CHAPTER 05

Biological Control for Fall Armyworm Management in Asia

CASE STUDY: INDIA

Chandish R. Ballal*, A. Kandan, Richa Varshney, Ankita Gupta, A.N. Shylesha, Om Prakash Navik, T. Venkatesan, Gandhi Gracy, B. Ramanujam, R. Rangeshwaran, G. Sivakumar, M. Nagesh, Jagadeesh Patil, U. Amala, T.M. Shivalingaswamy, K. Subaharan, N. Bakthavatsalam, Y. Lalitha, B. Poornesha, V. Apoorva, T.R. Ashika, and Nishtha Nayyar

CASE STUDY: BANGLADESH

Syed Nurul Alam[1**], Debasish Sarker[2], and Timothy J. Krupnik[1]

* Corresponding author (ballalchandish@gmail.com); All the authors are affiliated with the Indian Council of Agricultural Research (ICAR)–National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru, India.

** Corresponding author (alamsn09@gmail.com); [1]CIMMYT, Dhaka, Bangladesh; [2]Entomology Division, BARI, Gazipur, Bangladesh.

1. Overview

Biological control, as mentioned in **Chapter 1**, is an important pillar of integrated pest management (IPM). The various components of IPM are not meant to act independently, and in some cases, for example with the use of parasitoids and predators, extra precautions may be needed when using pesticide applications. This is especially true in maize, where an IPM program must address several pests and not just the fall armyworm (FAW; *Spodoptera frugiperda* (J.E. Smith)). Biological control should be integrated into an IPM program with a clear understanding of its interactions with the other components of the IPM crop strategy such as habitat manipulations, host plant resistance, and pesticide use practices (Figure 2 in **Chapter 6**, Agroecology) (Orr 2009). In this chapter, biological control efforts for management of FAW are discussed using two case studies—India and Bangladesh. The principles and protocols could be applicable across the FAW-affected countries in Asia. A summary of the current status of biocontrol worldwide and issues regarding its adoption can be found in Barratt *et al.* (2018).

CASE STUDY: INDIA

2. Introduction

Increased international trade has resulted in a continuous influx of alien arthropod species globally. In the last 13 years, India has formally recorded the entry of 17 invasive insect pests, including the fall armyworm (FAW; Gupta *et al.* 2017; Shylesha *et al.* 2018; Selvaraj *et al.* 2020). The scientific community, in collaboration with other stakeholders including farming communities, has succeeded in tackling these invasive pests using an IPM framework, which includes classical biological control (*i.e.*, the introduction of a new predator/parasitoid of an exotic pest to a new location where the pest has invaded) as well as augmentative biocontrol (the rearing and release of biocontrol agents as a treatment). A central goal of IPM is conservation biological control, which seeks to minimize harm to biocontrol agents occurring naturally in the cropping system. Finally, an increased demand for use of less-toxic pesticides, and in some cases a demand for biointensive pest management (BIPM) approaches (Anitha and Parimala 2014; Dufour 2001; Prakash and Rao 2017; Ranga Rao *et al.* 2007), is a driving force for greater use of biocontrol options where they are cost-effective and efficacious.

The focus of research at the Indian Council of Agricultural Research–National Bureau of Agricultural Insect Resources (ICAR-NBAIR), Bengaluru, India, was to search for indigenous natural enemies of FAW and to develop, promote, and deploy validated sustainable BIPM technologies against FAW. During 2018-19, 22 indigenous natural enemies of FAW were identified from infested fields, which comprised 13 parasitoids, five predators, and four microbials. This information on indigenous natural enemies is of paramount importance in designing conservation or augmentation biological control strategies for management of FAW, particularly because of the regulatory restrictions on bulk import of biopesticides.

Further, systematic studies were taken up to identify those bioagents that are amenable for multiplication and field utilization against FAW. Instead of testing the available commercial biopesticides, those potential bioagents/biopesticides available at the NBAIR macrobial (parasitoids and predators) and microbial repositories were evaluated against FAW—initially through laboratory trials to identify the best agents, which were followed by field testing in different agroecological zones of the country. Three egg parasitoids and one egg-larval parasitoid were observed to be amenable to rearing and release. Two exotic parasitoids, *Trichogramma pretiosum* Riley and *Telenomus remus* Nixon (which had been imported into India several decades ago and were already available in the insectary at NBAIR), which have proven to be efficient bioagents in Latin American (Figueiredo *et al.* 2015) and African trials, were evaluated against FAW. It was also interesting to record that *T. remus,* which was imported and released several decades ago to target *Spodoptera litura* (Fabricius) in India and which had never been recorded in release and recovery studies, was now observed to parasitize FAW eggs in nature. The indigenous species *Trichogramma chilonis* Ishii was also recorded as a dominant parasitoid of FAW eggs in nature.

A total of six microbials—one strain of *Metarhizium anisopliae,* one strain of *Pseudomonas fluorescens*, two strains of the entomopathogenic nematode *Heterorhabditis indica* from the NBAIR microbial repository, and two microbials isolated from infected field-collected FAW larvae, which included a strain of *S. frugiperda* nucleopolyhedrovirus (SpfrNPV) and a strain of *Bacillus thuringiensis* (*Bt*)—were identified for final large-scale field trials. These were validated in the fields in different parts of the country in collaboration with the All India Coordinated Research Project on Biological Control. A BIPM strategy was developed that comprised nano-based pheromone traps for monitoring and mass trapping, egg parasitoids for targeting the egg stage of the pest, and microbials to target the early and late larval stages.

3. Indigenous Natural Enemies of FAW Reported from Indian Maize Fields

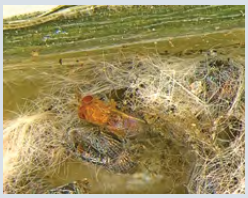
Molina-Ochoa *et al.* (2003) reported approximately 150 species of parasitoids and parasites of FAW, which belonged to 14 families—nine in the Hymenoptera, four in the Diptera and one in the Nematoda—throughout the Americas and the Caribbean Basin. Ichneumonids and braconids were the most diverse families in Hymenoptera. The Tachinidae was the most diverse family among the parasitoids, with 55 species.

In July 2018, when FAW was first reported in India, an extensive search was made for the presence of indigenous natural enemies. As expected, a number of such parasitoids and predators were found. Shylesha *et al.* (2018) have documented natural parasitism by parasitoids (Table 1), namely, the egg parasitoids *Telenomus* sp. (Platygastridae) and *Trichogramma* sp. (Trichogrammatidae), the egg-larval braconid parasitoid *Chelonus* spp., the gregarious larval parasitoid *Glyptapanteles creatonoti* (Viereck) (Braconidae), the solitary larval parasitoid *Campoletis chlorideae* Uchida (Ichneumonidae), and an indeterminate larval-pupal Ichneumoninae parasitoid.

Gupta *et al.* (2019) reported *Cotesia ruficrus* (Haliday) as a larval parasitoid of FAW in the maize fields of Karnataka, Tamil Nadu, Rajasthan, Uttar Pradesh, Punjab, and Meghalaya. Gupta *et al.* (2020a, b) additionally reported *Chelonus formosanus* Sonan and *Coccygidium transcaspicum* (Kokujev), respectively, from FAW.

Two parasitoids, namely, *Phanerotoma* sp., and *Coccygidium transcaspicum* (Table 1), which were found associated with FAW, were less frequently encountered than *T. remus*, *Trichogramma chilonis*, *Chelonus formosanus*, *Chelonus* spp. (see Section 5.3.3), *Campoletis chlorideae* Uchida, and *Cotesia ruficrus*. *Forficula* sp. (Dermaptera: Forficulidae) (Table 1) was also observed feeding on FAW larvae in some of the fields that were free from insecticidal spray.

Table 1. Parasitoids and predators reported on FAW in India.

Scientific name	Biological attribute	Photograph	Collection locality	Reference(s)
Telenomus sp.# Hymenoptera: Platygastridae	Egg parasitoid		Karnataka and Tamil Nadu	A
Trichogramma sp.## Hymenoptera: Trichogrammatidae	Egg parasitoid		Karnataka	A
Chelonus formosanus Sonan Hymenoptera: Braconidae	Egg-larval parasitoid		Karnataka; Telangana	B
Coccygidium transcaspicum (Kokujev) Hymenoptera: Braconidae	Larval parasitoid		Karnataka; Telangana	C

Scientific name	Biological attribute	Photograph	Collection locality	Reference(s)
Cotesia ruficrus (Haliday) Hymenoptera: Braconidae	Larval parasitoid		Karnataka; Tamil Nadu; Rajasthan; Uttar Pradesh; Punjab; Meghalaya	D
Glyptapanteles creatonoti (Viereck) Hymenoptera: Braconidae	Larval parasitoid		Karnataka	A
Campoletis chlorideae Uchida Hymenoptera: Ichneumonidae	Larval parasitoid		Karnataka	A,E
Eriborus sp. Hymenoptera: Ichneumonidae	Larval parasitoid		Karnataka	E
Odontepyris sp. Hymenoptera: Bethylidae	Larval parasitoid		Tamil Nadu	E
Phanerotoma sp. Hymenoptera: Braconidae	Larval parasitoid		Karnataka	F
Exorista sorbillans (Wiedemann) Diptera: Tachinidae	Larval parasitoid		Karnataka	E
Forficula sp. Dermaptera: Forficulidae	Predator		Karnataka	A,E
Harmonia octomaculata (Fabricius): Coleoptera: Coccinellidae	Predator		Karnataka	E
Coccinella transversalis Fabricius Coleoptera: Coccinellidae	Predator		Karnataka	E
Indeterminate Ichneumoninae Hymenoptera: Ichneumonidae	Larval-pupal parasitoid		Karnataka	A

Source of all photos in Table 1: Ankita Gupta, ICAR-NBAIR.
Reported in Shylesha *et al.* (2018) as *Telenomus* sp.; now identified as *Telenomus remus* Nixon
Reported in Shylesha *et al.* (2018) as *Trichogramma* sp.; now identified as *Trichogramma chilonis* Ishii
References (for discovery on FAW in India): A: Shylesha *et al.* (2018); B: Gupta *et al.* (2020a); C: Gupta *et al.* (2020b); D: Gupta *et al.* (2019); E: Sharanabasappa *et al.* (2019); F: Present report.

3.1. Natural Field Parasitism Rates in Various Countries

A number of natural enemies of FAW including parasitoids, predators, and pathogens have been reported from African countries (Huesing *et al.* 2018) and Latin America (Molina-Ochoa *et al.* 2003). From African countries, *Cotesia icipe* Fernandez-Triana & Fiobe (Hymenoptera: Braconidae) was the major parasitoid recorded in Ethiopia, with a parasitism rate of 37.6% (Sisay *et al.* 2018). Another braconid, *Coccygidium luteum* (Brullé), was found parasitizing over 20% of larvae found in Ghana and Benin (Agboyi *et al.* 2020; Koffi *et al.* 2020). *Chelonus curvimaculatus* Cameron (Hymenoptera: Braconidae) was the only egg-larval parasitoid recorded in Kenya, with a 4.8% parasitism rate; however, *Chelonus bifoveolatus* Szépligeti was a dominant parasitoid in West Africa (Agboyi *et al.* 2020; Koffi *et al.* 2020). In 2018, six species of egg and larval parasitoids were recovered with *C. icipe* being the dominant larval parasitoid, with percentage parasitism ranging from 16% to 42% in the three surveyed countries. In Kenya, *T. remus* (Hymenoptera: Scelionidae) was the dominant egg parasitoid, causing up to 69.3% parasitism as compared to only 4% by *C. curvimaculatus.* Pomari *et al.* (2013) reported that a ratio of 0.165 female *T. remus* parasitoids per FAW egg can be recommended to be released in maize since this release rate resulted in ≥80% parasitism. In Latin America, inundative releases of *T. remus* resulted in 90% parasitism, providing control of FAW (Cave 2000; Ferrer 2001).

Telenomus remus was originally imported into India and field-released several decades ago for the management of *S. litura* (Sankaran 1974). Parasitism of FAW eggs by *T. remus* was recorded in Karnataka, Maharashtra, and Telengana states in India (Navik *et al.* 2021; ICAR-NBAIR 2020). *Trichogramma chilonis* parasitism was recorded in Karnataka and Maharashtra (Navik *et al.* 2021; ICAR-NBAIR 2020). Parasitism by *T. chilonis* in the early stage of FAW invasion in 2018 ranged from 1.08% to 1.20%; however, in the following year (2019-20), parasitism by *T. chilonis* increased and ranged from 2.28% to 20% in different localities of Karnataka. In Maharashtra, the parasitism rate by *T. chilonis* ranged from 7.5% to 18%. Parasitism by *T. remus* was 1.0% to 7% during 2018 and 1.2% to 8% during 2019-20 in Karnataka. Both parasitoids were observed to parasitize eggs in the same egg mass, indicating their complementarity. In India, laboratory studies at ICAR-NBAIR indicated 100% parasitism by *T. remus* on FAW eggs. *Telenomus remus* was observed to be the dominant parasitoid when both *T. pretiosum* and *T. remus* were released together.

3.2. Parasitism Potential of *Trichogramma* Species against FAW

Laboratory screening of field-collected *Trichogramma chilonis* and lab-reared *T. pretiosum*, *T. chilonis*, and *Trichogrammatoidea armigera* indicated that field-collected and lab-reared *T. chilonis* and lab-reared *T. pretiosum* could provide high rates of parasitism (74%, 69%, and 67%, respectively) (ICAR-NBAIR 2020; Om Prakash Navik, unpublished data).

4. Entomopathogens

4.1. Entomopathogenic Fungi

Attempts were made to record the microbial pathogens infecting FAW in different maize-growing areas in India. The aim was to identify potential entomopathogens and further develop alternative pest management strategies using microbials. *Metarhizium rileyi* (=*Nomuraea rileyi*) was observed to cause epizootics (Figure 1A) with around 10-62% infection in natural field situations in different locations in the states of Karnataka (Shivamogga, Chikkaballapur, Hassan, Davanagare, Chitradurga, and Bangalore districts) (Shylesha *et al.* 2018; Mallapur *et al.* 2018), Andhra Pradesh (Anantapur, Visakhapatnam, and Vijayanagaram districts), Telangana (Ranga Reddy and Medak districts), and Maharastra (Ahmednagar, Pune, and Solapur districts).

Two strains of *Metarhizium rileyi* were isolated from FAW cadavers collected from Chikkaballapur and Anantapur (designated NrSf-4 and NrSf-5, respectively). Molecular characterization was done using the internal transcribed spacer (ITS) region and the sequences were deposited at NCBI GenBank (accession number MN602591). *Beauveria felina* has been found to cause natural infection (Figure

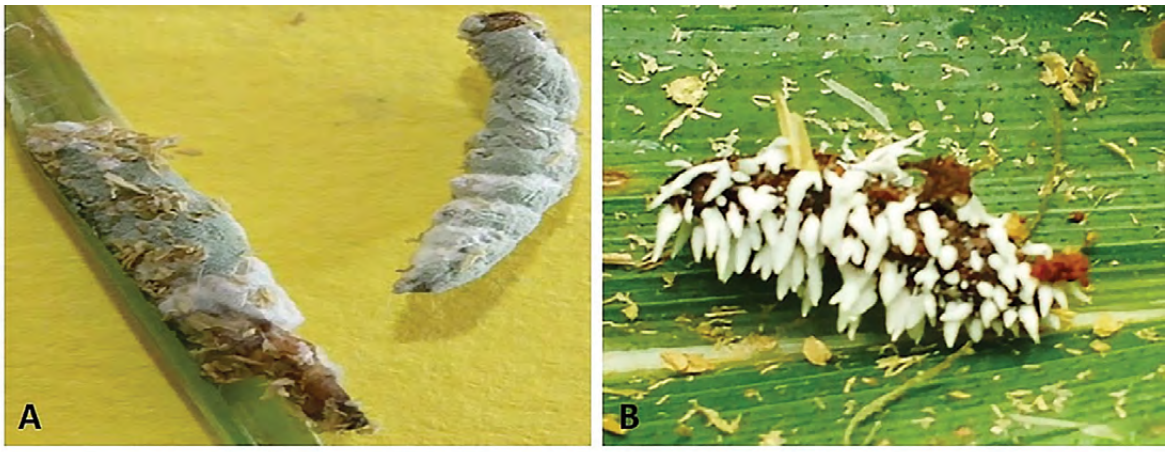


Figure 1. (A) Epizootics of *Metarhizium rileyi* on FAW; (B) natural infection of *Beauveria felina* on FAW.

1B), recorded as around 30% infection in Chikkaballapur, Karnataka (ICAR-NBAIR 2020). The organism has been isolated, molecular characterization was done using the ITS region, and the sequences were deposited at NCBI GenBank (accession number MN833071).

ICAR-NBAIR holds a repository of promising isolates of entomofungal pathogens. After initial laboratory studies, field evaluations of *Beauveria bassiana* (ICAR-NBAIR Bb-45) and *Metarhizium anisopliae* (ICAR-NBAIR Ma-35) were carried out against FAW during the *rabi* (winter) season in 2018 and the *kharif* (monsoon) season in 2019 (Bangalore and Chikkaballapur in Karnataka) and at Anakapalle in Andhra Pradesh during *rabi* 2018. Three foliar sprays @ 5 g/L (talc formulation containing 1×10^8 spores/g) were applied at 15, 30, and 45 days after maize germination. The results indicated that ICAR-NBAIR Ma-35 and ICAR-NBAIR-Bb-45 could cause 50-80% reduction in pest damage. Field evaluation against FAW in Dharwad district of Karnataka resulted in 58-62% pest reduction 15 days after spraying (Mallapur *et al.* 2018). The Bb-45 and Ma-35 isolates have been field-tested so far in different maize-growing areas of India covering an area of 40 hectares in Karnataka, Andhra Pradesh, Telangana, Tamil Nadu, Maharashtra, and Orissa during the *rabi* and *kharif* seasons of 2018 and 2019.

4.2. Entomopathogenic Bacteria

4.2.1. *Bacillus thuringiensis*

Bacillus thuringiensis Berliner (*Bt*) is a bacterium that forms insecticidal proteins and other toxins, and is used as a biopesticidal spray. Most *Bt* crystal (Cry) toxins are specific to lepidopteran insects, and *Bt* strains with activity against FAW can be found all over the world. For example, *Bt aizawai* HD 68 and *Bt thuringiensis* 4412 have shown 80% and 80.4% mortality, respectively, against FAW in laboratory studies (Polanczyk *et al.* 2000). de Souza *et al.* (2009) identified *Bt (israelensis* type) showing toxicity to FAW (LC_{50} of 76.58 µg cm^{-2}). Monnerat *et al.* (2007) isolated three indigenous *Bt* isolates from Brazil showing higher toxicity (LC_{50} of 18-25 ng cm^{-2}) against FAW. Similarly, Cerqueira *et al.* (2016) isolated four indigenous isolates from Brazilian soils having LC_{50} of 44-108 ng cm^{-2} against FAW. At ICAR-NBAIR, researchers have succeeded in identifying an effective indigenous *Bt* isolate, NBAIR-BT25, and further developing it into a formulation for FAW management (Figure 2). This isolate has been submitted to GenBank as MN327970 (AICRP-BC 2020). In a replicated trial in Orissa, a combination of *Trichogramma* sp. releases to target the egg stage followed by NBAIR-BT25 sprays to target the larval stage resulted in a green cob yield of 16.05 t/ha, significantly higher than that of the untreated control (8.14 t/ha) and comparable to that obtained with emamectin benzoate (17.54 t/ha) (AICRP-BC 2019). (Green cob yield includes the wet weight of cob + kernels + husks sold as “sweet corn” for human consumption, which would be higher than typical yields of dried corn used for grain.) The percentages of plant damage in these three treatments followed the expected trend, *i.e.*, more damage was associated with lower yield. Additional testing will be required to confirm these effects.

Note that uncharacterized strains (*i.e.*, for which the specific insecticidal protein(s) are unknown) cannot be commercialized in most jurisdictions, but *Bt* formulations active against FAW can be obtained commercially in many world areas. Care must be taken to ensure that commercially available products are authentic. In India, ICAR-NBAIR is evaluating indigenous *Bt* strains with the goal of commercialization. More information on use of *Bt* and its characteristics relative to those of other pesticides can be found in **Chapter 3**. Genetically modified (GM) maize expressing one or more *Bt* proteins is also available in some countries (see **Chapter 4**).

Figure 2. Dead larvae observed after foliar application of NBAIR-BT25.

4.2.2. *Pseudomonas fluorescens* Strain NBAIR-PFDWD against FAW

Pseudomonas fluorescens is well-known for plant-growth-promoting effects that improve crop health and increase agricultural production, and it exhibits potent insecticidal activities along with fungicidal activities (Flury *et al.* 2016). The *Pseudomonas fluorescens* group of bacteria is very diverse, and it comprises members that form part of the beneficial rhizosphere microbiota that cooperates with the plant to act against pests and diseases (Venturi and Keel 2016). Loper *et al.* (2016) reported the identification of additional insect pathogenicity factors of a representative insecticidal pseudomonad, *P. protegens* Pf-5, in an oral infection model against dipteran and lepidopteran insects. In our study, *P. fluorescens* strain NBAIR-PFDWD exhibited potential insecticidal activity against FAW in *in vitro* bioassays. Different dosages of NBAIR-PFDWD were tested, and 100% mortality of FAW was observed at a dosage of 10^8 colony-forming units (CFU)/ml within 72 h after treatment. An increase in dosage (to 2×10^9 CFU/ml) of this strain led to 100% mortality in 48 h.

Under field conditions, a talc-based formulation of NBAIR-PFDWD was sprayed on FAW-infested hybrid maize at ICAR-NBAIR, Yelahanka campus, Karnataka. Treatments were initiated after the observation of more than 80% FAW infestation on 25-day-old hybrid maize seedlings. Four post-infestation sprays (20 g/L of water) at weekly intervals effectively controlled FAW infestation under field conditions. One hundred percent recovery of the hybrid maize plants was observed, suggesting that the treatment promoted plant growth as well as removing the insects. This NBAIR-PFDWD strain could effectively manage FAW infestation on maize under field conditions.

4.3. Entomopathogenic Viruses

SpfrNPV belongs to the family Baculoviridae, a family of viruses that are recognized for controlling insect pests in an efficient and environmentally sustainable manner. Baculoviruses are reported to be highly specific, virulent, and safe to non-target organisms (Moscardi 1999; Fuxa 2004; Barrera *et al.* 2011). Worldwide, different isolates of SpfrNPV have been used for biological control of FAW, with efficacies higher than 80%, demonstrating its potential to control the pest (Martínez *et al.* 2012; Behle and Popham 2012; Gómez *et al.* 2013). A commercial formulation of SpfrNPV called CORPOICA

was developed in Colombia for the biological control of FAW (Villamizar 2015). Fawligen is another commercial baculovirus-based biopesticide produced by AgBiTech, USA, and released into the market for the management of FAW (https://www.prnewswire.com/news-releases/agbitech-and-upl-enter-distribution-agreement-for-baculovirus-insecticide-products-for-africa-300991442.html).

Surveys were conducted by NBAIR in maize fields of Chikkaballapur (Karnataka), Coimbatore, and Jolarpettai (Tamil Nadu) and diseased FAW larvae, which were showing characteristic viral infection symptoms, were collected (Figure 3). Naturally NPV-infected larvae were observed in the field with characteristic infection symptoms of hanging from the leaves, eventually oozing viroid particles and fluids. Observation of discharged body fluid of diseased larvae under a phase-contrast microscope revealed numerous spherical particles resembling occlusion bodies (OBs) of baculovirus, especially SpfrNPV (Figure 4). OBs of nucleopolyhedrovirus were extracted from the diseased larvae by differential centrifugation. The OBs were enumerated using a Neubauer's hemocytometer and mounted on a phase-contrast light microscope at 10× and 40× magnification.

Figure 3. Diseased FAW larvae showing characteristic viral infection symptoms.

Under scanning electron microscopy (SEM), the OBs of SpfrNPV appeared as tetrahedral shapes (Figure 5A). Transmission electron microscopy (TEM) of the OBs revealed the tetrahedral-shaped occlusion body, with a size of 1.64 µm (Figure 5B) (Sivakumar *et al.* 2020).

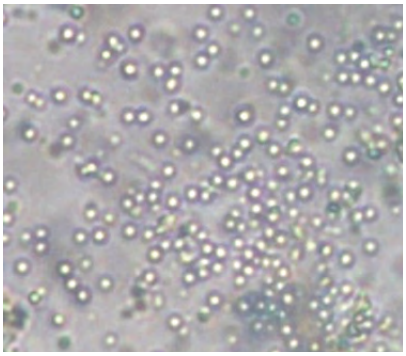


Figure 4. Light micrograph of body fluid obtained from diseased FAW larvae showing nucleopolyhedrovirus occlusion bodies (OBs).

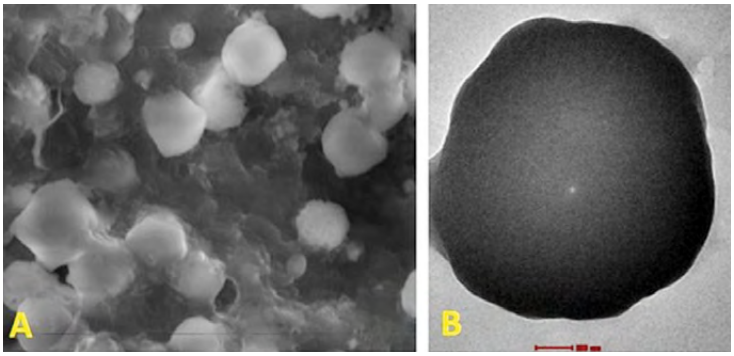


Figure 5. Scanning (A) and transmission (B) electron micrographs of tetrahedral occlusion bodies of *S. frugiperda* NPV.

Field experiments were conducted by NBAIR in farmers' fields in the Chikkaballapur district of Karnataka to evaluate the bioefficacy of SpfrNPV NBAIR1 (ICAR-NBAIR 2020). Field data revealed that a prophylactic spray of aqueous suspension of SpfrNPV NBAIR1 twice @ 3 ml/L, at a concentration of 1.5×10^{12} polyhedral occlusion bodies (POBs)/ha, on the 20th and 35th day after sowing reduced the FAW infestation by 80.4% during the *rabi* season and 68-72% during the *kharif* season and increased yield and general growth of the maize plants (G. Sivakumar, personal communication). Thus, for the first time, a new virulent indigenous novel isolate of NPV associated with FAW was recorded from India and evaluated against FAW. This product is in the process of being commercialized.

4.4. Entomopathogenic Nematodes

A less explored but promising biocontrol strategy is the use of entomopathogenic nematodes (EPNs) for management of lepidopteran pests and root grubs. EPNs belonging to the families Steinernematidae and Heterorhabditidae are ubiquitous generalist parasites of several economically important insect pests. They are symbiotically associated with the bacteria *Xenorhabdus* spp. and *Photorhabdus* spp. When these bacteria are released into the insect hemocoel, they cause septicemia and death of the insect in 24-48 h (Kaya and Gaugler 1993). EPNs have a great potential as biological control agents against agricultural and horticultural insect pests because of their wide host range. Furthermore, they can be easily mass-produced, formulated, and applied as biopesticides (Kaya and Gaugler 1993). The effects of different application technologies were evaluated, with focus on the appropriate concentration, viability, and efficiency of infective juveniles of the nematodes *Heterorhabditis indica* Poinar, Karunakar and David and *Steinernema* sp. (IBCB-n6) to control FAW on maize plants. It was reported that the efficacy of *H. indica* was enhanced against FAW when mixed with an insecticide, lufenuron (Negrisoli *et al.* 2010). Viteri *et al.* (2018) reported that when *S. carpocapsae* was used in combination with chlorantraniliprole or spinetoram, this combination caused more than 90% mortality in 5th-instar larvae of FAW in 72-h in laboratory tests.

4.4.1. Application of EPNs for FAW Management

Four isolates of EPNs were examined for their efficacy against FAW in maize fields at the research farm of ICAR-NBAIR, located at Yelahanka, Bengaluru, during *kharif* and *rabi* 2018-19. Based on the lethal concentration (LC) values obtained, time and method of application were standardized. Consolidated results indicated that prophylactic application of a wettable powder (WP) formulation of *H. indica* at 15 days after plant emergence reduced FAW infestation by 65-72% during *kharif* and 58-64% during *rabi*, compared to 45-65% in maize treated with emamectin benzoate. The relatively high FAW incidence in the maize treated with emamectin benzoate indicates that this treatment did not perform as well as expected based on global experience (see **Chapter 3**), which might be due to application timing, application technique, or possibly the development of insecticide resistance. Yields per plant were on par in EPN-treated and insecticide-treated plants. Due to plant mortality and stand/m^2, significant differences in productivity were observed in treated and untreated maize. Field trials on EPN doses, efficacy in combination with conventional insecticides, and formulations of EPN against FAW (*kharif* 2019-20) indicated dose-dependent control of FAW larvae with an optimum dose of 4-6 kg ha^{-1} either in the form of WP or granular formulation of *H. indica* NBAII Hi101. Split doses and combination of *H. indica* with emamectin benzoate at split doses could reduce the infestation by 80-88%. A field study on application of WP formulations of EPN on the maize whorl indicated that WP formulations of EPN reduced FAW populations by 60-72% and produced plant growth and yield comparable to emamectin benzoate and chlorpyriphos sprays, both in *kharif* and *rabi*. Another field trial in Pachora, Maharashtra, in black cotton soils demonstrated that application of a WP formulation of EPN to the plant root zone in combination with whorl application in the first fortnight followed by split dose 30 days later prevented secondary infestation of field populations.

ICAR-NBAIR researchers demonstrated and validated the delivery of EPN to whorls using WP formulations that reduced FAW populations to approximately 60-72% of the original in maize and sustained plant growth and yield comparable to emamectin benzoate and chlorpyrifos sprays at experiments done at four Krishi Vigyan Kendras (KVKs; “Farm Science Centers”) in Telangana, six in Tamil Nadu, three in Maharashtra, and four in Karnataka. The area covered under the supply of WP of *Heterorhabditis indica* NBAII Hi101 provided to farmers, KVKs, AICRP centers, etc., for the management of FAW was about 58 ha, whereas 112 ha was covered through supply of WP of *H. indica* NBAIIH38. Training was imparted to 165 farmers, KVK workers, and trainers and the technology was commercialized by several companies. The technology for WP formulation of EPNs developed at ICAR-NBAIR is available for commercialization.

4.5. Biointensive Pest Management (BIPM) Trial

A combinatorial efficacy trial was evaluated in farmer's fields during the *rabi* and *kharif* seasons (2018-2019), which comprised installation of controlled-release FAW pheromone traps (developed by ICAR-NBAIR), four releases of the egg parasitoid *T. pretiosum*, two sprays of neem oil, and one spray each of two indigenous microbial biopesticides: *Bt* (NBAIR-BT25) and *M. anisopliae* (NBAIR Ma-35). There was a significant reduction in egg masses (76% and 71.64%) and larval population (80% and 74.44%) at 60 days after treatment during the *rabi* and *kharif* seasons, respectively. This was accompanied by an increase in maize cob (ear) yield compared to the farmers' fields, where 6-7 sprays of emamectin benzoate 5% SG were applied (Varshney *et al.* 2020). As in the experiments with EPN (Section 4.4.1), the emamectin benzoate treatment did not reduce larval infestation as well as expected, though the reasons are unclear. While the major challenge lies in making the biocontrol agents available to farmers, this module needs to be validated in larger areas under different agroecological regions of the country, with replicated yield comparisons to highly effective treatments and modifications in the components of the module based on regional requirements. In addition, the cost-benefit ratio of such a treatment regime needs to be assessed.

The results of a study by Amala *et al.* (2020) in India indicate that maize-legume intercropping with a border crop of napier grass can be very effective in managing FAW, as it could significantly reduce the pest population and increase the abundance of natural enemies. It would be important to evaluate the effects of these treatments on yield and whether the above "push-pull strategy" can be integrated into the combination of treatments described above. Additional information on agroecological control of FAW can be found in **Chapter 6**.

5. Protocols for Mass Production of Natural Enemies under Laboratory Conditions

5.1. Mass Production of Egg Parasitoids *Trichogramma* and *Telenomus*

Trichogramma species used for management of FAW are mass-produced on eggs of laboratory host *Corcyra cephalonica*. *Telenomus remus* can be easily mass-produced on eggs of FAW and *S. litura*. In Africa, rearing of both these parasitoids for targeting FAW was initiated in the International Center of Insect Physiology and Ecology (*icipe*) in 2019 with support from the United States Agency for International Development (USAID) Feed the Future Innovation Lab for Integrated Pest Management, through the "Rice, Maize, and Chickpea IPM for East Africa" project. *Trichogramma* cultures were initiated at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) facility in Niger in 2015, and both FAW and *Telenomus* cultures were initiated in 2018 (Kenis *et al.* 2019). In Colombia, many private sugarcane plantations maintain small insectaries for production of trichogrammatids. During the 1990s, there were 30 commercial mass-production facilities for parasitoids and predators in Colombia, but by 2000 the number had decreased to nine (Van Lenteren and Bueno 2003). The live-insect repository at the NBAIR, Bangalore, India, holds 130 live-insect cultures, which are multiplied and maintained year-round. Pest cultures such as *C. cephalonica, S. litura, Helicoverpa armigera* (Hübner), and FAW; parasitoid cultures such as *Trichogramma* spp., *T. remus, Goniozus nephantidis* (Muesbeck), and *Chelonus* spp.; predators such as *Chrysoperla zastrowi sillemi* Esben-Peterson and *Cryptolaemus montrouzieri* Mulsant; and some species of anthocorid predators and predatory mites are maintained in the repository.

ICAR-NBAIR supplies parasitoids, predators, and microbials to farmers (primarily for rice, coconut, vegetables, maize, and sugarcane) free of charge. In India, there are very few commercial entities producing parasitoids and predators. The major role of NBAIR is to provide start-up cultures to government and private-sector labs and units, who in turn supply farmers. ICAR-NBAIR also trains officials from government departments and private units to mass-produce macrobials and microbials, as availability of biocontrol agents is a major concern in India.

One of the challenges facing producers of parasitoids is the need for rapid distribution and use following production. As mentioned below (Section 5.3.1), ICAR-NBAIR has developed a cold-storage method for *Trichogramma* that allows storage for up to 3 months, which could provide satisfactory adult emergence, longevity, and parasitism (Ghosh and Ballal 2017).

5.1.1. Mass Production of Host Insects

5.1.1.1. Rearing of tobacco caterpillar Spodoptera litura (Fabricius) (Lepidoptera: Noctuidae)

Spodoptera litura (Fabricius) is a polyphagous pest attacking tobacco, cole crops, castor, cotton, sunflower, tomato, pigeon pea, and other crops and is distributed throughout India. The adult moth has a longevity of 10 to 24 days. Eggs are laid in batches on tender leaves and are covered with scales. The caterpillars are about 3.7 cm long and pale greenish brown in color with dark markings. A black ring encircling the body is present at both ends. The egg, larval, and pupal stages last for 3-4, 18-20, and 9-10 days, respectively. The pupation occurs in soil.

Male and female pupae of *S. litura* can be distinguished by the distance between the genital and anal pores, which in females is more than twice that in the males. In addition, in the female pupae, on either side of the genital pore, a `V' shaped depression or fold extending up to the tenth segment is visible (Figure 6).

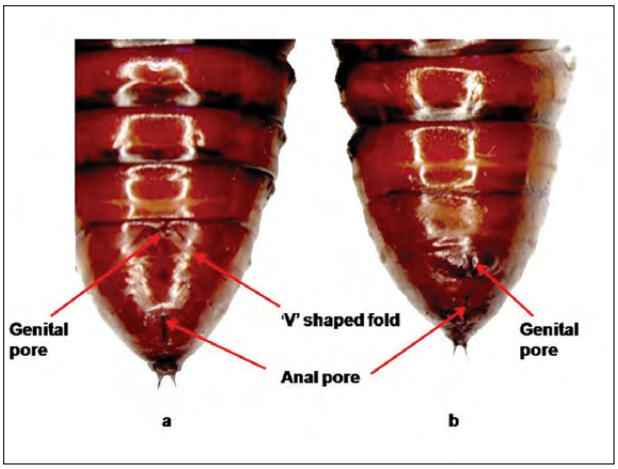


Figure 6. Spodoptera litura pupae: (a) female; (b) male.

Spodoptera litura is the most suitable host for multiplying *Telenomus remus.* The rearing protocol for this host insect has been standardized. The oviposition cage is a plastic container with a ventilated lid (Figure 7).

1. Line the inner wall and roof of the container with ordinary paper.
2. Release moths @25 pairs/container. Moths will lay eggs on the paper lining.
3. Collect eggs by cutting out portions of the lining where eggs are laid, and place the eggs in glass vials with cotton plugs. Eggs hatch in 3 to 4 days.
4. Group-rear the initial larval stages on bunches of castor leaves or on semi-synthetic diet (Section 5.1.2) in ventilated plastic boxes. These boxes can be conveniently stacked in racks to save space.
5. After 1 week, transfer the larvae to individual vials of semi-synthetic diet or multicellular larval rearing trays.

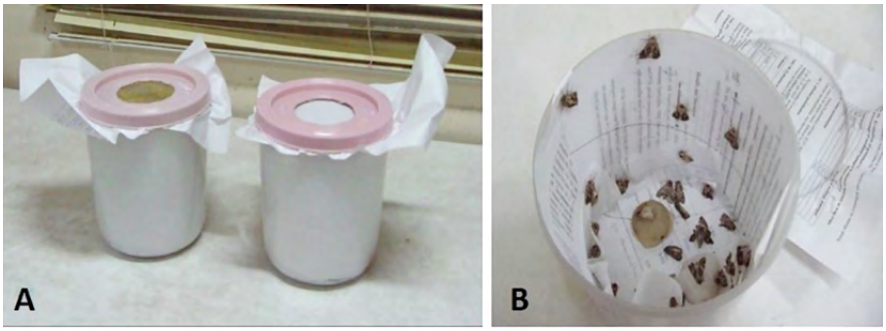


Figure 7. *Spodoptera* egg-laying cage.

5.1.2. *Spodoptera litura* Diet Preparation

Ingredients for the four diet parts are given in Table 2.

1. Mix 390 ml of water with part A of the diet. Run blender for 2 minutes.
2. Boil part B in 390 ml of water. Add this to part A in blender. Run blender for 1 minute.
3. Add part C and run blender again for 1 minute.
4. Add part D and run blender for a minute. Pour the diet into sterilized glass vials before the diet cools.
5. Transfer one larva to each tube and plug tube tightly with cotton wool.
6. After 20-25 days, collect the pupae formed inside the vials.
7. Sterilize pupae in 0.1% sodium hypochlorite solution and dry before placing into adult emergence cages.

Table 2. Composition of the semi-synthetic diet used for rearing *S. litura.*

Part	Ingredients	Quantity
A	Chickpea (Kabuli gram) flour	105 g
	Methyl para hydroxy benzoate	2 g
	Sorbic acid	1 g
	Yeast tablets	10 g
B	Agar	12.75 g
C	Ascorbic acid	3.25 g
	Multivitaplex	2 caps
	Vitamin E	2 caps
	Streptomycin sulphate	0.25 g
D	10% formalin	5 ml

5.2. Mass Multiplication of *Corcyra cephalonica*

The rice meal moth, *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) is a host insect highly amenable to rearing because of its adaptability to varied rearing conditions and its positive influence on the progeny of the natural enemies. *Corcyra cephalonica* is used as an alternative host to mass-multiply natural enemies such as trichogrammatid egg parasitoids, several braconids, and anthocorid and chrysopid predators. *Corcyra* can be mass-multiplied throughout the year in all the ecological zones of India at 28 ± 2°C and 65 ± 5% relative humidity (RH).

The eggs are oval and measure 0.5 × 0.3 mm. Full-grown larvae are creamish white with a brown head and prothoracic tergite and well developed prolegs on abdominal segments 3-6 and 10. The last-instar larva spins a closely woven, very tough, double-layered cocoon in which it develops into a dark-brown pupa. Adults are greyish-brown with thin vague lines of darker brown color along the wing veins. The males are smaller than the females. The pre-oviposition period is 2 days. Egg laying mainly occurs during the night and more eggs are laid on the second and third days after emergence, though oviposition continues throughout the adult lifespan. The incubation period is 2-3 days. Optimum conditions for larval development of *C. cephalonica* are 28-30°C and 70% RH. Under these conditions, the developmental period from egg hatch to adult emergence is 26-27 days. Males generally have 7 larval instars and females have 8 instars.

5.2.1. Mass Production of *Corcyra* in the Laboratory

Table 3 provides a list of the chemicals and equipment needed for raising *Corcyra*. Methods are described in the following sections.

Table 3. Materials required for mass production of *Corcyra*.

Coarsely milled bajra (pearl millet) grain	Ground nut (peanut) (*Arachis hypogaea*) kernels
Yeast	Streptomycin sulphate (Ambystrin)
Honey	Vitamin E capsules (Evion)
Storage racks	Formaldehyde (2%)
Wooden rearing box	Vacuum cleaner (used as a suction pump)
Oviposition drums	Sulphur (WP)
Enamel tray	Face masks
Detergent	Mesh (see details in protocol)
Filter paper	Cotton wool
Thread	

5.2.1.1. Preparation of rearing boxes

Corcyra is reared in a rectangular rearing box (15 × 30 × 45 cm) made of 10-mm-thick plywood. The lid is provided with six ventilated holes (1 cm^2), covered on both sides with brass mesh of 100 mesh size (Figure 8). The boxes used for *Corcyra* multiplication are thoroughly cleaned with 0.5% detergent, rinsed, and dried. Whenever the boxes are emptied after a cycle of rearing, they have to be cleaned, preferably with 2% formaldehyde solution.

Figure 8. *Corcyra* rearing box.

5.2.1.2. Preparation of rearing medium and rearing of Corcyra

1. Coarsely mill the required quantities of bajra grains (each grain broken into 3-4 pieces).
2. Heat-sterilize the broken grains at 70°C for 1 h to eliminate residual populations of stored product insects such as *Rhizopertha dominica*, *Sitotroga cerealella*, *Tribolium castaneum*, and fungal contaminants. Cool the grains (in the shade or under a fan) in a clean area.
3. Transfer the grains to the rearing box @ 2.5 kg/box. Add 75 g of groundnut seed powder, 5 g of yeast powder, 0.7 g of streptomycin sulphate (Ambystrin), and 0.125 cc of *Corcyra* eggs (~2000 eggs). One cc of eggs contains approximately 16000-18000 eggs.
4. Place the inoculated boxes on racks at room temperature. Write the date of inoculation in each box. The larvae that hatch out in 3-4 days begin to feed on the fortified bajra medium. At this stage, light webbings are noticed on the surface of the medium. As the larvae grow, they move into the medium. During this period, the boxes and their contents should not be disturbed.
5. Moths emerge 40 days after inoculation and emergence can continue even up to 2 months. Collect moths daily using a suction pump (modified vacuum cleaner) and transfer to an oviposition drum (Figure 9A). Workers involved in the collection of moths should wear masks to avoid inhalation of scales (Figure 9B). The oviposition drums are made of plastic, with a wire-mesh base to enable collection of eggs. The walls of the drums have two vents (ventilation holes) opposite each other that are covered with wire mesh (Figure 9C). The lids of the drums have slots for introducing the moths and one vent with mesh for providing adult feed. The oviposition drums holding the moths are maintained for 4 to 7 days for egg collection, after which they are emptied and cleaned for the next cycle of use.
6. Provide the adults with honey solution as feed, which is prepared by mixing 50 ml honey with 50 ml water and 5 capsules of vitamin E (Evion). Store the prepared feed in the refrigerator until use. Soak a piece of cotton wool in the honey solution, attach it to a thread, and hang it from the lid inside the drum. The moths lay loose eggs in large numbers.
7. To minimize the risk of inhalation of scales in the culture rooms, place the oviposition drums on sheets of filter paper placed in enamel trays, which trap the scales effectively. Place sets of oviposition drums in a well-ventilated area. Every morning, lift up the oviposition drums and gently clean the wire-mesh base from the outside using a brush so that the eggs and remnants of scales and insect parts settled on the mesh are collected along with those on the filter paper. Pass the eggs through 15-, 30-, and 40-mesh sieves to remove scales. Quantify the eggs using a measuring cylinder. Retain some eggs for building up the host stocks; the rest can be used for natural enemy production.
8. For *Trichogramma* production, expose eggs to ultraviolet (UV) rays (30 W UV tube) for 45 minutes at a distance of 2 feet.

Figure 9. (A) Moth collection device; (B) moth collection using suction pump; (C) oviposition cage.

5.2.1.3. Precautions during Corcyra rearing

1. Line the doors and windows of the *Corcyra* production rooms with 60- to 100-size brass mesh to prevent entry of *Habrobracon* (= *Bracon*) *hebetor* (Say), which can completely destroy the culture.
2. Note the date of charging in each *Corcyra* box to enable the staff to initiate collection of moths on the correct day.
3. Dispose of the contents of rearing boxes after complete emergence.
4. Clean boxes properly before reuse.
5. Spray the rearing boxes with Dicofol and dry them before use to prevent mite infestation.
6. Use a light trap for monitoring *Habrobracon* infestation.

5.3. Production of Egg Parasitoids

5.3.1. Production of Trichogrammatids

1. As described above (Section 5.2.1.2), treat the eggs of *C. cephalonica* with UV rays for 45 minutes (to prevent hatching) and then glue them uniformly onto a card (15 × 10 cm) within a marked area of 12 × 8 cm. Gently remove the excess eggs from the cards by using a brush. Allow the cards to dry before exposing to parasitoids.
2. Expose the eggs to adult female *Trichogramma* in a tube (2.5 ×15 cm) or in plastic covers. If cards with unparasitized eggs are exposed to female parasitoids in tubes, small card strips (1.5 ×10 cm) are used and the egg-to-female ratio should be 8:1. If large cards (15 × 10 cm) are exposed in polythene covers, the eggs-to-female ratio should be 30:1, allowing the females to parasitize till mortality. One nucleus card of *Trichogramma* can be used to parasitize six additional cards. Provide a mixture of 50% honey and vitamin E (see Section 5.2.1.2 for preparation) in a soaked cotton swab as feed for the adults. Remove the parasitized cards ("tricho cards") after 2 days. Parasitized *Corcyra* eggs turn black on the 5th day after exposure, which indicates parasitization of the eggs.
3. Prepare 6-day-old parasitized cards for shipment or field release. Staple each parasitized card in such a way that the eggs do not rub against each other and get damaged. Twenty or more tricho cards can be packed in each polythene bag depending on the size of the bag. Provide a fine honey streak on the inner side of polythene bag as feed for adults that may emerge in transit. A technology has also been developed for long-term storage of tricho cards for up to 3 months through diapause induction (Ghosh and Ballal 2017). Tricho cards can be stored for shorter durations in a normal refrigerator at 10°C for up to 21 days.

5.3.1.1. Release of trichogrammatids into the field

1. Cut each tricho card into 16 pieces. Staple each piece onto the under-surface of a leaf, so that the eggs are not exposed directly to sunlight.
2. Perform releases at weekly intervals as long as pest eggs are available in the field.
3. Initial release of trichogrammatids could be timed based on pheromone trap catches or visual observation of the target pest.

5.3.2 Production of *Telenomus remus* (Scelionidae: Hymenoptera)

Telenomus remus (Figure 10) is primarily produced on the eggs of the tobacco caterpillar, *S. litura,* which is the preferred host. It can also parasitize eggs of *S. exigua*, *Helicoverpa armigera*, *Plusia signata, Agrotis segetum, Agrotis biconica, Agrotis ipsilon, Mythimna loreyi, Trichoplusia ni, Achaea janata,* and *Corcyra cephalonica*. Out of the total production of eggs, about 90% are utilized for *T. remus* production, the rest for continuation of the host culture. The ideal conditions for rearing of *T. remus* are 26-27°C and 60-70% RH. The protocol below describes use of *S. litura* as the host.

Figure 10. *Telenomus remus*.

5.3.2.1. Materials required

The materials include *S. litura* egg masses (Section 5.1.1.1), nucleus culture of *Telenomus remus*, cards, test tubes, polythene bags, scissors, stapler, water-soluble gum, honey solution (50%), and a refrigerator.

5.3.2.2. Protocol

Telenomus remus males have filiform antennae and short abdomens whereas females have clubbed antennae and longer and wider abdomens with the ovipositor clearly visible under a 10× hand lens. The males emerge before the females.

1. Pair the adults for 24 h for mating.
2. After a pre-oviposition period, confine the adult females in 25 × 150 mm glass tubes. Stick a small piece of cotton swab soaked with 50% honey solution on the inner side of the glass tube as feed for the adult parasitoids.
3. Glue ~6000 freshly laid (0-24 h old) *S. litura* eggs onto a thick paper card (10 × 2 cm) and expose to 100 parasitoids for 24-48 h for parasitization. On the 3rd and 4th days, 3,000 eggs may be provided, and on the 5th day, 1500 eggs may be provided. The parasitized eggs turn black in 4 to 5 days. Remove larvae hatching from unparasitized eggs from the card.
4. Transfer cards carrying only parasitized eggs into fresh, clean tubes for the emergence of the parasitoids. The adult parasitoids emerge in 9 to 10 days from the date of parasitization and can be used for field release. Retain 10% of the adults for continuation of the cultures.

5.3.3. Rearing of *Chelonus* spp. (Egg-Larval Parasitoid)

Chelonus spp., egg-larval parasitoids, were recorded frequently from field samples collected from different districts of Karnataka since March 2018, when the incidence of FAW was initially reported across the country. The parasitoid specimens were processed for identification (both morphological and molecular) and the genus was confirmed as *Chelonus* spp. There appeared to be two different species of *Chelonus*: one small and arrhenotokous (Figure 11A), one large and arrhenotokous (Figure 11B). (In arrhenotokous species, unfertilized eggs develop into males.) The insect in (B) has been identified as *Chelonus formosanus,* first reported from India (Gupta *et al.* 2020a). Species-level identification of (A) is in progress. Adults of *Chelonus* spp. can be reared by allowing them to parasitize the eggs of natural hosts such as FAW and *S. litura,* or the laboratory host, *C. cephalonica*. Adult longevity varied from 2 to 7 days. The developmental period was 20 to 25 days on natural host and 25 to 50 days on *C. cephalonica*. The parasitism rate was 10 to 19.4%. Adult emergence was 45 to 57.5% from cocoons reared on natural host and 85 to 98% from those on *C. cephalonica*. Successful rearing on *C. cephalonica* eggs enabled field evaluation of *Chelonus* spp. against FAW. Field trials are in progress.

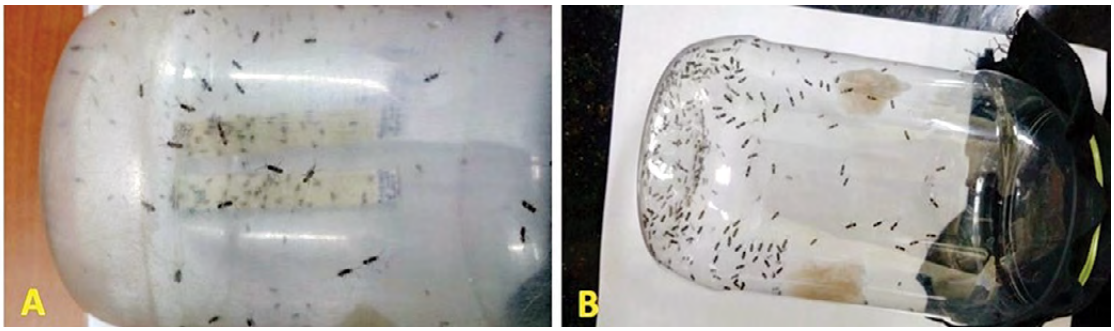


Figure 11. Two different species of egg-larval parasitoid *Chelonus* spp. recorded on FAW in Karnataka, India: (A) *Chelonus* sp., (B) *Chelonus formosanus*.

6. Conclusion

Indian agriculture is facing a variety of challenges, which include climate change and onslaughts by invasive pests. Researchers have realized that management strategies, particularly biological control practices, have to be tailored to adapt to these challenges within an IPM framework. In addition, all management strategies have to be based on the understanding of the genetic diversity of the pest. The Indian research team at ICAR-NBAIR, which identified FAW through molecular characterization when it first invaded India in 2018, went on to analyze the genetic diversity of FAW in India two years after the initial introduction. Apart from the ancestral rice and corn strain haplotypes, 14 novel haplotypes unique to India, as well as inter-strain recombination, were recorded. The study also suggested that the invasive FAW populations in Asia and Africa have a common origin and that the FAW population in India might be undergoing expansion (Nayyar *et al.* 2021). Details on additional studies of FAW strain biology are provided in **Chapter 1**.

The identification of promising indigenous macrobials and microbial isolates and development of a nano-based pheromone technology culminated in the development and validation of a BIPM module. The major challenge faced in adoption of this approach by farmers is the availability of macrobial and microbial biocontrol agents. Commercial entrepreneurs have to be trained to mass-rear egg parasitoids and EPNs, which are exempt from registration. The efficacy of the indigenous microbial isolates discovered by NBAIR has been evaluated in the lab and field and the production protocols have been developed. NBAIR is in the process of generating toxicology data and preparing the regulatory dossiers, which can then be provided to the private sector for commercialization. Other important challenges include the complexity of obtaining and applying biocontrol agents at the proper time and the need to assess the cost-benefit ratio for treatments being considered.

Areas for further research, development, and commercialization

- Tracking the future evolution of FAW with respect to pesticide resistance and host range.
- Assessment of the efficacy of different management options on FAW in India.
- Comparative performance of the egg parasitoids and their competitive/complementary interactions.
- Comparative efficacy of the indigenous microbial isolates to target the larval stage of FAW infesting different crop stages.
- Evaluation of the BIPM module in different agroecological zones of the country.
- Large-scale validation of habitat manipulation strategies in farmers' fields and their integration within the BIPM module.
- Ensuring commercial availability of biocontrol agents for the farmers to effectively tackle FAW.
- Cost-benefit analysis of treatments using the CESAS (cost, efficacy, safety, accessibility, and scalability) model described in **Chapter 1**.

CASE STUDY: BANGLADESH*

7. Introduction

Fall armyworm (*Spodoptera frugiperda*; FAW) was recorded in November 2018 for the first time in Bangladesh and since then has spread throughout the country. Infestation at the vegetative stage of maize was estimated at 40 to 50% while ear damage was estimated at around 23-30% in the summer maize (BWMRI 2020). An effective IPM strategy for this devastating pest is very much essential to minimize the maize production losses. Until now famers have depended mainly on synthetic chemical pesticides to combat the pest. However, to control the FAW, in an environmentally sustainable, socio-economically acceptable manner, IPM strategies are very much needed. Fortunately, FAW has been effectively controlled in the Americas for over 100 years so the goal in Bangladesh is to adopt these proven approaches to the Bangladesh farming system. Such strategies may include augmentative/inundative (periodic release of natural enemies against the target pest) biocontrol and use of technologies that support conservation biological control (*e.g.,* manipulation of environment and agronomic practices in a way that favors natural enemies).

8. Promising Biocontrol Agents against FAW in Bangladesh

Several studies have been initiated in Bangladesh to characterize natural enemies of FAW along with development of methods for their bioassay testing, standardizing of mass production, and field trials. Several biocontrol agents, such as larval parasitoid *Habrobracon* (= *Bracon*) *hebetor* (Say) and egg parasitoid *Trichogramma* spp., are being used as a component of an IPM package against different insect pests. Several public and private companies are also undertaking mass-rearing of different biological control agents and making them available to the farming communities on a limited scale. As FAW is a recently introduced pest in the country, limited work has been done on its biological control. So far, several predators including earwig (*Euborellia annulipes* Lucas) and ladybird beetle (*Coleomegilla maculate* De Geer), and several parasitoids including *Trichogramma* sp., *Telenomus remus, H. hebetor, Chelonus* sp., *Campoletis chlorideae*, etc., have been identified as effective biocontrol agents against FAW in the country.

8.1. Efficiency of *Habrobracon hebetor* as a Larval Parasitoid of FAW

Habrobracon hebetor is a medium-sized wasp that is parasitic on later instars of larvae lacking seta (hairs on their body) of a wide range of insect pests. Female *Habrobracon* first inject venom and thus paralyze host insect larvae. As little as one part of venom in 200 million parts of host blood (hemolymph) was sufficient to cause permanent paralysis and death of insect larvae, and one female *Habrobracon* can paralyze 500-1000 larvae. Female *Habrobracon* then lay their eggs on the paralyzed host larvae; the emerging *Habrobracon* larvae multiply therein and thus destroy the pest. In Bangladesh, *Habrobracon* is considered as an effective parasitoid and it is being successfully and commercially applied to control of many devastating pests such as *Prodenia* caterpillar (*Spodoptera litura*), tomato fruit worm (*Helicoverpa armigera*), tea looper (*Biston suppressaria*), etc. Laboratory and field efficacy studies against FAW larvae were undertaken by Bangladesh Agricultural Research Institute (BARI) in collaboration with the Centre for Agriculture and Biosciences International (CABI).

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8.1.1. Laboratory Study

Laboratory studies were carried out in the IPM laboratory of the Entomology Division, BARI, Gazipur, during December 2019 to evaluate the efficacy of *H. hebetor* as a larval parasitoid of FAW larvae. Five 4th-instar larvae of FAW and one pair of adult *H. hebetor* were placed in a test tube (replicated 10 times). Adult *B. hebetor* females sting the FAW larvae and inject venom inside their body by inserting the ovipositor, which paralyzes the larvae and lead to death (Figure 12). After 24 hours of exposure, all the host larvae were transferred to Petri plates. It was recorded that 100% of the FAW larvae were killed. All the larvae became black, dead and dry within 24 h after *H. hebetor* venom injection. However, no *H. hebetor* emerged from the dead FAW larvae due to desiccation of the dead host. The dry-matter content of FAW larvae is less than that of other insect larvae, and the water content is high. For that reason, the larvae killed due to parasitization by *H. hebetor* dried up very quickly. Thus, it can be concluded that *H. hebetor* can kill FAW larvae efficiently, but no new *H. hebetor* will be released from those larvae. For effective results, inundative release of *H. hebetor* should be considered.

Figure 12. (A, B) Stinging and injecting venom of *B. hebetor* on FAW larva; (C) dead and dry larvae of FAW after 24 hours.

8.1.2. Field Study

An efficacy study of *H. hebetor* against FAW was carried out in the maize fields of farmers in Shibganj, Bogra, during June-July 2019. *Habrobracon hebetor* were applied at a rate of 800-1200 adults per ha (one jar contained 800-1200 adults, costing US$3.00). Releases of *H. hebetor* were done at 15-day intervals. The study was replicated three times in a disperse manner (200 m distance from one replication to another). Many dead larvae were recorded in the treated areas due to the venom injection by the parasitoid. FAW larval populations were reduced by 32-45% in the treated areas. Use of this organism in IPM management approaches with other technologies for effective control of FAW needs further evaluation, particularly in terms of yield protection.

8.2. Mass-Rearing Protocol for *H. hebetor*

BARI-Bangladesh has developed and validated a unique mass-rearing protocol for *H. hebetor* (Figure 13) which has been commercialized by a private company, Ispahani Agro Limited. Late-instar (5th-6th instar) wax moth (*Galleria mellonella*) larvae were used as the host of *H. hebetor*. First, a parent stock of wax moth was developed in honeycomb in glass jars. First- to second-instar larvae of wax moth were released into the artificial diet (made with proprietary proportions of wheat flour, maize flour, milk, animal fat, sugar, and yeast and autoclaved at 125°C and 1.5 PSI for 70 minutes). When the larvae attained full growth length (18-20 days later), they were transferred into a plastic bottle (200 larvae /bottle) containing a corrugated paper sheet. The full-fed larvae took position on the corrugated paper sheet for pupation. After pupation, 40 adult *H. hebetor* (30 female and 10 male) were released into the plastic bottle with a honey cube for their food. The open end of the jar was closed with black cloth. The wax moth larvae and *H. hebetor* were then kept in racks for 8-10 days for parasitism, egg laying, pupation and adult emergence of *H. hebetor*.

Figure 13. Mass-rearing protocol for *H. hebetor*. (A) Parent stock of wax moth larvae; (B) artificial diet for wax moth larvae; (C) full-grown larvae on the diet; (D) full-grown larvae put in plastic jar with a corrugated paper sheet; (E) parasitization of *H. hebetor* in the dark; (F) adult *H. hebetor* ready to use in the field.

8.3. Efficiency of *Trichogramma evanescens* Westwood and *Trichogramma chilonis* as Egg Parasitoids of FAW

Trichogramma are minute wasps that are parasites of lepidopteran insect pests. This species is considered as an effective egg parasitoid against many insect pests and is used worldwide in biocontrol-based pest management. The *Trichogramma* lays its eggs in the host insect eggs, and multiplies therein, thus preventing hatching of host insect larvae. In Bangladesh, it is commercially used against many destructive insect pests such as eggplant shoot and fruit borer (*Leucinodes orbonalis*) and tomato fruit worm (*Helicoverpa armigera*), among others.

Laboratory efficacy studies of two species of *Trichogramma* for activity against FAW eggs were undertaken by BARI in collaboration with CABI. The study was undertaken in the IPM laboratory of the Entomology Division, BARI, under controlled conditions at a temperature of 25±2°C, relative humidity (RH) of 60±5% to evaluate the efficacy of two species of egg parasitoids—*Trichogramma evanescens* and *T. chilonis*. FAW eggs were collected from a laboratory-reared population of 24-h age. Approximately 100 eggs were glued onto light blue-and-white paper (10 × 1.5 cm) with gum acacia diluted in distilled water. The paper strips with FAW eggs were kept in test tubes. Then, 10 pairs of laboratory-reared 24-h-old female wasps of *T. evanescens* or *T. chilonis* were released inside the test tubes containing FAW eggs and allowed to parasitize on the eggs for 24 h (Figure 14).

The parasitism rates of *T. evanescens* and *T. chilonis* on FAW eggs after 24-h exposures were 56% and 47%, respectively, and the adult emergence rates were 89% and 92%, respectively. Parasitoid egg-to-adult duration was 10 days for both parasitoids.

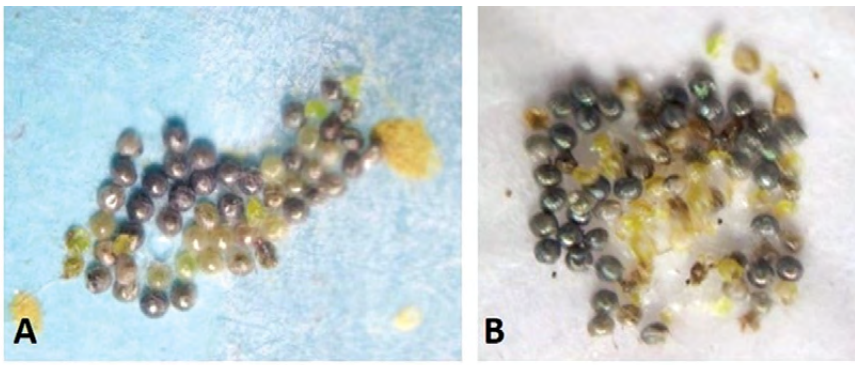

Figure 14. Parasitization of FAW eggs by Trichogramma species.
(A) FAW eggs parasitized by *T. evanescens*; (B) FAW eggs parasitized by *T. chilonis.*

8.4. Mass-Rearing Protocol for *Trichogramma* and its Hosts *Corcyra cephalonica* and *Sitotroga cerealella*

Easy and cost-effective mass-rearing is very important for augmentative biocontrol using an egg parasitoid such as *Trichogramma*. Mass-rearing of *Trichogramma* is generally done on eggs of rice meal moth, *Corcyra cephalonica*; however, it is not very cost effective. Therefore, the BARI team developed a cost-effective mass-rearing protocol of *Trichogramma* sp. on *Sitotroga cerealella* eggs, which has been commercialized by a private company, Ispahani Agro Ltd. in Bangladesh.

To rear *S. cerealella*, 5 kg wheat flour can be poured into boiling water and cooked for 2-3 minutes. Then the treated wheat is placed in steel trays (50 cm × 60 cm), each tray containing 2.5 kg and 1 gm of *S. cerealella* eggs and kept untouched for 5-6 days. After that, around 100-150 ml water per kg wheat flour is added and mixed properly with gentle stirring. After 22-25 days, the infested wheat with *S. cerealella* larvae is put into a 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 with the opening covered by 32-gauge mesh net. Adults were kept in the cylinder for one day for mating and subsequent egg laying. On the following day the eggs laid on the wall of the cylinder were brushed off and then sieved to collect fresh eggs. The adults and their body parts and scales were removed from the eggs by holding the cylinder near an exhaust fan to obtain the fresh eggs. Five grams of fresh eggs of *S. cerealella* were then put in a long, moist glass cylinder (glass cylinders were moistened by keeping them inside a freezer for few minutes) and the eggs were spread over the cylinder. A vial containing 1 g of eggs parasitized with *Trichogramma* was then placed inside the glass cylinder. The glass cylinders were then kept continuously in fluorescent light at 25.0±2.0°C for 9-11 days (Figure 15). Within 9-12 days, parasitism of almost all eggs of *S. cerealella* had occurred. 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 parasitized eggs can also be collected and kept in desiccators at 3-4°C and 75-85% RH for 1-1.5 months.

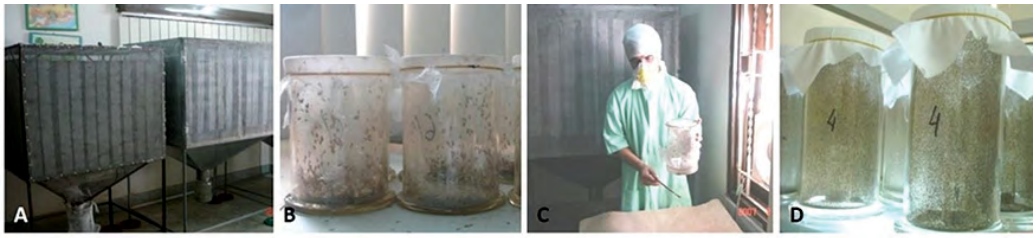

Figure 15. Mass-rearing protocol for host insects of *Trichogramma* parasitoids.
(A) Mass-rearing chamber for host insects; (B) egg laying of host insects in glass cylinders; (C) collection of host eggs; (D) parasitization of host eggs.

8.5. Augmentative Biological Control for FAW Management

Table 4 provides a summary of the current status of augmentative biocontrol in Bangladesh. This information is also likely to be useful for other countries in subtropical Asia where FAW has become a problem.

Table 4. Status of augmentative biological control against FAW in Bangladesh.

Specific biological control agent identified against FAW	Institution(s) involved	Stage of development (Proof-of-concept / Piloting / Scaling)	Efficacy (= % crop yield protected from the loss due to FAW)
Habrobracon hebetor (larval parasitoid) Commercial name: I-bracon	BARI and Ispahani Agro Ltd.	Scaling for different destructive insect pests; proof-of-concept for FAW	Laboratory efficacy 100%; Field efficacy: 32-45%. Data on percent crop yield protected was not obtained
Trichogramma evanescens and *T. chilonis*	BARI and Ispahani Agro Ltd.	Scaling for different destructive insect pests; proof-of-concept for FAW in the laboratory has been done	Laboratory efficacy 47-56%; Field efficacy: not yet done. Data on percent crop yield protected was also not obtained

Cost analysis of *Bracon hebetor* was undertaken by Ispahani Agro Limited, one of the primary private companies involved in the commercialization of the biocontrol agent in Bangladesh (Table 5).

Table 5. Cost analysis of *Habrobracon hebetor* as a biocontrol agent in Bangladesh.

Item	Cost (in US$)	Remarks
Mass-rearing of the biological control agent (I-Bracon)	**2.35 × 4 = 9.4**	Application: 1 Jar for 15 Days and 4 Jars in a crop season per hectare
Personnel (Technicians/Skilled helpers)	0.71 × 4 = 2.84	Seasonal cost
Infrastructure/Facilities	0.71 × 4 = 2.84	Seasonal cost
Supplies & expendables (*e.g.*, diet, cages etc.)	0.94 × 4 = 3.76	Seasonal cost
Transport (including cold chain, where relevant) and distribution through local dealers	**0.47 × 4 = 1.88**	Seasonal cost
Augmentative release of the biological control agent in the farmers' fields	**0.12 × 4 = 0.48**	Seasonal cost
TOTAL	**11.76/Crop Season**	

9. Areas for Further Research, Development, and Commercialization

- The following topics are key areas for future research that—if well positioned with private partners—could aid in commercial availability of biocontrol agents to assist farmers in an IPM response to FAW.
- Regular field surveys to identify effective predators and egg and larval parasitoids of FAW.
- Evaluation of efficacy of field predation/parasitization of different predators and parasitoids against FAW.
- Development of cost-effective mass-rearing protocols for newly identified parasitoids, especially *Trichogramma pretiosum* and *Telenomus remus.*
- Development of IPM strategies against FAW with biological control as one of the major components.
- Storage, transport, and field application of different biocontrol agents for cost-effective management of FAW.
- Cost-benefit analysis of treatments using the CESAS (cost, efficacy, safety, accessibility, and scalability) model described in **Chapter 1**.

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