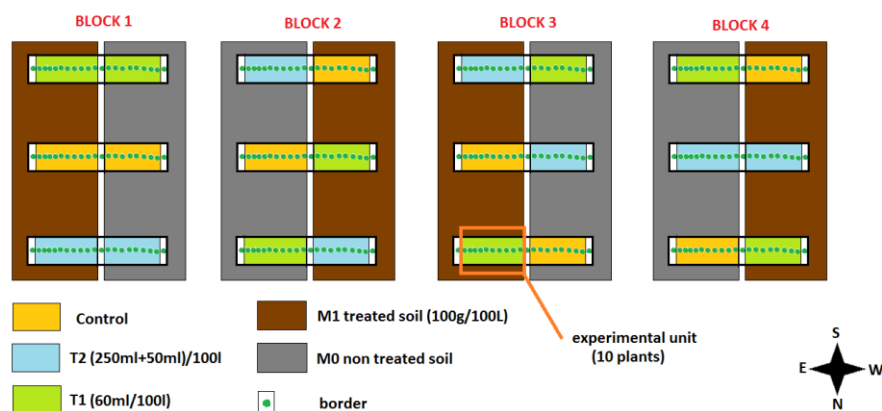


Fig. 12: Monitoring delta trap changing lure and sticky trap

2. Experimental design



The experimental unit of this design is the half of the row (11m length) excluding the 1 m considered as border. In each row, the combination of the soil treatment (Recharge) and the foliar treatments on the 10 plants is assessed to give an observation data.

Data assessment

The tomato plants can be attacked from seedling stage to the mature plants. The parameters used to assess the damage of *T. absoluta* were the leaves and fruit mines, and fruits on which the black frass is visible. Leaves of the ten plants of the experimental unit are investigated and observed carefully, counting the number of leaves mined by the moth. A random of two infected leaves from five different plants in each treatment (ten leaves in total) was harvested weekly. The collected samples were investigated in the laboratory under stereomicroscope to determine the mortality of larvae.

Data analysis

Leaves and fruit mines, larvae and damages data will be analyzed by statistical method analysis of variance using the MINITAB statistical software (Leora Software, 1987). This software calculates the following parameters: Fiducial Limits, Standard Error and Slope of regression. The average mortality was processed and compared using MINITAB software.

Results of the insecticide bioassays were reported as percentage of mortality. Prior to analysis, data were transformed to a square root function.

Results and discussion

In order to confirm the laboratory results, a greenhouse trial was carried out to evaluate the efficacy of the biorational insecticides Abamectin 5% (Biotrine®) and the mixture Azadirachtin 0.1% (Fytomax®) plus Abamectin 5% in combination with the soil treatment *Metarhiziumanisopliae* (Recharge®). Basing on the results of the screening test on the laboratory, the concentrations used are respectively of 10ml/20L, (50+10) ml/20L and 150g/20L. The experimental design was a split plot where foliar treatments are randomized on the small plots, and the largest plots concern the soil treatment. Leaves damage and number of death larvae and the yield is assessed on the 3th, 5th 8th and 12th cluster harvest.

In addition, no significant difference was obtained between plots treated with *Metarhiziumanisopliae* 2% and non-treated soil plots $p=0.513$. This does not agree with obtained results in the laboratory assay that showed mortality of 12.8% to 66.2% on pupae. therefore, (Elmaghawry M., 2015) found that *M. anisopliae* have a significant efficacy when compared to untreated plants but

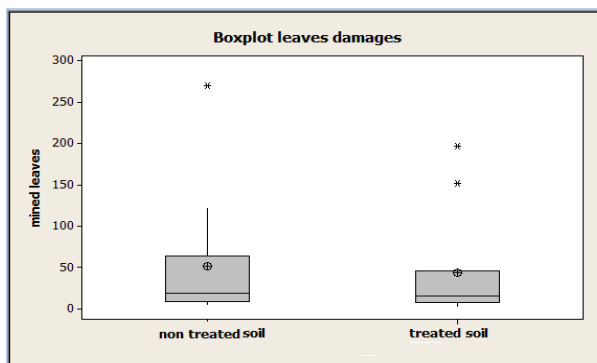


Fig. 13: Leaves damage of treated soil plots and control

still not satisfactory to increase the mortality of larvae. That is could be explained by the application mode (soil or foliage) and the target of the treatment (pupae or larvae).

The failure of *M. anisopliae* could also be explained by results of (Lopes R. B. et al., 2013) who stated that high relative humidity, temperature and/ or O₂ (factors that boost the metabolic activity) have a negative impact on germination of *M. anisopliae*.

Regarding foliar treatments, significant to highly significant difference between foliar treatments is obtained in all the observations with (df=2 pp=0.001). Similar to the laboratory results when Biotrine® at the rate of 0.4ml/L and the mixture Biotrine® plus Fytomax® at the rate of (0.4+4)ml/L gives the highest mortality respectively 84.8% and 100%. Thus, the application of Abamectin and the mixture Azadirachtin plus Abamectin reduced the average percentage of infected leaves to respectively 5.96% and 2.5% throughout the cycle of the crop. The obtained results are in accordance with results found by (Moussa S. et al., 2013) who found Azadirachtin the most effective bio-insecticide in the control of *T. absoluta*. Moreover, (Elmaghawry M., 2015) had up to 82.26% of larval reduction by applying Azadirachtin under greenhouse condition. (Brunherotto R. et al., 2010; Tomé H. et al., 2012) observed the delayed development of larvae and high insect mortality allowing few larvae to reach the pupa stage. Concerning Abamectin (Sallam A. et al., 2015) recommended its use to control the leaf-miner. However, some authors reported the resistance of *T. absoluta* toward this compound (Siqueira H. et al., 2000; Lietti et al., 2005). The obtained results could be explained by the synergistic blend Abamectin and plant extract in Biotrine® in order to trigger the

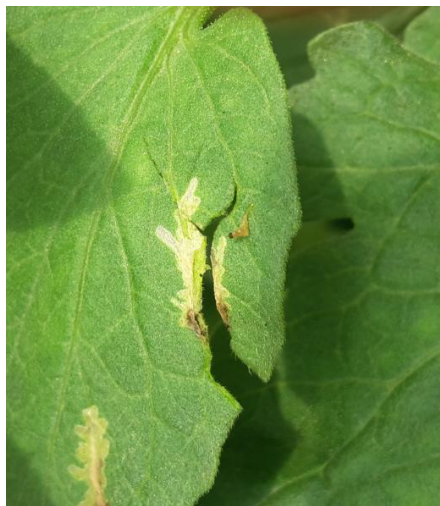


Fig. 14: Dead larva inside the mine after treating with Abamectin Biotrine®



Fig. 15: Fruit damage on control plots



Fig. 16: Leaf damage on control plots



Fig. 17: Healthy foliage in treated plants with the mix azadirachtin plus abamectin



Fig. 18: Healthy cluster in plots treated with abamectinbiotriner®

plant defense and protect it from pests (Siqueira H. *et al.*, 2000; Russell IPM, 2016). This comes in harmony with results obtained by (Salem N. M., 2015), who used the same Biorationals (Biotrine®, Fytomax®) in combination with mass trapping in Egypt. As a result, reduced infestation to 11.1% had been recorded.

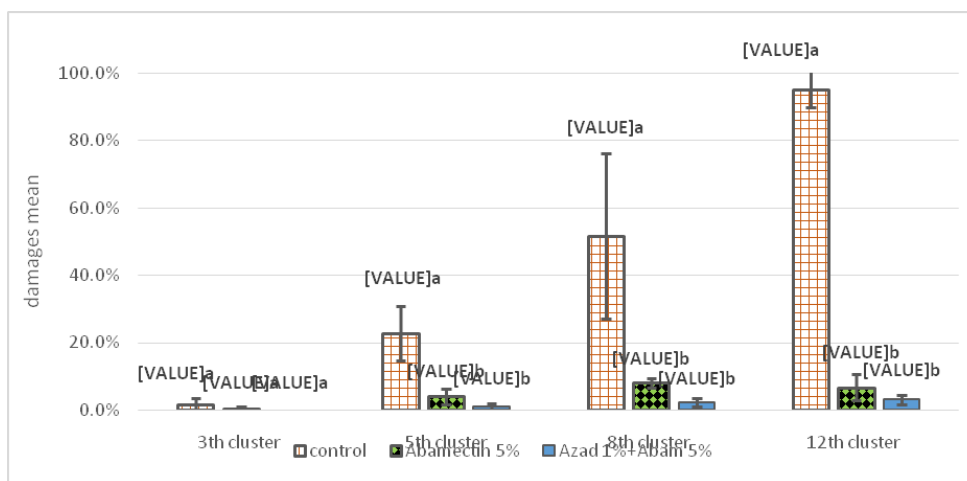


Fig. 19: Leaves damage percentage per treatment

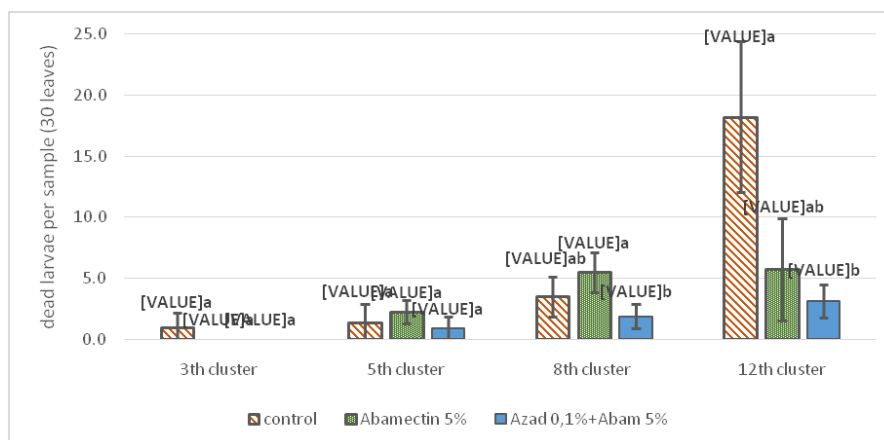


Fig. 20: Dead larvae per treatment leaves sample (30 leaves)

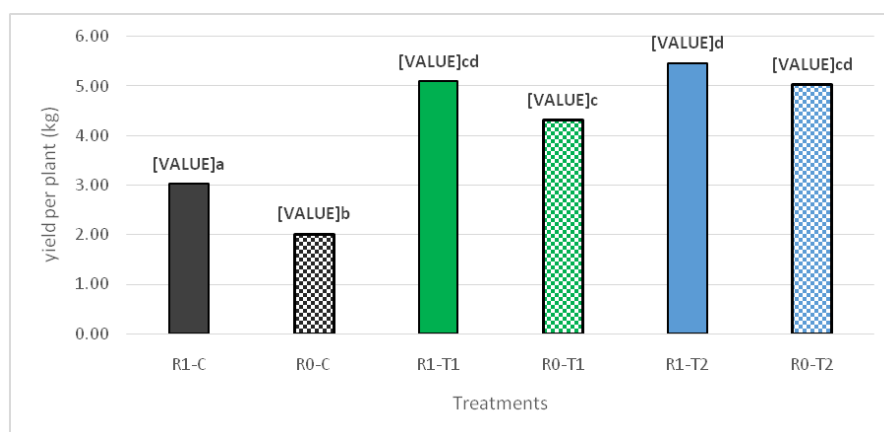


Fig. 21: Yield mean per plant over the different treatments plots

Regarding the yield a highly difference on the yield has been recorded on the treated plants by the biorationals comparing to the control (foliar $p \leq 0.001$ soil $p = 0.04$). In addition, an increase was clear on plants treated with the mix of Azadirachtin and Abamectin on the foliar and *Metarhizium anisopliae* on the soil. This could be explained by the limitation of damages to the minimum by these foliar treatments and the biostimulant effect of *M. anisopliae*.

In conclusion soil applications of *M. anisopliae* did suppress the damage due to *Tuta absoluta*. Moreover, Abamectin were able to keep the damage between 4% and 8% compared to the mixture Azadirachtin plus Abamectin that kept the damage between 1% and 3%. This study proved combination of biorational studies is pivotal to control *Tuta absoluta*.

CONCEPT NOTE

SAARC Regional Training on Integrated Pest Management

Jointly organized by

SAARC Agriculture Centre (SAC), Dhaka, Bangladesh
Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh and
Asia-Pacific Association of Agricultural Research Institutions (APAARI)

28-31st May 2018 in BARI, Gazipur, Bangladesh

Agricultural impacts of climate change will manifest in terms of changes in land and water resources, insect pest populations, diseases, etc. which ultimately translate into a change in productivity and profitability of agriculture. In this connection, IPM is an ecosystem approach to crop protection and production that combines different management strategies and practices to grow healthy crops and minimize the use of pesticides. IPM is not a single pest control method but encompasses a series of pest management evaluations, decisions and control methods. It is based on the concept that it is not necessary to eliminate all pests but to reduce pest populations to levels where pests cannot cause significant loss. An IPM strategy includes the use of pest-resistant crop varieties, modification of agronomic practices to reduce pest incidence, biological control, other innovative pest suppression approaches and need-based, judicious use of chemical pesticides. IPM can make a key contribution to not only addressing the challenge of food insecurity but also meeting the growing consumer demand in the Asia-Pacific region for safe food¹.

Several kinds of pests can infest the different parts of plants/crops, and damage them partially or sometimes completely, seriously affecting the quantity and quality of the yield. Moreover, certain crop production intensification practices (e.g. early season spraying of pesticides in rice fields) can even increase the impact of pests on plants. In order to protect their plants from those pests attack, farmers are mostly depending on the toxic synthetic pesticides. But in many cases indiscriminate uses of synthetic pesticides have been observed, especially in the high valued crops like vegetable, fruits etc. In rice, generally farmers are applying pesticides 4-5 times per season but in high valued vegetables like brinjal, cabbage, cauliflower, cucurbit crops indiscriminate use of pesticides are going on. There are also evidences for daily applications of toxic pesticides especially during the hot and humid rainy season. Brinjal and also other vegetables are often harvested within 6-12 hours of spraying of chemical pesticides.

These excessive uses of pesticides have no doubt adverse effects on environment like killing of beneficial insects, birds, fish, environmental pollution, development of pesticide resistance, resurgence of pest population and human health hazards by leftover residues of pesticides. Different crops especially vegetables and fruits are harvested and marketed without knowing any residue status. This is most likely to cause serious health hazard to the consumers. Crops with thin skins or those eaten whole are the most risky for consumers. Although some pesticides are broken down during cooking, but some are temperature-stable and unaffected by cooking, while some can become more hazardous on cooking, for example, the fungicide, *mancozeb*, which on heating break down into carcinogenic *ethylene thiourea*. Moreover, the repeated applications have induced multiple resistances of different pests against various pesticides. It is also suspected that the fish populations in the open water bodies as well as in the rice fields have been reduced due to the adverse effects of the pesticide use. The serious consequences of pesticide use are well

¹ <http://www.fao.org/agriculture/crops/thematic-sitemap/theme/spi/scpi-home/managing-ecosystems/integrated-pest-management/en/>

documented all over the world. Global use of pesticides creates substantial health impact in all parts of the world, although the exact toll is difficult to pinpoint. In Bangladesh a survey on brinjal farmers at Jessore region revealed that more than 98% of the farmers are suffering from different side effect of pesticides.

To get rid from this measurable pest management system, some new avenues must have to be developed. One of the ways may be to develop eco-friendly, sustainable, socio-economic acceptable integrated pest management or IPM packages. IPM is one of a number of integrated approaches that are gaining credence for use in sustainable agriculture development. It involves the integration by farmer of the most appropriate management strategies for pest control where sole dependency on pesticides can be avoided. According to FAO "Integrated Pest Control is a pest management system that in the contest of the associated environment and the population dynamics of the pest species, utilizes all suitable techniques and methods in as compatible manner as possible and maintains the pest population at the levels below those causing economic injury". IPM also includes the necessary Phytosanitary measures, monitoring and diagnostic system, good agricultural practices and the management of natural enemies with the minimum amount of pesticides (when needed and of good quality). IPM is thus an important part of Integrated Plant Production Management (IPPM) and sustainable crop production intensification. By enhancing the ecosystem function, by making the agricultural ecosystem healthier, more ecosystem services are provided as pest control.

One of the most important elements of any robust Integrated Pest Management strategy is making optimal use of biological control or bio-pesticides. Biological control of pests is the use of natural enemies to regulate pest populations to a level where these will not cause yield loss. The need for more optimal use of biological control is recognized at national and international levels. However, throughout most of South Asia access to technical knowledge and skills with regards to production and sustainable utilizations of bio-control or bio-pesticides options are still inadequate to warrant its widespread adoption. In this connection, scientists of Bangladesh Agricultural Research Institute (BARI) have already developed Bio-rational based IPM packages against several destructive insect pests of different crops, where emphasis has been given on use of biological control, mass trapping with pheromones, use of different microbial and bio-pesticides, use of pest-resistant crop varieties, modification of agronomic practices etc. to reduce pest incidence.

Therefore, it is very much important to review the concepts and principles of bio-control and bio-rational based IPM, to share experiences and discuss opportunities and challenges for quality production and sustainable utilization and to identify best options for ensuring better access and utilization of bio-control and bio-rational options by IPM farmers of the SAARC Member countries. Thus, there is a need to organize regional level capacity development through training program and field visit to address different issues of bio-rational based IPM and take advantage of the investment in practical training for Integrated Pest Management (IPM) for the young scientists and extension officer of SAARC Member States.

Objectives

- Raise awareness on the potential use of different bio-control agents and provide hands on training on their mass rearing and release in the laboratory and field conditions and on monitoring and evaluation of their effectiveness in the field;
- Scale out the potential use of bio-pesticides for effective management of destructive pests of different crops especially high valued crops to facilitate the availability of safe food.
- Teach the best IPM technologies to reduce the use of toxic chemical pesticides for sustainable crop production in SAARC Member States;

- Identify opportunities and develop models for linking the stakeholders like extensionist, researchers, input providers and ensuring private sector involvement in commercialization of bio-control agents, bio-pesticides for pest management in SAARC Member States.

Methodology

- Organize Regional IPM Training in coordination with potential partners for NARS and Extension officials of Ministry of Agriculture/Department of Agriculture of SAARC Member States.
- Hands on training on the identification, mass rearing, field release, quality control of different bio-control agents for pest management.
- Extension Approaches to create awareness and demonstration of technology at field level to convince and gain their confidence leading to adoption
- Monitoring and evaluation of different components of IPM in the field.
- Facilitate to organize exposure visit for improved technologies of healthy production, value chain development activities for SAARC Member States.
- Group Discussions and hands-on activities/practice in the laboratory and in the field.

Schedule

28-31st May 2018

- 27th May – Regional participants arrive in Dhaka, Bangladesh
- 28th May – Travel to Gazipur and attend the Opening ceremony and training program
- 29-30th May – Participate in Training Program
- 31st May – Field Visit and interaction with IPM farmer
- 1st June – Travel back to respective countries

Venue: Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur, Bangladesh

Target participants: 20

- 7 - Young Scientists of NARS from SAARC MSs
- 7 - Officials of NAES (DoA/MoA) from SAARC MSs
- 6 -Bangladesh (BARC, BARI and BRRI)

Collaborating Institutions

- SAARC Agriculture Centre (SAC), Dhaka, Bangladesh
- Bangladesh Agricultural Research Institute (BARI)
- Department of Agriculture Extension (DAE), Bangladesh
- Asia-Pacific Association of Agricultural Research Institutions (APAARI)
- Centre for Agriculture and Bioscience International (CABI)

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Training Program
SAARC Regional Training on Integrated Pest Management
BARI, Joydepur, Gazipur, Bangladesh, 28-31st May 2018

Day 1 (28 May 2018)

08.00 Arrival of the participants from Dhaka

09.00 -10.00 Inaugural Ceremony

10.00- 10:30 Tea Break/photo session

10:30 -13:00 Technical Session: I

10:30 -11:30 Bangladesh Agriculture: Key features, achievements and emerging challenges (Lecture)

11.30-12.30 Integrated Pest Management – An Overview (Lecture)

12.30-13.30 Strengthening Biocontrol by transboundary exchange of tools, techniques and expertise amongst SAARC countries (Lecture)

13.30-14.30 Lunch Break

14.30-17.00 Technical Session: II

14.30-15.30 Sampling Procedures of Different Arthropods emphasize on biological control agents. (Lecture)

15.30-16.30 Use of different Bio-pesticides for pest management and their quality control. (Lecture)

16.30 -17.30 Insect pests of vegetable crops and their integrated management. (Lecture)

Day 2 (29 May 2018)

09.00 -10.00 Technical Session: III

09.00 -10.00 Insect Pests of fruit crops and their integrated management (Lecture).

10.00-11.00 Biological Control of insect pests and mass rearing techniques of predators and parasitoids (Lecture)

11.00- 11:30 Tea Break

11.30-17.00 Join the “Discussion Meeting” at Ministry of Agriculture, Bangladesh Secretariat, Dhaka

Day 3 (30 May 2018)

09.00 -13.00 Technical Session: IV

09.00 -10.00 Rice insect pests and their integrated management (Lecture)

10:00 -11:00	Tomato leaf miner, <i>Tuta absoluta</i> an invasive pest of South Asia and their bio-rational solutions
11.00- 11:30	Tea/coffee Break
11:30 -12:30	Commercial formulations of <i>Trichoderma</i> and their use in disease management: Perspective Bangladesh (Lecture)
12:30-13:30	Mass rearing protocol of different microbial pesticides and their quality control (Lecture)
13.30-14.30	Lunch Break
14.30-17.00	Technical Session: V
14.30-16:00	Mass rearing, field release, efficacy testing techniques for different predators and parasitoids (Practical).
16:00-17:00	Mass rearing protocol of different microbial pesticides and <i>Tricho</i> compost (Practical).

Day 4 (31 May 2018)

09.00 -13.00 Technical Session: VI

08.00 -11.00	Visit of Ispahani Bio-control Laboratory, Konabari, Gazipur: A private bio-pesticide company
11.00- 11:30	Tea/coffee Break
11:30 -13:00	Field studies on bio-rational management on different vegetables & fruits
13.00-14.00	Lunch Break

14.00-17.00 Technical Session: VII

14:00-15:00	Preparation of action plans of IPM for different SAARC countries
15:00-17:00	Evaluation and Closing ceremony

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