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# Green Technologies for Sustainable Management of Invasive and Transboundary Pests



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# Green Technologies for Sustainable Management of Invasive and Transboundary Pests

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## MESSAGE

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National Institute of Agricultural Extension Management (MANAGE), Hyderabad is an autonomous organization under the Ministry of Agriculture & Farmers Welfare, Government of India. The policies of liberalization and globalization of the economy and the level of agricultural technology becoming more sophisticated and complex, calls for major initiatives towards reorientation and modernization of the agricultural extension system. Effective ways of managing the extension system needed to be evolved and extension organizations enabled to transform the existing set up through professional guidance and training of critical manpower. MANAGE is the response to this imperative need. Agricultural extension to be effective, demands sound technological knowledge to the extension functionaries and therefore MANAGE has focused on training program on technological aspect in collaboration with ICAR institutions and state agriculture/veterinary universities, having expertise and facilities to organize technical training program for extension functionaries of state department.

Identification of invasive cross-border insects as a significant danger intensified by factors like the widespread movement of individuals (travel) and goods economic, commercial, and ecological influences like climate change. Ensuring access to food has now become a well-established reality. Transboundary insect pests management must promptly prioritize attention to addressing nature interventions implemented in other parts of the globe and acknowledging a coordinated endeavor for biological control, whether it involves local species or introduced ones maintaining a classical style, while effectively managing their aggressive attack. The present article explores the potential and necessity of biological control when it comes to dealing with invasive insects in the current decade. Initiating efforts as a primary and crucial measure for their control and administration establish a system of environmentally-friendly management.

This e-book covers an array of subjects, Green Technologies for Sustainable Management of Invasive and Transboundary Pests. We are confident that this compilation of cutting-edge knowledge and industry insights will be a valuable resource for those looking to optimize their operations and contribute to a more sustainable future. I would like to extend my appreciation to, ICAR-NIBSM, Raipur & MANAGE, Hyderabad organizers and for the tremendous effort in compiling this ebook. I also thank the authors, editors, and designers who have contributed to this ebook creation.

Dr. P. Chandra Shekara  
(Director General, MANAGE)



## FOREWORD

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Invasive and transboundary pests are currently seen as major dangers to Indian agriculture since they aim to significantly reduce crop productivity while also harming biodiversity and causing significant ecological, cultural, and economic losses. Up to 1.4 trillion USD in annual crop losses are attributed to invading insects. It is noteworthy that since 1847, there have been reports of the introduction of 33 insect and non-insect pests in India. Globalization, free trade, and misconceptions about quarantining imported bulk and research materials, among other things, are considered to be the main factors that opened the door to numerous foreign pests and diseases and will continue to support the burden brought on by invasiveness in the future. Another danger is transboundary plant pests (TPPs), which have been generating major agricultural losses, jeopardising food security, harming the local ecosystem and biodiversity, and having negative socioeconomic and public health effects. In terms of India, a few TPPs, such as "Desert Locust" and "Carambola fruit fly," are on the rise in the geopolitical sphere. By either preventing an epidemic from starting in the first place or by spotting it early and preventing it from establishing and spreading further, early detection of invasive species is the key to effectively controlling any outbreak and so minimising the harm they cause.

It is crucial to comprehend that "Green Technologies," such as microbial biopesticides, nano-pheromones with enhanced efficacy, endophytes for biotic and abiotic stress tolerance etc., are considered to be ecologically feasible when it comes to management. The nano-version of a number of these tactics helps to multiply their effectiveness in the field against invasive and transboundary pests. The fact that 970 registered biopesticide formulations are currently available in India for the control of insect pests is proof that "Microbial Biopesticides" are recognised to gain relevance in accordance with the changing policies of the Government of India and stakeholders' preferences.

Although it is a concise book, "**Green Technologies for Sustainable Management of Invasive and Transboundary Pests**" could introduce a few cutting-edge technologies with real-time improvements in their efficacies under field conditions in the years to come. It is my privilege to appreciate the authors who compiled and edited it for the benefit of researchers, policy makers, scientists, students, corporate, stakeholders etc.

(P. K. Ghosh)  
Director and Vice-Chancellor  
ICAR-NIBSM, Raipur

## PREFACE

Invasive Alien Pests (IAS), because of their extensive capacity for adaptation, and polyphagous and voracious attitudes that had been conducive for continuous invasion in India since 18<sup>th</sup> Century. Of fact, since 1847, some 33 insect and non-insect pests have entered India and taken up residence in a variety of crop eco-systems, posing serious ecological, cultural, and economic challenges. Due to a number of factors, including free commerce, globalization, and insufficient quarantine systems at the border, India may continue to receive additional pests in the future. Transboundary plant pests (TPPs) are migratory insects, plant diseases, and weeds that can spread to multiple countries and reach epidemic proportions, cause significant losses to crops, threaten food security, harm local biodiversity and the environment, and have serious socio-economic and public health consequences in addition to causing global famine. This is another aspect of the threat that India is currently facing. The effects of climate change significantly complicate how severe IAS and TPPs are.

Effective management of IAS entails employing early identification, warning, and containment of the spread of pests from the locations of outbreak to the nearby landscape. The logical way to do rid of TPPs would be to continuously monitor geopolitical boundaries with geospatial technologies like GPS, GIS, remote sensing, etc., and build policies based on satellite-oriented data. From a management standpoint, possible "Green Technologies" services can significantly improve IAS and TPP management.

A few crucial tactics that were mentioned above compelled the writers to organize a three-day online course in association with MANAGE, Hyderabad in order to convey their message to the various participants and provide supporting literature. For the benefit of a broad audience of readers, including scientists, researchers, students, field workers, policymakers, corporations, etc., the resource materials gathered from knowledgeable deliberators are compiled, edited, and presented in the form of an e-book titled "Green Technologies for Sustainable Management of Invasive and Transboundary Pests."

### **Editors**

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## Chapter 1

### Invasive and Transboundary Pests: Indian scenario

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#### Abstract

A total of 33 invasive pests including insect and non-insect pests have been reported to enter and establish in various crop habitats in India during pre- and post-independence era, green and post-green revolution era. It continued till date with the 2<sup>nd</sup> decade of 21<sup>st</sup> century and has caused heavy yield losses, leading to a threat for food security. Out of the invasive pests encountered so far, 18 insect species belong to Hemiptera (06 whiteflies, 05 mealybugs, 03 aphids, 03 scale insects, 01 psyllid), six Lepidoptera, three Coleoptera, two Hymenoptera, one Diptera and three non-insect pests (01 Acarina, 01 Mollusca, 01 root-knot nematode). Trade policies, free trade, globalization *etc.*, invited maximum number of invasive pests in to India during the post-green revolution era and the 2<sup>nd</sup> decade of 21<sup>st</sup> century. The COVID-19 induced lockdowns are responsible for few insects to attain dreadful status in causing significant damage and yield losses in crops. Two insects *viz.*, desert locust and carambola fruit fly are considered as transboundary pests in India. The ICAR institutes/SAUs have developed efficient Integrated Pest Management (IPM) for almost all invasive pests of India which are available for practice in many public domains.

**Keywords:** Invasive alien pests, Transboundary pests, Eras, Introduction, Nature of damage

#### 1. Introduction

Invasive pests when they occupy new ranges/new habitats have the potential to manipulate ecological and economic systems on a global basis by altering eco-system, tritrophic interactions, threaten biodiversity *etc.* (Noar et al. 2021). Additionally substantial reduction in host plant fitness, lack of co-evolution between natural enemies and pest species, wide spread mortality *etc.*, are recorded, which capitalize the ecological, cultural and economic losses (Rigling and Prospero 2018). The invasive insects-induced global crop losses is estimated for up to 1.4 trillion USD annually (Bradshaw et al. 2016). Increasing international trade has created diverse pathways for the entry/outbreak of the invasive species, which accelerate the rate at which introductions occur. As globalization intensifies, the worldwide economic and ecological burden of invasive species is expected to increase. In India, eight invasive pests during pre- and post-independence era, 14 during green and post-green revolution era and 11 during 2<sup>nd</sup> decade of 21<sup>st</sup> century were recorded in various crop habitats.

Transboundary Plant Pests (TPPs) are those migratory insects, plant diseases and weeds that can spread to several countries and reach epidemic proportions, cause significant losses to crops, threaten food security, damage the local biodiversity and environment and create serious socio-economic and public health consequences. TPPs-induced decline in agricultural productivity contributes to poverty and hunger, particularly of small farmers, besides acting as

the barriers to trade. Three major reasons for the increased risk of TPPs that spread to challenge the world's food security include i) Global movement of agricultural goods, ii) Global movement of tourists and migration, and iii) Global climate change.

TPPs contribute substantially in pest and disease-induced global food loss which is approximately 1/3<sup>rd</sup> of annual food production. Across the world, more specifically the Asia-Pacific region faces severe threats from a number of TPPs that occur as epidemics or are endemic in national boundaries. Climate change is creating new ecological platform for the entry and establishment of pests and diseases from one geographical region to another. This expansion will continue to result in huge financial losses, therefore require large eradication programmes and effective control measures. In India, desert locust and carambola fruit fly are reported to be the major TPPs which are under surveillance in co-ordination with FAO. This chapter retrieves on various invasive insect and non-insect pests and transboundary insect pests which had/have been posing problem to India during pre- and -post independence era, green and post-green revolution era and 2<sup>nd</sup> decade of 21<sup>st</sup> century.

## **2. Invasive pests of Pre- and -Post Independence Era**

### **2.1. Giant African Snail (GAS), *Achatina fulica* (Ferussac) (Mollusca: Achatinidae)**

GAS is a native of East Africa and was introduced from East Africa to India through living specimens from Mauritius to Calcutta in 1847 and in 1907, it multiplied in large numbers in Calcutta and spread to Bihar, Assam, Madhya Pradesh, Maharashtra, Kerala, Tamil Nadu and Karnataka in gardens and ornamental nurseries. In 1946-48, it appeared as epidemic in Balasore, Orissa in vegetable fields, especially, cabbage, cauliflower and pumpkin (Behura 1955).

### **2.2. San Jose Scale (SJS), *Quadraspidiotus perniciosus* (Comstock) (Hemiptera: Diaspididae)**

SJS is a native of Northern China but it attained threat status at San Jose in California in 1873. It entered in to India through Kashmir state in 1879 along with the flowering plants of *Cydonia japonica* and became a serious pest of temperate fruits especially apples of North Western India viz., Jammu and Kashmir, Himachal Pradesh and Uttar Pradesh states in 1921. It was reported to attack 51 hosts including apple, plum, peach and peas in Jammu and Kashmir, Himachal Pradesh, Uttar Pradesh, Tamil Nadu, Karnataka, West Bengal, Sikkim, Assam and Meghalaya states, besides causing 100% fruit infestation in Khunmoh, Srinagar district (Tuhan et al. 1979).

### **2.3. Coffee green scale (CGS), *Coccus viridis* (Green) (Hemiptera: Coccidae)**

CGS is a major pest of Arabian coffee and was introduced in India in 1889 via Ceylone (Singh and Rao 1977). It is very common in coffee growing areas of Karnataka, Tamil Nadu and Kerala states, besides attacking guava, mango, orange etc.



#### **2.4. Potato tuber moth (PTM), *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae)**

PTM is native to the Western South America and was introduced in India in 1900 A. D., along with seed potatoes from Italy. The actual damage was first reported in 1906 in East Bengal. PTM became destructive on potato both in field (51.5-56.4%) and storage (70%) conditions in Karnataka and Uttar Pradesh (Lefroy 1968).

#### **2.5. Apple woolly aphid (AWA), *Eriosoma lanigerum* (Hausmann) (Hemiptera: Aphididae)**

AWA is a native to the America and was introduced to Coonoor, Tamil Nadu, India in 1889 and in 1909 on the nursery stock imported from England to Shimla district, Himachal Pradesh and other apple growing areas of India (Thakur and Dogra 1980).

#### **2.6. Diamond back moth (DBM), *Plutella xylostella* L. (Lepidoptera: Yponomeutidae)**

DBM is a native to Southern Europe. It appeared destructive on cruciferous crops (Gandhale et al. 1982). In India, it was reported in 1914 and distributed in all crucifer growing areas to cause average crop damage of 52% (David and Kumaraswami 1978).

#### **2.7. Cottony cushion scale (CUS), *Icerya purchasi* Maskell (Hemiptera: Margarodidae)**

CUS is a native to Australia and was a threat to citrus industry of Southern California (Caltagirone and Doult 1989). It was first recorded in 1928 at Nilgiris and Madurai districts of Tamil Nadu and believed to be introduced through imported orchard stocks or flowering plants, besides through apple cuttings imported from Ceylone (Rao and Cherian 1944). A further spread was reported in many parts of Tamil Nadu, Karnataka, Kerala and Maharashtra and reported to feed on 38 hosts including *Acacia*, *Casuarina*, *Citrus* sp. and *Rosa* sp. (Rao 1944).

#### **2.8 Sugarcane woolly aphid (SWA), *Ceratovacuna lanigera* Zehntner (Hemiptera: Aphididae)**

SWA was reported for the first time in West Bengal in 1958 on sugarcane (Basu and Banerjee 1985). Both nymph and adults suck the phloem sap and affect photosynthesis by excreting copious honey-dew, which in turn results in the development of sooty mould fungus.

### **3. Invasive pests in green and post-green revolution era**

#### **3.1. Pine woolly aphid (PWA), *Pineus pini* (Macquart) (Hemiptera: Adelgidae)**

PWA was first recorded in Nilgiris, Tamil Nadu on pine trees in 1970 and entered from Western and Central Europe (McAvory et al. 2007).

### **3.2. Litchi longhorn beetle (LLB), *Aristobia reticulator* (Viet) (Coleoptera: Cerambycidae)**

LLB was first reported in Tamil Nadu in 1997 and is a native of China (Shylesha et al. 2000). It attacks guava, China rose, redgram, citrus and mango. It was found attacking on litchi in Arunachal Pradesh, causing 76% loss to the crop.

### **3.3. Subabul psyllid (SP), *Heteropsylla cubana* Crasford (Homoptera: Psyllidae)**

SP is native to temperate South America. It is reported to enter India through wind currents from Sri Lanka in 1988 (Singh and Jalali 1990). Its infestation was first reported in Kattupakkam, Chengalpattu, Tamil Nadu in February 1988. Thereafter, it moved to Bengaluru, Pune and Nagpur cities. It was further spread to Andhra Pradesh and Neapanagar of Madhya Pradesh (Singh and Bhandari 1989). It is reported to cause heavy damage to subabul.

### **3.4. Codling moth (CM), *Cydia pomonella* (L.) (Lepidoptera: Tortricidae)**

CM is a serious pest of temperate fruits including apple, walnut, peach, pear, apricot etc. and was first introduced in the Ladakh region of Jammu and Kashmir from Pakistan in 1989 (Pawar and Kapil 1982), causing 95.6% loss to apple.

### **3.5. Coffee berry borer (CBB), *Hypothenemus hampei* (Ferrai) (Coleoptera: Scolytidae)**

CBB is a native to Central Africa and occupied several coffee cultivating countries including Central and South America, South East Asia, Pacific countries etc. (Waterhouse and Norris 1987). In India, it attained the pest status in 1990 in the estates of Gudalur of Nilgiris district, Tamil Nadu. It is believed that the route of spread of this pest through infested coffee berry brought from Sri Lanka by Tamil refugees (Kumar *et al.* 1990). It entered in to Wayanad district of Kerala and was also detected in Kutta estate of Kodagu district of Karnataka in February 1991 and Siddapur areas in August 1993. The CBB infestation affected US \$ 300 million worth coffee berry export of India (Sreedharan 1995).

### **3.6. Serpentine leaf miner (SLM), *Liriomyza trifolii* (Burgress) (Diptera: Agromyzidae)**

SLM is a native of Florida and Carribean Islands. It entered in to India (Hyderabad) through infested Chrysanthemum cut flowers during 1991 and occupied in Karnataka, Andhra Pradesh, Maharashtra, Gujarat and Delhi. It was recorded on cotton in Karnataka, then spread in to tomato and cucurbits, causing damage to the extent of 30-40%. Severe infestation of SLM was reported on ridge gourd and tomato in Andhra Pradesh, cotton, tomato and castor in Karnataka and okra, smooth gourd, field bean, hybrid cotton and castor in Tamil Nadu (Pawan et al. 1996).

### **3.7. Spiralling whitefly (SWF), *Aleurodicus disperses* Russell (Hemiptera: Aleyrodidae)**

SWF is native to the Caribbean region and Central America. It was first recorded in cassava in November 1993 in Trivandram district, Kerala. It spread to tapioca, rubber and in another years, it occupied 72 hosts in Kerala (Prathapan 1996) and 96 hosts in Karnataka (Murali, 1999), besides attacking guava, pomegranate, banana and ornamental crops.

### **3.8. Coconut eriophyid mite (CEM), *Aceria guerreronis* (K.) (Acarina: Eriophyidae)**

In India, CEM was first reported in 1997 in the coconut plantations of Ernakulam district of Kerala (Sathiamma et al. 1998). It was reported in Alleppey, Kottayam and Trichur of Kerala, Udumalpet and Pollachi of Tamil Nadu and coconut growing areas of Karnataka and Andhra Pradesh (Shivarama Reddy and Naik 2000). It is reported to spread through the winds and transport of infested nuts (Ramarethinam and Marimuthu 1998).

### **3.9. Silver leaf whitefly (SLWF), *Bemisia tabaci* B-biotype (Homoptera: Aleyrodidae)**

SLWF is a native to Israel and its occurrence was first reported in Kolar district of Karnataka in vegetable crops during 1999; thereafter the spread was noticed in cauliflower, tomato and chillies of Hosur area, Tamil Nadu. It is a highly dangerous sucking pest as it spreads virus diseases to crops indirectly (Mishra et al. 2020).

### **3.10. Eucalyptus gall wasp (EGW) or Blue gum chalcid, *Leptocybe invasa* Fisher & La Salle (Hymenoptera: Eulophidae)**

EGW was first introduced in to Karnataka and Tamil Nadu in 2001 from Australia. It causes extensive damage to leaves of eucalyptus trees by producing galls (Jacob et al. 2007).

### **3.11. Lotus lily midge (LLM), *Stenochironomus nelumbus* (Diptera: Chironomidae)**

LLM was reported in Thirunavaya, Kerala in 2005. It was introduced from China. The newly hatched grub causes leaf rotting in lotus by mining of sub-cuticular parenchyma (Deepu and Habeeburrahman 2008).

### **3.12. Erythrina gall wasp (EGW), *Quadrastichus erythrinae* Kim (Hymenoptera: Eulophidae)**

EGW was introduced from Tanzania, East Africa in to Kerala, India in 2006 (Faizal et al. 2006).

### **3.13. Cotton mealy bug (CMB), *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae)**

CMB was first recorded in cotton crop of Gujarat in 2006. It was introduced from Central America (Tanwar et al. 2007).

### **3.14. Papaya mealybug (PMB), *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae)**

PMB is a native to Mexico and was first recorded in Coimbatore, Tamil Nadu in 2007 in papaya gardens (Tanwar et al. 2010). A classical biological control for this pest was launched in 2010 by importing an Ecyrtid parasitoid, *Acerophagus papayae* from Puerto Rico, Latin America with the help of United States Department of Agriculture-Animal and Health Inspection Services (USDA-APHIS) (Shylesha et al. 2011).

#### **4. Invasive pests in the 2<sup>nd</sup> decade of 21<sup>st</sup> century**

##### **4.1 Jack Beardsley mealybug (JBMB), *Pseudococcus jackbeardsleyi* Gimple and Miller (Hemiptera: Pseudococcidae)**

JBMB was first reported at Karnataka in 2012 in banana crop and is a native of USA (Shylesha 2013).

##### **4.2. Madeira mealybug (MMB), *Phenacoccus madeirensis* Green (Hemiptera: Pseudococcidae)**

MMB is a native Neotropical and was recorded in Hibiscus plants of Karnataka in 2012 (Shylesha and Sunil Joshi 2012)

##### **4.3. South American tomato moth (SATM), *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)**

SATM had been first reported in 2014 in Karnataka (Sridhar *et al.* 2014) and turned a regular pest of India due to its fast spread and fecundity. During the subsequent years, threat of *T. absoluta* was noticed at several parts of India including Bengaluru (Karnataka), Rajendranagar (Telangana) (Kumari *et al.* 2015), Ludhiana (Punjab) and Varanasi and Mirzapur (Uttar Pradesh) and attained the key pest status.

##### **4.4. Coconut spindle leaf beetle (CSLB), *Wallacea* sp. (Coleoptera: Chrysomelidae)**

CSLB was introduced from the Oriental region, Australia in to the coconut plantations of Andaman Islands in 2014 (Prathapan and Shameem 2015).

##### **4.5. Rugose spiralling whitefly (RSW), *Aleurodicus rugioperculatus* Martin (Hemiptera: Aleyrodidae)**

RSW, a native of America was first noticed on coconut from Pollachi, Tamil Nadu and Palakkad, Kerala in 2016, and subsequently occupied almost all the southern parts of India including Tamil Nadu, Karnataka, Kerala, Andhra Pradesh, Goa and Assam, besides extending its habitats in banana, mango, sapota, guava, cashew, maize, ramphal, oil palm, Indian almond, water apple, jackfruit and many ornamental plants (Anonymous 2019).

Aphelinid parasitoid, *Encarsia guadeloupa*e Viggiani (Hymenoptera: Aphelinidae) has been reported to cause 56-82% parasitisation on spiralling whitefly under natural condition. Growing banana and *Canna indica* along with coconut can conserve the parasitoid. Entomopathogenic fungus, *Isaria fumosorosea* under various field testing in Andhra Pradesh, Karnataka, Kerala, Tamil Nadu, West Bengal and Maharashtra is evident to cause 91% control on RSW (Anonymous 2020c).

#### 4.6. Fall armyworm, *Spodoptera frugiperda* (J. E. Smith); (Lepidoptera: Noctuidae)

FAW was first reported in Shivamoga, Karnataka in 2018 (Kalleshwaraswamy et al. 2019) in maize and established as a regular insect pest of maize with its attack also reported on sorghum and bajra. It spreads rapidly in various parts of India because of its peculiar behaviours of fast flying capacity, voracious and polyphagous in nature. During May to July 2018, it occupied the entire Karnataka, Tamil Nadu, Telangana and Andhra Pradesh. In Maharashtra, Madhya Pradesh, Chhattisgarh and Odissa, the spread took place in August 2018; Gujarat, West Bengal in September 2018; Jorkhand in October 2018; Tripura and Mizoram in November 2018; Nagaland in March 2019; Sikkim, Meghalaya, Manipur in May 2019; UP, Bihar, Assam in June 2019; Uttarkhand in July 2019 and Haryana, Punjab in August 2019 while the FAW incidence was not reported in Kerala and Union Territories (Andaman and Nicobar Islands, Dadra and Naga Haveli, Daman and Diu, Chandigarh, Lakshadweep, Puducherry, Delhi, Ladakh, Jammu & Kashmir) and Himachal Pradesh. The neighboring countries including Bhutan, Pakistan and Afghanistan are still free from FAW occurrence (Fig. 1).

Hymenopteran parasitoids viz., *Telenomus* sp. (Platygastridae), *Trichogramma* sp. (Trichogrammatidae), *Chelonus* sp., *Glyptapanteles creatonoti* (Viereck) and *Cotesia* sp. of Braconidae, *Phanerotoma* sp. and *Campoletis chloridae* Uchida of Ichneumonidae (Shylesha et al. 2018) and Nuclear polyhedrosis virus (NPV of Sf) (Sivakumar et al. 2020) and *Nomuraea rileyi* of *S. frugiperda* larvae have been documented (Shylesha et al. 2018) to have good biocontrol potential on FAW. Web-based FAW portals of FAO (FAO 2020); CABI-based news, research, practical extension materials, videos and other resources; mobile app on FAW Monitoring and Early Warning System (FAMEWS); Global Action for Fall Armyworm Control' in 2020 (CABI 2020b) and ASEAN Action Plan on FAW Control (Anonymous 2020e) are the recently established systems for early detection and management of FAW.

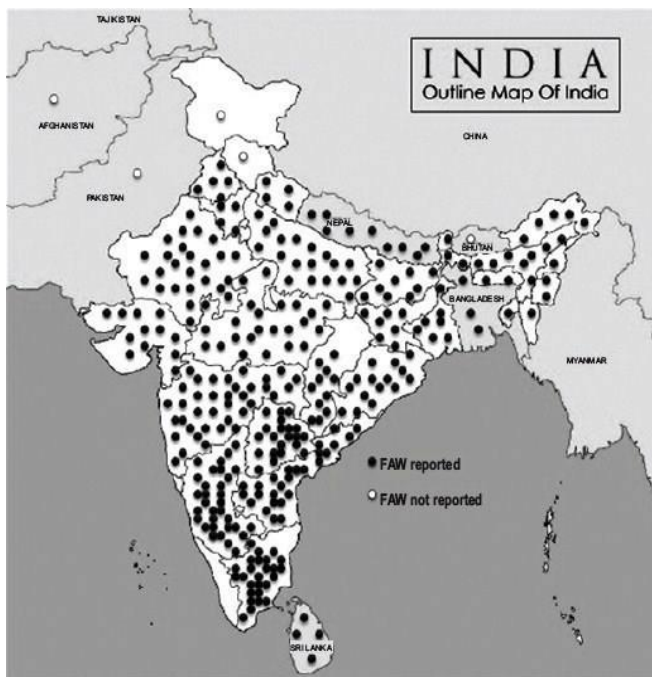


Fig 1. Distribution of FAW in south East Asia

#### **4.7. Nesting whitefly (NWF), *Paraleyrodes minei* Iaccarino (Hemiptera: Aleyrodidae)**

NWF was first reported in Kerala on coconut in 2018 and is a native of Syria (Chandirka Mohan et al. 2019).

#### **4.8. Bondar's Nesting whitefly (BNSF), *Paraleyrodes bondari* Peracchi (Hemiptera: Aleyrodidae)**

BNSF was reported on coconut at Kerala in 2018 and is a native of Central America (Joseph Rajkumar et al. 2019).

#### **4.9. Neotropical whitefly (NTWF), *Aleurotrachelus atratus* Hempel (Hemiptera: Aleyrodidae)**

NTWF was recorded on coconut for the first time at Mandya, Bengaluru in 2019. It is a native of Brazil (Selvaraj et al. 2019).

#### **4.10. Cassava mealybug (CMB) *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae)**

Invasions of CMB was first recorded in Thrissur, Kerala followed by its widespread infestations in the neighbouring state of Tamil Nadu in Southern India (Anonymous 2020d). CMB-specific encyrtid parasitoid *Anagyrus lopezi* De-Santis (Hymenoptera: Encyrtidae) Karyani et al. 2016 and *Anagyrus papayae* (Shylesha et al. 2018) have been reported to be in association with CMB under natural field condition for parasitisation.

#### **4.11. Root-knot nematode (RKN), *Meloidogyne enterolobii* (Yang and Eisenback, 1988) (Tylenchida: Heteroderidae)**

RKN is posing a serious threat to guava (*Psidium guajava*) cultivation throughout the world. More recently it was reported in the guava orchards of Tamil Nadu (Poornima et al. 2016; Suresh et al. 2019), Uttarakhand (Kumar and Rawat 2018) and Luni basin of Rajasthan (Bhati and Parashar 2020) where trees declined, showing yellowing and stunted growth. It was also identified in the guava orchards of Andhra Pradesh and Telangana states. This nematode causes severe root galling, thereby reduce the nutrient uptake ability of plants.

### **5. Transboundary pests of India**

#### **5.1. Desert locust (DL), *Schistocerca gregaria* Forskål (Orthoptera: Acrididae)**

DL is considered as one of the pandemics across the world due to its inter-continental flight rate, high fecundity and voraciousness which can cause a huge damage to several economically important crops (Cressman 2016). Out of several desert locust plagues occurred in India between 1964 and 2010, the severest one was those occurred during 2020, resulting huge crop losses. Till now since 1993, no such severity was faced by India. During 2020, India received the spread and frequent cyclones-associated unusual rainfalls-induced DL attacks which had a profound

negative impacts on all sectors of India including Agriculture, resulting dearth of Indian economy substantially (Anand 2020).

The excess rainfalls during March-April 2020 in India favoured rapid multiplication of desert locust with 20% excess population which could facilitate desert locust to form 3-4 plaques and reached up to Central India including Maharashtra, Madhya Pradesh and the bordering districts of Chhattisgarh which had never been experienced for past 27 years in Central India. The excess rainfalls induced locust attacks was compounded with inadequate control of locust due to lockdown associated restricted movement of officials, extension officers, farmers and spray-men and poor access of plant protection inputs, which resulted that the nascent populations of locust had not been contained (Anonymous 2020a,b).

Aerial application of ULV formulations of pesticides (Malathion 96%) are in practice in several countries however, due to huge side effects on the environment and non-target organism, an integrated pest management program using bio-pesticides from plants and microorganisms are greatly reliable for successful control of desert locust (Kooyman 2003; Githae and Kuria 2021).

Early research on the use of fungi as a bio-control agent against desert locust was focused on *Metarhizium flavoviride* (Youssef 2014; Reda et al. 2018). The oil formulation of *M. flavoviridae* was reported to reduce the hopper bands tremendously (Lomer 1997). However, the negative effect of this formulation on bees and other non-target organisms discouraged its widespread use against desert locust control (Ball et al. 1997).

The fungus, *Metarhizium acridum* (Ascomycota: Hypocreales) isolated from Africa and Australia during 1990s was considered as the best alternative to *M. flavoviridae*. *Metarhizium anisopliae* var. *acridum*, commonly known as *Metarhizium acridum* (Bischoff et al. 2009), is an environmentally safer commercial bio-pesticide that has been developed for ultra low volume spraying (Van Huis 2007). The bio-pesticide kills about 70–90% of treated locusts within 14–20 days with no measurable impact on non-target organisms (Lomer et al. 2001).

In 2020, FAO recommended the commercial product of *M. acridum* (Green Muscle™) as a biological solution for DL in Somalia, Kenya and Ethiopia (CABI 2020a). It is the time of the hour that India imports and uses the right formulation of *M. acridum* for effective management of DL. Biological control of desert locust though it is a safe method that will take several days to bring under control. Rational use of pesticides along with biological control of desert locust is suggested, considering natural-risk management plans for locust outbreaks as well as the benefit and cost of proposed control measures and their environmental and health impact.

## **5.2. Carambola fruit fly, *Bactrocera carambolae* Drew & Hancock (Diptera: Tephritidae)**

CF is a native to Asia and specifically to Malaysia, southern Thailand and western Indonesia (Muller 2008). In past years, this species has invaded South America *via* the trade of fruits from Indonesia. It is a polyphagous species which attacks 100 different host plants including avocado, guava, mango, papaya and orange, among several others (Pasinato et al. 2019).

Application of green muscardine fungi, *Metarhizium* spp., in soil, wrapping of fruits with news paper, brown paper bag or a sleeve and bait spray are some of the eco-friendly methods that are in practice to manage this pest (Muller 2008). The occurrence of CF has been reported in severe form in Bangladesh, Vietnam, Malaysia, Andaman Nicobar islands; localized occurrence in India, Thailand and Indonesia and absent in Afghanistan, Pakistan, Nepal Bhutan, Burma and few north eastern states of India (Fig. 2). The Indian Council of Agricultural Research, New Delhi is in vigil in monitoring the movement of CF in boundaries of India especially in the geo-boundaries between India and Bangladesh.



**Fig 2. Distribution of Carambola fruit fly, *Bactrocera carambola***

## 6. Conclusion

Invasive pests and TPPs are threat to plant health which reflects on food security globally. Thirty-three invasive insect and non-insect pests reported in India so far since 1847 to till date have already entered, occupied new crop habitats and established. The COVID-19 induced lockdowns aggravated the spread and outbreaks of certain invasive pests including fall armyworm, cassava mealybug, root-knot nematode etc., due to restricted movement of field level plant protection workers, non-availability of insect mitigation inputs like pesticides, biopesticides, botanicals etc. The ICAR institutes/State Agricultural Universities of India have developed well equipped Integrated Pest Management (IPM), pest-wise based on pest-biology and -ecology of the invaded pests in the new habitats which are readily available in public domains for the stake-holders to adapt. However, rigorous/rampant monitoring of the entry of new invasive pests through sea- and air-ports should be a continuous exercise of the officers, extension and field level workers etc., as the free trade policies, globalization etc., are favourable for frequent entry of invasive pests.



Biocontrol agents including parasitoids, predators, entomopathogenic fungi, bacteria, virus *etc.*, are reported to have good control potential on the invasive pests. Many such natural enemies which had/have been moving along with host insects in to new ranges/habitats are being identified and utilized for biological control. However the natural enemies of many invasive pests are not reported to move along with host insects wherein, the importation of potential natural enemies from the native/origin of invasive pests is recommended. The desert locust and Carambola fruit fly are considered as potential transboundary insect pests to India. The Locust Warning Organization, Faridabad is the premier agency to monitor and forewarn the movement and occurrence of desert locust in India, using the geo-spatial technologies including GPS, GIS, remote sensing etc. The Carambola fruit fly reported to prevail in Bangladesh may be likely to invade India which requires rigorous monitoring of geo-boundaries.

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## Chapter 2

### Trends in Biopesticides in India

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#### Abstract

Chinese farmers were the first to apply biological control of agricultural pests when they used red ants to manage pests of fruit crops. Neem-based products were then used as fertiliser and as a barrier against pests of stored goods. Later, biopesticides surpassed macro-biocontrol agents in prominence and were added as one of the elements of integrated pest management. The interventions and directives of the United States Environmental Protection Agency and European Food Safety Authority were for way forward to the evolution of pesticides derived from naturally occurring organisms and plant materials. The fungal based biopesticides especially *Trichoderma* spp., are abundant in Indian market than other microbe based formulations. It is *vice-versa* in Western countries wherein *Bacillus thuringiensis* based products are being used in wide spread control of Lepidoptera pests of temperate crops. Talc and oil based biopesticide formulations are most common in India as they as fillers could be able to prolong the pathogenicity by enhancing shelf-life. List of biocontrol laboratories and important companies along with their products are tabulated and furnished to understand the scope of biopesticide in India.

**Keywords:** Biopesticide, Classification, Formulations, Biocontrol labs, Field applications

#### 1. Introduction

In 1901, Japanese biologist Shigetane Ishiwata discovered spores of the bacterium, *Bacillus thuringiensis* (*Bt*) from a sick silkworm. This bacteria is still the most often used biopesticide today (Chen 2014; Glare and O'Callaghan 2000). Sporeine, the first *Bt* product to be sold commercially, debuted in 1938. The extensive usage of biopesticides started in the 1950s in the US. A low level of research and development was maintained in the second half of the 20<sup>th</sup> century as a result of the widespread use of synthetic chemical insecticides and World War II that were less expensive but more poisonous. The Pacific Yeast Product Company created the submerged fermentation industrial process in 1956, allowing for the large-scale manufacturing of *Bt* (Glare and O'Callaghan 2000). In 1973, *Heliothis* NPV was granted exemption from tolerance and the first viral insecticide, Elcar received a label in 1975. In 1977, *B. thuringiensis* var. israelensis, which is poisonous to flies, was reported in 1977, while the strain tenebrionis, which is poisonous to beetles, was found in 1983.

In Europe, Aristotle was the first to mention that bees suffered disease and in 1835, Agostino Bassi showed that animal disease could be caused by a microorganism, when he found that the fungus *Beauveria bassiana* causes the muscardine disease of silkworms. Early observations were largely concentrated on two domesticated insects, the honey bee and silkworm. Gradually these studies were extended to pest species too, and the concept of utilizing disease to control these insects was born. In 1879, the Russian, Metchnikoff, conducted the first

significant experiments in the destruction of injurious insects with micro organisms by infecting larvae of the beetle *Anisoplia austriaca* with the fungus *Metarhizium anisopliae*.

Authorities from the US Environmental Protection Agency (USEPA) and the European Food Safety Authority (EFSA) revised the pesticide laws when these issues arose in order to protect human and animal health as well as the environment from the risks associated with pesticides. They proposed the ideas of the ideal pesticide, which include i) a high selectivity to target species but a minimal toxicity to non-target organisms, ii) a high effectiveness at a low application rate, and iii) a low environmental persistence. Thus a new concept on ‘Biopesticide’ had evolved to fight with pests effectively but have minimal impacts on humans, animals, and the environment. Active biopesticide research has expanded in the latter decade of the 20<sup>th</sup> century along with a notable increase in publications (Shukla et al. 2019) (Fig. 1).

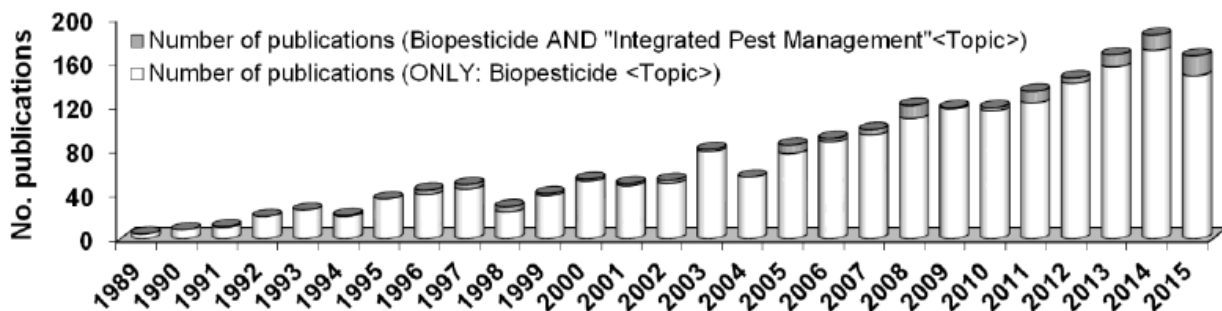


Fig 1. Publications on biopesticide research from 1989 to 2015 (Web of Science 2015).

Around 1400 biopesticide products were sold globally at the start of the twenty-first century, making up around 2.5% of the entire pesticide business (Marrone 2007; Chandler et al. 2012). From 2012 to 2017, the demand for biopesticides was predicted to increase at a faster compound annual growth rate (GAGR) of 16.1% (compared to 3%) than that of synthetic pesticides, resulting in an estimated \$ 5.2 billion global market in 2017 (Lehr 2010). Europe, Oceania, and Latin America accounted for 20, 20, and 10% of worldwide biopesticide consumption, respectively, whereas North America consumed roughly 40% of them (Leng et al. 2014). Numerous legislative initiatives for the sustainable use of pesticides were implemented, and they all emphasized how crucial it is to use less chemical pesticides overall to avoid potential environmental damage.

In India, Bio-Control Research Laboratories (BCRL), a division of Pest Control India (PCI) Limited under contract with Plant Protection Research Institute (PPRI) was the pioneer in commercial production of biocontrol agents (Manjunath 1992). Currently, the BCRL is manufacturing and selling formulation of antagonistic fungi (*Trichoderma viride*, *Trichoderma harzianum*, *Beauveria bassiana*) and bacteria. Later, National Policy on Agriculture (2000) and National Policy for Farmers (2007) also supported IPM. In India, a total of 970 biopesticide formulations have been registered in Central Insecticide Board and Registration Committee (CIB&RC) as on 1.1.2020 in which *Trichoderma* and *Pseudomonas* based formulations dominate (<http://164.100.161.213/divisions/cib-rc/bio-pesticide-registrant>). A compendium consisting of 31 microbial formulations which are in different stages of registration and



commercialization were reported to possess 3 to 25 months shelf-life at 25°C to 35°C (Saxena et al. 2021).

Three kinds of biocontrol products are being used in India which include 1) Microbial biopesticides, 2) Plant-derived botanical pesticides, and 3) Pheromones or other natural insect growth regulators. Microbial biopesticides consist majorly of fungi, bacteria, viruses or entomopathogenic nematodes as bioactive principles. In India, fungal based biopesticides share in the market is maximum (Mishra et al. 2020; Fig. 1,2,3) while *Bacillus thuringiensis* based products rank first in usage in USA, Europe etc. Among fungal biopesticides, *Trichoderma* (only two species) based products approximately around 355 are readily available in the Indian market for the field applications (Kumar et al. 2019). *Pseudomonas fluorescens* based products are in wide spread usage over *Bacillus* based products globally with reference to bacterial biopesticide (Mishra et al. 2020). In India, strains of *B. thuringiensis*, *Bacillus sphaericus* and *Bacillus subtilis* are registered as biopesticides. Other non-spore forming bacteria like, *Serratia entomophila* and *Chromobacterium subsugae* though have efficacy on limited range of insects are not evaluated systematically (Martin et al. 2007). In India, two nucleopolyhedron viruses (NPVs) of *Helicoverpa armigera*, and *Spodoptera litura* are in use under field conditions in a smaller extent. Though the natural occurrence of granulovirus (GVs) of sugarcane pests in southern and northern states of India was reported, their commercial production are not yet started (Easwaramoorthy and Jayaraj 1987).

The fate of using two most effective entomopathogenic nematodes belonging to the family Heterorhabditis and Steinernema is questionable, however their applications against different soil-borne pests under field conditions are scarce (Sankaranarayanan et al. 2006) as no registered product is available in the market to date in India.

## **2. Biopesticide formulations**

CIBRC registration guidelines emphasized few quality parameters of biopesticide formulations for registration and before entering into market which include safe and effective, easy delivery with prolonged shelf-life. At present there are only few biocontrol products that strictly adhere to CIBRC guidelines. In India, wettable powder (WP), wettable granules (WG), suspension concentrates (SC) and aqueous suspension (AS) formulations are being used. Currently, biocontrol products are formulated in solid carriers which include talc, peat, lignite, clay, wheat husk, rice bran, grinded corn cob, fly ash and sawdust. *Bacillus* based products are being sold in the form of aqueous suspension, dust, WP and granules, charcoal, plaster of paris and fly ash (Tikar and Prakash 2017). Currently, the liquid formulations of *Bt* products and viral biopesticides are very popular in the market. New Nanotechnology-based biopesticide formulations such as nanoencapsulation and nanoemulsions can improve the large scale applicability of bioagents under field conditions (Koul 2019). However, these techniques are still in infancy and yet to be commercialized at the industrial level. However, the survival of infecting units of microbe in various carriers is remarkably debatable.

### 3. Biopesticide mass-production units in India

The number of bio-production units has currently increased to 361, of which 141 are in the private sector without GOI grant aids and 38 with GOI grant aids. Moreover, the Ministry of Agriculture and Farmers Welfare has assisted about 35 IPM centers to produce biopesticides since 2010 (Keswani et al. 2016). A total of 98 State Biocontrol Laboratories were established by the State Departments of Agriculture and Horticulture of Gujarat, Uttar Pradesh, Karnataka, Tamil Nadu, Andhra Pradesh and Kerala as well as the production of microbial pesticides (Pathak et al. 2017) by the Institutions of the Indian Council of Agricultural Research (Mishra et al. 2020; Fig. 4).

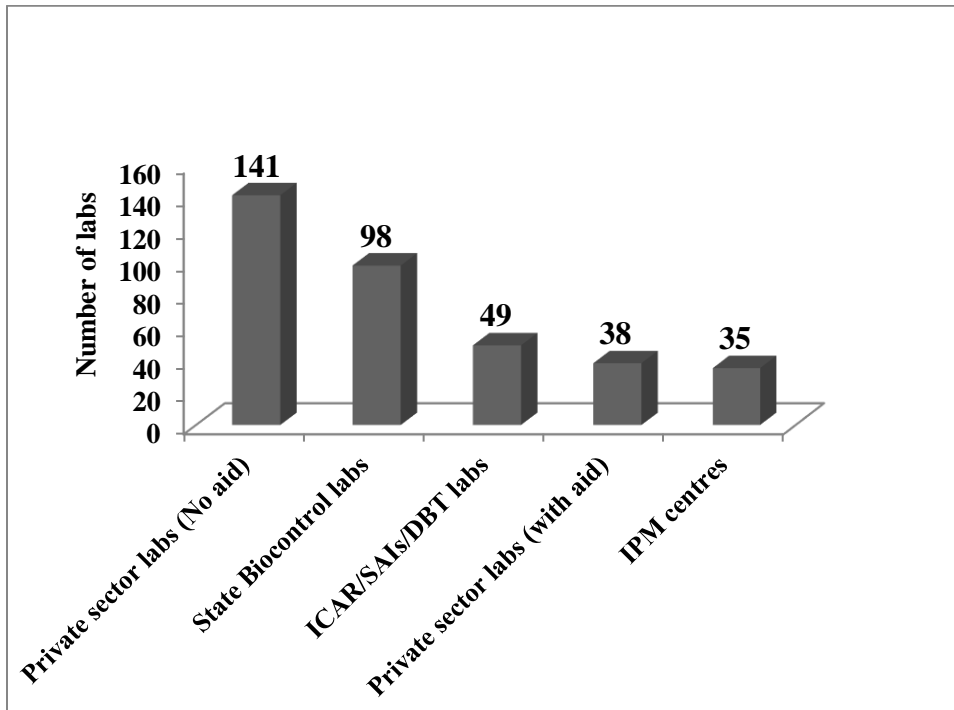


Fig 1. Current status of biocontrol labs in India

Central and state agricultural universities and various ICAR institutes including Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, ICAR-Central Plantation Crops Research Institute (CPCRI), Kayamkulam, Kerala, Kerala Agricultural University (KAU), Kerala, ICAR-Indian Institute of Horticultural Research, Bengaluru, ICAR-Central Research Institute for Dryland Agriculture, Hyderabad, ICAR-Directorate of Oilseed Research (ICAR), Hyderabad are popular biopesticide production units in Southern parts of India. In the northeast, Assam Agriculture University and Central Agricultural University, Manipur are producing biopesticides against invasive pests. In north India, ICAR-Indian Agricultural Research Institute (IARI), New Delhi, Punjab Agricultural University (PAU), Punjab, G.B. Pant University of Agriculture and Technology (GBPUA & T), Uttarakhand are involved in the production of biopesticides. ICAR-Indian Institute of Sugarcane Research (IISR), ICAR-Central Institute for Subtropical Horticulture, and Directorate of Plant Protection Quarantine & Storage in Lucknow, are major government agencies which are mass producing biopesticides in Uttar Pradesh. Many ICAR sponsored Krishi Vigyan Kendras (KVK), State Government sponsored state biocontrol

labs and National Agricultural Co-operative Marketing Federation of India (NAFED) are in the full time job in production of biopesticides.

In India, public sectors contribute 70% of the biopesticides production. Major companies are Biotech International Ltd., New Delhi, International Panaacea Ltd, New Delhi, Ajay Biotech (India) Ltd, Pune, Bharat Biocon Pvt. Ltd., Chhattisgarh, Microplex Biotech and Agrochem Pvt., Mumbai, Excel Crop Care Ltd., Mumbai; Govinda Agro Tech Ltd., Nagpur, Jai Biotech Industries, Satpur, Nasik, Ganesh Biocontrol System, Rajkot, Gujarat Chemicals and Fertilizers Trading Company, Baroda, Gujarat Eco Microbial Technologies Pvt. Ltd., Vadodara, Chaitra Agri-Organics, Mysore, Deep Farm Inputs (P) Ltd., Thiruvananthapuram, Kerala; Kan Biosys Pvt. Ltd., Pune; Indore Biotech Inputs and Research Pvt. Ltd., Indore, Romvijay Biotech Pvt. Ltd., Pondichery; Devi Biotech (P) Ltd., Madurai, Tamil Nadu; T. Stanes and Company Ltd., Coimbatore, Tamil Nadu; Harit Bio Control Lab., Yavatmal and Hindustan Bioenergy Ltd., Lucknow. Few Indian companies which work in biopesticide production in collaboration with foreign companies are Lupin Agro-chemicals, Mumbai; Sugar and distillery companies such as KCP Sugar and Industries Corporation Ltd., Andhra Pradesh, Rajshree Sugars and Chemicals Ltd., Tamil Nadu; New Swadeshi Sugar Mills, Bihar, and Bannari Amman Sugars Ltd., Tamil Nadu have also started to produce biopesticides mainly of *Pseudomonas fluorescens* and *Trichoderma harzianum*.

#### 4. Biopesticide registrant in India

As on 1.1.2021, a total of 970 biopesticides registered in India by CIB&RC under the 1968 Insecticide Act which include microbial biopesticides of *Bacillus thuringiensis* var. kurstaki (42), var. israelensis (22), var. sphaericus (05), var. galleriae (01), *Pseudomonas fluorescence* (196), *Bacillus subtilis* (04), *Trichoderma viride* (289), *T. harzianum* (51), *Ampyliomyces quisqualis* (02), *Beauveria bassiana* (106), *Metarhizium anisopliae* (30), *Verticillium lecani* (93), *Verticillium chlamydosporium* (03), *Helicoverpa armigera* NPV (30) and *Spodoptera litura* NPV (03) and only 38 biopesticidal formulations (Kumar et al. 2018; Keswani et al. 2019; <http://ppqs.gov.in/divisions/cib-rc/biopesticide-registrant>) (Table 1).

Table 1. List of microbial biopesticide formulations registered in Indian

S. No.	Name of Company	Organism	Trade name	Target biotic stress
1.	M/s Rallis India Ltd., Bengaluru	<i>Bacillus thuringiensis</i>	Bobit IIWP	Lepidoptera insects
2.	M/s Sandoz (I) Ltd., India	<i>Bacillus thuringiensis</i> var. kurstaki	Deflin WG	Lepidoptera insects
3.	M/s Aventis Crop science Ltd., Bengaluru	<i>Bacillus thuringiensis</i> var. israelensis (H-14)	Vectobac 12 AS	Diptera insects
4.	M/s T. Stanes and Company Ltd., Cotore, India	<i>Beauveria bassiana</i>	Biopowder WP	Sucking insects, bollworms
5.	M/s Biotech Industries Ltd., New Delhi	NPV	Biovirus-H	<i>Helicoverpa armigera</i>

6.	M/s Pest control India Ltd., Bengaluru	NPV	Spodocide 0.50% AS	<i>Spodoptera liutura</i>
		NPV	Helicide 0.50% AS	<i>Helicoverpa armigera</i>
7.	M/s Anshul Agrochemicals, Bengaluru	<i>Beauveria bassiana</i>	Green Heal	Sucking insects, borers, bollworms
		<i>Beauveria bassiana</i>	Beveroz-L	Sucking insects, borers, bollworms
		<i>Beauveria bassiana</i>	Almax	Sucking insects, borers, bollworms
		<i>Pseudomonas floescens</i>	Pseudomax	Soil and seed borne diseases
		<i>Trichoderma viridi</i>	Trichomax	Soil borne diseases, plant parasitic nematodes
8.	M/s Deepa Farm inputs Private Limited	<i>Pseudomonas floescens</i>	Bio-Plus Pseudo	Soil and seed borne diseases
		<i>Trichoderma viridi</i>	Bio-Plus Tricho	Soil borne diseases, plant parasitic nematodes
		<i>Verticillium lecanii</i>	Bio-Plus Verticillium	Sucking pests, plant parasitic nematodes
		<i>Metarhizium anisopliae</i>	Bio-Plus Metarhizium	Beetles, soil arthropods
9.	M/s Kan biosys Pvt. Ltd., Pune	<i>Trichoderma harzianum</i>	Nemastin 1% WP	Root knot nematode
		<i>Trichoderma viridi</i>	Combat 1% WP	Root rot, damping off, wilt
		<i>Beauveria bassiana</i>	Brigade-B 1.15% WP	Rice leaf folder
		<i>Pseudomonas floescens</i>	Sudo 0.5% WP	Late leaf spot of groundnut
10.	M/s Peak Chemical Industries Ltd., West Bengal	<i>Paecilomyces fumosoroseus</i>	Bardan	Spider mite, parasitic mites
		<i>Metarhizium anisopliae</i>	Moti	Termite
		<i>Beauveria bassiana</i>	Badsha	Sucking pests
		<i>Verticillium lecanii</i>	Victor	Parasitic nematodes, whitefly, thrips, aphids
11.	M/s Uttam Chemicals Industries, Rajasthan	<i>Trichoderma harzianum</i>	Trichoderma harzianum 1% WP	Parasitic nematodes
		<i>Trichoderma viridi</i>	Trichoderma viridi 1.5% WP	Nematicide, crop diseases
12.	M/s Ambic Organic, Surat	<i>Paecilomyces fumosoroseus</i>	Almite	Mites, DBM, sucking pests
13.	M/s Criyagen Agri & Biotech Pvt. Ltd.,	<i>Trichoderma viridi</i>	Trichoderma	Soil borne fungus

	Bengaluru			
14.	M/s Biotech International Ltd., New Delhi	<i>Bacillus thuringiensis</i> var. kurstaki	BIOLEP WP	<i>Helicoverpa</i> , <i>Spodoptera</i> , DBM, borers, hairy caterpillars, cut worms, army worms, leaf rollers & miners, skeletonizers & Defoliators
		<i>Beauveria bassiana</i>	BIORIN WP/AS	<i>Helicoverpa</i> , <i>Spodoptera</i> , DBM, leaf borer, hairy caterpillars, mites, spidermites, whiteflies, aphids, scale insects, locusts & colorado beetles
		<i>Verticillium lecanii</i>	BIOLINE WP/AS	White fly, green leaf hopper, thrips, mealy bug, brown plant hopper, leaf miner, aphids, mites, jassids
		<i>Metarhizium anisopliae</i>	BIOMET WP/AS	White grubs, termite, cut worm, caterpillars, semiloopers, sucking pests, mealybugs & aphids
		NPV	BIOVIRUS-H AS	<i>Helicoverpa armigera</i>
		NPV	BIOVIRUS-S AS	<i>Spodoptera litura</i>
		<i>Pseudomonas fluorescens</i>	BIOMONAS WP/AS	Bacterial wilt, black rot, bacterial spot, sheath blight; blast, anthracnose, powdery & downy Mildew, panama wilt, panama wilt, sigatoka, bacterial leaf spot
		<i>Trichoderma viride</i>	BIODERMA WP/AS	Root rot, stem rot, damping off, fusarium wilt and verticillium wilt, all types of leaf spot, leaf & blight
		<i>Trichoderma harzianum</i>	BIODERMA-H WP/AS	Root rot, stem rot, damping off, fusarium wilt and verticillium wilt, all types of leaf spot, leaf & blight
		<i>Bacillus subtilis</i>	BIOSUBTILIN WP/AS	Fusarium wilt, Macrophomina, damping

				off, Pythium, Rhizoctonia Black scarf of Potato, root rot, leaf spot, powdery and downey mildew, bacterial spot & bacterial leaf blight
		<i>Ampelomyces quisqualis</i>	ARMOUR WP/AS	Powdery Mildew in pulses, vegetables, fruits & ornamental crops
		<i>Paecilomyces lilacinus</i>	BIONEMAT WP/AS	Root-knot nematodes, reniform nematodes, cyst nematode, golden cyst nematodes, citrus nematodes, lesion nematode
		<i>Bacillus firmus</i>	NEMATO CURE WP/AS	Root-knot nematodes, reniform nematodes, cyst nematode, golden cyst nematodes, citrus nematodes, lesion nematode
		<i>Hirsutella thompsonii</i>	NO MITE WP/AS	Various types of crop mite – Scoulet, purple, red spider mite
15.	M/s International Panaacea Ltd, New Delhi	<i>Trichoderma viride</i>	Sanjeevni 1.0% WP	<i>Fusarium</i> , Charcoal rot, Black scurf, Karnal bunt, Silver leaf of plum & peach, <i>Rhizoctonia</i> , <i>Pythium</i> , <i>Schlerolims</i> , <i>Verticillium</i> , <i>Alternaria</i>
		<i>Trichoderma harzianum</i>	Bioharz 2% AS	<i>Fusarium</i> , Charcoal rot, Black scurf, Karnal bunt, Silver leaf of plum & peach, <i>Rhizoctonia</i> , <i>Pythium</i> , <i>Sclerolims</i> , <i>Verticillium</i> , <i>Alternaria</i>
		<i>Pseudomonas flourescens</i>	Rakshak 1% WP	Soil and seed borne diseases
		<i>Trichoderma viride</i>	Bokashi Bran	Soil and seed borne diseases
		<i>Trichoderma viride</i>	Seed2plant	Soil and seed borne diseases
		<i>Trichoderma viride</i>	Tricho-PEP H 1% WP	Diseases
			Tricho-P 1.5% WP	root rot, wilt, stem rot

		<i>Paecilomyces lilaecinus</i>	Nematofree 1% WP	<i>Helicoverpa, Spodoptera</i> , borers, hairy caterpillars. pest of vegetables and fruits, whitefly, aphids, DBM, scale insects, locust, Colorado potato beetles
		<i>Pseudomonas fluorescens</i>	Bactvipe 2% AS	Root rot, stem rot, collar rot, wilt, blights, leaf spots, anthracnose, Alternaria and downy & powdery mildews
		<i>Ampelomyces quisqualis</i>	MilGO 2% AS	Powdery mildew, <i>Alternaria solani</i> , <i>Colletotrichum</i> , <i>Coccodes</i> , <i>Cladosporium</i> , <i>cucumerinum</i>
		<i>Beauveria bassiana</i>	DAMAN 1% WP	<i>Helicoverpa, Spodoptera</i> , borers, hairy caterpillars, pest of vegetables and fruits, whitefly, aphids, DBM, scale insects, locust, Colorado potato beetles
		<i>Bacillus subtilis</i>	MILDOWN 2% AS	<i>Pythium, Alternaria, Xanthomonas, Rhizoctonia, Botrytis, Scelerotiana, Phytophthora</i>
16.	M/s Ajay Biotech (India) Ltd, Pune	<i>Bacillus thuringiensis</i> var. Kurstaki	Bio Dart	Insecticide
		<i>Metarhizium anisopliae</i>	Meta Guard WP/AS	Termites, white grubs, soil insects
		<i>Beauveria bassiana, Metarhizium anisopliae, Verticillium lecanii</i>	AJAY VBM	Insect pests
		<i>Paecilomyces</i> spp.	Nemadart	Parasitic nematodes
		Consortia of microbes	TERMINIL	Termites
		<i>Trichoderma viride</i>	Trident 1.5% WP	Soil and seed borne diseases
		<i>Trichoderma harzianum</i>	Trichoshield	Soil borne fungus
		<i>Trichoderma viride</i>	ROM Trichoderma	Rhizome rot of turmeric and cardamom, fusarium

				wilt of banana, wilt disease of pepper, beetelvine, chillies, tomato and vegetables, <i>Phytophthora</i> , <i>Pythium</i> , <i>Sclerotium</i>
17.	M/s Bharat Biocon Pvt. Ltd., Chhattisgarh	<i>Pseudomonas fluorescens</i>	Pseudocon 0.5% WP	Crop diseases
		<i>Trichoderma harzianum</i>	TRICHOCON 1% WP	Crop diseases
		<i>Beauveria bassiana</i>	BASICON 1.15%	Lepidoptera insects
		<i>Verticillium lecanii</i>	VERTICON 1.15%	Plant parasitic nematodes
		<i>Metarhizium anisopliae</i>	METACON 1.15%	Beetle pests, locust
18.	M/s Jai Biotech Industries, Satpur, Nasik	<i>Verticillium lecanii</i>	Vertimust 1.1% WP	Sucking pests
		<i>Beauveria bassiana</i>	Beauvera 1.15% WP	Lepidoptera pests
		<i>Trichoderma viride</i>	Jaimold 1% WP	<i>Sclerocium</i> , <i>Rhizoctonium</i> , <i>Pythium</i> , <i>Fusarium</i>
19.	M/s Ganesh Biocontrol System, Rajkot	NPV	Biokills	<i>Spodoptera litura</i>
		<i>Pseudomonas fluourescens</i>	Monas	<i>Rhizoctinia</i> , <i>Sclerotini</i> , blights & <i>Alternaria</i> , <i>Ascochyta</i> , <i>Cercospora</i> , <i>Macrophomina</i> , <i>Xanthomonas</i> , <i>Erwinia</i>
		<i>Beauveria bassiana</i>	BASS 1.5% WP	<i>Helicoverpa</i> , <i>Spodoptera</i> , DBM, borers, hairy caterpillar of vegetables & fruit plants, mites & spidermites of vegetables & ornamentals, whiteflies on cotton & vegetables, aphids & scale insects, locust, potato and Coffee pod - borer
20.	M/s Gujarat Eco Microbial Technologies Pvt. Ltd., Vadodara	<i>Trichoderma viride</i>	NR-III	Soil & air borne pathogens, sucking pest
		<i>Trichoderma viride</i>	TRIOJEET	Damping off, wilt, root rot
21.	M/s Indore Biotech Inputs and Research	NPV	Helicop AS	<i>Helicoverpa armigera</i>
		<i>Metarhizium</i>	BIO-MAGIC	Grub, termite, fruit flies,



	Pvt. Ltd., Indore	<i>anisopliae</i>		hopper, wire worms, vegetable worms, aphids, jassids
		<i>Beauveria bassiana</i>	BIO-WONDER 1.15% WP	Hairy insects, aphids, white flies, mealy bugs, grasshoppers, thrips, stem borer, termites, beetles, caterpillars
		<i>Verticillium lecanii</i>	Vercitile 1.15% WP	Aphids, whiteflies, thrips, mealy bugs, scale insects, leaf hopper, mango hopper
		<i>Bacillus thuringiensis</i> var. kurstaki	Cezar 0.5% WP	Castor semilooper, soybean and gram pod borer, <i>Spodoptera litura</i> , Bihar hairy caterpillar, sphinx moth
		<i>Paecilomyces lilacinus</i>	BioAce 1% WP	Plant parasitic nematodes
		<i>Trichoderma viride</i>	Biohit WP	<b><i>Pythium, Fusarium oxyforum, Rhizoctonia solani, Alternaria, Sclerotinia rolfsia</i></b>
		<i>Pseudomonas fluorescens</i>	Biomonarch	<b><i>Macrophomina, Fusarium, Rhizoctonia, Sclerotium, Pythium</i></b>
22.	M/s Romvijay Biotech Pvt. Ltd., Pondichery	<i>Hirsutella thompsoni, Verticillium lecanii</i>	Biomite	All species of mites
		<i>Beauveria, Bacillus subtilis</i>	ROM Grub kill	Beetles, borers
		<i>Metarhizium anisopliae</i>	ROM Meta kill	Beetles, borers
		<i>Beauveria bassiana</i>	ROM Beevicide	Beetles, borers
		<i>Paecilomyces lilacinus</i>	ROM Pelicide	Plan parasitic nematodes
		<i>Verticillium lecanii</i>	VERELAC	Sucking pests
		<i>Trichoderma viride</i>	ROM Trichoderma	Wilt, rot, plant parasitic nematode
		<i>Paecilomyces lilacinus</i>	ROM TRY PAE MIX	Wilt, plant parasitic nematode
		<i>Ampelomyces quisqualis</i>	ROM No-Mildew	Mildew diseases
		<i>Pseudomonas fluorescens</i>	ROM Pseudomonas	Foliar and soil borne diseases
23.	M/s Devi Biotech (P) Ltd., Madurai, Tamil Nadu	<i>Trichoderma viride</i>	Boom Derma 1.5% WP	Damping off, wilt, collar rot, root rot, leaf blights spots
		<i>Paecilomyces lilacinus</i>	Boom Nemo 1%	Root knot nematodes,

			WP	cyst nematodes, reniform nematode, burrowing nematode, citrus nematode, golden cyst nematode and lesion nematodes
		<i>Pseudomonas fluorescens</i>	Boom Monas 1% WP	Root rot, wilt, blast, sheath blight, damping off, leaf spot and rhizome rot
		<i>Verticillium lecanii</i>	Boom Vert 1.5% WP	Aphids, thrips, mealy bugs, white flies, jassids, Hoppers, scales and all types of mites.
		<i>Beauveria bassiana</i>	Boom Bass 1.15% WP	Root grubs, boll worms, <i>Spodoptera</i> , coffee berry borers, pod borers, hoppers and weevils.
24.	M/s T. Stanes and Company Ltd., Coimbatore, Tamil Nadu	<i>Verticillium lecanii</i>	BIO CATCH 1.15% WP	whiteflies, jassids, aphids, thrips, mealybugs
		<i>Beauveria bassiana</i>	Bio-Power 1.15% WP	Borers, cutworms, root grubs, leafhoppers. whitefly, aphids, thrips, mealybug
		<i>Metarhizium anisopliae</i>	Bio Magic 1.15% WP	Leaf hoppers, grasshoppers, root grubs, corn root worms, bugs, beetles, palm weevils, borers, cutworms, termites
		<i>Paecilomyces lilacinus</i>	Bio Nematon 1.15% WP	Root knot nematodes, burrowing nematodes, cyst nematodes, lesion nematodes
		<i>Trichoderma harzianum</i>	Bio Wrap 1% WP	Root-knot nematode, wilt disease of tomato, okra crops
		Entomopathogenic nematode	Crown	Root grub
		<i>Streptomyces</i> spp.	Stanomyte 1.5% LF	Mites
25.	M/s Harit Bio Control Lab., Yavatmal	NPV	Helistop	<i>Helicoverpa armigera</i>
		<i>Trichoderma viride</i>	Haritderma 1% WP	Damping, wilting, root spots, leaf spots and blights
		<i>Verticillium lecanii</i>	Versatile 1%	Sucking pests

			WP	
		<i>Beauveria bassiana</i>	Wow 1.5% WP	Leaffolders
26.	M/s Bannari Amman Sugars Ltd., Tamil Nadu	<i>Bacillus licheniformis</i>	LEAF GUARD	<i>Actinopelte</i> Leafspot, Alternaria, Leafspot, leaf blight, Anthracnose, leaf blotch, Drechslera ink spot, Bipolaris Leaf spot, <i>Rhizoctonia</i> blight
		<i>Trichoderma viride</i> <i>T. harzianum</i>	ROOT CARE	Soil borne diseases
		<i>Pseudomonas fluorescens</i>	Pseudo Care	Crop diseases
		<i>Bacillus subtilis</i>	LEAF CARE	Fungal diseases

**Table 2. List of biopesticides in pipe-line for registration and licensing in India**

S. No	Entomopathogenic fungi	Formulation	Shelf life	Trade name	Target pests	Dose
1.	<i>Beauveria bassiana</i> (Bals.-Criv.) Vuill. (1912) (Bb-5a)	Oil formulation (1 × 10 <sup>8</sup> cfu/ml)	12 months at 25-35°C	Shatpada Aphid Kill	Chilli and brinjal aphids, <i>Aphis gossypii</i> Glover, 1877, cabbage aphid, <i>Brevicoryne brassicae</i> (Linnaeus, 1758); cowpea aphid, <i>A. craccivora</i> C. L. Koch, 1854	5 ml/lit. of water at 15 days interval
2.	<i>Isaria fumosorosea</i> Wize (1904)(Pfu5)	Talc (1 × 10 <sup>8</sup> cfu/g); oil formulation (1 × 10 <sup>8</sup> cfu/ml)	12 months at 25-35°C	Shatpada Rugose Whitefly Kill	Coconut and oil palm Rugose spiraling whitefly, <i>Aleurodicus rugioperculatus</i> Martin	2-3 foliar spray at 5 ml or 5 g/lit. of water at 15 days interval
3.	<i>Lecanicillium lecanii</i> R. Zare & W. Gams, 2001 (VI-8)	Oil formulation (1 × 10 <sup>8</sup> cfu/ml)	12 months at 25-35°C	Shatpada Sucking pest Hit	Chilli aphids, <i>A. gossypii</i> , cowpea aphid, <i>A. craccivora</i>	Three foliar sprays at 5 ml/lit. of water at 15 days interval
4.	<i>Metarhizium anisopliae</i> (Metchnikoff) Sorokin (1883) Ma 4	Talc (1 × 10 <sup>8</sup> cfu/ml)	12 months at 25-35°C	Shatpada Grubicide	Sugarcane white grub, <i>Holotrichia</i> spp.	Soil application twice in a year during June/July, July/August at 30 days interval at 2.5 kg mixed with 250 kg FYM/ha
5.	<i>M. anisopliae</i> Ma 35	Talc (1 × 10 <sup>8</sup> cfu/g); oil formulation (1 × 10 <sup>8</sup> cfu/ml)	12 months at 25-35°C	Shatpada Larvicide	Maize fall armyworm, <i>Spodoptera frugiperda</i> (J. E. Smith)	Three foliar sprays at 5 ml or 5 g/lit. of water at 20,30,40 days after sowing
6.	<i>Trichoderma reesei</i> Simmons, 1977 CST-T-3	Wettable powder (1 × 10 <sup>8</sup>	12 months at 25-35°C	ICAR-Fusicont	Fusarium wilt of banana, <i>Fusarium oxysporum</i> f. sp. <i>cubense</i> E. F. Sm., W. C. Snyder & H. N.	Soil drenching at 1 lit/, four times on 3 <sup>rd</sup> , 5 <sup>th</sup> , 9 <sup>th</sup> , 12 <sup>th</sup> month after

		cfu/g)			Hansen (1940) Tropical race 4 and race 1	planting
7.	<i>T. harzianum</i> Rifai, (1969) + <i>Bacillus amyloliquefaciens</i> Priest <i>et al.</i> , 1987	Talc ( $1 \times 10^7$ cfu/g)	12 months at 25-35°C	Bio-Pulse	Wilt of chickpea, lentil, pea, pigeonpea; damping off/seedling mortality in papaya; Target fungi ( <i>Rhizoctonia</i> , <i>Sclerotium</i> , <i>Sclerotinia</i> , <i>Fusarium</i> , <i>Pythium</i> , <i>Ralstonia</i> , <i>Macrophomina</i> , <i>Bipolaris</i> , <i>Phoma</i> )	Seed treatment (10 g/kg seed)
8.	<i>T. harzianum</i> AZNF-5	Carrier based ( $1 \times 10^8$ cfu/g)	4 months at 55°C	Maru Sena 1	<i>Fusarium oxysporum</i> f. sp. <i>cumini</i> in cumin	Seed treatment (4 g/kg seed); soil application (1 kg/ha with 50 kg FYM) before sowing
9.	<i>T. harzianum</i> (ICAR-CAZRI AZNF-5 and <i>Bacillus firmus</i> Bredemann and Werner 1933, ICAR-CAZRI AZ-1	Carrier based ( $1 \times 10^8$ cfu/g each)	4 months at 55°C	Mishrit Maru sena	<i>Macrophomina phaseolina</i> (Tassi) Goid. (1947) in legumes and oils seed crops	Seed treatment (10 g/kg seed with jiggery) and soil application (1 kg/ha with 40 kg FYM)
10.	<i>B. bassiana</i> RF6	Talc ( $1 \times 10^9$ cfu/g)	8 months at 25-35°C	NRRI-BBLF	Rice leaf folder, <i>Cnaphalocrocis medinalis</i> (Guenée, 1854)	Foliar spray at 2 g/lit. of water
11.	<i>M. anisopliae</i> TF 19	Talc ( $1 \times 10^9$ cfu/g)	8 months at 25-35°C	NRRI-Malf	Rice leaf folder, <i>C. medinalis</i>	Foliar spray at 2 g/lit. of water
12.	<i>T. harzianum</i> *Th4d	Liquid suspension concentrate ( $1 \times 10^9$ cfu/ml)	18 months at 25-35°C	Triguard Th-L	Phytophthora seedling blight, <i>Macrophomina</i> root rot and <i>Fusarium</i> wilt of safflower and gray mold of castor, <i>Alternaria</i> aster leaf blight and powdery mildew of sunflower	Seed treatment at 1 ml/kg seeds, foliar spray at 1-2 ml/lit. of water
13.	<i>T. harzianum</i> *Th4	Wettable powder	18 months at 25-35°C	Triguard Th-P	Phytophthora seedling blight, <i>Macrophomina</i> root rot; <i>Fusarium</i>	Seed treatment at 10 g/kg of seeds

		(1 × 10 <sup>9</sup> cfu/ml)			wilt of safflower and castor, Aspergillus root rot in groundnut	
14.	<i>T. asperellum</i> *Samuels, Lieckf. & Nirenberg 1999 Ta DOR 7316	Wettable powder (1 × 10 <sup>9</sup> cfu/ml)	18 months at 25-35°C	Triguard Ta-P	Phytophthora seedling blight, Macrophomina root rot; Fusarium wilt of safflower and castor	Seed treatment at 10 g/kg seeds
15.	<i>B. bassiana</i> (ITCC 4513)	Liquid suspension concentrate (1 × 10 <sup>12</sup> cfu/ml)	24 months at 25-30°C	Mycoguard Bb-L	<i>Helicoverpa armigera</i> (Hubner) in pigeonpea	Two to three foliar sprays at 0.3 ml/lit of water at 10 days interval
16.	<i>T. harzianum</i> *ICAR-IIHR Th-2	Wettable powder (2 × 10 <sup>6</sup> cfu/g)	10 months at 25-35°C	Arka Krishi Vriddhi	<i>Meloidogyne incognita</i> (Kofoid & White, 1919), <i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i> , <i>S. rolfsii</i> , <i>F. solani</i> (Mart.) Sacc. (1881) in brinjal, tomato, carrot, okra	Seed treatment at 20 g/kg seed, nursery bed treatment at 50 g/m <sup>2</sup> for transplantable crops, soil application at 5 kg/ha after enrichment in 5 tons FYM before sowing or transplanting
17.	<i>T. viride</i> *ICAR-IIHR Tv-5	Wettable powder (2 × 10 <sup>6</sup> cfu/g)	10 months at 25-35°C	Arka Krishi Veera	<i>M. incognita</i> , <i>Ralstonia solanacearum</i> (Smith 1896), <i>Erwinia carotovora</i> Winslow <i>et al.</i> , 1920, <i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i> , <i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i> , <i>F. solani</i>	Seed treatment at 20 g/kg seed, nursery bed treatment at 50 g/m <sup>2</sup> for transplantable crops, soil application at 5 kg/ha after enrichment in 5 tons FYM before sowing or transplanting
18.	<i>Pochonia chlamydosporia</i> *Zare and Gams IIHR-Vc-3	Carrier based (2 × 10 <sup>6</sup> cfu/g)	10 months at 25-35°C	Arka Krishi Rakshak	<i>M. incognita</i> in brinjal, tomato, carrot, okra	Seed treatment at 20 g/kg seed, nursery bed treatment at 50 g/m <sup>2</sup> for transplantable crops, soil application at 5 kg/ha after enrichment

						in 5 tons FYM before sowing or transplanting
19.	<i>T. asperelloides asperelloides</i> 5R	Liquid formulation (5 × 10 <sup>11</sup> cfu/ml)	3 months at 25-35°C	Manjari Vineguard	Grapes powdery mildew	Soil drenching at 2 ml/lit. of water
20.	<i>T. afroharzianum</i>	Liquid (5 × 10 <sup>8</sup> cfu/ml)	3 months at 25-35°C	Manjari Rakshak	Grapes powdery mildew	Foliar spray at 2 ml/lit. of water
21.	<i>T. harzianum</i> IARI P4	Wettable powder (10 <sup>8</sup> cfu/g)	25 months at 25°C	Pusa 5 SD	<i>F. oxysporum</i> f. sp. <i>ciceris</i> , <i>S. rolfsii</i> , <i>S. sclerotiorum</i> (Lib.) de Bary (1884) in chickpea; <i>R. solani</i> J.G. Kühn 1858, <i>R. bataticola</i> (Taub.)Butl. in chickpea and mugbean, <i>F. oxysporum</i> f. sp. <i>lycopersici</i> in tomato, <i>P. ultimum</i> , <i>R. solani</i> in fresh bean, major seed borne fungal pathogens	Seed treatment at 4 g/kg of seeds
22.	<i>Purpureocillium lilacinum</i>	Wettable powder (2 × 10 <sup>6</sup> cfu/ml)	10 months at 25-35°C	ARKA Krishi Kawach	<i>Meloidogyne incognita</i> in brinjal, tomato, carrot, okra	Seed treatment at 20 g/kg seed, nursery bed treatment at 50 g/m <sup>2</sup> for transplantable crops, soil application at 5 kg/ha after enrichment in 5 tons FYM before transplanting or sowing
23.	<i>Bacillus thuringiensis</i> var. <i>kurstaki</i>	Liquid (1 × 10 <sup>8</sup> cfu/ml)	12 months at 25-35°C	Shatpada Armour	Maize fall armyworm	Two to three foliar sprays at 10 ml/lit of water at 25, 35, 45 days after sowing
24.	<i>B. thuringiensis</i> var. <i>kurstaki</i>	Liquid (1 × 10 <sup>8</sup> cfu/ml)	12 months at 25-35°C	Shatpada Terminator	<i>H. armigera</i> , <i>Plutella xylostella</i> , <i>Chilo partellus</i> , <i>Cnaphalocrocis medinalis</i> , <i>Leucinodes orbonalis</i> , <i>Amsacta albistriga</i>	Two to three foliar sprays at 20 ml/lit of water at pre-flowering and post flowering

						stages
25.	<i>Pseudomonas fluorescens</i>	Talc based (1 × 10 <sup>8</sup> cfu/ml)	12 months at 25-35°C	Shatpada All Rounder	<i>Thrips</i> spp., in capsicum and Fusarium wilt of red gram	Foliar application at 20 g/lit of water at 20,30,40,50 days after transplanting for the management of thrips in capsicum; soil application in the root zone during 25,40,55 days after sowing at 2.5 kg/ha for management of red gram wilt; mix 2.5 kg of formulation in 250 kg farmyard manure and apply
26.	<i>B. albus</i>	Talc based (1 × 10 <sup>8</sup> cfu/ml)	12 months at 25-35°C	Shatpada Master Blaster	<i>S. frugiperda</i> , <i>Tuta absoluta</i> , <i>Fusarium oxysporum</i> f. sp. <i>cucumerinum</i>	Foliar application at 20 g/lit of water at 20,30,40,50 days after sowing for fall armyworm and tomato pin worm; soil application in the root zone during 25,40,55 days after sowing at 2.5 kg/ha for management of cucumber wilt; mix 2.5 kg of formulation in 250 kg farmyard manure and apply
27.	<i>P. fluorescens</i>	Talc based (1 × 10 <sup>8</sup> cfu/ml)	12 months at 25-35°C	Eco-Pesticide	Spot blotch of wheat, sheath blight of rice and wilt of tomato and chickpea	Seed treatment (10 g/kg seed)
28.	<i>B. firmus</i>	Carrier based	6 months	Maru sena	<i>Macrophomina phaseolina</i> in	Seed treatment (30 g/kg



		(1 × 10 <sup>8</sup> cfu/ml)	at 25-35°C	3	legumes and oil seed	seed with jiggery solution) before sowing
29.	<i>B. thuringiensis</i> var. kurstaki	Liquid suspension concentrate (1 × 10 <sup>11</sup> cfu/ml)	24 months at 25-35°C	Bioguard Bt-L	<i>Spodoptera litura</i> in soybean	Two foliar sprays at 3 ml/lit of water at 10 days interval.
30.	<i>P. fluorescens</i>	Wettable powder (2 × 10 <sup>8</sup> cfu/ml)	10 months at 25-35°C	ARKA Krishi Samarakshak	<i>Meloidogyne incognita</i> , <i>Ralstonia solanacearum</i> , <i>Erwinia carotovora</i> , <i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i> , <i>Fusarium solani</i> in brinjal, tomato, carrot, okra	Seed treatment at 20 g/kg seed, nursery bed treatment at 50 g/m <sup>2</sup> for transplantable crops, soil application at 5 kg/ha after enrichment in 5 tonns FYM before transplanting or sowing
31.	<i>P. fluorescens</i>	Liquid (2 × 10 <sup>8</sup> cfu/ml), carrier based (2 × 10 <sup>8</sup> cfu/ml)	10-12 months at 25-35°C	ARKA krishi All Rounder and ARKA Plant Growth Booster	<i>Meloidogyne incognita</i> , <i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i> , <i>Fusarium solani</i> in several vegetable and fruit crops	Seed treatment at 20 g or 20 ml/kg seed, substrate treatment with 10 ml or 10 g/kg of cocopeat, soil application at 5 kg or 5 li/ha after enrichment in 5 tons FYM before transplanting or sowing

\*Licensed to private companies

## 5 Conclusions

Government regulations and the detrimental effects of chemical pesticides force a shift to alternate plant protection measures. As a result, microbial biopesticide, one of the environmentally friendly techniques, has become more significant in agriculture both globally and in India. Although a few factors, such as quality control and the identification of effective organisms, predispose the market and widespread use of biopesticide, central and state government initiatives, such as the establishment of assisted and non-aided biocontrol laboratories and intense R&D activities, support the growth of biopesticide steadily.

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## Chapter 3

### Novel Formulations of Bio-pesticides

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#### Abstract

The pests and pathogens attack are frequently responsible for reducing agricultural production yields and causing sustainable losses, which poses a grave threat to food security. Further, the danger of mosquito vectors accountable for the life-threatening disease has led to an enormous global burden and challenged the health sector worldwide. Due to the unavailability of drugs and vaccines, mosquito controls remain an alternative solution. The management of agricultural pests and mosquito vectors currently relies upon synthetic pesticides. However, the excessive use of synthetic pesticides does not necessarily guarantee improved crop yield and desired pest control, although it is causative for several other issues like environmental contamination, soil and water pollution, substantial loss in soil health, and non-target toxicity. Therefore, establishing an approach incorporating safer alternatives to synthetic ones can benefit sustainable agricultural concepts and public health, which can reverse the harmful effects of conventional synthetic pesticide practices. The beneficial Pesticides necessitate effective, safe, and low-cost techniques to combat the effects of various agents (Agriculture and stored grain pests, mosquito vectors, etc.) with minimal impact on nature and humankind. The products derived from natural sources like plant phyto-chemicals and microorganism (bacteria and fungi) is considered safe and effective as bio-pesticides. Plants are a source of multiple phyto-chemicals and microorganisms that have been demonstrated to be effective against pests and vectors; thus, utilizing natural bio-pesticides is one method of indigenous pest management. Despite the enormous benefits of bio-pesticides, their potential needs to be highlighted, as in their unformulated state, they possess numerous disadvantages. The formulation technologies have opened new avenues for overcoming these disadvantages and providing effective solutions for bio-pesticide usage. The combination of bio-pesticides and formulation technology can effectively solve agricultural production losses caused by pests and disease threats caused by vectors. This chapter describes the potential botanical pesticide formulations to comprehend the fundamentals of their development.

**Keywords:** Biopesticides, Novel formulations, Field applications

#### 1. Introduction

Plants have been utilized as traditional folk treatments, current medications, insect repellents, etc. The existence of diverse phytochemicals in plants has offered a natural method for combating pests responsible for crop loss and disease-causing vectors. Herbal products generated from plants are currently trending as bio-pesticide in all industries, including pharmaceuticals, cosmetics, and agrochemical industries. Similarly, microorganisms-based bio-pesticides, including fungi, bacteria, and viruses, are emerging as effective tools in pest management in

agriculture and public health sectors. Although, the popularity of botanical and microbial pesticides is increasing due to good bio-efficacy, target specificity, and safety to non-target organisms and environment. However, most bio-pesticides suffer shelf life and infectivity issues during application. The formulation is essential for effectively implementing plant and microbial bio-pesticides against various vectors and pests.

Unlike synthetic pesticides, natural pesticides offer various advantages, including biodegradability, non-persistence, and eco-friendliness (Govindarajan et al. 2011, Philip C. Stevenson et al. 2017). The bio-pesticides of plant origin, often known as "botanical pesticides" can be generated from various plant components; similarly, microbial pesticides are effective and provide a long-term solution against the damaging pest (Pant et al. 2014). The bio-pesticides are native, abundantly accessible, inexpensive, and have a broad spectrum of activity, including insecticide, antifeedants, repellents, larvicidal, and ovicidal properties (Murgan et al. 2012). Through crude extracts, oils, de-oiled cakes, bacteria, and entomopathogenic fungi, bio-pesticides' potential against a wide variety of pests has been reported. This chapter discusses bio-pesticides potential and their formulations in agricultural and public health pest control.

## **2. Review of Plant and Microbial-based Bio-pesticides**

Crop losses caused by insects are a big challenge to farmers' incomes and global food security (Avelino et al. 2015). Due to biotic or abiotic influences, crop loss can occur in the field (pre-harvest) or during storage (post-harvest) (Cerda et al. 2015). Neem (*Azadirachta indica*) is registered under the Insecticide Act of 1968 as a botanical (Bhandari et al. 2016), extensive research showed that neem-based pesticides help safeguard crops and control mosquito vectors (Chermenskaya et al. 2010; Dua et al. 2009). Azadirachtin (tetranortriterpenoids), present in the seeds and leaves of neem, is responsible for the plant's insecticidal properties (Ahamed et al. 2012). Lopez et al. (2010) evaluated the insecticidal activity of the seed and leaf extract of castor (*Ricinin communis*) against *Spodoptera frugiperda*; they concluded that ricinine is the primary component responsible for the insecticidal activity of seed extracts against the pest. *Alpinia galangal* (Linnaeus), a medicinal plant belonging to the Zingiberaceae family, was discovered to be efficient against *Spodoptera litura*, a polyphagous insect that causes economic losses to a wide variety of agriculturally significant crops (Datta et al. 2019). The diamondback moth (*Plutella xylostella*) (Lepidoptera: Plutellidae) and the aphid (*Brevicoryne brassicae*) (Hemiptera: Aphididae) are the most damaging pests of cultivated brassicas, accounting for over 90 percent of crop loss. *Maerua edulis* and *Bobgunnia madagascariensis* crude aqueous extracts were found to be beneficial in reducing cabbage pests (Mazhawidza et al. 2017). The essential oil of Hemp (*Cannabis sativa*) was discovered to be efficient against phytophagous insects (M. persicae) aphids, which are significant agricultural pests responsible for crop loss (Benellia et al. 2018).

In underdeveloped nations, it is estimated that 43% of global yearly losses in stored products are caused by stored grain pests (Pant et al. 2014). In recent years, research has concentrated on using plants and their phytochemicals as prospective synthetic pesticide alternatives. (Kys et al. 2017) discovered that powdered *Olax zeylanica* leaves had repellent properties against *Sitophilus oryzae*, the rice weevil. *Tribolium castaneum* (red flour beetle), a pest of stored grains, was significantly inhibited by Karanja (*Pongamia glabra*) and Jatropa

(*Jatropha curcas*) seedcakes formulated with an aqueous extract (Pant et al. 2014). Karanjin and Phorbol esters are the principal insecticidal components of Karanja and Jatropha, respectively (Pant et al. 2014). Saponins are a steroidal or triterpenoidal substances found in plants. They are distinguished by their high molecular weight and non-polar aglycone in addition to polar sugar molecules. Saponins have attracted interest as insecticidal chemicals due to their toxicity against a variety of significant crop-stored grain pests (Singh et al. 2018). According to reports, castor oil has insecticidal efficacy against *Zabrotes subfasciatus* (Mushobozy et al. 2009).

Bio-pesticides as a nematicide, fungicide, and weedicide have been proven; castor oil derived from the seed of the castor plant (*Ricinus communis*) is widely cultivated in India, East Africa, and other tropical and warm-temperate regions of the world. It has insecticidal properties against a wide range of pests, including mosquitoes (Ali et al. 2018, Sogan et al. 2018a,b). Root-knot nematode is one of the most devastating pests that cause significant production loss in rice crops (Kavitha et al. 2016). Castor plant has been reported to exhibit nematicidal activity against root-knot nematode (Prasad et al. 2005; Mohamed et al. 2013). De-oiled Karanja (*Pongamia glabra*) seed cake also contains insecticidal and nematicidal properties (Sharma et al. 2011). Hydro-alcoholic crude extracts from several parts (leaves, stems, rhizomes) of the hop plant (*Humulus lupulus*), as well as hops essential oil, were found to be active against the *Z. tritici* fungus. (Laetitia et al. 2018). Extracts were found to include polyphenols (Apigenin, Gallic acid, Catechin, Quercetin, and Tannic acid), which are well-known antifungal substances (Sittisart et al. 2017). The effectiveness of a *Magnolia officinalis* bark extract against pathogens, such as *Plasmopara viticola* (which causes grapevine downy mildew) and *Venturia inaequalis* (which causes apple scab), was described (Thueriga et al. 2018).

In the present scenario, bioactive compounds of biological origin from bacteria, fungi, plants, and entomopathogenic microbes have the potential to be used as alternatives to chemical insecticides. The Fungal based products exhibited high toxicity against mosquitoes, with relatively less side toxicity towards non-target organisms, and are biodegradable; these characteristic features make them ideal eco-friendly tools which can be exploited for mosquito vector control. The *Verticillium lecanii* is a well-known and unique EPF with a wide range of pests, including arthropods (scale insects and aphids), plant parasitic nematodes (root-knot nematode, *Meloidogyne incognita*; cysts and eggs of *Heterodera glycines*) and plant pathogens (biotrophic powdery mildew and rust fungi) (Ghaffari et al. 2017).

### **3. Application of Bio-pesticides: Need for Formulations**

The effectiveness of bio-pesticides for pest management measures, as described in numerous pieces of research, is the use of bio-botanicals in their crude or unprocessed form; therefore, the projected favorable outcomes are not observed under realistic settings during field experiments. Degradation and volatilization of bioactive chemicals are the primary causes of decreased efficacy of bio-pesticides in the field (Borges et al. 2018). In addition, a higher concentration is required to attain the desired effect. Due to the disadvantages mentioned above, bio-pesticides' potential for application in pest management has yet to be widely recognized. To eliminate these limitations and achieve maximum performance, bio-pesticides formulations are sought.

## **4. Bio-pesticides Formulation Technology**

The "formulation" is the processing of Pesticide with inert substances, usually referred to as adjuvants, to induce specific favorable attributes to the technical pesticides. Processing a bio-pesticide into a formulation includes grinding, mixing with inert materials such as talc, silica, diatomaceous earth, etc., and adding adjuvants such as wetting agents, spreaders, emulsifiers, and stabilizers to impart particular and unique characteristics (Bhandari et al. 2016). Further, the formulation technology entails using various adjuvants (Polymers, emulsifying agents, surfactants, solvents, stabilizers, defoamers, etc.) to deliver safe and effective products that are easy to use and enhance the efficacy of raw bio-pesticides.

The formulation of Pesticides increases the self-life, safety, application, and efficacy of biopesticides and also improves their handling. Depending on the type of formulation, there could be adhesion to the target, and regulated release of the bioactive chemicals can be achieved with the formulation technology (Borges et al. 2018). Thus, the formulation technology gives advancements by enhancing the quality of existing bio-pesticide by creating a new product.

## **5. Classes of Bio-pesticides Formulations**

### **5.1. Conventional Formulations**

Most agrochemical formulation techniques in the recent past relied on straightforward solvent-based solutions or powder mixes. The inclusion of petroleum-based solvents and dust in these common formulations has a negative impact on both the environment and people (Hazara et al. 2015). These compounds may be phytotoxic to plants, are difficult to mix in spray tanks, and have poor compatibility. Emulsifiable concentrates and wettable powders are examples of the conventional category.

#### **5.1.1. Wettable Powder (WP)**

WP is the original formulation under the conventional classification. It is a dry bio-pesticide formulation where the biopesticide has been mixed with fine solid carriers as filler, wetting and dispersing agents (surfactants), and an insecticide. For WP, silica and china clay are the most frequently utilized fillers. In water, the WP disperses into a stable and uniform suspension. While formulating the WP of bio-pesticides, the china clay is gradually mixed with surfactants and the solid portion of the bio-pesticide. To formulate liquid bio-pesticide as WP it is first absorbed on the silica carrier before further ingredients are added. The mixture is homogenized using a mixer once the complete amount of the active ingredient has been added. The steps in preparation are as follows: Step I: Blending the ingredients (using a simple household mixer on a laboratory scale and a Ribbon Blender for large-scale production). Step II: Grinding (using Air jet milling and Air classifier miller) the average particle size required is in the range of four to five microns. Final step is packaging. [*Bacillus thuringiensis* WP (Arun Sri et al. 2003), *EPF Verticellium lecani* (Shina et al. 2006)].

### **5.1.2. Emulsifiable concentration (EC)**

EC is created by dissolving the Pesticide in a solvent with emulsifiers and surfactants. The solvents employed in EC preparations are distillates of petroleum. C-9, Aromax, and Solvoso are the most typical solvents utilized. Due to environmental concerns with EC formulations, a new trend is emerging that involves using biodiesel in EC formulations (Chin et al. 2012). EC derived from vegetable oil, as opposed to petroleum distillates, is currently on trend. As an alternate carrier solvent, ethylene glycol di-acetate has been reported in EC (Zhang et al. 2018). When diluted in water, EC formulations produce a stable "milky" emulsion. In an ideal EC formulation, the emulsion should be initially stable when diluted with water, and after 24 hours, neither creaming nor oil separation should be detected. The choice of surfactant is crucial to the stability of an emulsion. As an antifungal EC, phenolic chemicals (phlorizin, resveratrol, and alkylresorcinols) regarded natural alternatives were reported (Patzeek et al. 2018). Neem oil EC has been reported (Waghmare et al. 2007). The available biopesticide EC are Neem oil EC, clove bud essential oil (CEO) Emulsifiable Concentrate (EC) formulations (Milićević et al. 2022), *B. Bassiana* EC (Lorena et al. 2022), Avermectin EC (Zhou et al. 2019).

## **6. New Generation Bio-pesticides Formulation**

The new generation formulations are an enhancement over conventional/traditional formulations technologies and are devoid of dust and solvent. These advancements in formulation technology include novel formulation types that can give products a competitive edge, a longer shelf life, enhanced efficacy, and consumer safety (Hazara 2015; Bhandari et al. 2016). Different categories of new-generation bio-pesticide formulations are discussed as follows:

### **6.1. O/W Emulsions (EW)**

Oil in water emulsion, abbreviated as EW is the first category of next-generation formulations. In EW formulation, a solid active is dissolved in a water-immiscible solvent and distributed into a continuous water phase using high shear mixing. The two phases are stabilized by the selection of appropriate emulsifiers (Hazara 2015). Typically, the average particle size of EW is between 5 and 6 microns. EW formulations are highly susceptible to creaming, sedimentation via coagulation, flocculation, and Ostwald ripening. The factors that can influence the stability of EW are susceptible to creaming, sedimentation, coagulation, flocculation, and Ostwald ripening. Abamectin is a macrocyclic lactone that has been reported as EW (Zhang 2014). Neem oil EW has been reported as bio-insecticide formulation (Massaguni et al. 2016). Tobacco extract (*Nicotiana tabacum*) was made as a concentrated emulsion (EW), including palm oil and emulsifiers (Tween and Span), resulting in a more physically stable product (Puripattanavong et al. 2013).

### **6.2. Microemulsions (ME) and Nanoemulsions**

MEs are homogenous and isotropic dispersions with low viscosity, optical transparency, thermodynamic stability, and an interior (dispersed) phase with typical dimensions ranging from 10 to 100 nm. Aniseed essential oil microemulsion is reported as an insecticide (Pavela et al. 2019). The microemulsion formulation of norcantharidin for the control of the diamondback



moth, *Plutella xylostella*, a notorious pest of brassica crops worldwide has been reported (Saho et al. 2018). D-limonene-loaded nanoemulsions was investigated against fungal pathogens (Feng et al. 2020). Dodecanoic acid, fatty acid derived from papaya leavesoil-in-water microemulsion, has been reported against Papaya Ring Spot Virus transmitted by aphids (Ramaya et al. 2022). Microemulsions of neem oil have been reported (Singla et al. 2013). Due to the very fine size of ME, the absorption is very high, however, the use of a greater amount of surfactant and less insecticide loading is an issue (Singla et al. 2013).

Nanoemulsions are oil-in-water dispersions with droplet sizes between 1-200 nm (Solans et al. 2003). They are kinetically stable when combined with a natural oil, water, and surfactant (Bouchernal et al. 2004). The nanoemulsion of eucalyptus oil was found to be efficient against *Tribolium castaneum*, the red flour beetle (Pant et al. 2014). As an insecticide, castor oil nanoemulsion was described (Sogan et al., 2018). The nanoemulsion of bio-waste cashew nut shell liquid has been reported as larvicide against mosquito vectors (Kala et al. 2019). Enhanced absorption due to a reduction in droplet size Improved water solubility of poorly water-soluble substances are the advantages of this delivery system (Fernandes et al. 2014), however the use of a greater amount of surfactant is an issue (Sharma et al. 2018). Insecticidal nanoemulsion containing apolar fraction from fruits of Manilkara subsericea was effective against *D. peruvianus* (Fernandes et al. 2014). Essential oil Mint (*Mentha spicata* L), Basil (*Ocimum basilicum* L), and flowers of carnation (*Trifolium incarnatum*) as a nanoemulsion were effective against root-knot nematode (Hammad et al. 2022). The essential oil of *Myrtus communis* has been identified as an effective larvicide and categorized as an active larvicide. Compared to bulk essential oil, the nanoformulation of *M. communis* exhibited a considerable improvement in its residual efficacy (Frizoyan et al. 2022).

### **6.3. Controlled Release Formulations (CRF)**

The controlled release technique meant for pesticide discharge can be modified according to need. The foundation of CRF technology is the encapsulation of pesticides within any carrier material. Positive aspects of controlled release formulation

1. Extended duration of effectiveness of non-persistent insecticides (Dubey et al. 2011)
2. Reduced Pesticide is required for the same amount of time compared to standard formulations, resulting in less waste and fewer applications. Reduced environmental contamination, notably surface water and groundwater contamination. Reduced losses owing to environmental conditions (evaporation, photolysis, leaching with water, and chemical and microbiological degradation), resulted in active ingredient cost savings (Mulqueen et al. 2003).
3. Decreased toxicity to non-target species of plants, animals, birds, and fish; Enhanced pesticide efficiency due to improved targeting. Greater safety for pesticide formulation users and those who come in contact with them.

### **6.4. Microencapsulation/Nanoencapsulation**

Encapsulation involves coating microscopic solid particles, liquid droplets, or a thin coating with a layer of these substances. The capsules smaller than 1  $\mu$ m are categorized as Nanocapsules, and

those larger than 1000 m are classified as Microcapsules (Hesis, 2006; Maji, 2007; Mortti, 2005; Hussain, 2008). To protect seed quality, a formulation based on microencapsulated Boldo (*Peumus boldus*) essential oil (EO) was assessed on stored grain pests (*Arachis hipogaea*) (Girardi et al. 2018). (Peng et al. 2014) described encapsulated mustard essential oils and microencapsulation of Neem and Karanja oil in alginate beads has been described (Pant et al. 2012) against mosquito larvae. The nano-encapsulated cedar wood oil and lemon grass oil against immature and adult mosquitoes, respectively, with lost lasting efficacy, has been described (Kala et al. 2019b; Kala et al. 2020).

## 6.5. Tablets

Tablets are "preformed solids of regular shape and proportions, typically round, with flat or convex faces, the distance between faces being smaller than the diameter" (FAO specification). Tablet is an optimal combination of Active ingredients (AI) and adjuvants crushed into a solid mass of consistent shape and size. The benefits of tablet formulations: Because they are compressed, they are easy to handle and dust-free. The tablet formulation provides premeasured dose rates. Tablet formulation eliminates the necessity for weighing and other measurements for dose preparation, as doses can be determined simply by counting the number of tablets (Sharma et al. 2005). Tablet formulation is ideally suited for situations with minimal dose requirements. Varieties of tablets included; Tablet dispersible in water/ Effervescent Extremely rapid dispersion in water yields a homogeneous and stable suspension. The tablet is dissolved in water to create a suspension.

During the previous decades, numerous pesticide tablets for mosquito control have been studied. The usage of synthetic insecticides to control mosquito vectors has resulted in resistance and negative environmental impact (Dua et al. 2009). However, they are harmless to humans and the environment; pesticides originating from natural sources, such as the microbial insecticides *Bacillus sparacus* and *Bacillus thuringiensis*, are widely utilised in public health pest management (Zhang et al. 2016). *Bacillus thuringiensis* (*Bt*) is a bacterium used commercially to control agriculture- and public-health-threatening insects (Ibharim et al. 2010). Slow release tablets of *Bacillus thuringiensis* are described by (Mulla et al. 2004). Unlike WT or ET tablets, slow-release tablets do not disintegrate quickly in water but continue to release active slowly in water and can be effective for a more extended period with a single application. These were effective against *Aedes aegypti* larvae for around 90 days. Spinosad-containing extended-release dispersible tablets based on CRF technology were described (Heltrin et al. 2010). Controlled discharge Floating Tablets (Neem oil) (number of patent application: 880/DEL/2012) has been described. The controlled release of tablets for application directly in an aquatic environment, after application to water bodies, these float on the water's surface and release their active ingredient at a slow rate. The advantages of floating tablet are that they are directly applied to water bodies, regulate the release of active from the tablet matrix, and reduce treatment frequency and dosage. Tablets made from waste biomass for store grain pest control (Application number: 2705/DEL/2012) have been described.

## 7. Conclusions

Bio-pesticides have the potential to lessen the environmental impact of synthetic pesticides and provide sustainable pest control. With the multiple formulation technologies available to target pests, the demand for new, safer, and more effective formulation technologies is leading in the research and development of bio-pesticides formulation. Formulation technology focuses primarily on cost reduction and environmental effects. Bio-pesticide formulation techniques can effectively resist the problematic effects of synthetic pesticides and give a cost-efficient and effective option for pest control and vector control. The delivery of bio-pesticides in the new generation formulation can have the potential to provide better efficacy and user safety; however, the primary issues of new formulation technology like nanoemulsions are; product performance, efficacy, environmental and user safety; therefore, the formulation of bio-pesticides must be improved with these considerations in mind.

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## Chapter 4

### Effect of *Metarhizium* spp. on Desert Locust

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Desert locust (*Schistocerca gregaria* Forskål) is considered as one of the pandemics across the world due to its inter-continental flight rate, high fecundity and voraciousness which can cause huge damage to several economically important crops (Cressman 2016). Aerial application of ULV formulations of pesticides are in practice in several countries however, due to huge side effects on the environment and non-target organism, an integrated pest management program using bio-pesticides from plants and microorganisms are greatly reliable for successful control of desert locust (Kooyman, 2003; Githae and Kuria 2021).

Early research on the use of fungi as a bio-control agent against desert locust was focused on *Metarhizium flavoviride* (Youssef 2014; Reda et al. 2018). The oil formulation of *M. flavoviridae* was reported to reduce the hopper bands tremendously (Lomer, 1997). However, its negative effect on bees, non-target organisms etc., discouraged the widespread use against desert locust control (Ball et al. 1997).

The fungus, *Metarhizium acridum* (Ascomcota: Hypocreales) isolated from Africa and Australia during 1990s was considered as a best alternative to *M. flavoviridae*. *Metarhizium anisopliae* var. *acridum*, commonly known as *Metarhizium acridum* in modern science [Bischoff et al. 2009], is an environmentally safer commercial bio-pesticide that has been developed for ultra low volume spraying (Van Huis 2007). The bio-pesticide kills about 70–90% of treated locusts within 14–20 days with no measurable impact on non-target organisms (Lomer et al. 2001) (Table 1).

Table 1. Various effects of *Metarhizium* spp. on desert locust

S. No.	Entomopathogenic fungi	Effect on desert locust	Reference
1.	<i>Metarhizium anisopliae</i> var. <i>acridum</i> Driver & Milner	Increased acidic phosphatase (AcP) for autophagy and defense Altered behavioral changes Changed biochemistry and antimicrobial defenses Less energy reserves and poor flight capability	Xia et al. (2000) Blanford and Thomas (2001) Gillespie et al. (2000) Seyoum et al. (2002)
2.	<i>Metarhizium anisopliae</i> var. <i>acridum</i> + Pheromone Phenyl Aceto Nitrile (PAN)	Reduced feeding and mobility	Mohamed et al. (2011)

3.	<i>Metarhizium anisopliae</i> var. <i>acridum</i> + <i>Paranosema</i> (Nosema) <i>locustae</i> (Protozoa)	Mortality happened sooner than those infected with only one of the pathogens	Tounou et al. (2008)
4.	<i>Metarhizium anisopliae</i> var. <i>acridum</i> + <i>Beauveria bassiana</i> (Bals-Criv) Vuill.	Significant reduction in total proteins and hemocyte counts	Milat-Biassaad et al. (2011)
5.	<i>Metarhizium flavoviride</i> Gams & Rozsypal	Reduction dispersal of hopper bands into small groups Reduced daily food consumption Significant reductions in flight activity and food consumption High mortality in sparse vegetation than in dense vegetation	Langewald et al. (1997) Moore et al. (1992) Seyoum et al. (1994) Lomer (1997)
6.	<i>Metarhizium robertsii</i> J.F. Bisch., S.A. Rehner & Humber	Produced Destruxin A which inhibited fever	Bundey et al. (2003)
7.	Oil-based formulation of <i>M. acridum</i> (Green Muscle)	80% reduction in population in 21 days application at 107 g viable conidia ha <sup>-1</sup>	Mullie et al. 2021

Biological control of desert locust though it is a safe method will take several days to bring under control. Rational use of pesticides along with biological control of desert locust is suggested, considering natural-risk management plans for locust outbreaks as well as the benefit and cost of proposed control measures and their environmental and health impact.

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## Chapter 5

### Endophytes and their Role in Mitigation of Biotic Stresses in Plants

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#### 1. Introduction

Plants are constantly involved in interactions with a wide range of microbial communities including bacteria and fungi. These plant associated microbes colonize the rhizosphere, the phyllosphere (epiphytes) and the inside of the plant tissues (endophytes). Plant microbiomes are generally shaped by both the factors namely plant genotype *viz.*, organ, tissues, species and health status, as well as plant's environment such as soil, soil condition and climatic factors. Endophytes refers to a group of microorganisms that colonize the internal tissues of plant without causing any immediate symptom of infection and/or visible manifestation of disease, and live in mutualistic association with plants for at least a part of their life cycle (Kumar *et al.* 2020). The concept of an endophyte was originally proposed by De Bary in 1866 as “any organism that grows within plant tissues” and is distinct from an epiphyte living on the surface of plants. Bacterial and fungal endophytes are ubiquitously inhabitants’ in plant tissues without causing any adverse effect and colonize all plant tissues and compartments, mainly the intercellular and intracellular spaces of inner tissues (Morelli *et al.* 2020). Acquisition and transmission of endophytes mainly occurs through horizontal and vertical modes. They infect plants by means of horizontal transmission, when their inoculum is transported to another plant, or vertically when they infect the seed progeny of an infected plant.

Endophytic microorganism live inside the host plants and is known to promote host plant growth and development, increase in nutrient uptake, inhibition of pathogen growth, reduction of disease severity and enhancing tolerance to various environmental stresses. The detail of application of plants and endophyte interaction is depicted in the Figure 1. Endophytic bacteria belong to a diverse group ranging from Gram’s-positive to Gram’s-negative bacteria, such as *Bacillus*, *Pseudomonas*, *Enterobacter*, *Brevibacterium* etc. The bacterial endophytic diversity in plants is affected mainly by the environment and host related factors such as genotypes of the host, growth and developmental stages of the host, geographical location, climatic conditions, soil type and temperature etc. Bacterial endophytes have been isolated from the different plant tissues namely root, leaf, stem and seed tissues of legume crops *Lathyrus*, pigeonpea and rice (Kumar *et al.* 2018; Mahto *et al.* 2019; Kumar *et al.* 2020a). The composition and diversity of microbes were analyzed in the different plants and tissues and the majority of culturable endophyte species were found belonging to the phylum Proteobacteria, class Firmicutes, Gram-negative and whereas Bacteroides were less common (Reinhold-Hurek and Hurek 2011).

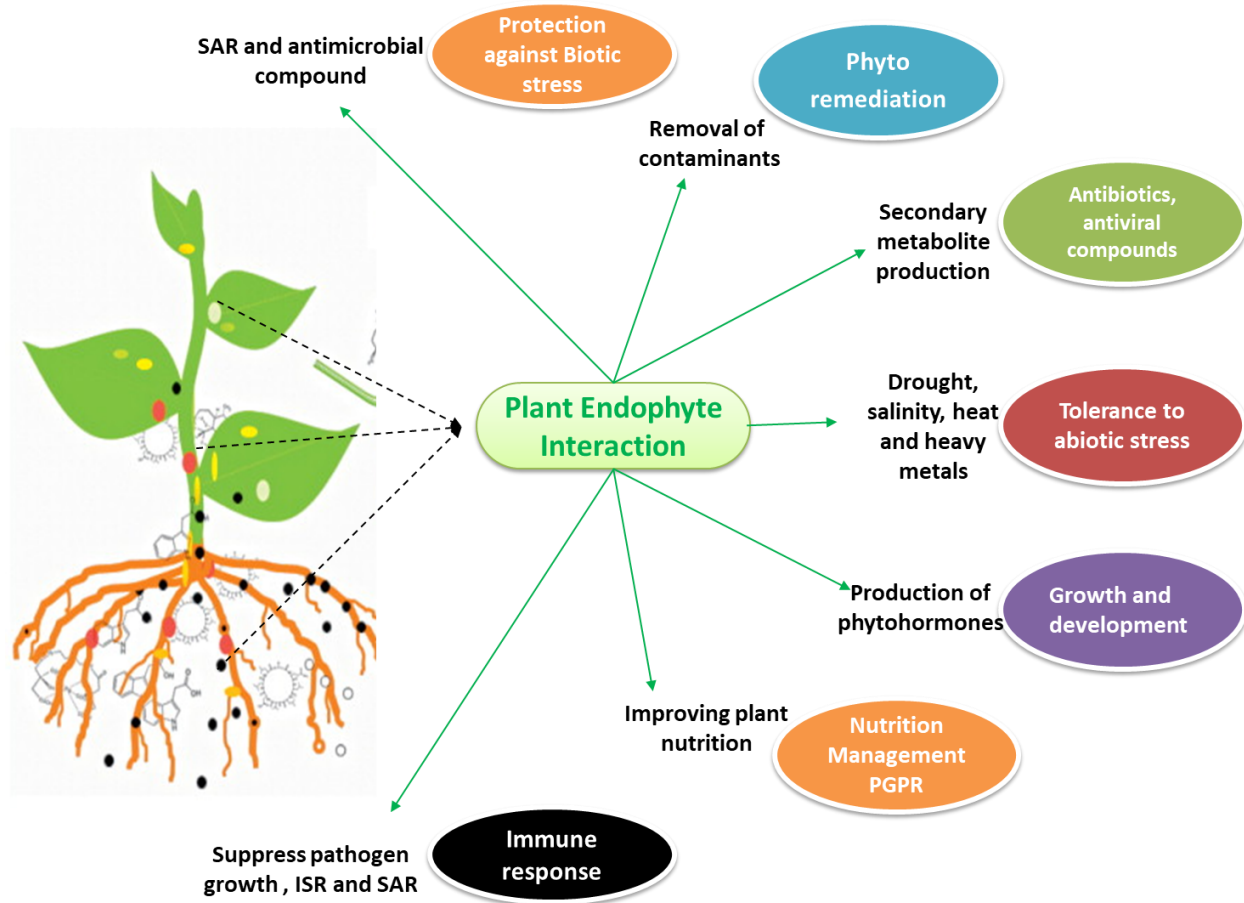


Fig 1. Plant microbe interaction for the beneficial functions of endophytes

## 2. Application of Endophytes

Plants have evolved to form complex, beneficial relationships with the microorganisms in their surroundings and these associated microbes are known as plant microbiome. The associated microbiome plays an essential role in maintaining microbial diversity and balance in sustaining the growth and health of host organisms. Microbial species have the ability to contribute multiple aspects to the system including essential functions such as (i) growth promotion, through nutrient supply by nitrogen fixation and solubilization and mobilization of phosphorus and minerals; enhancing seed germination; (ii) imparting resistance/ tolerance against various biotic stress; (iii) influencing physiology and production of bioactive metabolites; (iv) competing with pathogens for niche and nutrition inducing systemic resistance in host plants; (v) producing phytohormones and plant growth promoting regulators thus modulate plant growth and development; (vi) protect plants from pathogens and insects; (vii) tolerance to abiotic stresses; and (viii) removal of contaminants, phytoremediation's (Truyens et al. 2015; Berg et al. 2017). The contribution of plant associated microbes in such a way that the any disturbance in the microbial composition/ may led to the microbial dysbiosis led to the appearance of disease (White et al. 2019).

Plant diseases caused by pests and pathogens result in crop losses and are a significant threat to food security. Agrochemicals are efficiently used for managing the plant diseases and

insects. However, the intensive application of chemical fertilizers and pesticides has created negative effects not only on the ecosystem and human beings but also develop serious environmental pollution, ecological imbalance and enhancing resistance to the pathogen. The use of beneficial microorganisms, their products and metabolites as bio-controls are environment friendly and safe for human beings. Endophytes and their bioactive metabolites have received considerable attention nowadays due to their potential as biological control agents (Xia et al. 2022). Endophytes receiving attention as a sustainable alternative to synthetic pesticides and antibiotics to control plant-pathogenic bacteria and fungi and mitigating plant stress tolerance. These microbes utilize host plants for their production, development, and colonization in return they provide a variety of benefits to the host plants by interacting with numerous co-occurring microbes and provide beneficial effects to plants through their different lifestyles *viz.*, mutualistic, commensalistic and/ or symbiotic association.

### **3. Biological control**

Biological control is a mechanism that promotes plant growth by protecting against the attack of phytopathogens, mediated by the production of siderophores, bacteriocins or antibiotics or other bioactive compounds. It refers to a process in which one or more beneficial microorganisms in the rhizosphere unfavourably affect the survival or activity of a plant pathogen called as bio-control. As microbial endophytes occupied the same ecological niches as occupied by plant phytopathogens and proposed as effective biocontrol agents and can be applied as an ecofriendly alternative to chemical pesticides (Compant et al. 2013; Goel et al. 2018b; Dash et al. 2019). The application of endophytic microbes in managing the biotic stresses is a smart choice for management of plant diseases and insects (Sapak et al. 2008) as they offer the added advantage over the rhizospheric microbes due to their ability to colonize plant's internal tissue and live inside the plants, and not influenced by the external factors like temperature, time of application etc.. Microbial endophytes influences growth and development of plants by using two mechanisms either directly or indirectly (i) In the direct mechanism endophytes helps the plants in the acquisition of nutrients by synthesis, solubilization and mobilization of available nutrients such as nitrogen, phosphorous, iron and synthesis of phytohormones, whereas Indirectly through production of siderophore, metabolites, HCN and antibiotics against pathogenic microbes (Tzec-Interian et al. 2020). Endophytes may control phytopathogen growth through competition for nutrients and resources, production of antimicrobial metabolites and activation of immune responses in plant (Islam et al. 2015; Desgarences et al. 2020).

### **4. Mechanism of endophytes for pathogen inhibition**

Endophytes may adopt several strategies to attenuate the negative impacts of pathogens and pests on their host (Santoyo et al. 2016; Khare et al. 2018). Those activities may be achieved by direct inhibition of pathogens since they share similar colonizing patterns and are in intimate contact with plants. Microbial endophytes have been isolated from different plant species and have been tested to the inhibition of growth of phytopathogens and the detail of isolates and phytopathogens is mentioned in the Table 1. Events for direct inhibition of pathogens are mainly mediated by inhibitory allelochemicals including siderophores, antibiotics, cell wall degrading enzymes, volatile organic compounds (VOCs), alkaloids, steroids, quinines, terpenoids, phenols, and flavonoids (Gunatilaka 2006; Yu et al. 2010; Brader et al. 2014; Chung et al. 2016; EK-

Ramus et al. 2019), or by a quenching signal of pathogens (Miller et al. 2001). Indirect interactions associated with endophytes comprise the induction of plant defenses. This induction is performed by stimulation of defenses through induced systemic resistance (ISR) or endophyte induced resistance (E-IR) (De Kesel et al. 2021). The detail of endophytes mediated biotic stress management is mentioned in the Figure 2. The production of different types of enzymes, metabolites and anti-oxidants and plant microbe interaction is discussed below.

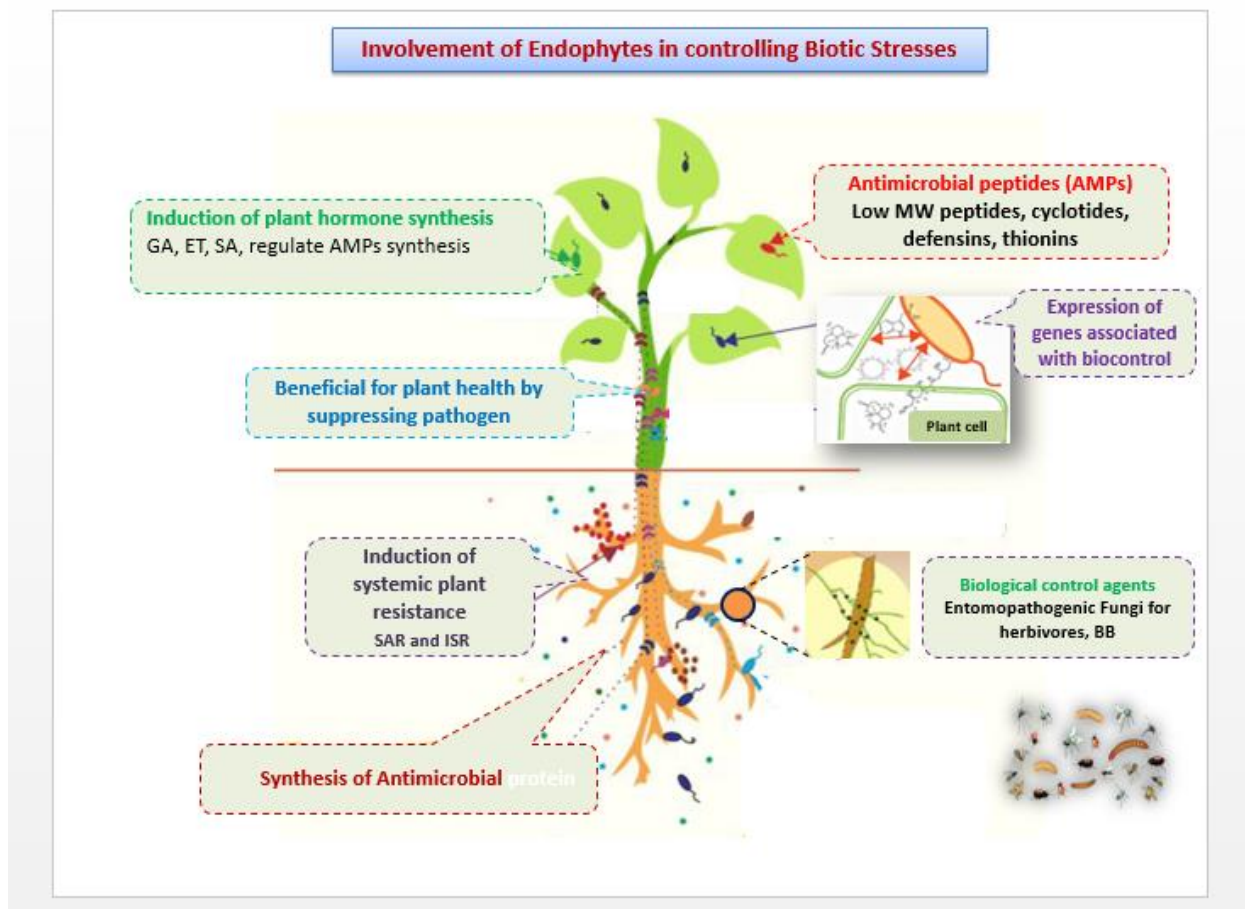


Fig 2. Representative image showing role of endophytes in controlling different biotic stresses and their probable mechanisms.

#### 4.1. Production of antimicrobial peptides (Lipopeptides)

Endophytic bacteria are known to produce several antimicrobial compounds. Lipopeptides are one of the most important classes of antimicrobial compounds. It was reported that the bacterial isolates, *Bacillus amyloliquefaciens* and *Paenibacillus* spp. are the potent producers of lipopeptides (Ongena and Jacques 2008; Villarreal-Delgado et al. 2018). In addition to lipopeptides, other antibiotic compounds, such as polyketides (surfactin, bacillomycin, fengycin, iturin, lichenysin, mycosubtilin, plipastatin, pumilacidin) produced by *Bacillus subtilis*, cyclic cationic lipopeptide, polymyxins found to be synthesized by *Paenibacillus polymyxa* (Stein 2005). Similarly, Kumar et al. (2020a) identified the presence of surfactin and iturin in the bacterial endophytes of rice having antimicrobial activities against soil borne fungal pathogens,



*Sclerotium rolfsii*, *Fusarium* and *Rhizoctonia*. Miller et al. (1998) found that the *Pseudomonas viridiflava* was able to produce ecomycins, a lipopeptide containing unusual amino acids including homoserine and  $\beta$  hydroxy aspartic acid.

#### **4.2. Volatile Organic Compounds (VOCs)**

VOCs are another group of antimicrobial compounds produced by endophytes having broad-spectrum antagonistic activities against phytopathogens including bacteria, fungi, and nematodes (Fernando et al. 2005; Khare et al. 2018). *Pseudomonas putida* BP25, an endophyte associated with black pepper have been reported to inhibit the proliferation of different fungus and nematodes by the emission of VOCs (Sheroan et al. 2016). Fungal endophyte produces various antimicrobial compounds such as alkaloids, flavanoids, steroids, peptides and phenol with antifungal activity (Kusari and Spiteller 2012; Campos et al. 2008). Furthermore, bioactive metabolites synthesized by endophytes can be used directly or indirectly as bio-control agents against plant diseases management (Park et al. 2017; Saad et al. 2019). Bioactive compounds synthesized by the fungal endophytes viz., *Acremonium* spp. and *Fusarium* spp. exhibited potential antimicrobial property (Powthong et al. 2012). *Pestalotiopsis* species isolated from leaf tissues of *Pinus caneriensis* exhibited antimicrobial activities against broad range of pathogenic bacteria (Bagyalakshmi et al. 2012).

#### **4.3. Synthesis of Secondary Metabolites**

Secondary metabolites are biologically active compounds that are very useful source of anticancer, antioxidant, antidiabetic, immunosuppressive, antifungal, antioomycete, antibacterial, insecticidal, nematocidal, and antiviral agents (Gunatilaka 2006). Fungal endophytes are capable of producing plant related metabolites and their analogs with beneficial value. Moreover, endophytes produce metabolites those are involved in mechanisms of signaling between host and partner fungi, defense, and genetic regulation for the establishment of symbiosis (Sun et al. 2014; Hardoim et al. 2015). Furthermore, these compounds can provoke the production of some novel biologically active secondary metabolites (Firakova et al. 2007; Rodriguez et al. 2009) that can be exploited and useful for human beings as important medicinal resources (Table 1).

**Table 1. List of bacterial endophytes isolated from different plants having the antagonistic potential against phytopathogens**

S.No.	Host plant	Endophytes	Phytopathogens inhibited	References
1.	<i>Ocimum sanctum</i>	<i>Macrophomina phaseolina</i>	<i>Sclerotinia sclerotiorum</i> and <i>Fusarium oxysporum</i>	Chowdhary and Kaushik (2015)
2.	<i>Catharanthus roseus</i>	<i>Diaporthe</i> spp.	<i>Alternaria alternata</i> , <i>Botrytis cinerea</i> , <i>Colletotrichum gloeosporioides</i> , <i>Fusarium graminearum</i> and <i>Phytophthora cinnamomi</i>	Yan et al. 2018
3.	<i>Withania somnifera</i>	<i>Talaromyces trachyspermus</i>	<i>Sclerotinia sclerotiorum</i>	Sahu et al. 2019
4.	<i>Artemisia nilagirica</i>	<i>Trichoderma viride</i> , <i>Penicillium atrovenetum</i> , <i>Aspergillus fumigatus</i> and <i>Cladosporium cladosporioides</i>	<i>Phytophthora infestans</i>	Myrchiang et al. 2014
5.	<i>Chlorophytum borivillianum</i>	<i>Fusarium proliferatum</i> , <i>Rhizoctonia bataticola</i> , <i>Setosphaeria rostrata</i> , <i>Bipolaris maydis</i> , <i>Diaporthe phaseolorum</i> , <i>Fusarium solani</i> , <i>Macrophomina phaseolina</i>	<i>Botrytis cinerea</i> , <i>Fusarium oxysporum</i> , <i>Sclerotinia sclerotiorum</i> and <i>Rhizoctonia solani</i>	Chowdhary and Kaushik, 2017
6.	<i>Tylophora indica</i>	<i>Alternaria tenuissima</i> , <i>Colletotrichum truncatum</i> and <i>Alternaria</i> sp.	<i>Sclerotinia sclerotiorum</i> and <i>Fusarium oxysporum</i>	Kumar et al. 2011
7.	<i>Triticum aestivum</i>	<i>Chaetomium</i> sp, <i>Phoma</i> sp.	<i>Puccinia recondite</i>	Dingle and McGee, 2003
8.	<i>Hordeum vulgare</i>	<i>Acremonium blochii</i> , <i>Aspergillus fumigatus</i> , <i>C. destructans</i> , <i>Dactylaria</i> sp., <i>Fusarium equiseti</i> , <i>Phoma herbarum</i>	<i>Gaeumannomyces graminis</i> var. <i>tritici</i>	Macia'- Vicente et al. 2008
9.	<i>Gossypium herbaceum</i>	<i>Penicillium simplicissimum</i>	<i>Verticillium dahliae</i>	Yuan et al. 2017
10.	<i>Brassica napus</i>	<i>Chaetomium globosum</i> CanS-73, <i>Clonostachys rosea</i> CanS-43, <i>Leptosphaeria biglobosa</i> CanS-51 <i>Aspergillus flavipes</i> CanS-34A,	<i>Sclerotinia sclerotiorum</i> , <i>Botrytis cinerea</i>	Zhang et al. 2014
11.	<i>Theobroma cacao</i>	<i>Gliocladium catenulatum</i>	<i>Crinipellis perniciosa</i>	Rubini et al., 2005
12.	<i>Zingiber officinale</i>	<i>Rhizopycnis vagum</i>	<i>Rhizoctonia solani</i> , <i>Colletotrichum</i>	Anisha et al., 2018

			<i>acutatum, Fusarium oxysporum, Sclerotium rolfsii</i>	
13.	<i>Cucumis sativus</i>	<i>Chaetomium</i> Ch1001	Root-knot nematode <i>Meloidogyne incognita</i>	Yan et al. 2011
14.	<i>Beta vulgaris</i>	<i>B. mycoides</i>	<i>Cercospora beticola</i> Sacc.	Barbagus et al. 2002
15.	<i>Solanum lycopersicum</i>	<i>B. pumilus</i> SE34	<i>Fusarium oxysporum</i> f. sp. <i>radicis lycopersici</i>	Benhamou et al. 1998
16.	<i>Lactuca sativa</i>	<i>B. amyloliquefaciens</i> strain FZB42	<i>R. solani</i>	Chowdhary et al. 2015
17.	<i>Litchi chinensis</i>	<i>B. amyloliquefaciens</i> strain TB2	<i>Peronophthora litchi</i>	Cai et al. 2010
18.	<i>Vitis vinifera</i>	<i>B. pumilus</i>	<i>P. chlamydospora</i>	Haider et al. 2016
19.	<i>Musa paradisiaca</i>	<i>Fusarium oxysporum</i>	<i>Radopholus similis</i>	Vu et al. 2006
20.	<i>Olea europaea</i>	<i>Pseudomonas fluorescens</i> PICF7	<i>Verticillium</i> sp.	Gómez-Lama Cabanás et al. 2014
21.	<i>Anthurium andreaeanum</i>	<i>Bacillus</i> B014	<i>Xanthomonas axonopodis</i> pv. <i>dieffenbachiae</i>	Li et al. 2012

#### 4.4. Secretion of Lytic Enzymes

Microbial endophytes secrete a wide variety of lytic enzymes including  $\beta$ -1,3-glucanase, chitinase, cellulase and protease. These lytic enzymes hydrolyze various polymeric compounds such as chitin, cellulose, proteins, and lipids. Chitinase mediates the degradation of chitin, which is the major cell wall component of fungal pathogens, thus altering the integrity of fungal cell wall and compromising the survival of the pathogen in the plants and soil. It was reported that chitinase produced by endophytic *Streptomyces hygroscopicus* found to inhibit the growth of different fungus namely *Alternaria alternata*, *Aspergillus flavus*, *Botrytis cinerea*, *Ralstonia solani*, *Fusarium oxysporum*, *Aspergillus niger*, *Sclerotinia sclerotiorum* and *Hyaloperonospora parasitica* (Haggag and Abdallah 2012). Similarly, *Bacillus cereus* producing chitinase enzymes were showed to protect the cotton seedlings from root disease caused by *R. solani* (Pleban et al. 1997).

#### 4.5. Production of Siderophore

Bacterial endophytes are **known** to secrete siderophores in soil while interacting with host plants, where siderophores chelate iron from the environment for use by plant and microbial cells (Gamalero and Glick 2015; Hardoim et al. 2015). Siderophore production contributes to an uptake of enhanced nutrient by the host plants and helps in the controlling growth of pathogenic microbes by depriving them from iron (Fe) (Saha et al. 2016).

#### 4.6. Quorum quenching

An additional direct mechanism of endophytes against pathogens is the disruption of quorum sensing (QS) molecules required for the regulation of various physiological activities such as cell–cell crosstalk, reproduction, biofilm formation, adaptation, mutualism, and pathogenesis (Hosni et al. 2011). Endophyte hampers the signaling pathways of phytopathogens, resulting in decreased survivability. For instance, the endophytic bacteria harbored in *Cannabis sativa* were reported to disrupt the cell-to-cell communication of *Chromobacterium violaceum* (Kusari et al. 2014). Additionally, *Stenotrophomonas maltophilia*, *Pseudomonas aeruginosa* and *Rhodococcus corynebacterioides* isolated from the xylem of different plant species could degrade the 3-hydroxy palmitic acid methyl ester (3OH-PAME), a QS molecule of *R. solanacearum*, and reduce bacterial wilt in eggplant (Achari and Ramesh 2014).

#### 4.7. Pathogenesis-related proteins (PRs)

Pathogenesis-related proteins (PRs) are triggered due to necrotic lesions in plants. Studies revealed that some endophytes induces PR proteins and increase resistance against a wide range of pathogens. *Bacillus pumilus* SE34 triggered ISR in tomato by production of toxic substances such as phenolic compounds and  $\beta$ -1,3-glucanases enzyme. Similarly, *B. amyloliquefaciens* strain TB2 produces PR proteins against *Peronophthora litchi* responsible for causing downy blight disease in litchi (Cai et al. 2010). In addition to this, the endophytic actinobacteria isolated from wheat tissues showed an upregulation of PR-1, PR-4, PDF1.2, and HEL defense genes in response to *Erwinia carotovora* subsp. *carotovora* challenge (Conn et al. 2008). Moreover,

recently, endosymbiotic bacteria *Bacillus* spp. was reported to produce antifungal lipopeptides (iturin and fengycin) and induce PR genes, including PR-1 and PR-4 in maize (Gond et al. 2015).

#### **4.8. Accumulation of Antioxidant**

Besides PPRs, endophytes are also known to induce several defense-related enzymes, including antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL). A significant increase in antioxidant enzymes such as SOD, CAT, PPO, POD, catalase and peroxidase, was observed after 24 hours of inoculation of endophytic bacterium, *B. subtilis*, in tomato plants (Chandrasekaran and Chun, 2016). Similarly, inoculation of banana plantlet with the endobacterium *Serratia marcescens* strain UPM39B3 induced the production of host defense enzymes such as peroxidase, PPO, PAL, total soluble phenols and lignothioglycolic acid, and protected plants against Fusarium wilt disease (Ting et al. 2010).

#### **4.9. Induction of immune response**

In addition to the direct mechanism endophytes play a crucial role in mitigating biotic stresses by inducing ISR against pathogen and/or suppressing the growth of phytopathogens and improving agricultural productivity (Kloepper and Ryu 2006; Gunatilaka et al. 2006), synthesis phytohormones such as Jasmonic acid and Salicylic acid and ethylene which is known to play a crucial roles during the mitigation of plant stress response (Khare et al. 2016). The modulation of signaling and defense components during endophytic colonization may result in the activation of the enhanced resistance and crosstalk between endophytic communities and thus the host plant can activate gene clusters, leading to the synthesis of novel secondary metabolites. Numerous studies indicated that ISR can be triggered through several compounds produced by endophytes such as phytohormones, lipopeptides, pyocyanin, siderophore, and VOCs (Ryu et al. 2004). ISR triggered by endophytic bacteria can protect the host against a wide range of pathogens. Recent studies have shown the endophyte-mediated resistance (EMR) which seems to be different from Induced Systemic Resistance and Systemic Acquired Resistance, as salicylic acid, jasmonate and ethylene are not involved (Pieterse et al. 2014; Constantin et al. 2019).

### **5. Application of endophytes for managing biotic stress**

- Development of formulation of individual endophytes.
- Development of effective consortium of microbial endophytes for PGP and antimicrobial activities.
- Identification and isolation of secondary metabolite for use as biological control.
- Lipopeptide based control of pathogens.
- Induction of Endophytes Mediated Resistance (EMR).
- Elucidation of molecular mechanism involved in endophyte mediated plant stress tolerance for engineering of resistance in plants.

With the above mentioned benefits of microbial endophytes there is an urgent need to explore the untapped potential of bacterial endophytes for growth promoting and mitigating the biotic stresses in sustainable manner.

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## Chapter 6

### Role of Entomopathogenic Nematodes in Pest Management

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#### 1. Introduction

Concerns about the harmful effects of pesticides on the environment were raised by the use of chemical pesticides in agriculture. Methods that are environmentally friendly are urgently needed. Another worry about employing chemical pesticides is the introduction of novel biotypes and the development of resistance, which motivates us to adapt our approach to pest management. A viable and appealing alternative for crop protection, biological management is safe for the environment. Application of bioagent-based products in various crop environments depends heavily on an understanding of the biology and adaptation of bio-control agents. For the management of insect pests, especially soil-dwelling insects, entomopathogenic nematodes (EPNs) are efficient bio-control agents. *Steinernema* and *Heterorhabditis* are two very pathogenic EPN genera that can kill their insect hosts in just one to two days. Both *Steinernema* and *Heterorhabditis* contain the symbiotic bacteria *Xenorhabdus* and *Photorhabdus* in their stomachs, which they release when they penetrate an insect (<https://vikaspedia.in>).

#### 2. Entomopathogenic Nematodes

Entomopathogenic nematodes are soft bodied, non-segmented roundworms that are obligate or sometimes facultative parasites of insects, causes death to insects. Naturally occurring in soil conditions, entomopathogenic nematodes seek their host by sensing vibrations, carbon dioxide, and other chemical cues (Kaya and Gaugler 1993). *Heterorhabditidae* and *Steinernematidae* species have been employed successfully in pest management programmes as biological pesticides (Grewal et al. 2005). Because they are thought to be non-toxic to people, very specific to their target pest(s), and can be sprayed with regular pesticide equipment, entomopathogenic nematodes fit well into integrated pest management, or IPM, programme (Shapiro-Ilan et al. 2006).

The family Steinernematidae is comprised of two genera *Steinernema* Travassos, 1927 (Poinar, 1990) and *Neosteinerema* (Nguyen and Smart, 1994). There is only one species of *Neosteinerema*, *Neosteineremalongicurvicauda*, which diverged from the termite *Reticulitermes flavipes* (Koller). *Heterorhabditis* is the single genus in the family Heterorhabditidae (Poinar 1976). EPNs are mutually associated with bacteria of the Enterobacteriaceae family; the bacteria carried by Steinernematidae is typically a species of the genus *Xenorhabdus*, while the bacteria carried by Heterorhabditidae is a species of *Photorhabdus* (Table 1).

Table 1. Diagnostic characters between *Steinernema* and *Heterorhabditis*

<i>Steinernema</i>	<i>Heterorhabditis</i>
<b>Symbiotic Bacteria</b>	
<i>Associated Species – Xenorhabdus</i>	<i>Associated Species – Photorhabdus</i>
Location of bacteria- within specialized intestinal vesicle	Location of bacteria- inside the anterior part of the intestine
<b>Adults</b>	
Excretory pore anteriorly located to nerve ring. Spicule ventrally acute. Bursa absent. Genital papillae 10-11 pairs.	Excretory pore posteriorly located to nerve ring. Spicule nearly straight. Bursa present. Genital papillae 9 pairs.
<b>Infective juveniles</b>	
Excretory pore anterior to nerve ring	Excretory pore posterior to nerve ring
Luminescence - No	Luminescence - Yes
Colour of dead larva- Black	Colour of dead larva- Red, pink

## 2.1. Biology of Entomopathogenic Nematodes

The only free-living stage of entomopathogenic nematodes is the infective juvenile stage, or IJ. The cycle begins with an infective juvenile, whose only function is to seek out and infect new hosts. When a host has been located, the juvenile stage enters the hemocoel of the host insect through the spiracles, mouth, anus, or in certain species, intersegmental membranes of the cuticle (Bedding and Molyneux 1982). Mutualistic relationships exist between *Heterorhabditis* and *Steinernema* with bacteria belonging to the genera *Photorhabdus* and *Xenorhabdus*, respectively (Ferreira and Malan, 2014). The juvenile stage releases cells of their symbiotic bacteria into the hemocoel from their intestines. Within 24 to 48 hours, the infected host normally passes away as a result of the bacteria multiplying in the insect haemolymph. Nematodes carry on feeding on host tissue after the host has passed away, developing, and reproducing. The progeny nematodes develop through four juvenile stages to the adult. One or more generations may develop inside the host cadaver depending on the resources available, and a significant number of infectious juveniles are finally released into the environment to infect further hosts and complete their life cycle (Kaya and Gaugler 1993).

*Steinernematid* and *Heterorhabditid* nematodes have different reproduction processes. Infective juveniles of heterorhabditid nematodes become hermaphroditic adults but individuals of the next generation produce both male and females whereas in steinernematid nematodes all generations are produced by males and females (gonochorism) (Grewal et al. 2005). When insects are killed by heterorhabditids, the insect cadaver turns red, while steinernematids cause the insect cadaver to turn brown or tan (Kaya and Gaugler 1993). The colours produced by the monoculture of mutualistic bacteria growing in the hosts are indicative of the colour of the host's body.

## 2.2. Searching behaviour

The two search methods used by entomopathogenic nematodes are ambushers and cruisers (Grewal et al. 1994). In order to target moving insects (nictitating) in the upper soil, ambushers

like *Steinernema carpocapsae* lay in wait and conserve energy. Cruisers such as *Steinernemaglaseri* and *Heterorhabditisbacteriophora* are extremely active and typically underground, moving great distances using volatile signals and other strategies to locate their host beneath. They are therefore useful against pests that are less mobile, like white grubs (Scarab beetles). *Steinernemafeltiae* and *Steinernemariobrave* are two nematode species that use an intermediate foraging strategy (a combination of ambush and cruiser type) to locate their host (Grewal et al.1994) (Table 2).

Table 2. Commercial use of Entomopathogenic Nematodes as Biopesticides

EPN species	Targeted pests
<i>Steinernema carpocapsae</i>	Turfgrass pests- billbugs, cutworms, armyworms, sod webworms, chinch bugs, crane flies. Orchard, ornamental and vegetable pests - banana moths, codling moths, cranberry girdlers, dogwood borers and other clearwing borer species, black vine weevils, peach tree borers, shore flies ( <i>Scatella</i> spp.)
<i>Steinernema kraussei</i>	Black vine weevil, <i>Otiorynchus sulcatus</i>
<i>Heterorhabditis marelatus</i>	White grubs (scarabs), cutworms, black vine weevils
<i>Heterorhabditis bacteriophora</i>	White grubs (scarabs), cutworms, black vine weevils, flea beetles, corn root worms, citrus root weevils
<i>Heterorhabditis indica</i>	Fungus gnats, root mealybugs, grubs
<i>Steinernemaglaseri</i>	White grubs (scarabs, especially Japanese beetle, <i>Popillia</i> sp.), banana root borers
<i>Heterorhabditis zealandica</i>	Scarab grubs
<i>Steinernemascapterisci</i>	Mole crickets ( <i>Scapteriscus</i> spp.)
<i>Heterorhabditis megidis</i>	Weevils
<i>Steinernema feltiae</i>	Fungus gnats ( <i>Bradysia</i> spp.), shore flies, western flower thrips, leafminers
<i>Steinernema riobrave</i>	Citrus root weevils ( <i>Diaprepes</i> spp.), mole crickets

### 2.3. Advantages of EPN's over chemicals

- Highly lethal to significant insect pests with the fewest off-target effects.
- Very safe for both people and the environment. There is no need for safety equipment, there are no aftereffects, there is no groundwater contamination, and pollinators are safe. Eliminates insects in 24-48 hours
- They are simple to mass produce and may be used with irrigation systems and common pesticide application equipment.

### 2.4. Factors influencing the efficacy of EPNs

A number of factors influence the activity, survival, infection rate, virulence of the EPNs such as abiotic factors (temperature, soil pH, humidity, soil porosity, UV radiation, soil moisture), biotic factors (Other parasites, predators, pathogens, plant exudates, rhizosphere characters etc.) and host related factors such as host species, host immunity, host developmental stages).Abiotic

factors influence the activity of EPNs the most than any other factors. Being a soil inhabited organism, EPNs affect from soil related parameters. Soil temperature is one of the factors which influence the efficacy of EPN. The optimum soil temperature for EPN survival and infection may vary between species and strains (Grewal et al. 1994). In general, extreme temperatures of 0°C and 40°C were lethal; whereas, temperatures below 10-15°C can resist their mobility (Manjunatha et al. 2022). Some nematodes such as *H. indica*, *S. glaseri*, and *S. riobrave* are relatively heat tolerant whereas others, such as *H. megidis*, *S. feltiae*, and *Heterorhabditis* are more tolerant to cooler temperatures (Shapiro-Ilan et al. 2012).

Application of EPN for the management of insect pests of above ground plant parts severely affected by the UV radiation or desiccation (Manjunatha et al. 2022). To minimise the effect of UV radiation or desiccation, it is highly recommended to apply EPN during evening hours or early morning hours and/or subsurface application (Cabanillas and Raulston 1995) to increase the efficiency and efficacy of EPNs. Soil moisture is one of the important factors which affect the activity and survival of soil applied EPNs. Soil moisture is very much essential for EPNs activity and survival but at the same time higher soil moisture deprive oxygen and effect the EPNs activity drastically. Optimum soil moisture varies between different EPN species. According to Molyneux and Bedding (1984) “in sandy soils, *Heterorhabditis* sp. and *S. glaseri* infect at water potentials between -0.003 to -0.4 bars, whereas in sandy-loam soil, infection of these nematodes occurred at water potentials between -0.0 1 to -100 bars.

Soil properties also main factors influencing the activity and survival of EPNs. Soil texture is one of the important soil properties which affects the EPN the most. In general, the soils with higher clay content significantly reduce the activity and efficiency of EPNs in comparison to lighter soils or soil with less clay content. Soil pH is another factor which influence the activity of EPNs. Soil with more than 10 pH is highly detrimental to EPNs and a range of 4-8 soil pH is ideally suitable for survival, reproduction and enhanced activity of EPN (Manjunatha et al. 2022) (Table 3).

Table 3. Recent studies investigating the effect of entomopathogenic nematodes against insect pests (Labaude and Griffin, 2018).

<b>Pest group</b>	<b>Pest Species</b>	<b>Nematode Species Tested</b>	<b>Reference</b>
<b>Coleoptera</b>	<i>Anomalagraueri</i> (white grub)	<i>H. bacteriophora</i> , <i>S. carpocapsae</i> , <i>S. longicaudum</i>	Kajuga et al. (2018)
	<i>Curculio elephas</i> (chestnut weevil)	<i>H. bacteriophora</i> , <i>S. glaseri</i> , <i>S. weiseri</i>	Demir et al. (2015)
	<i>Holotrichiaoblita</i> (whitegrub)	<i>H. bacteriophora</i> , <i>S. longicaudum</i>	Guo et al. (2015)
	<i>Hylobiusabietis</i> (large pine weevil)	<i>S. carpocapsae</i> , <i>S. downesi</i>	Kapranas et al. (2016)
	<i>Phyllotretacruciferae</i> (crucifer flea beetle)	<i>H. bacteriophora</i> , <i>H. indica</i> , <i>S. feltiae</i> <i>S. carpocapsae</i>	Antwi and Reddy (2016)

	<i>Polyphyllafullo</i>	<i>H. bacteriophora</i> , <i>S. glaseri</i> , <i>S. weiseri</i>	Demir <i>et al.</i> , 2015
	<i>Rhynchophorus ferrugineus</i> (red palmweevil)	<i>H. bacteriophora</i> , <i>S. carpocapsae</i> , <i>S. feltiae</i>	Manzoor <i>et al.</i> , 2017
	<i>Strategusaloeus</i> (oil palm chiza)	<i>H. bacteriophora</i> , <i>H. indica</i> , <i>S. feltiae</i> , <i>S. colombiense</i> , <i>S. websteri</i>	Gómez and Sáenz-Aponte 2015
<b>Diptera</b>	<i>Aedes aegypti</i> (yellow fever mosquito)	<i>H. baujardi</i> , <i>S. carpocapsae</i> , <i>H.indica</i>	Cardoso <i>et al.</i> , 2015
	<i>Bactrocera dorsalis</i>	<i>H. indica</i> , <i>H. tayserae</i>	Godjo <i>et al.</i> , 2018
	<i>Bactroceratryoni</i> (Queensland fruit fly)	<i>H. bacteriophora</i> , <i>S. carpocapsae</i> , <i>S. feltiae</i>	Langford <i>et al.</i> , 2014
	<i>Bradysiaodoriphaga</i> (chive maggot)	<i>H. bacteriophora</i> , <i>S. carpocapsae</i> , <i>S. feltiae</i> , <i>H. indica</i> , <i>S.longicaudum</i>	Bai <i>et al.</i> , 2016; Wu <i>et al.</i> , 2017
	<i>Chironomus plumosus</i>	<i>H. bacteriophora</i> , <i>S. carpocapsae</i> , <i>S. feltiae</i> , <i>S. kraussei</i>	Edmunds <i>et al.</i> , 2017
	<i>Drosophila suzukii</i> (spotted wing drosophila)	<i>H. bacteriophora</i> , <i>S. carpocapsae</i> , <i>S. feltiae</i> , <i>S. kraussei</i>	Cuthbertson and Audsley 2016; Hübner <i>et al.</i> , 2017; Garriga <i>et al.</i> , 2018
	<i>Musca domestica</i> (housefly)	<i>H. indica</i> , <i>S. abbasi</i> , <i>S.carpocapsae</i> , <i>S. feltiae</i> , <i>S. glaseri</i>	Archana <i>et al.</i> , 2017
	<i>Rhagoletiscerasi</i> (European cherry fruitfly)	<i>H. bacteriophora</i> , <i>H. marelatus</i> , <i>S.carpocapsae</i> , <i>S. feltiae</i>	Kepenekci <i>et al.</i> , 2015
	<i>Stomoxyscalcitrans</i> (stable fly)	<i>H. bacteriophora</i> , <i>H. baujardi</i>	Leal <i>et al.</i> , 2017
<b>Hemiptera</b>	<i>Eriosomalanigerum</i> (wooly apple aphid)	<i>H. bacteriophora</i> , <i>H. megidis</i> , <i>S.carpocapsae</i> , <i>S. feltiae</i> , <i>S. glaseri</i> , <i>S. kraussei</i>	Berkvens <i>et al.</i> , 2014
	<i>Planococcusficus</i> (vinemealybug)	<i>S. yirgalemense</i>	Le Vieux and Malan 2013
	<i>Trialeurodes vaporariorum</i> (greenhouse whitefly)	<i>H. bacteriophora</i> , <i>S. feltiae</i>	Rezaei <i>et al.</i> , 2015



Hymenoptera	<i>Cephuscinctus</i> (wheat stem sawfly)	<i>H. bacteriophora</i> , <i>H. indica</i> , <i>S. carpocapsae</i> , <i>S. feltiae</i> , <i>S. glaseri</i> , <i>S. kraussei</i> , <i>S. riobrave</i>	Portman <i>et al.</i> , 2016
<b>Isoptera</b>	<i>Coptotermes formosanus</i> (formosan subterranean termite)	<i>S. kariii</i>	Wagutu <i>et al.</i> , 2017
	<i>Macrotermes bellicosus</i> (termite)	<i>H. indica</i> , <i>H. sonorensi</i>	Zadji <i>et al.</i> , 2014
	<i>Trinervitermes occidentalis</i> (termite)	<i>H. indica</i> , <i>H. sonorensi</i>	Zadji <i>et al.</i> , 2014
<b>Lepidoptera</b>	<i>Cydiapomonella</i> (codling moth)	<i>H. bacteriophora</i> , <i>S. feltiae</i> , <i>S. jeffreyense</i> , <i>S. yirgalemense</i>	Odendaal <i>et al.</i> , 2016
	<i>Ectomyeloisceratoniae</i> (carob moth)	<i>H. bacteriophora</i> , <i>S. carpocapsae</i> , <i>S. feltiae</i>	Memari <i>et al.</i> , 2016
	<i>Ephesiakuehniella</i> (millmoth)	<i>S. carpocapsae</i> , <i>S. feltiae</i> , <i>S. riobrave</i>	Ramos-Rodríguez <i>et al.</i> , 2006
	<i>Heliothis subflexa</i>	<i>H. bacteriophora</i> , <i>S. carpocapsae</i> , <i>S. feltiae</i> , <i>S. websteri</i>	Bolaños <i>et al.</i> 2016
	<i>Paranthrenediaphana</i> (clearwing moth)	<i>H. bacteriophora</i> , <i>S. carpocapsae</i> , <i>S. feltiae</i>	Azarnia <i>et al.</i> , 2018
	<i>Plodia interpunctella</i> (Indian meal moth)	<i>S. riobrave</i> , <i>S. feltiae</i> , <i>S. carpocapsae</i>	Ramos-Rodríguez <i>et al.</i> , 2006
	<i>Plutella xylostella</i> (diamondblack moth)	<i>S. carpocapsae</i>	Sunanda <i>et al.</i> , 2014
	<i>Spodopteralitura</i> (tobacco cutworm)	<i>H. bacteriophora</i> , <i>S. glaseri</i>	Safdar <i>et al.</i> , 2018
	<i>Synanthedon exitiosa</i> (peachtree borer)	<i>S. carpocapsae</i>	Shapiro-Ilan <i>et al.</i> , 2016
	<i>Thaumetopoea wilkinsoni</i> (pine processionary moth)	<i>S. affine</i> , <i>S. feltiae</i>	Karabörklü <i>et al.</i> , 2015
<i>Tuta absoluta</i> (tomato leaf miner)	<i>H. bacteriophora</i> , <i>S. carpocapsae</i> , <i>S. feltiae</i>	Van Damme <i>et al.</i> , 2016; Kamali <i>et al.</i> , 2018	
<i>Zeuzerapyrina</i> (leopard moth)	<i>H. bacteriophora</i> , <i>S. carpocapsae</i>	Salari <i>et al.</i> , 2015	

## Case Study 1

### **Control of the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) larvae in laboratory using entomopathogenic nematodes from subtropical environment (Faculty of Agriculture, University of Eswatini., 2020).**

- In the study, the pathogenicity of two subtropical EPN species was examined in relation to *T. absoluta* larvae. *Steinernema yirgalemense* and *S. jeffreyense* at different concentrations (0, 20, 40, 60 IJs/insect) were screened for efficacy (i.e. mortality rate) against larvae of *T. absoluta* in laboratory bioassays.
- According to the results, neither *S. yirgalemense* nor *S. jeffreyense* significantly differed from the other in their ability to kill *T. absoluta* larvae in 24-well bioassay trays. Mortality was much higher ( $p < 0.05$ ) when 60 IJs/insect were utilised.
- In comparison to the other four combinations of EPN and concentration examined, the combination of *S. yirgalemense* at 60 IJs/insect (100%) and *S. jeffreyense* at 60 IJs/insect (98.3%) caused a considerably greater ( $p < 0.05$ ) mortality rate.
- In the leaf bioassay, *Steinernema yirgalemense* at 60 IJs/insect caused noticeably higher mean mortality than the other EPN and concentration combinations.
- Both of the tested EPNs were successful in killing *T. absoluta* larvae, according to the results.
- However, large-scale field trials are advised to show the biocontrol agent's potential for use in integrated pest management programmes. *Steinernema yirgalemense* can successfully detect *T. absoluta* larvae inside leaf mines at 60 IJs/insect (Source: Dlamini et al. 2020)
- Mean percentage mortality (95% confidence level) of *Tuta absoluta* larvae inoculated with 0, 10, 20, and 40 IJs/insect of *Steinernema yirgalemense* and *Steinernema jeffreyense* in a laboratory trial. Different letters above the vertical bars indicate significant differences ( $p < 0.05$ ).
- Mean percentage mortality (95% confidence level) of *Tuta absoluta* larvae inoculated with 0, 10, 20, and 40 IJs/insect of *Steinernema yirgalemense* and *Steinernema jeffreyense* in a leaf trial. Different letters above the vertical bars indicate significant differences ( $p < 0.05$ ).

## Case Study 2

### **Susceptibility of Various Developmental Stages of the Fall Armyworm, *Spodoptera frugiperda*, to Entomopathogenic Nematodes (Gyeongsan, Gyeongbuk province, Korea, 2020).**

- Entomopathogenic nematodes (EPNs) are a potential biological control agent that could be employed to manage *S. frugiperda*'s underground late larval and pupal phases. A comparison of the virulence of seven EPNs against the larval and pupal stages of *S. frugiperda* included *H. bacteriophora*, *H. indica*, *S. arenarium*, *S. carpocapsae*, *S. longicaudum*, *Heterorhabditis* sp., and *S. kushidai*.
- In a Petri dish trial, *Steinernema carpocapsae* and *Heterorhabditis indica* both were extremely virulent toward younger larvae, while *S. arenarium* and *S. longicaudum* were

extremely virulent toward older larvae. *Heterorhabditis* sp., *S. kushidai*, and *H. bacteriophora*, on the other hand, exhibited minimal virulence against all larval stages.

- When compared to the other EPN species, *H. indica*, *S. carpocapsae*, and *S. longicaudum* were extremely virulent against late larval and pupal stages in soil column and pot assays. Therefore, *H. indica*, *S. carpocapsae*, and *S. longicaudum* are suggested for *S. frugiperda*'s biological control (Acharya et al. 2020)

### Case Study 3

#### **Potential of South African entomopathogenic nematodes to control the leaf miner, *Holocacistacapensis* (Lepidoptera: Heliozelidae), (At Stellenbosch, South Africa, 2019)**

- In South African table and wine grape vineyards, the Cape grapevine leafminer, *Holocacista capensis*, is a sporadic pest of economic significance. The purpose of the study was to identify the vulnerability of leaf-mining *H. capensis* larvae to seven Steinernematidae and Heterorhabditidae entomopathogenic nematode (EPN) species. Using concentrations of 0, 25, 50, 100, 200, and 400 IJs/leaf-mining larva, concentration experiments were carried out to estimate the lethal dose for the three most virulent species (*Heterorhabditisbaujardi*, *H. indica*, and *H. noenieputensis*).
- With *H. baujardi* (92%), *H. noenieputensis* (85%), and *H. indica* (83%), high mortality of leaf-mining larvae was attained. Almost double the number of *H. noenieputensis* (34 nematodes/insect) penetrated the insect larvae, in comparison with the other two EPNs. However, *H. baujardi*'s relative potency was 3.56 times greater than that of *H. indica*, while *H. indica*'s relative potency was 2.57 times more than that of *H. noenieputensis*. The laboratory results were considered to be promising, particularly in terms of the nematode's capacity to enter the leaf-mining galleries and successfully infect the larvae (Steyn et al. 2019).

### Case Study 4

#### **Virulence of four *Steinernema* species as a biological control agent in controlling the termite, *Coptotermesheimi* (Wasmann) (Isoptera: Rhinotermitidae), (University of Karachi, Karachi, Pakistan, 2020)**

- Subterranean termites are a widespread, ancient group of social insects that are widely distributed. They are mostly recognised as agricultural pests and as important economic pests for destroying wooden structures.
- Four *Steinernema* strains of entomopathogenic nematodes (EPN) were tested against *Coptotermes heimi* (Wasmann).
- These EPN included *Steinernema pakistanense* NNRC-AS.04, *S. siamkayai* NNRC-As.12, *S. bifurcatum* NNRC-As.65, and *S. maqbooli* NNRC-As.88. The virulence of each strain was assessed at three different EPN inocula in sand-filled plastic containers.
- All of the investigated EPN species showed a nematode inoculum impact that was statistically significant.

- At 150 IJs/ml, NNRC-AS.04 and NNRC-As.65 demonstrated the largest virulence effects, with 95 and 100%, respectively (Khanum et al. 2020).

### Case Study 5

#### **Combined Effect of Entomopathogens against *Thrips tabaci* Lindeman (Thysanoptera: Thripidae): Laboratory, Greenhouse and Field Trials, (Faisalabad, Punjab, Pakistan, 2021).**

- Onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) is one of the most destructive insect pests of onions, *Allium cepa* L.
- The effectiveness of applying both entomopathogenic nematodes and entomopathogenic fungi against various *T. tabaci* soil-dwelling stages was assessed. All four paired combinations (nematode + fungus) were present; the nematodes included *Heterorhabditis bacteriophora* (VS strain) and *Steinernema feltiae* (SN strain), and the fungi included *Beauveria bassiana* (WG-11) and *Metarhizium anisopliae* (WG-02).
- Only *H. bacteriophora* and *B. bassiana* applied together (WG-11) in a small cup bioassay resulted in a synergistic interaction against pre-pupae; all other combinations were compatible with pupae and late second instars only additively.
- Again, in a larger arena, a potted soil bioassay, the use of both pathogens together increased mortality when compared to the use of each pathogen alone. All the combinations showed additive interactions, with the highest mortality seen in pre-pupae, followed by pupae and late second instar larvae using *H. bacteriophora* and *B. bassiana* (WG-11).
- In the potted plant bioassay, it was also found that treated groups' adult emergence was lower than that of control groups.
- In comparison to controls, fewer adults and larvae were found in treated groups under field circumstances. Overall, pupae and late second instar larvae were the stages most responsive to pathogen treatments, and combination applications of both diseases also decreased the adult population.
- Combined application of entomopathogenic nematodes and fungi could be used for integrated pest management (IPM) of *T. tabaci* in onion production systems (Gulzaret *al.*, 2021).

### Case Study 6

#### **Efficacy of Entomopathogenic Nematodes against Soil-Dwelling Life Stages of Western Flower Thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae), (Kiel University, Germany, 2001).**

- In a laboratory investigation, the effectiveness of six entomopathogenic nematode (EPN) strains against soil-dwelling life stages of the western flower thrips (WFT), *Frankliniella occidentalis* (Pergande), (Thysanoptera: Thripidae) was evaluated.
- The EPN strain collections screened included the *S. carpocapsae* strain DD136, three *Steinernema feltiae* species, including *S. feltiae* Sylt (S.f S), *S. feltiae* OBSIII (S.f O), and *S. feltiae* strain CR (S.f C), as well as two *Heterorhabditis bacteriophora* species, *H. bacteriophora* HK3 (H.b (S.c D).

- The studied EPN strains were capable of infecting all stages of WFT that live in soil. The strains S.f S, S.c D, and H.b H were the most virulent. Under high soil moisture conditions, the S.f O strain was extremely virulent against late second instar larvae and prepupae of WFT, but less so against pupae under relatively drier soil conditions.
- Results from dose rate experiments show that high mortality in all soil-dwelling life stages of WFT required a rather high concentration of 400 infective juveniles (IJs) per cm<sup>2</sup>. However, WFT already experienced 30–50% mortality at dose rates of 100–200 IJs/cm<sup>2</sup> (Ebssa et al. 2001).

## Conclusion

Biological control is a technique used to reduce the negative impacts of insect pests, such as the destruction of valuable crops and plantations, the damage of plant growth, or diseases brought on by pests (Flint and Dreistadt 1998).

- ❖ EPNs are a group of organisms that live in soil and target soilborne insect pests that are close to, on, or in the soil itself. They can be employed to efficiently control economically significant insect pests. Different nematode species and strains have variations in their ability to survive, carry out searches, and spread infection, which makes them more or less suitable for various insect pest control programmes (Garcia del Pino and Palomo 1996).
- ❖ Finding wild populations to collect new species and strains for potential application in biological control is of great interest. One promising non-chemical strategy for controlling insect pests is the use of EPNs. EPNs have numerous hosts and are geographically dispersed extensively. Currently, they are being used as biological control agents in numerous researches to eradicate a number of significant insect pests globally (Shields *et al.*, 1999; Gozel and Gunes, 2013; Gozel and Kasap, 2015).
- ❖ It is made clear that more, in-depth basic knowledge on the biology of EPNs, including ecology, behaviour, and genetics, is required to comprehend the underlying causes of both their successes and failures as biological control organisms. Abiotic elements like soil type, soil temperature, and soil moisture are crucial for success, as well as the most suitable nematode species or strain (Kaya and Gaugler, 1993).
- ❖ Before being applied to the field, nematode virulence, host discovery, and ecological parameters are crucial for a proper match to the host. Poor host appropriateness has been the most frequent error made in the application of EPNs, therefore it is crucial to match the right nematode host-seeking technique with the pest. Additionally, application strategies such field dose, volume, irrigation, and suitable application techniques are crucial. In determining if nematodes are potential control possibilities, plant shape and phenology must also be considered (Georgis et al. 2006).

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## Chapter 7

### Chemoecological Approaches in Pest Management

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#### **Abstract**

The need to produce an inexpensive and abundant food supply for a growing population is a great challenge and warrants higher use of inputs like fertilizers and pesticides. Increasing use of pesticides has negative impact on farmers, consumers, non-target organism and environment and this prompts the search for safe alternative for pest control. The challenge to balance between crop production and environmental protection can be achieved by adopting eco-friendly pest management strategies like chemoecological methods. Understanding the chemoecological approaches using cutting-edge technologies involving chemical detectors (GCMS) and electrophysiological tools resulted in developing robust behavioral manipulations in pest management. The compounds identified to be used in behavioral pest management will aid to decline the dependence on xenobiotics.

**Keywords:** Biodetectors, Behavioural manipulation, Eco-friendly strategy

#### **1. Identification and Separation of Semiochemicals**

The increasing scientific interest in the biochemistry, physiology, ecology and atmospheric chemistry of VOCs has led to the development of a variety of systems for the collection and analysis of volatiles (Millar and Haynes 1998). Volatile analysis has improved by the design of relatively sensitive bench-top instruments for gas chromatography-mass spectrometry (GC-MS) (Dorothea et al. 2006).

Volatiles surrounding the airspace (headspace) around the insects are sampled and concentrated prior to analysis. Headspace sampling is a non-destructive method for collecting volatiles. When compared with solvent extractions of pheromone glands from insects, headspace analysis gives a more realistic picture of the volatile profile emitted by insects. It provides real time information for ecologically relevant applications. Materials like glass, metal and special plastics such as Teflon that are inert are to be used for volatile trapping (Dorothea et al. 2006).

An important advance in static headspace analysis is the development of solid phase microextraction (SPME) which is a fast and simple method for collecting volatiles at detection limits in the ppb (parts per billion by volume) range. Solid phase microextraction is based on ad/absorption and desorption of volatiles from an inert fiber coated with different types of ad/absorbents. The fiber is attached within the needle of a modified syringe and volatiles can be sampled by inserting the needle through a septum of a headspace collection container and pushing the plunger to expose the fiber. Following equilibration between the fiber and the volatile sample, the fiber is retracted into the needle and can be transferred to a gas

chromatograph for direct thermal desorption. Solid phase microextraction fibers can be reused approximately 100 times. Thermal desorption of VOCs from the fiber eliminates the need for solvents that may contain impurities which will interfere with sample analysis (Dorothea et al. 2006).

## **2. Determination of semiochemicals**

Insect semiochemical adsorbing matrices are analyzed by the standard technique of GC. GC analysis protocols and recent advances in GC analysis technology are described (Handley and Adlard 2005; Lockwood 2001; Merfort 2002). For GC analysis of semiochemicals, samples are either injected as solvent extracts into the heated injector in a split or split less mode or desorbed from the adsorbent by placing it directly in a thermal desorption tube, heated to 250-300°C. In a two stage thermal desorption, the thermally released volatiles are concentrated by a cold trap (or cryotrap) prior to their injection into the GC column (Dorothea et al. 2006). For analytical purposes, volatiles are commonly separated on fused silica capillary columns with different stationary phases, such as the non-polar dimethyl polysiloxanes (e.g. DB-1, DB-5, CPSil 5), and the more polar polyethylene glycol polymers, including Carbowax\_ 20M, DB-Wax, and HP-20M.

Following separation on a GC column, semiochemicals are analyzed by a variety of detectors. Flame ionization detector (FID) is commonly used for quantitative analysis because of their wide linear dynamic range, their very stable response and their high sensitivity with detection limits of the order of picograms to nanograms per compound. Mass spectrometry (MS) detectors are used for routine semiochemical GC analysis. GC-MS bench top instruments, compounds exiting the GC column are ionized by electron impact (EI) and where in the positively charged molecules and molecule fragments are selected based on their mass-to-charge ( $m/z$ ) ratio on hitting the a quadrupole ion trap. Total ion chromatograms are obtained, provide information on retention time and its mass spectrum consisting of a characteristic ion fragmentation pattern of a compound. Identification of compounds in GC-MS analysis is done by using the popular mass spectral libraries such as Wiley and NIST MS databases (Dorothea et al. 2006). The orientation response of an insect to semiochemicals is evaluated by olfactometry and electrophysiological assays.

## **3. Establishing the physiological response of semiochemicals**

Different electrophysiological techniques have been employed to study the nervous system of insects since the late 1950s. In 1957, Schneider for the first time announced that it was possible to measure the electrophysiological responses from the antennae of an insect, *Bombyx mori*, using an electroantennogram (EAG). The EAG technique has since been developed for several insect species of various insect orders (Millar and Haynes, 1998). Despite its usefulness, the method is limited in its use. For instance, the sensitivity of the technique is low, a shortcoming often observed in insects whose antennae have e.g.a limited number of sensilla (Millar and Haynes 1998). To overcome this problem, a technique called single sensillum recordings (SSRs) was developed, which can measure responses of single ORNs (Millar and Haynes 1998). Single sensillum recordings allow for the analyses of specific types of sensilla and for the determination of the mechanisms by which insects code for different odors (Majid 2007).

In SSRs, two sharpened tungsten electrodes are used: a ground electrode is in contact with the hemolymph while the recording electrode is inserted near the base or in the shaft of a single sensillum. Voltage differences generated between the electrodes, when amplified, can be viewed on an oscilloscope when placed within the circuit (de Bruyne et al. 2001).

The introduction of gas chromatography (GC) coupled SSRs, first used in moths and aphids allowed for the first detection of active components within a complex blend of compounds. This technique has later been used to identify novel ligands of ORNs in a large number of insect species. At the GC part an extract likely to contain odorants detected by the ORNs is injected onto the GC-column. The column is located in an oven where it is possible to regulate the column temperature. As the temperature of the column is increased the components of the extract are separated while traveling down the column and exit the GC set-up. The separated components of the extracts encounter the single sensillum from which a stable electrical contact is established. Responses of the ORNs housed in a single sensillum to the extract components are recorded. The chemical identity of the response eliciting component(s) can be further identified using mass spectrometry (MS). The olfactory receptors respond to compounds that are attractive and repulsive. Hence the behavioral experiments have to be carried out to ascertain the nature of compounds using olfactometers and wind tunnels.

In Indian agriculture, the traditional technique of controlling important insect pests is to use synthetic insecticides at regular intervals from planting through harvest. Insecticide resistance, pest resurgence, and toxic effects on non-target organisms, including natural enemies, resulted from the indiscriminate and injudicious use of broad-spectrum insecticides. (Deshmukh et al. 2010). Semiochemistry is a new science that is being hailed as the best alternative to chemical pesticides for regulating tri-trophic interactions involving host plants, herbivores, and their natural enemies (El-Ghany 2019).

Insect pheromones are natural substances that suit modern pest control standards, such as species specificity, absence of toxicity to mammals, environmental friendliness, and use in agricultural pest management. As a result, the practical application of insect pheromones, particularly sex pheromones, has had a great deal of success in managing low-density pest populations and reducing pest numbers over time with minimum impact on natural enemies (Rizvi et al. 2021). Mass trapping, sex pheromone-based mating disruption, and push-pull strategies have greatly reduced the usage of traditional insecticides, resulting in more sustainable and environment-friendly pest management in agricultural crops (Tiwari et al. 2014).

In India, there are about 24 insect pheromones easily available, most of which are sold as rubber septa saturated with pheromone chemicals. Traps baited with pheromone lures are commonly employed to attract and kill male insects, causing subsequent mating with the opposite sex to be disrupted (Nandagopal et al. 2008). In 2021, the market for pheromone technology in India was estimated to be worth USD 3.54 million. During the projection period, the market is expected to grow at a 15.40 per cent CAGR from USD 4.05 million in 2022 to USD 11.03 million in 2029.

Despite the fact that existing pheromone formulations are widely available in the Indian market, their cost ineffectiveness limits stakeholder affordability. According to the survey,

sustainable approaches will gain significant traction in the home market during the next few years. The rural region is projected to be a centre for pheromone research and development. The current state of pheromone technology still requires fine-tuning in terms of cost reduction and more controlled release capability.

In March 2020, scientists and researchers from the Jawaharlal Nehru Centre for Advanced Scientific Research in Bengaluru and the Indian Council of Agricultural Research (ICAR) unveiled a matrix-powder-like substance fueled by the nano-enabled regulated release of pheromones for agricultural pest management. Nano-formulation of pheromones have been developed for key pests of crops including tomato pinworms, rice yellow stem borer, diamondback moth, fall armyworm *etc.*; evaluated, and commercialized with the help of a few leading companies of India such as Barrix Agro Sciences Pvt. Ltd. (Bangalore, India), Russell IPM (Deeside, U.K.), Pheromone Chemicals (Hyderabad, India), ATGC Biotech Pvt. Ltd. (ISCA Global) (Hyderabad, India), Agri Phero Solutionz (Hyderabad, India), Jaydev Chemical Industries (Mumbai, India), Green Revolutions (Kolhapur, India), Gaiagen Technologies Pvt. Ltd. (Mumbai, India), Rentokil PCI (Mumbai, India), Provivi Inc. (Santa Monica, U.S.).

Nano-pheromone is reportedly said to be marketed for 30 cents in India, making the pest control technology nearly eight times cheaper. Although nano-pheromone technology has been marketed by a small number of Indian private enterprises, further validations and demonstrations are needed across the country to increase its visibility and awareness among farmers.

#### **4. Conclusions**

Though the adoption rate of semiochemicals in pest management is at a lower level as compared to pesticides, considering the shift in the policy by Government to scale down the use of pesticides due to health and environmental concerns there is a scope to increase the adoption rate. A note of caution is that semiochemicals may also face the same fate related issues raised over toxicity issues, as very little effort has been made to test the chemicals that are used in behaviour manipulation. Another problem is that natural enemies use the cues used by the insect pest to identify its host and if the cues from the host plant to alter to bring in desirable pest control it would have a negative impact on natural enemies as it's devoid of the cue to orient itself to its host. Hence a proper understanding of the volatiles role in various trophic levels has to be understood prior to attempting a pest management method. The demand for environmentally safe alternatives to broad-spectrum is on rise. The adoption of behavioral manipulation techniques can help to meet this demand, since the amount of chemicals released into the environment by behavioral manipulation is relatively small and are relatively nontoxic to vertebrates and are selective to the target pest species.

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## Chapter 8

### Biocontrol of Plant Diseases

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#### 1. Introduction

Plant diseases are the biotic constraints that continue to pose threat to crop production and productivity all over the World. Historically, yield losses caused by plant diseases in different crops and their impact are well documented and caused serious losses in the past. For example, the Bengal famine in India in 1943 due to the destruction of rice by *Helminthosporium oryzae*, the Irish potato famine, caused by *Phytophthora infestans* in Ireland in 1840s and wheat stem rust (*Puccinia graminis* f. sp. *tritici*) in the USA in 1917 wiped out the wheat fields.

Various methods and strategies are being used for the management of plant diseases including use of agrochemicals. However, extensive and excessive use of chemicals resulted in negative effects on environment and also on beneficial organisms (non-target microbes), pesticide residues in food, feed and fodder. Because of the detrimental effects of the chemicals on environment, introduction of organic farming and integrated disease management systems led to efforts on developing alternative methods to synthetic chemicals for controlling pests and diseases. Among these alternatives, biocontrol is one of them. Biocontrol or biological control is the alternative approach for disease management that is eco-friendly and reduces the amount of harmful chemicals and their residues.

The concept of bio-control initiated for the management of insects with the term “Biological control” which was coined by Harry Smith in 1919 who defined it as “the suppression of insect population by the action of their native or introduced enemies”. Baker and Cook (1974) described it as “the reduction of inoculum density or disease producing activities of a pathogen or a parasite in its active or dormant state by one or more organisms”. Thus, biocontrol refers to the purposeful utilization of introduced specific organism or resident population of microbes to suppress the activities and populations of one or more plant pathogens. This may involve managing soils to promote the combined activities of native soil- and plant-associated organisms that contribute to general suppression. Mostly biocontrol involves the deliberate introduction of antagonistic micro-organisms to control a pathogen although it occurs to some extent in the ecosystem naturally. The term Biological Control Agents (BCAs) is referred to microbial antagonists which suppresses the growth of pathogens and the bio-formulations of these antagonists can be used to control plant diseases.

BCAs are effective in managing a number of plant diseases like root rots, wilts, damping-off etc. caused by a number of soil borne plant pathogens in several crops. *Trichoderma* spp. and *Pseudomonas fluorescens* are the two most widely used BCAs. Several commercial formulations of these antagonists are available in the market for controlling plant diseases. Two formulations of *Aspergillus niger* strain AN27 (*Kalisena* SD and *Kalisena* SL) have worked par excellence as biofungicide controlling destructive plant diseases caused by soil borne pathogens (viz.,

*Fusarium oxysporum*, *F. solani*, *Macrophomina phaseolina*, *Pythium* spp., *Rhizoictonia solani*, *Sclerotinia sclerotiorum*), increasing growth and crop yield in different agricultural crops (Sen 2000).

Several potential microorganisms effective against different plant pathogens have been registered and the Central Insecticide Board (CIB) permits biocontrol agents for registration and commercialization in India. The list of BCAs for plant disease management included in CIB for registration are *Bacillus subtilis*, *Pseudomonas fluorescens*, *Gliocladium* spp., *Trichoderma* spp., *Verticillium lecanii*, *Verticillium chlamydosporium*, *Streptomyces griseoviridi*, *Streptomyces lydicus*, *Fusarium oxysporum* (non-pathogenic), *Burkholderia cepacia*, *Coniocytrium minitans*, *Agrobacterium radiobacter strain 84*, *Pythium oligandrum*, *Erwinia amylovora* (hairpin protein), *Penicillium islandicum*, *Chaetomium globosum*, *Aspergillus niger*-strain AN27, VAM fungi, *Serratia marcescens* GPS 5, *Piriformospora indica* among others (Singh, 2014). In addition, plant endophytic microorganisms and plant growth promoting rhizobacteria (PGPR) have been identified as BCAs. Bacteriophages are viruses that infect and replicate in bacteria. Studies on bacteriophages as biocontrol options has regained interest in 21<sup>st</sup> century with promising results and in the US, bacteriophages are registered as biopesticides (Holtappels et al. 2021) (Table 1).

Table.1 Some examples of BCAs for the management of plant diseases of important crops

Crop/Disease	Pathogen	Reported BCAs
Rice Blast	<i>Pyricularia oryzae</i>	<i>P. fluorescens</i> , <i>Trichoderma</i> spp.
Rice Sheath blight	<i>Rhizoictonia solani</i>	<i>P. fluorescens</i> & <i>P. putida</i> , <i>T. harzianum</i> , <i>T. viride</i> , <i>T. virens</i> , <i>Aspergillus niger</i> AN27
Rice Bacterial leaf blight	<i>Xanthomonas oryzae</i> <i>pv. oryzae</i>	<i>Bacillus</i> spp.
Wheat Root rot	<i>Sclerotium rolfsii</i> , <i>Fusarium oxysporum</i>	<i>T. harzianum</i>
Maize Charcoal rot, Banded leaf & sheath blight	<i>Macrophomina</i> <i>phaseolina</i> , <i>R. solani</i>	<i>Trichoderma</i> spp.
Pigeonpea Wilt	<i>Fusarium udum</i>	<i>T. viride</i> , <i>T. hamatum</i> , <i>T. harzianum</i> , <i>T. koningii</i> , <i>Bacillus subtilis</i>
Chickpea Wilt	<i>F. oxysporum</i> f.sp. <i>ciceri</i>	<i>T. viride</i> , <i>T. harzianum</i> , <i>T. virens</i> , <i>B. subtilis</i> , <i>A. niger</i> AN27
Chickpea Root rot	<i>R. solani</i> , <i>M. phaseolina</i>	<i>T. viride</i> , <i>T. harzianum</i>
Chickpea Collar rot	<i>Sclerotium rolfsii</i>	<i>T. viride</i> , <i>T. harzianum</i> , <i>P. fluorescens</i>
Soybean Dry root rot	<i>M. phaseolina</i>	<i>T. viride</i> , <i>T. harzianum</i>
Mungbean Root rot	<i>M. phaseolina</i>	<i>T. harzianum</i> , <i>T. viride</i>
Groundnut Crown rot	<i>Aspergillus niger</i>	<i>T. viride</i> , <i>T. harzianum</i> , <i>B. subtilis</i>
Groundnut Stem & pod rot	<i>Sclerotium rolfsii</i>	<i>T. harzianum</i>
Groundnut Late leaf spot	<i>Phaeoisariopsis</i>	<i>Penicillium islandicum</i> ,

	<i>personata</i>	<i>P. fluorescens, T. harzianum, B. subtilis</i>
Groundnut Root and stem rot	<i>R. solani</i>	<i>T. virens, T. longibrachiatum</i>
Groundnut Rust	<i>Puccinia arachidis</i>	<i>Verticillium lecanii, T. harzianum</i>
Mustard Damping-off	<i>Pythium aphanidermatum</i>	<i>T. harzianum, T. viride</i>
Sesamum Wilt	<i>F. oxysporum</i> f. sp. <i>sesami</i>	<i>A. niger</i> AN27
Root rot	<i>M. phaseolina</i>	<i>Trichoderma</i> sp., <i>Gliocladium</i> sp., <i>B. subtilis</i>
Sunflower Blight	<i>Alternaria helianthii</i>	<i>T. virens</i>
Root/collar rot	<i>S. rolfsii, R. solani, Scerotiniasclerotiorum</i>	<i>T. harzianum, T. hamatum</i>
Bottlegourd Wilt/ Root rot	<i>F. oxysporum, R. solani</i>	<i>A. niger</i> AN27
Cauliflower Damping off	<i>P. aphanidermatum</i>	<i>A. niger</i> AN27
Chilli Root rot	<i>S. rolfsii</i>	<i>T. harzianum</i>
Chilli Fruit root and die back	<i>Colletotrichum capsici</i>	<i>T. viride, T. harzianum, T. konningii, T. hamatum, T. longibrachiatum, T. pileatus</i>
Cucumber Seedling diseases	<i>Phytophthora</i> or <i>Pythium</i> sp., <i>F. oxysporum</i> f. sp. <i>cucumerinum</i>	<i>T. harzianum, A. niger</i> AN27
Brinjal Wilt, Damping off	<i>F. solani, P. aphanidermatum</i>	<i>T. viride, T. konningii</i>
Collar rot	<i>S. sclerotiorum</i>	<i>T. viride, T. virens</i>
French bean Root rot	<i>R. solani</i>	<i>T. viride, T. hamatum</i>
Pea Seed & Collar rot, White rot	<i>Pythium</i> sp., <i>R. solani S. sclerotiorum</i>	<i>T. harzianum, T. hamatum T. viride</i>
Potato Black-scurf	<i>R. solani</i>	<i>T. viride, T. viride, B. subtilis</i>
Tomato Damping-off and wilt	<i>F. oxysporum</i> f. sp. <i>lycopersici</i>	<i>T. harzianum, P. fluorescens</i>
Banana Fusarium Wilt	<i>Fusarium oxysporum</i> f. sp. <i>cubense</i> Tropical Race 4	<i>T. reesei</i>
Banana Fusarium Wilt	<i>Fusarium oxysporum</i> f. sp. <i>cubense</i> Tropical Race 4	<i>Bacillus licheniformis</i>

Source: Singh (2014); Damodaran et al. (2020), Yadav et al (2021)

## 2. Mechanisms contributing towards Biocontrol

BCAs employ a number of mechanisms for their antagonistic activities. The most common mechanisms employed by BCAs are parasitism, antibiosis, competition, induction of resistance and plant growth promotion (Kohl et al. 2019). Increasing studies have shown that the antagonistic behaviour often involves the synergistic action of more than one mechanism. For example, species of *Trichoderma* may act as mycoparasite, metabolite producer, competitor and/or modulator of defence response (Mukherjee et al. 2013).

### 2.1. Parasitism

In parasitism, the pathogen is directly attacked by a specific BCA that kills it or its propagules. The parasitism of one fungus by another (hyper-parasitism or mycoparasitism) is manifested as morphological disturbance, direct penetration of hyphae and hyphal lysis. Parasitism occurs when the antagonist invades the pathogens by secreting enzymes such as chitinases, cellulases, glucanases and other lytic enzymes. Several fungal parasites of plant pathogens which include those parasitizing sclerotia and others attack living hyphae. A single fungal pathogen can be attacked by multiple parasites. *Trichoderma* spp. are the most common mycoparasites which penetrate resting structures such as sclerotia or may parasitize growing hyphae by coiling round them. *T. harzianum* degrades fungal cell walls by the lytic action of glucanases and chitinases, while other species also produce cellulase. *Aspergillus niger* AN27 application parasitized and killed the population of *Sclerotinia sclerotiorum* pathogen and checked the sclerotial formation in the soil (Sen 2000). On the phylloplane, several fungi, including *Verticillium lecanii*, and *Cladosporium* sp., are known to attack rust fungi. Bacteria which occur on the phylloplane and in the rhizosphere are also known to parasitize plant pathogens. There are also many examples of bacteria causing lysis of fungal spores. Isolates of *Bacillus* obtained from the surface of cereal rust urediniospores can lyse germ tubes of these rust spores and when bacterial suspensions are sprayed onto cereal leaves, pustule development is reduced. Many rhizobacteria produce hydrogen cyanide (HCN) and may contribute to pathogen suppression. Certain fluorescent pseudomonads produce HCN and are able to suppress the pathogens. HCN effectively blocks the cytochrome oxidase pathway and is highly toxic to all aerobic microorganisms. *P. fluorescens* CHA0 produces antibiotics, siderophores and HCN, but suppression of *Thielaviopsis basicola* (black rot of tobacco) appeared to be due primarily to HCN production (Voisard et al. 1989).

### 2.2. Competition for nutrients and space

Competition between pathogens and BCAs for nutrient resources or ecological niches is important for limiting disease incidence and severity (Kohl et al. 2019). BCAs establish themselves in the environment through the physical occupation of the site, biofilm formation resulting in reduced colonization of roots by the pathogen, and secretion of the essential micronutrients chelating such as siderophores. In general, soilborne pathogens, such as *Fusarium* spp. and *Pythium* spp. that infect through mycelial contact are more susceptible to competition than those pathogens that germinate directly on plant surfaces and infect through appressoria and infection pegs. Competition has been suggested to play a role in the biocontrol of species of *Fusarium* and *Pythium* by some strains of fluorescent pseudomonas. Fungal BCAs with highest

number of propagules or with good mass of mycelial growth have competitive advantage such as *Trichoderma* spp. One of the best documented examples of nutrient competition in biocontrol involves competition for iron between fluorescent pseudomonads and soilborne fungal pathogens such as *Fusarium oxysporum*. Bacterial BCAs like *Pseudomonas fluorescens* and *P. putida* produce siderophores that bind iron and facilitate its transport from the environment into the microbial cell. The siderophores pyoverdine and pseudobactin have a high affinity for the soluble ferric iron and inhibit the growth of pathogens by limiting the availability of iron. Competition for the same carbon source between *Pythium* sp. (seedling damping-off) and rhizosphere bacteria has resulted in effective biological control of *P. ultimum* in several crops.

### 2.3. Antibiosis (Production of antimicrobial substances)

Antibiosis plays an important role in biocontrol. BCAs produce antibiotic substances that suppress pathogens. Antibiotics are microbial toxins that can, at low concentrations, kill other microbes. Most microbes produce and secrete one or more compounds with antibiotic activity resulting in suppression of plant pathogens and the diseases they cause. Several biocontrol strains are known to produce multiple antibiotics which can suppress one or more pathogens and which can enhance the biocontrol ability of the antagonist. Some examples of antibiotics produced by the BCAs are:

BCA	Antibiotics	Target pathogen/ Disease	References
<i>Trichoderma virens</i>	Gliotoxin Gliovirin	<i>Rhizoctoniasolani</i> /Root rots	Wilhite et al. (2001)
<i>Agrobacterium radiobacter</i>	Agrocin 84	<i>Agrobacterium tumefaciens</i> /Crown gall	Kerr (1980)
<i>Bacillus amyloliquefaciens</i>	<i>Bacillomycin</i> , <i>fengycin</i>	<i>Fusarium oxysporum</i> /wilt	Koumoutsis et al. (2004)
<i>B. subtilis</i>	Mycosubtilin	<i>Pythium aphanidermatum</i> / Damping-off	Leclere et al. (2005)

### 2.4. Induced Systemic Resistance

Many BCAs show indirect mode of action for their antagonistic activities against plant pathogens by induction of resistance. The BCAs or non-pathogenic microbes activate defence mechanisms in the plant that lead to systemic resistance to a number of pathogens. This phenomenon is known as induced systemic resistance (ISR). This is different from systemic acquired resistance (SAR), which refers to the host reaction in response to localized infection by pathogens, manifested as broad range of protection against other pathogens. Several root-colonizing microorganisms are known to suppress diseases by ISR in plants (Mandal and Ray 2011).

## **2.5. Plant Growth Promotion**

Bio agents can reduce the disease incidence by increasing crop growth at least during the early stages of the life cycle by the way of disease escape. Chaubeet *al.*, (2003) reported that bio agents both fungal and bacterial help in managing the plant diseases by promoting the growth of plants through increased solubilisation of nutrients, increased nutrientuptake through enhanced root growth and sequestration of nutrients. *Aspergillus niger* strain AN-27 was reported to produce growth promoting compounds, 2- carboxy-methyl-3-hexyl-maleic anhydride and 2 methylene-3- hexyl-butanedioic acid (hexylitaconic acid) that were directly responsible for increased root and shoot length and biomass of crop plants (Selvakumar and Srivastava, 2000).

## **3. Methods of Application of BCAs**

Application of biocontrol agents can be made in two modes which are as follows:

### **3.1. Application to the infection site**

Application of BCAs directly to the infection site at a high population level to cover the pathogen which is also called as Inundate application. Seed coating and seed treatment with BCAs and application to soil at the site of seed placement are such treatments. These are the most commonly used procedures which have resulted in the successful control of several plant diseases.

### **3.2. One place application**

In this procedure, BCAs are applied at one place, but at lower populations which then multiply and spread to other plant parts and give protection which is also called as augmentative application. An Example of this method is Plant PGPR seed bacterization. Formulations of BCAs are generally found as wettable powders, dusts, granules and aqueous or oil-based liquid products. These formulations and their consortia can be delivered through different methods based primarily on survival nature and mode of infection of the pathogen. These application methods include seed treatment, seedling dip, soil application, foliar application, or through combination of different methods. Combined application of BCAs has been used to exploit their synergistic potential.

### **3.4. Seed and seedling treatment**

Seed treatment is the most effective method of applying BCAs, particularly against soil-borne pathogens. Generally 4 to 10 g of biocontrol formulation per kg of seed is used for treating the seeds. For shining skin seeds like pigeonpea, soybean etc., some sticky substance like gum, carboxymethyl cellulose should be mixed so that the BCA formulation sticks well and cover the surface completely. After treating the seeds, seeds should be kept for 48 hrs. at proper humidity and controlled environment so that BCA can grow and cover the seeds completely. This increases the efficacy of BCA as pathogen cannot attack the germinating seeds due to ready presence of BCA.

Seed bio-priming with BCAs is a promising approach of seed treatment to protect seeds from various seed- and soil-borne pathogens. This technique is able to incite changes in plant characteristics apart from facilitating uniform seed germination (Bisen et al. 2015). Callan et al. (1990) reported a 10-fold increase in the antagonist population load on the seeds as a result of seed bio-priming using bacterial antagonists, thus, protecting the crop from pathogen invasion. Priming of field pea seeds with *Pseudomonas fluorescens*, *P. aeruginosa*, and *Bacillus subtilis* causes nearly 20% decrease in incidence of *Uromyces fabae* under field conditions (Mishra and Pandey, 2010). Carboxymethyl cellulose and pectin can be used as adhesive polymers for the seed priming. In crops where transplanting is required, seedlings are dipped in the BCA solution. Uprooted seedlings from nursery first washed thoroughly in water to remove soil from the roots. Then, roots are dipped in the BCA solution for 15 to 30 minutes and transplant in the field.

### **3.5. Soil application**

BCAs powder formulation can be applied into the soil prior to sowing or drenched at initial stages of crop growth. For immediate application, 1 Kg powder formulation of the BCA is mixed in 100 Kg farm yard manure (FYM), compost or sand and spread in 1 acre of field. FYM or vermin-compost enriched with bio-agents can also be prepared in farmer's field and used for soil treatment. In this method, 100 kg of FYM or compost is spread under the shade and sprinkled with water for humidity. One Kg of BCA powder (eg. *Trichoderma*) is mixed well in this manure and cover with plastic sheet for 15-20 days. During the period, BCA multiply manifold in the manure and this enriched manure is used in nursery bed or in fields for soil treatment.

### **3.6. Foliar or spray application**

Foliar sprays of BCA solution are required for the management of pathogens attacking the above-ground plant parts. Generally 5 to 10 g BCA formulation per litre of water is used for sprays. Several BCAs have been used as seed and soil application to reduce the incidence of plant diseases caused by soil borne fungal pathogens. Consequently, suppression of the initial inoculum will be the first step in managing these pathogens. This should be followed by periodic spray applications to suppress foliar phases of these diseases for the inhibition of the secondary infection and dissemination of these pathogens.

## **4. Advantages of Biocontrol Agents**

1. BCAs are potentially self-sustaining, spread on their own after initial establishment.
2. Result in long-term disease suppression in an eco-friendly manner.
3. They do not cause toxicity to the plants.
4. BCAs multiply easily in the soil and leave no residual problem.
5. BCAs control plant disease as well as enhance plant growth.
6. Reduced input of non-renewable resources is required.

## **5. Limitations of Biocontrol**

The durability of BCAs depends on the persistence of its efficacy in space and time. However, inconsistency of efficacy of various BCAs against target pathogens under field conditions have

been reported. This variable efficacy can be attributed to environment variations encountered in field conditions, a lack of ecological competence i.e. survival & colonization ability of the BCA, essential traits of the BCA such as production of metabolites or enzymes and also lack of desired quality of the formulations (spurious products). Microbial products tend to act on more specific targets and have a shorter shelf life.

## **6. Field application of Biocontrol technology- ICAR-FUSICONT bioformulation for the management of Banana wilt caused by *Fusarium oxysporum* f. sp. *cubense* TR4**

Fusarium wilt / Panama wilt caused by *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (Foc TR4) is one of the most devastating diseases of bananas including cavendish group of bananas limiting banana production worldwide. Foc TR4 was found to inflict huge loss in Australia, China, Bangladesh, Indonesia, Jordan, Malaysia, Taiwan and India (Damodaran et al. 2019, 2020). Its severe outbreak in the northern region of India covering the states of Uttar Pradesh and Bihar threatening banana cultivation was reported which further spread to Gujarat and Madhya Pradesh. The ICAR-FUSICONT bioformulation has been proved to be very effective for the management of this disease. The Bio-preparation ICAR-FUSICONT comprising of consortia of growth promoting antagonistic agents *Trichoderma reesei* CSR-T-3 + *Lysnibacillus fusiformis* CSR-A-11 on an IPR protected modified CSR-BIO media. Nursery drenching of secondary hardened tissueculture banana plantlets with 1% ICAR-FUSICONT solution before planting in main field and further, soil drenching with 2% ICAR-FUSICONT mixture at the critical stages of the crop growth was the protocol adopted (Damodaran et al. 2019). Community based evaluation done at 2 locations each in Uttar Pradesh and Bihar. Adopters of the bioformulation recorded an average of 6.08 % disease incidence while non-adopters had 45.68 % disease incidence at harvest during two years evaluation 2018-2020 with about 85 to 92% success in the disease management.

## **7. Conclusion**

Biocontrol is one of the alternative strategies for the control of plant diseases which is environment friendly and fits well with the sustainable agricultural system as well as in the integrated pest management practices. Bio-control agents (BCAs) bring the disease suppression with no environmental hazards and also trigger plant growth. Promising achievements of biocontrol have emerged, however, field adoption needs more attention. The basic requirement is the suitable BCA capable of maintaining itself ahead of the pathogen. The crop environment also plays an important role in determining whether effective population levels of a BCA can be established in competition with the existing microbes. This will also affect the selection of the BCA. Many BCAs are available for use, however, understanding of the interactions among plant, pathogen and the environment will be significant for the effective adoption of biocontrol. Effective biocontrol can be achieved by selecting BCAs that are effective in different conditions, including soil texture, weather parameters including temperature extremes etc. Locally isolated strains of the BCA shall give better results that may adapt well. A consortium / combination of microbes will be more useful since microbial consortia make up a stable rhizosphere that offers more effective control against pathogens (Ram et al. 2018). Farmers are reluctant for using the bio-control strategy due to lack of awareness of technological features, therefore, training and field demonstrations are crucial.



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## Chapter 9

### Disease and Pest Dynamics in Protected Cultivation

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#### 1. Introduction

Protected cultivation is the concept of growing potential crops in the modified natural environment for ensuring optimum growth of the crop plants without any or least stress (Sabir and Singh 2013). In other words, protected cultivation can also be defined as controlled environment agriculture (CEA) which is highly productive, conservative of water and land and also protective of the environment (Jensen 2002).

Over the past 10 or so years, protected cultivation of high-value crops and cut flowers has demonstrated remarkable potential. Protected cultivation has increased in the area of agriculture globally with the development of a liberalised economy and the introduction of improved agricultural technologies. As a result of the greater productivity levels, these technologies are not only opening doors for producers with larger landholdings, but also for those with smaller holdings. By implementing protected farming techniques, growers can anticipate greater and supplementary compensation for superior produce. But in reality, greenhouse vegetable crops grown all over the world are susceptible to a variety of diseases and insect attacks because the covered crops offer stable and favourable microclimates for the growth of pest populations, which frequently limit the effectiveness of this crop production system (Sood 2010).

Pest damage results in significant losses for crops grown in greenhouses, including tomato, okra, capsicum, gerbera, carnation, cucumber, lettuce, and beans. Arthropod pests including mites, whiteflies, thrips, and aphids as well as diseases brought on by viruses, fungi, bacteria, and nematodes, among other arachnids, are the principal causes of crop losses. Among these numerous, insects play a significant role and must be carefully controlled to prevent crop losses and boost yields. The increasing need for higher crop yields both in the field and in greenhouses brings with it problems linked to large monocultures and pest attacks. Chemical pest control has to be reduced owing to its unwanted effects on non-targeted organisms (Biondini et al. 2012) and pest resistance (Liang et al. 2012). Therefore, the need for new, long-lasting, sustainable pest management techniques to increase the activity of beneficial organisms is important (Wratten et al. 2012) (Table 1).

**Table 1. Insect-pests Scenario under Protected Environment in India**

Group	Insect pests	Host	Distribution
Aphids	<i>Macrosiphum luteum</i>	Orchid	Sikkim
	<i>Macrosiphoniellasanborni</i>	Chrysanthemum	Karnataka, HP
	<i>Toxopteraaurantii</i>	Orchid	Sikkim
	<i>Myzusescalonicus</i>	Strawberry	New Delhi
	<i>Aphis gossypii</i>	Capsicum	Punjab, Delhi

	<i>Myzuspersicae</i>	Capsicum, Gerbera	Punjab, Maharashtra
<b>Caterpillars</b>	<i>Spodopteralitura</i>	Tomato, capsicum, rose, cucumber	Karnataka, Punjab, H.P.
	<i>Helicoverpaarmigera</i>	Capsicum, tomato, carnation	Punjab, Uttrakhand, H.P.
<b>Leaf-miner</b>	<i>Liriomyzatrifolii</i>	Chrysanthemum, cucumber, tomato, gerbera, and many ornamentals	Karnataka, H.P.
<b>Mites</b>	<i>Tetranychusneocalidonicus</i>	Cucumber	New Delhi
	<i>Tetranychuscinnabarinus</i>	Carnation	Maharashtra
	<i>Tetranychusurticae</i> (Spider mite)	Cucumber, tomato, capsicum, gerbera carnation,	H.P., Maharashtra
	<i>Stenotarsonemusfragariae</i>	Strawberry	New Delhi
	<i>Polyphagotarsonemus latus</i> (yellow mite)	Capsicum	Karnataka, Punjab, Delhi, H.P.
<b>Thrips</b>	<i>Thrips tabaci</i>	Gerbera	Maharashtra
	<i>Thrips palmi</i>	Gerbera	Karnataka
	<i>Scritothrips dorsalis</i>	Rose	Karnataka
<b>Whiteflies</b>	<i>Trialeurodesvaporariorum</i>	Tomato, cucumber, capsicum, beans, gerbera, and more than 30 hosts	H.P.and Nilgiri hills (TN)
	<i>Bemisiatabaci</i>	Gerbera, capsicum	Karnataka, Punjab

The abundance of food in protected conditions, along with the warm, humid weather, creates an ideal environment for the growth of pests. The natural enemies that keep pests in check outside are frequently absent in protected environment. Because of these factors, pest problems can arise indoors more quickly and severely than outside. The damage inflicted by arthropod pests on greenhouse crops varies with the pest and season.

Integrated pest management is a systematic approach of pest control which incorporates a number of methods and tactics in order to either lower pest populations or mitigate their negative economic effects. It is a site-specific pest management technique that depends on accurate pest identification and understanding of the biology of the pests. With a long-term perspective it is easier to see that an investment in IPM can pay for itself in a higher-quality crop and a cleaner environment (<http://agropedia.iitk.ac.in/>).

## 2. Pest Identification

Correct identification is important to selection of appropriate management practices. Different type of pests are

## 1. Aphids

- Aphids, often known as plant lice, are tiny, sedentary insects with soft bodies that build colonies on the leaves and stems of their host plants.
- They prefer to feed on tender, new growth and suck the cell sap from leaf or apical developing regions.
- Aphid feeding can cause leaves or stems to pucker or curl. In times of high atmospheric relative humidity, they also exude honeydew, on which black sooty mould grows and inhibits photosynthetic activity. They serve as a vector for a variety of viral infections.

## 2. Tobacco caterpillar

- One female lays about 300 eggs, which are grouped together and covered in brown hair.
- The larva is velvety black with lateral white bands and dorsal yellowish green stripes. They pupate in the ground.
- Adult moths have beautiful greyish brown patterns on their fore wings with grey wavy lines.
- They are primarily foliage feeders, but occasionally damage fruits.
- Heavy infestations may occur in isolated locations inside the greenhouse, and timely spot treatments can frequently provide effective control.

## 3. Tomato fruit-borer

- When fully grown, these caterpillars are stout-bodied, 3–4 cm long caterpillars with green or brown stripes. The moths have a little brown mark on each forewing and are light brown in colour.
- Tomato fruit borer consumes both fruits and flowers. The crop may be destroyed by severe infestations of these caterpillars.

## 4. Leaf miners

- Leaf miners are the larvae of tiny flies. By creating mines, they feed between the upper and lower surfaces of leaves, which harms plants. The mines widen as the larva develops.
- Damage is caused by larvae mining the leaves. If the infestation is severe, photosynthetic activity is decreased, which affects plant vigour.

## 5. Mites

- Mites are pests that feed on sap and harm a variety of greenhouse plants. Two species, the yellow mite (*P. latus*) and the two-spotted spider mite (*T. urticae*), are recognised as persistent problems. By puncturing tissue with their mouthparts and sucking out cell contents, these mites obtain their food.
- Two-spotted spider mites, which are pale to dark green in colour with two distinct black dots on the abdomen.
- The two-spotted spider mite can cover the entire plant with fine webbing when it is heavily infested. They often feed on the undersides of leaves, which causes the upper leaf

surface to look mottled or speckled. When mite infestations are severe, plants may lose their vigour and die.

- The yellow mites are tiny, oval, translucent, and greenish. They may infest the entire plant or only the buds, depending on the type of plant that was infected. The foliage of infected leaves may become darker than that of healthy leaves and become deformed, frequently curling inward. Because they are so little, infestations can go unnoticed until the damage is severe.

## **6. Thrips**

- Thrips are tiny, elongated insects. They range in a variety of color, from pale brown to black. Eggs are laid into slits in the tissue by female thrips. Nymphs eat similarly to adults and go through four moults as they grow. Winged adults either fly into the greenhouse or are transported in on contaminated plant material.
- The leaves, flowers, buds, and immature fruits of a crop are all infested by thrips. In order to obtain food, they rasp the plant's surface and sucking up the exuding sap. The appearance of leaves that have been severely affected is mottled or silvery.

## **7. Whiteflies**

- The cotton whitefly (*B. tabaci*) and the greenhouse whitefly (*T. vaporariorum*) are the two species of whiteflies that are most common in protected areas. The former is common in temperate areas, but the other is a significant pest in the nation's tropical and subtropical regions.
- Whiteflies are tiny, 2 mm long, and have a snowy white colour. They flutter from the undersides of leaves when the plants are disturbed. Whiteflies in all life stages are present on the lower surface of the leaves.
- Whitefly nymphs and adults feed on the sap of phloem cells and causes chlorotic patches. Plant growth is hampered by the premature drying of the leaves. Additionally, nymphs excrete a sticky liquid called honeydew, which coats the flower petals and leaf surfaces where the sooty growth develops in humid weather.

## **3. Management strategies**

The IPM programme for protected cultivation can be described as a pyramid constructed having three key components namely, Avoidance/preventive measures; Scouting and early detection; and Curative measures.

### **3.1. Preventive Strategies**

#### **Exclusion**

##### **Use of physical barriers**

Exclusion refers to the use of physical barriers, such as insect proof screens, to prevent insects from entering the greenhouse. Excluding insects is regarded as the first step in creating an

integrated strategy for greenhouse pest management (Bethke and Paine, 1991). Following are some exclusive mechanisms:

### **Use of insect-proof nets**

This includes common greenhouse pests such as thrips, aphids, leaf-miners, and whiteflies in addition to certain uncommon pests like fruit borers. To achieve full exclusion, a screen mesh with holes smaller than 200 micrometres is needed. Whiteflies *Trialeurodes vaporariorum* and *Bemisia tabaci* can be effectively excluded using insect-proof screens of 10 x 20 micron and 10 x 22 micron, respectively (Hanafi et al. 2007).

### **Provision of double door**

Since insects could enter the covered structure on clothing or be carried in by the wind, limited access to screened regions is advantageous. The issue of wind-carried insects is partially resolved by constructing a screened foyer to provide a doubledoor entry.

### **Use of reflective or metalized mulches**

These are mostly utilised for the ability to repel specific insects. Silverleaf whitefly entrance was effectively reduced by 90% using metalized mulch. The greatest overall reduction in whitefly entrance will be achieved by using metalized mulch in conjunction with screens (Hochmuth and Sprenkel, 2015). The entire greenhouse floor should be mulched to avoid weeds and to function as a mechanical barrier to some insect life stages (such as leafminers, thrips, and other lepidopteran pests), preventing them from moving into the soil for pupation.

### **Ultra-violet radiation absorbing sheets**

IPM programmes designed to protect crops from insects and insect-borne viral diseases have successfully exploited changing the visual behaviour of insects as a tactic. A variety of insect pests, including whiteflies, aphids, thrips, and leaf-miners, have been proven to be effectively prevented from migrating from the outside environment into the protected crop by manipulating the UV vision of insects using UV-blocking greenhouse cladding materials. Aphid (*Aphis gossypii*), greenhouse whitefly (*T. vaporariorum*), thrips (*Frankliniella occidentales* and *Scirtothrips dorsalis*) and leaf-miner (*Liriomyza* sp.) populations were found to be lower on tomatoes grown in a polyethylene plastic house treated to block UV rays than on crops grown in a regular plastic house.

### **Sanitation and cultural practices**

#### **Sanitation**

Sanitation includes the elimination of both infested materials and potential sources of infestation, followed by the disinfection of surfaces, and includes a variety of techniques such clean planting material, ventilation, clean or sterile soils tools, flats, and other equipment. Maintain a clean, closely mowed area around the greenhouse to avoid invasion by pests that emerge in outdoor

weeds; remove trash, boards, and old plant debris from the area; remove weeds and other plant waste; and completely clean the greenhouse after each production cycle.

## **Cultural Practices**

### **Pre-season cleanup**

It is crucial to get rid of pests from the previous crop before planting a fresh crop in the greenhouse. Take out all weeds and plant debris from the greenhouse. Many pests can also be found on broadleaf weeds or other crops. Due to this, it's crucial to keep other crops away from the greenhouse and to avoid having dense growths of broadleaf weeds close to the greenhouse's boundaries. Monoculture is advised in protected environments, but if polyculture is necessary, avoid staggered planting. A fallow period of two to four weeks significantly lowers the pest load. After watering, place yellow sticky cards and indicator plants to check for the presence of thrips, whiteflies, leaf-miners, or other insects. After two days, keep an eye out for any insects that are trapped on the cards, and keep watching until the activity comes to an end and only thereafter the decision regarding plantation of new crop be made.

### **Inspection upon arrival**

In protected cultivation, one of the most important points to keep in mind is to begin with planting materials that are insect-free. When new plants arrive at the greenhouse, examine them closely for signs of pest infestation. Remove lower or broken leaves if necessary to prevent the spread of pests.

### **Balanced use of fertilizer**

Follow fertilisation schedules based on a balanced usage of nutrients. Nitrogen should only be added when absolutely necessary for healthy growth. Periodic heavy sprays create nitrogen surpluses that promote excessive growth, which favour the population growth of aphids, and other pests. It has been discovered that applying potassium at the right quantities can lower insect pest incidence.

### **Pinching and Pruning**

The spread of pests in the greenhouse can be stopped extremely effectively by pinching off injured plant parts, blooms, and leaves that are spotted or have insect larvae or egg deposits. The plant waste must be immediately stored in a covered container before being discarded. All of the targeted pests' populations may be decreased by using this method. In order to get rid of numerous leaf-miners and whiteflies that are growing, lower leaves should be pruned after lower fruit clusters have been harvested.



## **Trap crop/Indicator plants**

For early detection and trapping of the target pests, some of the preferred hosts of the target pests can be used. As a trap crop for tobacco caterpillars in a protected environment, *Portulaca oleracea* can be planted in border rows with roses.

## **Plant Quarantine**

One of the reasons for the spread of insects and mite infestations in greenhouses is professionals or workers there. Transporting plants that have mites or thrips should be avoided, and clean, healthy plants should always be handled before moving infected ones.

## **Scouting and early detection**

Monitoring or scouting is the regular, systematic inspection of the plants and exteriors to identify and assess pest problems. Visual examination of the blooms and foliage as well as the usage of sticky or light traps are all part of it. In the greenhouse, isolated areas often serve as the starting point for pest infestations. Timely crop monitoring identifies instances where pests are absent or present at levels considerably below economic damage, hence avoiding needless control treatments and costs.

## **Scouting**

The majority of greenhouse-grown crops use scouting techniques that are based on visual observations to estimate the pest population in a controlled environment (Papaioannou et al. 2012). The typical pests that attack crops grown in greenhouses do not disperse uniformly across the crop. As a result, it is crucial to conduct a systematic, uniform search of the entire greenhouse. Look for any arthropod pests on the entire plant, including the soil's surface. Each time, give the plant a thorough inspection. Examine the more mature leaves, the fragile, young leaves, and the flush growth. It's crucial to examine every leaf on the plant when the crop is young. It's critical because most related arthropod pests prefer the underside of a leaf, it is important to turn the leaves over to check for pests.

## **Monitoring**

It is a relative approach of estimating insect populations in which no direct examination of the plants for insect pest presence is required. However, attractant traps are used to estimate the pest population. Yellow sticky cards (4"x12" or 8"x12") are a useful addition to pest observation in the protected environment for adults of whiteflies, aphids, thrips, and leaf-miners. Additionally, blue-colored sticky traps can be used to catch thrips.

### 3.2 Curative measures

#### Biological Control

The action of parasites, predators, or pathogens in maintaining another organism's population density at a lower average that would occur in their absence is known as biological control (Table 2).

Table 2. List of the key biocontrol chemicals used to combat pests in greenhouses

Target pest	Biocontrol agent	Scientific name
Spider mites	Predatory mites	<i>Amblyseiuscalifornicus</i> <i>Phytoseiulus longipes</i> <i>Phytoseiulus persimilis</i>
Serpentine leaf miner	Leafminer parasitoid	<i>Dacnuscasibiriica</i> , <i>Diglyphusisaea</i>
Various soft-bodied insects and eggs	Green lacewings	<i>Chrysoperlacarnea</i>
Various soft-bodied insects and eggs	Lady beetles	<i>Hippodamia convergens</i> ; <i>Cryptolaemus montrouzeri</i>
Whiteflies	Parasitic wasps	<i>Encarsia formosa</i>
Thrips	Predatory mites	<i>Amblyseius cucumeris</i> <i>Amblyseius mckenziei</i>

#### Chemical control

Chemical control is a well-known method of controlling insects. Insecticides are chemicals that kill insects. When insect pest populations become close to or cross the economic threshold, the only effective option for pest management is an insecticide (Table 3).

Table 3. Chemical formulations used to combat insect pests in greenhouses

Target pests	Chemicals	Reference
Thrips, Whiteflies, Aphids	Imidacloprid @ 0.4g/L, Acephate @ 1g/L or Acetamiprid @ 0.2g/L, Abamectin @ 0.5 ml/L,	Kumar (2007), Sabir (2012)
Caterpillars	Spinosad, Chlorantraniliprole @ 0.3ml/L, Flubendiamide @ 0.1ml/L	Sabir (2010)
Leaf miner	Spinosad @ 0.3ml/L, Abamectin @ 0.5ml/L	Sabir (2010)
Mites	Diafenthiuron, Fenpyroximate, Abamectin @ 0.5ml/L	Shah (2014)

## 4. Diseases under Protected Environment in India

### 4.1. Fungal Diseases

Late blight of tomato and bell pepper: *Phytophthora infestans* and *P. capsici*

Round or irregular lesions that are very big, greenish-black, and water-soaked appear on tomato or bell pepper leaves. These lesions grow quickly and transform from dark brown to purplish-black. Large, hard, brown lesions that seem leathery emerge when the disease affects green tomato fruit, and they are frequently confined to the sides or upper fruit surfaces. When circumstances are moist, infected regions on stems turn brown to black; whole vines may die within a short amount of time. (Tsitsigiannis et al. 2008; Rowe et al. 2010; Shishido, 2011)

**Management:** Following green manuring, within a week of ploughing and then apply *Trichoderma* powder @ 5 kg/ha. Before transplantation, seeds should be treated with Thiram @ 2–3 g/kg of seed (Shtienberget al. 2010). Spray Metalaxyl MZ, 72% WP @ 2 g/liter of water, Copper Hydroxy Chloride, 50% WP at 3 g/liter of water or Mancozeb, 75% WP at 2 g/liter of water. (Japan Green Horticulture Association, 2003; Shishido, 2011; Beniwal, 2015)

#### **Leaf mold of tomato: *Fulvia fulva***

Lower leaves are affected first, then younger leaves. On the upper leaf surface, pale green or yellowish areas appear. These spots later turn yellow in color. The developed fungal patches range in colour from velvety olive green to greyish purple. The spots develop brownish brown as the disease progresses. The leaves then prematurely curl, wilt, and fall (Tsitsigiannis et al. 2008; Mercure 2010; Shishido 2011).

**Management:** For managing the disease, protected horticulture facilities should be maintained warmer than the outdoors at night. Once the tomato crop has been harvested, crop residue should be collected and burned in order to prevent pathogen inoculums. Spray Copper hydroxide 77% WP or Mancozeb 75% WP or Chlorothalonil 75% WP at 2 gm/litre of water. (Japan Green House Association 2003; Shishido 2011).

#### **Graymold: *Botrytis cineria***

On the leaves or flower petals, light tan or grey patches first emerge before becoming covered with a gray-brown fungal growth. Where an infected leaf contacts the stem, elliptical patches may appear on the stem due to infection. Old flower petals are especially prone, usually starting with light watery spots. The fruit skin often splits over the rotted region, and a dark grey fungal growth forms over the area. (Kishi 1998; Tsitsigiannis et al. 2008; Shishido 2011)

**Management:** Adequate plant spacing and effective weed control should be done. Keep the air dry and don't let water accumulate on the leaves. Keep the greenhouse at a greater temperature at night than outside to avoid condensation on the leaves (Shishido, 2011). Registered fungicides, such as Difenconazole 25% WP @ 0.5ml/liter or Chlorothalonil 75% WP @ 2g/liter, should be applied every 5-7 days. (Japan Green House Association 2003).

**Downy mildew of cucurbits: *Pseudoperonosporacubensis*, attacks only cucurbit species, i.e. cucumber, melons, gourds, pumpkins and watermelons.**

The lower, older leaves typically have angular yellow spots on their surface. High humidity conditions may make a dark-gray fungal growth evident on the undersides of the spots. As the disease progresses, the yellow spots get larger, turn necrotic or brownish in the centre, and the browning spreads to the spot edges. These blotches could combine to create significant brown regions on the leaves (Ferguson et al. 2009; Shishido, 2011).

**Management:** All plant portions that have been affected should be removed and thrown away. Downy mildew may be controlled by growing cucurbits in conditions where humidity levels can be adjusted. Dew formation should be minimised by providing appropriate warmth and ventilation in sheltered horticulture facilities (Ushio and Takeuchi, 2006). According to Beniwal (2015), the bioagent *Bacillus subtilis* @ 10 g/liter of water is efficient for treating downy mildew on crops and ornamental plants. For the control of infections, registered fungicides such as, Cymoxanil 8% + Mancozeb 64% WP, Chlorothalonil 75% WP, Fosetylaluminium 80% WP @ 3g/liter, Dimethomorph 50% WP @ 1g/liter or Azoxystrobin 23% SC @ 0.5ml/liter, water should be sprayed.

**Powdery mildew of ornamentals and vegetables: *Oidiumviolae*, *Oidiopsissicula*, *Sphaerothecafuliginea* and *S. humuli***

Older leaves on the lowest parts are affected first. The leaves then develop a thin, white, powdery fungal growth on their surface. Spots are bright white or, in the case of *Oidiopsissicula*., yellowish white. With a severe infection, entire leaf blades might turn dark and dry up. However, fruit is unharmed and diseased leaves seldom fall off from the plant. (Tsitsigiannis et al. 2008; Mercures, 2010).

**Management:** Avoid excessive use of nitrogen in the crops. For the treatment of powdery mildew in vegetables and ornamental plants, Beniwal (2015) found that *Bacillus subtilis* @ 10 g/liter of water is effective. For the management of disease, some registered fungicides, such as Wettable sulphur 80% WP @ 2-3g/liter (Japan Green House Association, 2003), Chlorothalonil 75% WP @ 2g/liter, Azoxystrobin 23% SC @ 0.5ml/liter, Myclobutanil 10% WP, Pyraclostrobin 20% WG @ 1g/liter, or Tebuconazole 25.9% EC @ 1ml/liter water, should be used.

**Sclerotinia stem rot of ornamentals and vegetables: *Sclerotinia sclerotiorum***

Plants with diseased leaves begin to wilt, become gray-green, finally turn brown, curl, and die. To distinguish this disease from other stem and root rot infections, it is important to look for white mycelia and sclerotia on stems. In a few days, infected stem portions die, become tan, and ultimately bleach. This bleached stem is readily shredded and has a pithy texture. Infected plant parts generally have signs of the fungal pathogen as white, fluffy mycelia, and sclerotia on the surface of or embedded in the stem tissue (Kishi 1998).

**Management:** To avoid sclerotia from contaminating the soil, collect and remove all contaminated plant debris as soon it becomes noticeable. In order to get rid of any sclerotia that may have fallen to the ground, the soil around the base of heavily damaged plants should also be collected and thrown away (not composted). Registered chemicals such as Iprodione 50% WP, Thiophanatemethyl 70% WP, and Captan 75% WP are used to control the disease (Japan Green House Association, 2003).

### **Fusarium wilt: *Fusarium oxysporum***

Different host plants are attacked by special forms or races of the fungus: *F. oxysporum* sp. *lycopersici* attacks only on tomato, *F. oxysporum* sp. *cucumerinum* on cucumber, *F. oxysporum* sp. *melonis* on melon and *F. oxysporum* sp. *fragariae* on strawberry (Tsitsigiannis et al. 2008). First signs include a minor vein clearing on the outer leaflets and a drooping of the leaf petioles. Later, frequently before the plant reaches maturity, the older leaves begin to droop, become yellow, and necrotic, and the entire plant may die. In many situations, these symptoms start on one side of the stem and move up until the stem dies and all the foliage wilts. A brown ring can be seen in the region of the vascular bundles in a cross section of the stem close to the base of the diseased plant. The severity of the condition determines how far up the discoloration extends. Fruits that occasionally get an infection decay and fall off (Kishi 1998; Agrios 2005; Tsitsigiannis et al. 2008).

**Management:** Fusarium wilt is soil-borne. Therefore, the most effective and practical method of disease management is the adoption of resistant cultivars. It is essential to use healthy seeds and transplants. Some protected horticultural facilities where this disease has been detected severely presently use soil fumigation with chloropicrin, dazomet, or methyl-isothiocyanate. *Trichoderma* species (10 g/m<sup>2</sup>) and *Pseudomonas fluorescens* (10 ml/m<sup>2</sup>) were applied to the soil to control wilt, according to Beniwal (2015). The disease can be controlled by soil drenching with either copper oxychloride 50% WP at 3g/liter or carbendazim 50% WP @ 1g/liter water.

### **Bacterial diseases in protected horticultural crops**

#### **Bacterial wilt of solanaceous crops: *Ralstonia solanacearum***

The first symptom is chlorosis, stunting and then the entire plant suddenly wilts and dies. When the soil temperature is high (>30°C) and the soil moisture content is high, such extreme symptoms appear. In less favourable circumstances, wilt and decline occur more slowly, and the lower stems frequently develop a large number of adventitious roots. However, a reddish discoloration can be seen in both situations, initially in the vascular system and, in more severe cases, expanding into the pith and cortex, Plant Problem Clinic (2010).

**Management:** Soil fumigation using Chloropicrin is a useful strategy. Practice clean cultivation. Sprays 3 g/liter of copper oxychloride, 50% WP is successful at controlling the disease. Because the bacteria may persist for extended periods in the soil even without a host plant, crop rotation avoiding solanaceous crops often has to be done for 3 years. Additionally beneficial in combating this disease is the application of bleaching powder @ 15 kg/ha. Streptocycline (@ 40–100 ppm solution) seedling dip prevents early invasion and infection by the pathogen through

wounds created during transplantation. Using *Pseudomonas fluorescens* (10 g/m<sup>2</sup>), bleaching powder (300 g/200 m<sup>2</sup>), streptomycin (0.1 g/liter of water), and copper oxychloride (3 g/m<sup>2</sup>) bacterial wilt can be effectively managed (Beniwal 2015).

**Bacterial canker of tomato: *Clavibacter michiganensis* sp. *michiganensis***

Wilting, leaflet curling, and leaf browning are early symptoms of the disease. The petioles stay green and securely attached to the stem despite the leaves dying. The two main categories of symptoms are internal symptoms brought on by bacterial invasion of the vascular tissue and external symptoms brought on by bacterial colonisation of the outer tissues. Fruit may exhibit external symptoms at any age, although they often appear on green fruit initially. On the fruit's most exposed areas, white spots with a diameter of 2 to 3 mm appear. These spots have a dark brown core that rises and is ringed by a noticeable white halo. Thus, they are frequently referred to as "bird's-eye spots."

**Management:** Bacterial canker is particularly difficult to eliminate once developed in a greenhouse. Therefore, it is important to stop the disease from entering and spreading within a greenhouse. It is strongly advised to use treated seed and seed obtained through a "acid extraction" process. When diseased plants are discovered, they should be cut off at the ground line and removed together with the good plants that surround them. By cleaning hands, shoes, equipment, and crop-supporting wires, sanitation is crucial. To eliminate the bacteria, soils and seedbeds must be sterilised using steam or chloropicrin fumigation. In particular, if only exterior symptoms are present, registered compounds like Kasugamycin 3% SL @ 1.25ml/liter and Copper oxychloride 50% WP @ 3g/liter sprays might help in protecting healthy plants. (Shishido 2011).

**Bacterial spot/Angular leaf spot: *Pseudomonas syringae* pv. *lachrymans***

The bacteria creates microscopic, angular lesions on leaves that are water-soaked and eventually turn brown or straw-colored. Leaf lesions are delimited by the veins, causing the angular appearance of the lesions. Bacterial exudates that are white and milky in colour that grow on lesions in humid environments eventually dry to create a thin, white crust. Affected leaf tissue frequently dries up and falls away, leaving the leaves with irregularly shaped holes. Petioles and stems may also develop lesions. Fruit infections first appear as tiny, circular, wet, and soft lesions; but, as they age, they turn chalky and cracked. Fruit spots usually occur when fruits are about half grown. Below the lesions, flesh may be brown down to the seed layer. If attacked when very young, the fruit may fall off the plant (Kishi 1998).

**Management:** Restricting overhead irrigation, using disease-free seeds, rotating crops away from cucurbits for at least two years, and using resistant cultivars and preventative pesticides are all cultural measures that are highly advised. Copper oxychloride 50% WP @ 3g/liter (Hansen, 2009) or Streptomycin sulphate 9% SP + Tetracycline hydrochloride 1% SP (Agrimycin) @ 6g/liter at younger stages of crop growth (do not use during fruiting stages) or Copper hydroxide 77% WP @ 2g/liter or Cuprous oxide 4% DP @ 2 g/liter can be applied at the first sign of disease.

## **Viral diseases in protected horticultural crops**

### **Tomato mosaic virus (ToMV): Genus -Tobamovirus, Family- Virgaviridae**

Light and dark green mottled (mosaic) patches on leaves. On diseased plants, the leaves are frequently small, curled, and puckered. Early-stage infected plants are stunted and have a yellowish tint. TMV might decrease fruit size and production. (Cerkauskas 2004; Tsitsigiannis et al. 2008).

**Management:** Garden, field, greenhouse, and surrounding areas should be weed free. Surface-borne viruses can be removed from seed by dry heating it at 70 °C for four days or at 82–85 °C for twenty-four hours. By soaking seeds for 15 minutes in 100 g/l of tri-sodium phosphate solution (TSP), carefully washing, and spreading the seeds out to dry, ToMV on the seed coat can be removed. Diseased seedlings with twisted leaves, mosaic patterns, or aberrant growth should be removed. Before transferring from infected to healthy regions, clean your tools, stakes, and equipment.

### **Tomato yellow leaf curl virus (TYLCV): Genus- Begomovirus, Family- Geminiviridae**

Plants that are just beginning to grow exhibit more severe reduced internodes and stunted growth. The young leaves have margins that curve upward, giving them a cup-like appearance. They are also significantly smaller and wrinkled, with yellowed (chlorotic) leaf edges between the veins. Flowers may appear but usually will drop before fruit is set.

**Management:** Insecticides and resistant crop cultivars are the most effective measures for TYLCV control. Thiamethoxam 25% WG @ 200g/ha or imidacloprid 17.8% SL @ 250ml/ha should be used to control the disease (Poston and Anderson, 1997). Planting resistant/tolerant lines (such as Globe, Roma, Cherry, Tygress (Seminis), HA-3068, HA3073, HA-3074, HA-3371, TY02-1155, TY02-1184, TY02- 1276, TY02-1298, TY02-1314 (Hazera) etc.), crop rotation, and TYLCV resistance breeding are further approaches to prevent the spread of the virus.

### **Tomato spotted wilt virus (TSWV): Genus Tospovirus, Family Bunyaviridae.**

Wilting, stem mortality, stunting, yellowing, inadequate blooming, sunken patches, etches, or ring spots on leaves are all symptoms of diseased plant. Initially, leaves in the terminal region of the plant stop developing, get deformed and turn light green. The veins in young leaves thicken and become purple, giving the leaves a copper appearance. On diseased leaves, necrotic patches or ring spots usually appear. Infected plant stems frequently feature purplish-brown streaks. Immature, diseased fruit may show multiple rings, spots, and blotches in addition to being deformed. Yellow patches with concentric circles or necrotic streaks may appear on ripe fruit. (Tsitsigiannis et al. 2008).

**Management:** Remove and destroy diseased plant materials. Thrips are attractive to blue or yellow sticky cards, which are excellent for population management. To get rid of thrips linked with agricultural trash, soil can be fumigated if practical using metham sodium (Vapam) or 1, 3-

dichloropropene (Telone). Use systemic insecticides such as Thiamethoxam 25% WG at 200g/ha or Imidacloprid 17.8% SL at 250ml/ha to control the disease (Singh 2015).

### **Cucumber mosaic virus (CMV): Genus- Cucumovirus Family- Bromoviridae**

Symptoms initially appear on the youngest, still expanding leaves, developing a greenish yellow to dark green mottling. One of the most typical expressions is a severely stunted, nonproductive plant with dull, leathery pale green leaves that lacks any recognisable foliar patterns. Fruit from cucumbers may contain dark green "warts" or yellow and green speckling. Cucumber fruit produced in the later stages of the disease is sometimes smooth and pale whitish green (called "white pickle") and more blunted at the ends than fruit produced on healthy vines (Tsitsigiannis et al. 2008; Zitter and Murphy 2009).

**Management:** In and around fields, eliminate all biennial, perennial weeds and wild reservoir hosts. Keep weeds and other vulnerable plants, especially those on ditch banks, hedge or fence rows, and other sites, at least 10 yards away from susceptible crops. To keep aphids out, grow seedlings in a building or seedbed that is enclosed in netting with a mesh size of at least 32. Discard any seedlings or young plants that display viral symptoms. Rotating chemical spray with 2% neem seed kernel extract is also useful for controlling insects. Imidacloprid 17.8 SL sprayed at 1 mg per litre of water to suppress sucking insects

### **Conclusion**

The most intense method of crop production is greenhouse farming. This sector has the highest investment and labour expenditures than in any other, but also the highest yield and quality of produce. The production of seasonal and non-seasonal vegetable crops in greenhouses can maximise crop yield per unit area and improve food quality throughout the year around. Insect pests include aphids, silverleaf whiteflies, mites, and thrips, as well as a number of diseases brought on by fungi, bacteria, viruses, and other organisms, negatively impact crops. The warm, humid climate of a greenhouse is perfect for the growth of a variety of foliar and soil-borne plant diseases, as well as insect pests that are tough to eliminate. To stop the spread of diseases and insect pests in greenhouse soil and aerial environments, it is necessary to integrate cultural, physical, biological, and chemical control alternatives. An integrated strategy can be used to handle greenhouse pests effectively. In order to control insect pests and diseases, it is essential for greenhouse producers in India to employ as many IPM exclusion measures as they can.

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## Chapter 10

### Monitoring of Biotic Stresses and Estimation of Real Time Yield Losses through Modern Tools and Techniques

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#### Abstract

Effective crop protection requires early and accurate detection of biotic stress. Early detection, timely forewarning and crop yield loss estimation due to biotic stresses at regional scale is not only cumbersome, costly but also at times is difficult. In recent years, remarkable results have been achieved in the early detection of weeds, plant diseases and insect pests in crops. These achievements are related both to the development of non invasive, high resolution optical sensors space / air borne remote sensing and data analysis methods that is pattern recognition, machine learning that are able to cope with the resolution, size and complexity of the signals from these sensors. With the advent of hyperspectral radiometry, it has been possible to have insights into more details and better understanding of the crop stress induced by insect pests and diseases. Weather based crop simulation models integrated with remotely derived information may help in estimation of crop yield losses on real time basis.

#### 1. Introduction

Pests and diseases cause serious economic losses in yield and quality of many cultivated crops, which is estimated at 14% of the total agricultural production (Oerke et al. 1994). Timely detection, regional scale monitoring and forewarning of biotic stresses are important for effective crop protection. With the advent of hyper-spectral radiometry, it is possible to have insights into more details and better understanding of the crop stress induced by insect pests and diseases. Remote sensing of biotic stress is based on the assumption that stress interferes with photosynthesis and physical structure of the plant at tissue and canopy level, and thus affects the absorption of light energy and alters the reflectance spectrum (Prabhakar et al. 2012). High resolution optical sensors on air or space borne remote sensing along with data analysis methods i.e. machine learning/pattern recognition enhances the scope of accurate detection & forewarning. Reflectance data obtained by ground based remote sensing provides vital information to understand spectral interactions between pest damage on the host plants and also to collect fundamental ground-truth information required for interpretation of remote sensing data obtained from space/air borne platforms. Satellite remote sensing provides sufficient data for large scale studies, but has limitations of temporal, spatial resolution, and more importantly, availability of cloud free data. On the other hand, airborne systems have a high resolution, time flexibility and provide sufficient lead time for dissemination of crop protection advisory. In this lecture, an attempt has been made to present past experiences on use of modern tools i.e. remote sensing, GIS and machine learning for monitoring/analysis of biotic/abiotic stresses. Similar approach may be attempted in future to consolidate and improve monitoring/forewarn of biotic stresses at regional scale so that farmers can be advised timely for their management. The

application of such techniques in estimation of yield losses may also help policy makers in strengthening network for monitoring biotic stresses so as the yield losses may be minimised.

## **2. Examples of Monitoring Abiotic Stresses**

Most of the major irrigation canal commands, particularly in arid and semi-arid regions, are facing the problem of water scarcity in addition to land degradation due to waterlogging and salinity. A remote sensing based procedure for estimating water productivity in large areas or irrigation commands and diagnosing salt affected cropped lands have been illustrated in Sone Low Level Canal command and Western Yamuna Canal command, respectively (Ambast et al., 2008). Landsat-TM data have been used to evaluate the extent of cultivated area, water availability and its distribution, crop yield and water productivity in the winter season.

### **2.1 Monitoring of water stressed area:**

Regional Evapotranspiration through Surface Energy Partitioning (Ambast et al. 2002) was estimated on Landsat5 image of SLLC system to determine water stressed area in the canal command (Fig. 1,2). The model combines analytical and empirical relationships. Net radiation was obtained from distributed hemispherical surface reflectance and surface radiation temperature data in combination with spatially variable zenith angles for the determination of the clear sky incoming shortwave radiation. The incoming longwave radiation was hold areally constant. Soil heat flux density is computed from an empirical soil heat/net radiation flux density fraction. The area effective momentum flux density was obtained from the area effective aerodynamic resistance for dry land surface elements using the slope between surface temperature and surface hemispherical reflectance. The vertical near surface air temperature difference is coupled linearly to surface air temperature.

The vertical difference between two horizontal layers of different air temperature was obtained by an inversion of the equation for sensible heat transfer at specific partial areas corresponding with two extreme conditions i.e. where  $H = 0$  (wet) and one where  $\lambda E = 0$  (dry). These partial areas can be allocated by means of the temperature-albedo relationship. The momentum and sensible heat flux densities were based on the resistance version of the flux profile relationships. Finally, latent heat flux density was obtained as the residue of the land surface energy balance. The latent heat flux for free water surface was observed  $360 \text{ Wm}^{-2}$  whereas, for cropped land it was in the range of  $240\text{-}360 \text{ Wm}^{-2}$ . The frequency distribution indicates two distinct groups in the range of  $240\text{-}280 \text{ Wm}^{-2}$  within the cropped land. In absence of ground information on energy flux in the present study, the validation was made by comparing the actual evapotranspiration (ETa) for well water crop determined by energy balance and ETa measured by precision electronically weighing lysimeter for the wheat crop, which is a major crop of the region. The moving average for 5 days was preferred to compare the daily ETa because of use of historical data. For normal crop, ETa valued determined through energy balance was in the range of  $1.6\text{-}2.5 \text{ mm day}^{-1}$  whereas the 5-day moving average ETa for the day was  $1.5 \text{ mm day}^{-1}$ , which was considered quite comparable.

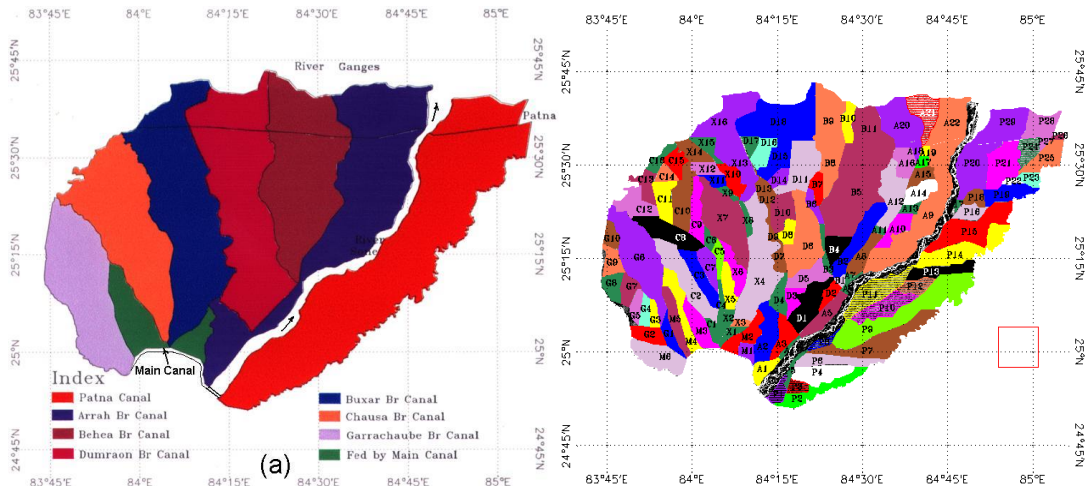


Fig 1. A digitized map of the Sone Low Level Canal system, India

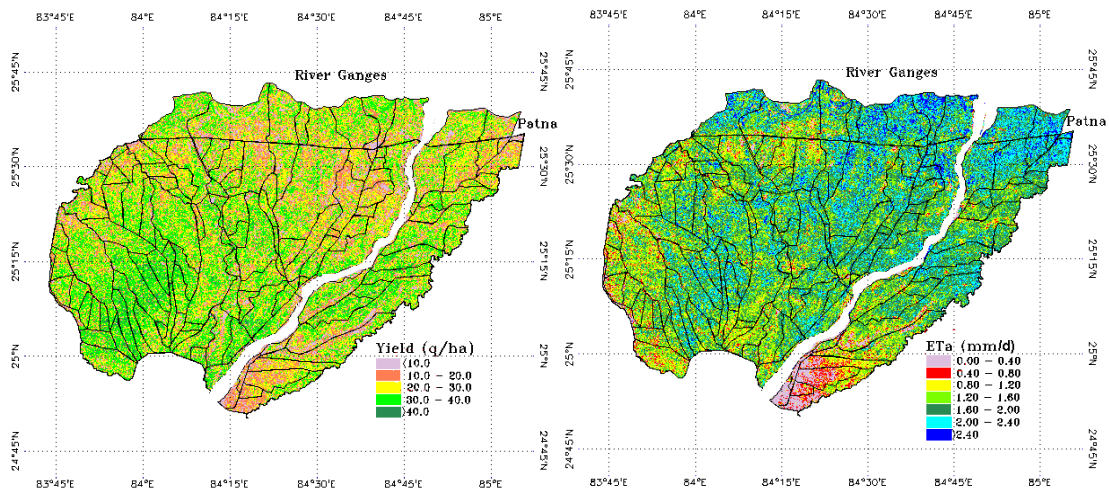


Fig.2. Images of (a) yield and (b)  $ET_a$  at distributary level

## 2.2 Monitoring of waterlogged and saline lands

Absence of total vegetation on severely waterlogged or salt-affected soil surface makes it possible to detect waterlogged and saline surfaces directly from the remote sensing, whereas it is difficult to identify slightly or moderately affected waterlogged and salt-affected cropped land directly. But such waterlogged and saline cropped land have very distinct reflectance characteristics due to patchy crop growth and can be identified using bio-physical approach with low <sup>(a)</sup>albedo-low NDVI and high albedo-low NDVI, respectively (Ambast et al. 1999). After evaluating the histogram of  $\alpha$  and NDVI, an approach, based on a guiding principle that the  $\alpha$  decreases with increase in water logging and increases with increase in salinity, has been developed (Fig. 3). The reduction in crop condition was considered in both the cases. The various signatures were generated assigning  $\alpha$  and NDVI values. The signature for bare soil and saline surface was assigned based on their spectral characteristics. The procedure has been applied on the satellite image of Bhalaut Canal command defining seven crop classes i.e. slightly, moderately and severely waterlogged crops, slightly, moderately and severely salt-affected crops, and normal crops (Fig. 4).

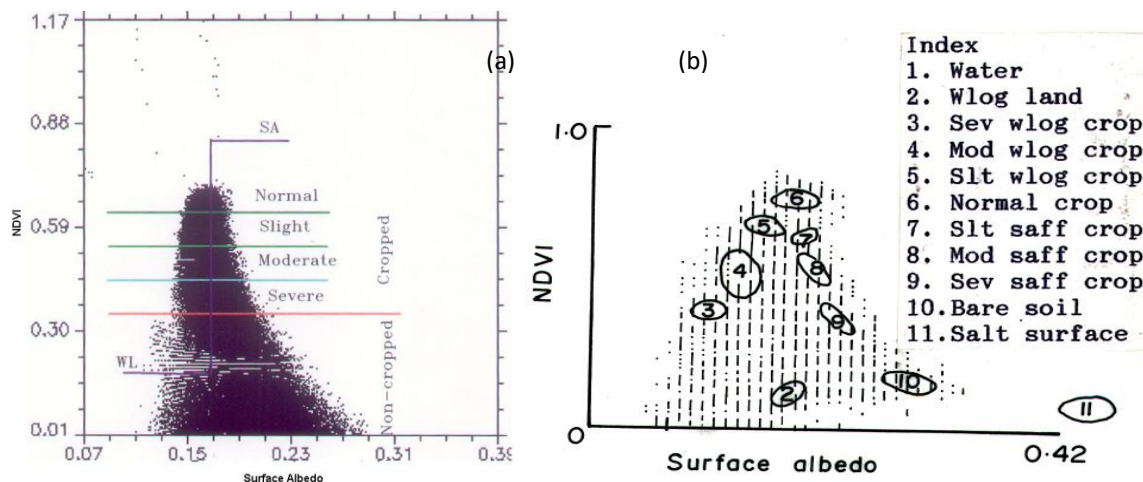


Fig 3. Scatter-gram between surface albedo and NDVI

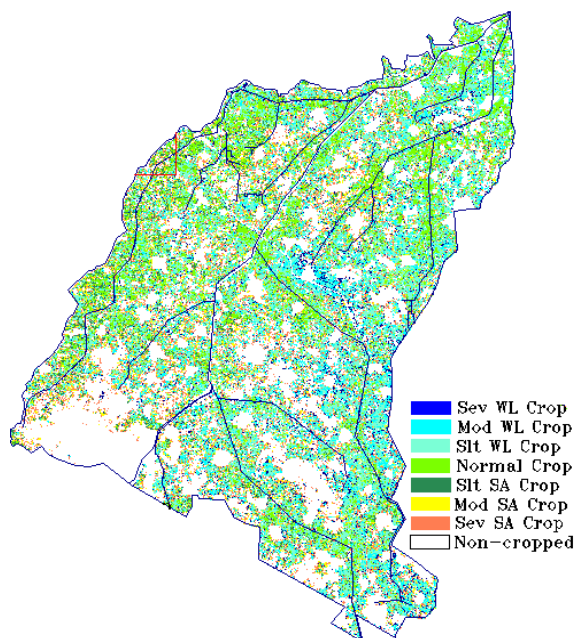


Fig.4. Waterlogged/salt-affected crops in the Bhalaut branch canal command, WYC Haryana

The statistical analysis of the canal command indicated 8% area as normal (more than 4 t/ha), 26% as slightly affected (3-4 t/ha), 18% as moderately affected (2-3 t/ha) and 13% as severely affected (1-2 t/ha) land under wheat crop. About 15% area is estimated as non-cropped (less than 1 t/ha) and 20% area as built-up and other area. The economic analysis indicated an average loss of 4320 Rs/ha due to water logging and soil salinity in the command, which is about 18% of the potential. The estimation of economic losses by remote sensing technique costs at 0.75 Rs/ha. The economic analysis indicated an average loss of about 18% of the potential due to waterlogging and soil salinity in the command (Table 1). Similar technique may be employed in monitoring of biotic stresses and economic losses caused due to it.

**Table 1. Estimation of economic losses in the Bhalaut branch canal command**

Class	Affected land (%)	Yield (t/ha)	Losses (t)
Normal crop	8.0	>4.0	--
Slightly affected crop	26.0	3.0-4.0	11258
Moderately affected crop	18.0	2.0-3.0	23382
Severely affected crop	12.8	1.0-2.0	27712
Non-cropped land	15.0	<1.0	51960
Total Loss (t)			62352*
Total Loss (M Rs)			377*
Total Loss (Rs/ha)			4320*
Total Loss (% to potential)			18*

\*Excluding non-cropped land

### 2.3. Approaches for Monitoring Biotic Stresses

Ample work has been done both at nation and international level on forecasting of biotic stresses on different crops and yield loss assessment (Dhar 2007; Kumar 2018). Statistical regression/correlation analysis were used for forecasting incidence of insect pests attack but were found of limited use (Trivedi 1998; Srivastava 2010) and weather based composite forecasting IASRI model which was found reasonably good (Mehta 2000; Agrawal 2007; Kumar 2018). Meteorological and satellite data based forewarning model for brown plant hopper has been attempted by Rana et al. (2017). Android based mobile apps for improved crop protection (Vennila et al. 2018). An information and communication technology (ICT) based intelligent pest and disease forewarning system for cotton based on microclimatic parameters was developed for providing forewarning on pests and diseases of cotton crop (Madasamy et al. 2020). ANN technique (BP) provided better model accuracy than the regression methods in prediction of pest incidence in rice (Yang et al. 2010). Artificial Neural Network assisted weather based plant disease forecasting system was developed with accuracy of 80-87% (Bhagawati et al. 2015). Machine learning techniques in disease forecasting: a case study on rice blast prediction (Kaundal et al. 2006). Satellite derived crop phenology, ground meteorological observation and machine learning has potential in forecasting of rice pest population occurrence as studied in Central Plain of Thailand (Skawasang 2019). A conceptual framework of integrated approach for timely forewarning and yield loss estimation is presented in Fig. 5.



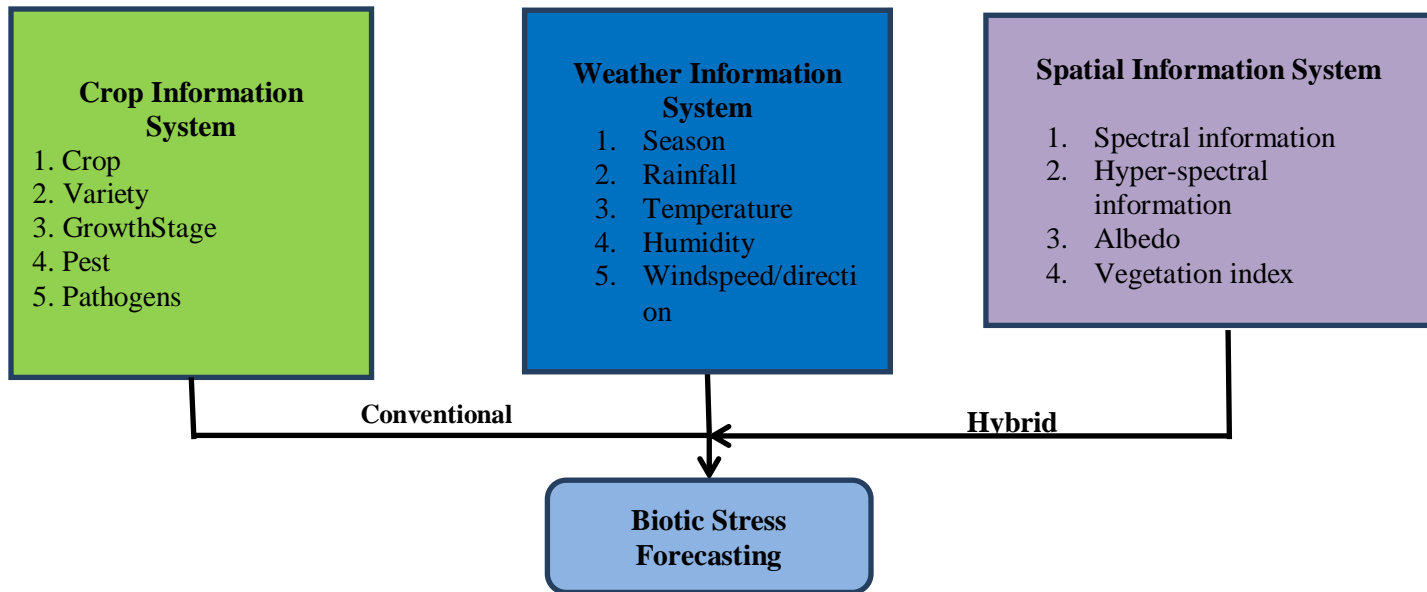


Fig 5. A conceptual approach for integrated model

### 3. Conclusions

With the advancement of air/space borne remote sensing along with machine learning techniques, analysis of spatial variability of biotic stresses and a synoptic view of the large area in a non-destructive and non-invasive way is possible. These achievements are related both to the development of noninvasive, high resolution optical sensors air or space borne remote sensing and data analysis methods that is pattern recognition, machine learning that are able to cope with the resolution, size and complexity of the signals from these sensors. With the advent of hyper-spectral radiometry, it has been possible to have insights into more details and better understanding of the crop stress induced by insect pests and diseases. Satellite remote sensing provides sufficient data for large scale studies, but has limitations of temporal, spatial resolution, and more importantly, availability of cloud free data. On the other hand, airborne systems have a high resolution, time flexibility and provide sufficient lead time for dissemination of crop protection advisory. Hence it can supplement many of the on-going field surveillance programs, which is often expensive, time consuming laborious and many a times error-prone.

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## Chapter 11

### **Plant Protection through Indigenous Traditional Knowledge (ITK) for Sustainable Agriculture**

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#### **Abstract**

The development of indigenous knowledge (IK) systems is cumulative, representing generations of experience, careful observations, trial and error experiments. The people have evolved ecologically sound technologies to deal with issues related to agro-ecosystem management. Indigenous technical/traditional knowledge (ITK) is community, local and rural in origin. The rural people have intimate knowledge of many aspects of their surroundings and adopt them based on needs to solve local problems in managing agricultural and related activities. It is used in management of insect pest and disease of plant. The ITKs are eco-friendly and even compatible to other management practices, contributing towards pesticidal residue-free agricultural products.

**Keywords:** Indigenous technical knowledge (ITK), Ecology, Disease, Insect, Residue-free

#### **1. Introduction**

Indigenous Technical Knowledge (ITK) is a term used to describe the distinct traditional local knowledge that exists within and is developed around specific conditions by indigenous women and men in a certain geographic location. Indigenous technological knowledge covers a wide range of fields, spanning crop production, animal raising, management of natural resource, preparation of food, healthcare, insect pest control, and so many more. Indigenous Technical Knowledge (ITK) is a population's actual knowledge that reflects traditional experiences as well as more recent encounters with new technologies. Indigenous agricultural practices (IAPs) are a body of knowledge that is not written down. There is no systematic record of who they are, what they do, and how they do it, as well as how they can be modified, their operations, boundaries, and applications. It exists in as many distinct brains, languages, and skills as there are now in as many diverse groups, civilizations, and circumstances. As a result, the Indian people are under tremendous pressure to collect, conserve, validate, and use IAPs in order to reduce reliance on external inputs, lower cultivation costs, and promote environmentally friendly agriculture.

Plant protection has become a serious matter due to changes in climate. The ecology and biology of different insect pests are also changing which makes pest control mechanisms more difficult and complex (Singh et al. 2019). Chemical pesticides are used in agricultural field indiscriminately which has resulted in resistance development in pests along with environmental degradation (Gill and Garg 2014). Pest management approaches are different among traditional farmers practicing traditional farming systems in different regions of the

country. Indigenous pest management practices generally involve use of locally available resources for the successful cultivation of crop plants and hence are eco-friendly and sustainable. ITK being applied in pest management have inherent characteristics of cultural and environmental compatibility as well as sustainability with cost-effectiveness (Pradhan et al. 2017).

## **2. ITK in Weed Management**

### **2.1. Sieving paddy seeds**

Before sowing, farmers sieve rice seeds in order to separate the seed of weeds. Since most of the weed seeds are smaller than rice seeds, they are filtered out in sieves. This is prevalent in Arunachal Pradesh (Singh 2003).

### **2.2. Control of wild rice by growing purple rice variety (R - 575)**

This practice is adopted to control the wild rice in rice field by growing the purple colored rice variety R-575. The practice is followed by farmer in Himachal Pradesh. The wild rice is locally called nirsan is a major problem in upland rice growing areas. The wild rice matures much earlier than the cultivated rice and shatters its grain in the field. Due to this, it becomes practically impossible to eradicate this weed from rice field. Since, this weed is of same species as that of cultivated rice, no herbicide can be used to control this weed. Farmers of the mentioned areas where this problem exists adopted a practice of growing purple colored rice variety (R-575) in the infested fields. This variety of rice has purple colored foliage, which is distinctly different from wild rice. During the weeding operation, farmers remove green colored wild rice from their fields, thus eradicating this weed from their field. The farmers are practicing this for the last 45 years (Sharma, 2003).

### **2.3. Use of paddy stalk to suppress weeds and conserve moisture in sandy loam soil**

During first week of June, paddy stalks are spread in a thick layer on sugarcane field. The layer acts as a cover to decrease the intensity of rain and suppress germination of weed seeds. After decomposition it is converted into organic matter, which is available to the succeeding crop, *i.e.* wheat or greengram. This technology is quite useful for the crop grown on sandy soil, and sugarcane crop can sustain moisture stress up to 20-30 days. About 70-80% weed population is controlled by this practice. Reported by Dr Ranjay K. Singh, Assistant Professor (Agricultural Extension and Rural Sociology), College of Horticulture and Forestry, Central Agricultural University, Pasighat (Arunachal Pradesh).

### **2.4. Control of *Parthenium hysterophorus* weed**

To control carrot-weed grass (*Parthenium hysterophorus*) in the agricultural fields, construction of pit is in practice. Digging a pit with 2 m length, 2 m breadth, and 4 m depth is recommended. Weeds collected from the fields are deposited in this pit. Since spores remain at the bottom of the pit, its spread is controlled resulting in check of Parthenium. Reported by Shri D. Kuppusamy, Gangavaram, Gingee, Villupuram (Tamil Nadu).

## **2.5. Wooden blade harrow for intercultivation of cotton:-**

Farmers of Manmarri village of district of RangaReddy in Andra Pradesh do inter cultivation in cotton by a blade harrow frequently. This tool is made of wooden beam of 3 m length, fixed to the main wooden frame that is tied to the yoke of one pair of bullocks. The farmers with this tool can cover intercultural operations of 1 ha in a day costing Rs 120. This tool is used not only to control the weeds in both directions but is also useful to increase the water-infiltration capacity in vertisols. Reported by Shri V. Srinivas, Research Associate, TARIVLP, Central Research Institute for Dryland Agriculture, Hyderabad (Andra Pradesh).

## **2.6. Use of *Sirangtheirong* leaves to control weeds in paddy field**

Farmers of Nagainga village of district Ukhrul, in Manipur collect fallen leaves of a local tree, called sirangtheirong (*Juglans regia*) and broadcast the leaves in paddy field. Thickness of layer is up to 1-2 cm. When the decomposition starts, weed growth is suppressed. Simultaneously many cutting and chewing type of insects are controlled. About 35-40% inhabitants use this old practice. Reported by Dr Ranjay K Singh, Assistant Professor (Agricultural Extension and Rural Sociology), College of Horticulture and Forestry, Central Agricultural University, Pasighat (Arunachal Pradesh).

## **2.7. Mini tractor-drawn ridger, weeder and sugarcane leafmulcher**

The ridger is developed as an attachment to the three-point hydraulic linkage of the mini tractor. The ridger comprises an upper main frame, a central shank and a ridger bottom. The distance between the outer edges of the rear wheels of the mini tractor is 1.2 m and total width of the implement is about 1 m. Hence in crop rows of 1.5 m normal spacing, the implement can be very easily used without damaging the crop. The cutting tool penetrates and cuts the soil. The soil is cut up to 22.5 cm depth and is lifted on the wing board on each side. The lifted soil is thrown on both sides. The ridger-weeder is unique as it does both weeding and earthing-up in a single operation in sugarcane crop. There is a saving in cost and time by more than 60%. Since it helps in trash mulching and obviates the need for its burning, the ridgerweeds also contributes towards conserving moisture and improves organic matter content in the soil. Reported by Shri P. K. Jeyakrishnan Facilitator: National Innovation Foundation, Ahmedabad (Gujarat).

## **2.8. Wooden blade harrow for inter cultivation of castor**

Farmers of Manmarri village frequently do intercultivation in castor by a blade harrow, which is sown by rectangular planting. This tool is made of wooden beam 45 cm wide, fixed to wooden pegs. Blade made of iron or mild steel is fixed to the wooden pegs. Two wooden beams with 3 m length are fixed to the main wooden frame which is tied to the yoke of one pair of bullocks. The farmers with this tool can cover intercultural operations of 1 ha in a day, costing Rs 120. This tool is useful not only to control the weeds in both directions but also to increase the infiltration capacity in vertisols. Reported by Shri V. Srinivas, Research Associate, TARIVLP. Central Research Institute for Dryland Agriculture, Hyderabad (Andra Pradesh).

## **2.9. Weedmanagement through salt application:**

Weed control in rice by using common salt. Common salt is dissolved in water and sprayed in rice fields for controlling major weeds. This practice is being used by 40 to 50% farmers of Makarbili village in Nawadpada district of Orissa with 60% effectiveness in controlling weeds. Reported by Sarbani Das (2003)

- The optimum dose for effective weed control is 1 kg of salt dissolved in 12 L of water
- The salt was applied in two sprays, one after sowing and another at active tillering stage
- Optimum rate as 120 kg/ha

## **2.10. Kopar Technology for weed management**

Kopar is a traditional method of farm practice in Chhattisgarh. Mr. P. C. Agarwal a progressive farmer of Kharora has used this technology for the management of biotic stresses (weeds, insect pests and diseases) in his own farm at Kharora and found very encouraging results over chemical control measures. As per Mt Agarwal, the technology could increase the yield by 20-30 per cent depending upon the variety, stage of the crop and number of Kopar operation in the crop. The Kopar technology has been demonstrated at farmer's field, Jaroda (Mr. Santosh Sahu farm) and ICAR-NIBSM farm in the paddy crop during *kharif* 2017. Mr. Sahu has used Kopar technology thrice (20, 30 and 40 DAT) in the paddy crop for the management of weeds in paddy (Cv. Swarna). The number of tillers was more (14-15/paddy plant) and the level of insect pests and diseases were negligible as compared to non-Kopared crop (8-10 tillers/ paddy plant). He recorded 26 q/1.5 acre in Kopared crop as compared to non-Kopared crop (20 q/1.5 acre). Whereas at ICAR-NIBSM farm, the yield was 21.36 q/acre (Cv. Swarna) and 19.82 q/acre (Cv. Mahamaya) in Kopared crop as compared to non-Kopared crop (16 q/acre in Mahamaya and 17.5 q/acre in Swarna). Reported by Dr. Anil Dixit, Dr. K.C. Sharma and Dr. P. Mooventhan.

## **2.11. Weed management in rice main field**

Preparing and leveling the main fields uniformly without undulations. Plough the land during summer to economize the water requirement for initial preparation of land. Flood the field 1 or 2 days before ploughing and allow water to soak in. Keep the surface of the field covered with water. Keep water to a depth of 2.5cm at the time of puddling. This practice help to reducing weed through puddling. Courtesy: TNRRI, Aduthurai (T.N.)

## **2.12. Biasi, Halod or Beushening in standing direct sown paddy**

Practice of ploughing in dry sown paddy in standing water at 25-30 days after sowing. This is useful to suppress the weeds and thinning the excessive plant population (H.P., Orissa and Jharkhand and Chhattisgarh). This ITK is practiced by all categories of farmers on individual basis. This is an age old practice. The area covered under this practice is 90 to 95%. In this method, furrows of depth ranging from 15 to 20cm are opened (when the crop is 30-40 days old) with animal drawn indigenous (desi) plough (Plate 14). This operation helps to maintain plant population, to reduce weed infestation by uprooting and buried into the soil, this will also help in

increase to prevent leaching, to induce tillering and to increase organic content. The area of coverage is about 0.5 ha/day. The cost of ploughing operation is Rs. 400 to 500/ha. (Sinha et al. 2015).

### **2.13. *Kharif* fallowing for assured *rabi* crop**

In *kharif* where raising of successful crops involves risk, farmers keep the land fallow. This practice, besides conserving moisture for *rabi* crops is useful to distribute peak demand of labour, draft power, build up native soil fertility and efficient management of weeds. Prevalent in deep black soils with low rainfall regions of Western Maharashtra and high rainfall areas of Madhya Pradesh. (Subba Reddy and Singh 2001).

### **2.14. Control of weeds in rice by using leaves of Karada**

Leaves of karada (*Cleistanthus collinus*) plants (100-150 kg/ha) are placed in the standing water as a result of which the water becomes blackish in colour. It is believed that it kills Gundhi bug and some aquatic weeds of paddy (Bhubaneshwar, Orissa) (Panda, 1992.)

### **2.15 Ploughing in standing maize for weed control**

The interspaces in standing maize field are ploughed when it has attained a height of 15-30 cm for weed control. Ploughing strengthens the root system of the maize crop by loosening the soil which enhances aeration and water filtration (H. P.) (Verma, 1998).

### **2.16. Control of weeds by crop rotation with wild sesamum**

For weed control, practices like interculturing and handweeding are not so convenient due to the reasons like broadcast sowing, large size of land holding and sloppy as well as undulating land topography. Ramtal (wild species of sesamum) is grown in the field for suppressing the growth of weeds. Growing it once can reduce more than 50% of weed population. Ramtal plant grows faster in the initial stage and suppresses the growth of weeds (Bharuch, Gujarat). (Vasava, 1992)

### **2.16. Use of dry leaves for mulching in cardamom**

Mulching with dry leaves in cardamom helps in retaining moisture in soil by reducing evaporation and to check weeds (Karnataka) (Anonymous, 1999).

### **2.17. Use of finger-millet husk as mulch**

Finger-millet husk used as mulch is less affected by termites, checks evaporation and weed growth. Termites fail to get a foot hold and hence their incidence is reduced (Karnataka) (Anonymous 1999).



### **2.18. Control of weeds through mango leaf mulching**

Farmers use mango leaves for mulching to control motha weed (Gonda, U.P.) (Anonymous, 2000).

### **2.19 Control of weeds through mango leaf mulching**

*C. dactylon* and *C. rotundus* in sugarcane, grapes and pomegranates is managed by growing *Amaranthus* (Sholapur).

### **2.20. Control of *Cynodondactylon***

Dry stalks of cumin plant are spread over the field in summer. In the monsoon, the rain water gets absorbed in the stalks before filtering down the soil. It is believed that water filtering through the cumin stalks prevents the germination and growth of this weed. It takes one year for the stalks to decompose. (Gujarat) (Thakor 1996).

### **2.21. Cotton shells for control of *Cyperusrotundus***

These cotton shells are broadcast in the field where patches of this weed are found. It is layered up to 3 inch thickness in these patches one month before rainy season begins. The field is then cultivated by plough to incorporate it well into the soil. This kills the root of the weed. By this practice, the field remains weed free for about 2-3 years. (Gujarat) (Gami 1996).

### **2.22. Control of Striga in Sorghum**

Seeds of suva (*Anthemumgraveolens*) are incorporated with sorghum seeds approximately 3-4 kg/ha at the time of sowing to avoid the emergence of striga. Suva is very slow growing and is suppressed at later stages by Sorghum. Farmers divert the harvested rainwater of adjoining fields into the striga infested fields in Saurashtra. Cut branches of *Calotropis* sp. are kept at the entrance of the rain water channel. This practice minimizes the striga population to a great extent, if repeated for several times during raining days ( Bharuch, Gujarat) (Ganchi 1992).

### **2.23. *Cuscuta* management in Niger**

Soil drenching with jackfruit leaf extract @ 1-2.5 litres diluted with 1000 litres of water 2-3 days after sowing restricts the germination of *Cuscuta*. (Koraput, Orissa) (Dixit et al. 2002).

## **3. ITK for Plant Disease Management**

- Soaking of paddy seeds in milk increases its resistance against 'tungro' virus and 'stunt' virus.
- For control of red leaf spot disease in paddy, the seeds are soaked in 'Pudina' leaf extract (*Mentha sativa*) for 24 hours.
- Make a solution with 2 kg fresh papaya leaves in 3-4 litres of water and leave overnight. After filtering, the solution is diluted with 50-60 litres of water and 250ml soap solution added to control rice brown spot disease.

- To treat fungal disease, such as damping off and dieback, farmers used to spray fresh cow dung in the chilli plant's collar region.
- Farmers used fresh cow dung slurry (1 kilogramme cow dung in 5 litre water) to treat ginger and turmeric seeds for disease management and enhanced germination.
- Control of nematodes in banana plantation by use of neem castor and ground nut cakes Before planting banana suckers, apply 60 cartloads of well rotten compost manure/acre. Prepare fields by repeated ploughings. Beds are formed and suckers are planted in a 6' × 6' geometry. Then neem cake and castor cake in equal proportion is applied (25-30 g) around each sucker after 60 days of planting. Field should be irrigated regularly. During the 8th month, 50 g of groundnut-cake is applied. This ensures protection of banana plants from nematodes (Virudhudunagar, Tamil Nadu).
- Tribal farmers use fermented solution of 5 kg of cow dung, 5 litre of cow urine, 150 gm lime + 100 litre of water to control khaira disease, bacterial and viral diseases in paddy.
- To control the papaha disease (tip burn) of paddy, farmers drain out the standing water from the field.
- Cowdung is mixed with water thoroughly and kept for 3- 4 hours till the coarse materials settle down. The solution on top is filtered and sprayed on paddy leaf for control of bacterial blight. Bactericidal action of cowdung helps reduce the population of the bacteria (*Xanthomonas* sp.)
- To control blight disease, mahua (*Madhukaindica*) cake (100 g) is mixed with 1 litre of water. The extract is mixed with washing powder and spraying is done. Smoke of mahua is also used for bacterial blight. About 3% neem oil extract and 3% mahua cake @ 1:1 ratio is also prepared and sprayed for control of pests.
- Soak the sorghum seeds in cow urine for half-an-hour and sun drying them before sowing to control head smut and to induce drought tolerance.
- Seed treatment to control wilt Use curd for seed treatment in pigeonpea/chickpea for 24– 48 hr to protect them against wilt disease (Gonda, Uttar Pradesh) (Anonymous 2000c).
- Control of late blight and bacterial wilt in potato tubers crop management In Orissa, late blight (*Phytophthora infestans*) and Bacterial wilt (*Ralstonia solanacearum*) are the two major diseases of potato. In coastal Orissa potato tubers are planted in between 'Kartik Purima' and 'Prathamastmi' to escape late blight at the maximum vegetative growth phase of the crop. To reduce bacterial wilt and black-leg (*Erwinia caratovora* sub sp. *caratovora*) incidence, they grow sesamum sugarcane or summer vegetables after harvest. The popular cropping patterns in coastal Orissa are (i) upland paddy-potato-sesamum (ii) potato-sugarcane and (iii) upland paddy-potato-summer vegetables in every 2 to 3 years interval (Orissa) (Biswal et al. 2002)
- Control of blight in cumin by botanical mixture Tender neem, kuvarpathu (*Aloe vera*) and aakdo (*Calatropis gigantea*) leaves are ground in equal proportion to make homogenous mixture. When the crop has grown for about 30 days, a mixture of 500 ml of the extract with 5 g of washing powder, diluted in 15 litres is applied. Water is sprayed at 8-10 days regular intervals which prevents blight (Surendranagar, Gujarat) (Rathod, 2001)
- Neem oil solution 4% or neem kernel extract 6% is sprayed to control rust disease in groundnut.
- 5 kg cow dung, 5 litre of cow urine, 150 gm of lime are mixed with 100 litres of water and then kept for one month for fermentation. After fermentation it can be sprayed to control aphids, bacterial and viral diseases of vegetable crops (Shakrawar et al. 2018)

- Some farmers of tribal areas of Madhya Pradesh used to apply fresh cow dung near the collar region of chilli plant to control fungal disease, viz. damping off and dieback.
- To prevent blight and fruit rot disease of brinjal and tomato, farmers used to spread rice straw at the base of the plant especially during fruiting stage. Due to straw the branches of the plants and fruits do not come in contact with the soil and thus fruit rot and blight disease is prevented (Saha and Dutta, 2013).

#### 4. ITK for Insect management

- Control of early stem-borer in rice by spraying neem oil Spray neem oil at 0.2%, applied thrice after appearance of pest, i.e. one month after transplanting. The active ingredients (limonoids), particularly azadirachtin and meliatriol are known to work as pest repellent (Ganjam, Orissa) (Das et al.1999)
- Control of stemborer and gall flies in rice with neem cake Neem-cakes are filled in gunny bags and immersed in irrigation channels to control stem-borer and gall flies (Nilgiri, Tamil Nadu) (Karthikeyan and Chandra Kandan, 1996)
- Control of brown plant hopper in rice Leaf extract of Mukudda (*Lasiosiphonerio cephalus*) is very effective in controlling brown planthopper menace in paddy. One kg of leaves is boiled in 10 litres of water. It is filtered and diluted to a ratio of 1:10 and is then sprayed on crop, once during nursery stage and again after transplantation (Karnataka) (Dinesh, 1998)
- Control of leafroller and paddy case-worm by bamboo shoots, kolathia, suan or wolf spider Insect pest of paddy, paddy leaf-roller and paddy caseworm can be controlled by cut pieces of bamboo shoots, kolathia (a local weed), suan (minor millet) and wolf spider (a predator) (Orissa) (Parasar, 1994)
- Control of gundhi pest of paddy by burning To save paddy crops from gundhi bug, farmers take a discarded tyre and burn it. They drag it bicycle tyre around the field and put it on the windward side. The smoke and smell is assumed to repel the pest (Faizabad, Uttar Pradesh). (Maurya, 1993a)
- Control of earhead bug in rice by using kerosene mixed rice Rice-bran (4–5 kg) is mixed with 1 litre of kerosene and sprayed in paddy field during early morning. The limitation of this method is that its bran cannot be used on a large scale (Pondicherry) (Ganeshan, 2001a)
- Stem borer control in paddy by use of branches of cashew nut Farmers in Tamil Nadu cut cashew tree branches and place it in field to prevent movement and boring action of stemborer larvae in rice fields boring action of stem-borer larvae in rice fields (Tamil Nadu) (Rath, 2002)
- Efficacy of the plant *Polygonum hydropiper* against brown planthopper of rice The tribal belt of Balasore and Mayurbhanj districts of Orissa represents a group of tribals known as Santhals who possess a rich knowledge of botanicals to be used for management of different pests. Water pepper (*Polygonul hydropiper*) widely used by tribals for different pest control purposes. They name it as Gotkinamaru (cattle-tickkiller).It is commonly known as Kalatadi or Galpudi by the locals. Leaves of the plant is used for killing ticks on cattles and buffaloes, for catching fish from muddy water and for controlling insect pests of rice. This is a lowland weed, grown annually in canal and river banks and swampy and marshy lands. Its leaf extract was found to exhibit insecticidal action.Results obtained from experiments showed that plant can prove itself a suitable tool for the management of brown plant cropper (Orissa) (Mayabini, 2002)

- Spray the decoction of tobacco waste to control sucking pests and caterpillars.
- Red gram seeds are mixed with red earth slurry, dried and stored to avoid storage pests.
- Castor seeds are fried, powdered and mixed with red gram seeds to reduce pest attack during storage.
- Five kg. of neem kernels are powdered and soaked in 100 lit of water for one day and filtered in the next day, diluted, mixed with soap solution and sprayed to control white flies.
- About 600g. of tobacco is soaked in water for 2-3 days, filtered and sprayed to control white flies.
- Sugar solution and neem oil are mixed with water and sprayed to control mealy bugs.
- For insect control, spraying of diluted onion or garlic juice is used by tribes of Chhindwara district of Madhya Pradesh to control grasshopper and other leaf inhabiting insects on Maize crop (Shakrawar et al. 2018).
- Kerosene oil mixed water is poured in kharif season standing crop field especially in paddy field, and plants are thrilled by a long rope synchronously. All the insects like hoppers fell down in that water and die.
- Hanging of dead frog, crab (*Carcinides* sp.), jack fruit (*Artocarpush eterophyllus*) or Outenga (*Dileniaindica*) to control Gandhi bug in paddy. These items act as attractant for the pest as well as for the predators of the pest such as tiger beetle.
- Broadcasting of rice husk (5-10 kg) or wood ash (8 kg) per acre mixed with 3-5 litre of kerosene during early morning to control rice stem borer. It acts as a chemical as well as physical poison.
- Broadcasting of goat's excreta at tillering stage to control rice hispa. Due to disagreeable odour pest fly away from the field (Saha and Dutta, 2013).

## 5. Conclusions

Indigenous technical knowledge provides valuable inputs to make efficient use of natural resources and extends relevant support for sustainable development. The above techniques are more beneficial, easily adaptable, less costly, economically viable, and help in insurance against insect pest and disease occurrence in field crops, vegetables, fruit orchards, and cattle and even in human beings. But, there is a need to explore, verify, modify and scientifically validate these practices for their wider use and application.

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## Chapter 12

### Role of Early Warning Systems (EWS) in Pest Management

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#### 1. Introduction

The United Nations Office for Disaster Risk Reduction (UNDRR) defines an early warning systems as “An integrated system of hazard monitoring, forecasting and prediction, disaster risk assessment, communication and preparedness, activities systems and processes that enables individuals, communities, governments, businesses and others to take timely action to reduce disaster risks in advance of hazardous events”. Biosecurity is defined by the Food and Agriculture Organization (FAO) of the United Nations (FAO) as a strategic and integrated approach that encompasses the policy and regulatory frameworks (*including instruments and activities*) for analyzing and managing relevant risks to human, animal and plant life and health, and associated risks to the environment. In agriculture, these measures are aimed at protecting food crops and livestock from pests, invasive species, and other organisms not conducive to the welfare of the human population. A plant biosecurity system requires early detection, accurate diagnosis and a rapid response to prevent the establishment and dispersal of pests, thus minimizing the subsequent impact.

**Keywords:** IAS, Early warning, Early warning systems

#### 2. Activities of Early Warning

- ❖ New technologies for data mining and fusion
- ❖ Tools for characterizing pests and diseases new to science, or variants of existing diseases
- ❖ Portable devices for on-the-spot identification of known diseases
- ❖ Methods for high-throughput screening for disease in areas of concentration, such as ports

To truly achieve early warning in bio-security requires close collaboration between developers and end-users to ensure that generated warnings are duly considered by decision makers, reflect best practice, scientific understanding and the working environment facing frontline actors. Progress towards this goal will rely on openness and mutual understanding of the role of EWS in IAS risk management, as much as on developments in the underlying technologies for surveillance and modeling procedures. Once plant pests and pathogens have spread and settled in a certain area, eradication is extremely difficult. Prevention by early detection at ports of entry is fundamental, especially in the context of growing global mobility and trade.

#### 3. Types of early warning systems

There are various ways of classifying early warning systems.



By type of hazard

Early warning systems have been developed and implemented for

- ❖ Geological hazards like tsunamis, earthquakes, volcanic activity, and landslides
- ❖ Hydrometeorological hazards including severe weather in land and at sea, floods, droughts, hurricanes, typhoons and cyclones, tornados, cold and heat waves, etc.
- ❖ Forest fires
- ❖ Biological hazards including insect plagues like locust outbreaks and harmful algae blooms
- ❖ Health hazards including vector-borne diseases, viruses and other types of diseases
- ❖ For pests and diseases on crops and livestock

#### **4. Early Warning System**

Though pest biology- and ecology-based efficient IPMs are available in public domains for mitigation of invasive and transboundary pests, early warning on their spread, distribution, outbreak *etc.*, is more important.

Early warning systems for plants pests are efficient systems that provide following services,

- ❖ Alert stakeholders, government authorities, and policy makers about contemporaneous high priority plant pest news.
- ❖ Several plant pest early warning systems (e.g., the EPPO Reporting Service, Pest Lens, ProMED) currently operate to gather and disseminate pertinent information on plant pest developments from a variety of sources.

Though the scope and methodology of these early warning systems differ, they have a common goal: to bring attention to high-risk pests before they are introduced to a new region, so that targeted inspection efforts and policy decisions may be implemented as needed. Early warning systems act synergistically to document the global movement of plant pests and offer a valuable resource for identifying high-risk pests and commodities.

- ❖ Understanding likely plant pest invasion pathways and current inspection protocols is a critical first step to reduce the number of invasive alien species introduced through trade pathways.
- ❖ By collecting and disseminating relevant plant pest information, early warning systems play a crucial role in maintaining high priority pest lists that inform port inspection and quarantine regulations.
- ❖ Early warning systems provide up-to-date, accurate information on emerging plant pests that may be a threat to agriculture or natural resources if they become established in new countries.
- ❖ For that reason, the target audience of each early warning system differs and may be associated with National Plant Protection Organizations (NPPOs) or Regional Plant Protection Organizations (RPPOs) to focus on the needs of the stakeholder regions. Early warning systems also differ in methodology and information sources.

Several early warning systems (EWSs) currently are functioning globally which include i) European and Mediterranean Plant Protection Organization (EPPO), ii) the North American Plant Protection Organization (NAPPO), iii) PestLens, iv) ProMED, v) International Plant Protection Convention (IPPC), and vi) International Plant Sentinel Network (IPSN).

## **5. Goals and processes of plant pest early warning systems**

### **5.1. PestLens**

PestLens serves as an early warning system for USDA-APHIS-PPQ concerning plant pests and pathogens that may pose a threat to United States agriculture and natural resources. Each week, PestLens analysts monitor a list of over 300 scientific journals, NPPO reports, Google alerts, newspapers, e-mail lists concerning invasive species, and other plant-health-related websites. The analysts evaluate content from each of these sources for its relevance to PPQ, which is determined by several factors, including:

- ❖ Whether the information is new to PPQ
- ❖ Whether the plant pest is of quarantine significance to the United States,
- ❖ Potential economic impact of the pest if they are introduced other countries
- ❖ Likelihood of a pathway for introduction, and Likelihood that action by PPQ may be needed to prevent their introduction

Scope of PestLens includes

- ❖ New host information
- ❖ New distribution records
- ❖ Detection of a pest in a new country where it is not yet established
- ❖ Eradication of a pest from a country,
- ❖ Descriptions of new species with pest potential
- ❖ Other research that may be of regulatory interest

Once important pest news is identified by the analysts

- ❖ They write a brief article that includes the new information about the pest as well as background information such as its previously known host range and distribution
- ❖ These articles are archived on the PestLens website and they are compiled into a weekly e-mail notification that is sent to PPQ employees and thousands of other subscribers
- ❖ The information is then used by PPQ to make regulatory decisions about affected plants and plant products, and these decisions are documented within the PestLens website

### **5.2. EPPO Alert List and EPPO Reporting Service**

- ❖ The European and Mediterranean Plant Protection Organization (EPPO) generates an EPPO Alert List, which is a list of plant pests and pathogens that may pose a risk to the 52 member countries of EPPO.

- ❖ The pests on the EPPO Alert List are chosen by the EPPO Secretariat based on the scientific literature and on suggestions by the NPPOs of the EPPO member countries.

Factors that may warrant inclusion into the EPPO Alert List include:

- ❖ Newly described pests
- ❖ Reports of spread to new geographical locations, and reports of major outbreaks in the EPPO region (for eg. recent outbreak of thrips in chilli crop of Telangana during November 2021).
- ❖ Each pest on the EPPO Alert List has a fact sheet which contains information, such as the known hosts and distribution, the type of damage, the mode of dissemination, and potential pathways for spread to new geographical locations.
- ❖ Some pests included in the EPPO Alert List may be selected for a Pest Risk Analysis
- ❖ Once a Pest Risk Analysis is completed, the pest may be placed on the EPPO A1/A2 list of pests that are recommended for regulation as quarantine pests
- ❖ Pests that are not selected for Pest Risk Analyses stay on the EPPO Alert List temporarily, typically about 3 yr, and then they are removed from the EPPO Alert List and archived to a list of previously listed pests
- ❖ This allows the NPPOs of the EPPO member countries to monitor the pests that are on the alert list and take any appropriate actions
- ❖ The European and Mediterranean Plant Protection Organization also produces the EPPO Reporting Service news-letter, which is a monthly bulletin that reports on phytosanitary events concerning pests that may threaten the EPPO region, including both quarantine pests and emerging pests. The EPPO Reporting Service reports on new hosts, new geographical locations, new pests, and new identification and detection methods.

### **5.3. North American Plant Protection Organization Phytosanitary Alert System (NAPPO)**

- ❖ The North American Plant Protection Organization Phytosanitary Alert System is a web-based system that provides official pest reports from the NPPOs of Canada, the United States, and Mexico.
- ❖ The Phytosanitary Alert System also provides Emerging Pest Alerts, which are news items about plant pests and pathogens that are not established in this region.
- ❖ These news items are obtained from public sources, including scientific journals, newspapers, records from port-of-entry interceptions, and domestic plant pest surveys.
- ❖ The official pest reports and the Emerging Pest Alerts are intended to provide early warning to member countries about pests of concern to the region.

### **5.4. ProMED**

- ❖ ProMED is an e-mail list that disseminates information pertaining to disease outbreaks that may affect human health, both directly, as in human pathogens or toxins, and indirectly, as in diseases of plants and animals that are important for agriculture.
- ❖ Subscribers can choose different ProMED mailing lists based on their areas of interest and their geographic region.

- ❖ ProMED differs from some of the other early warning systems both in its broader scope and also that it uses informal and non-traditional information sources to generate its reports, including local media, on-the-ground experts, and professional networks.
- ❖ ProMED focuses on outbreaks in new geographic regions, newly described diseases, and diseases for which the causal agent is unknown.
- ❖ In contrast, early warning systems associated with NPPOs often do not report on diseases for which the causal agent has not been identified, since identification of the pathogen species is typically necessary from a regulatory standpoint.

### **5.5. International Plant Protection Convention Pest Reports (IPPC)**

The International Plant Protection Convention is a plant health treaty signed by over 180 countries. National plant protection organizations of each member country submit official pest reports as needed concerning the occurrence, outbreak, spread, or eradication of organisms that are quarantine pests in that country or that are quarantine pests for neighboring countries and trading partners. These pest reports are posted on the International Plant Protection Convention website, enabling the NPPOs of other countries to respond with appropriate changes to phytosanitary requirements.

### **5.6. International Plant Sentinel Network (IPSN)**

The International Plant Sentinel Network is founded on the premise that historically, many devastating plant pests were either not known to science or not reported to be pests in their native ranges. Once these pests were introduced to new geographical regions, they caused severe damage to plants that did not co-evolve with them. For example, the fungus *Hymenoscyphus fraxineus* not known to be pathogenic in its native range but has caused widespread dieback of native European ash trees in Europe.

NPPO: National Plant Protection Organization; USDA-APHIS-PPQ: United States Department of Agriculture-Animal Plant Health Inspection Service-Plant Protection and Quarantine; CFIA: Canadian Food Inspection Agency; MPI: Ministry of Primary Industries; DEFRA: Department of Environment, Food and Rural Affairs; EWS: Early warning system; EPPO: European Mediterranean Plant Protection Organization; NAPPO: North American Plant Protection Organization; IPPC: International Plant Protection Convention; IPSN: International Plant Sentinel Network; RPPO: Regional Plant Protection Organization.

In India, the Plant Quarantine Organization, Ministry of Agriculture and Farmers Welfare, Government of India is the apex body for implementation of plant quarantine regulations and it provides plant protection advisories concerning the country through obtaining information from EWSs. The Department of Plant Protection, Quarantine and Storage (DPPQS), India has a national network of 29 plant quarantine stations at different sites which include airports (10), seaports (10) and land frontiers (9). In all, two categories of materials are being imported under the PQ Order, 2003: (a) bulk consignments for consumption and sowing/planting, and (b) samples of germplasm in small quantities for research purposes. The Plant Quarantine Stations under the DPPQS undertake quarantine processing and clearance of consignments of the first category (Fig 2).

The National Bureau of Plant Genetic Resources (NBPGR), ICAR, New Delhi undertakes the quarantine processing of all plant germplasm and transgenic planting material under exchange for which it has well-equipped laboratories and green house complexes and recently, a containment facility has also been established for processing transgenic. The NBPGR also has a well-equipped quarantine station at Hyderabad, which mainly deals with export samples of the International Crops Research Institute for The Semi-arid Tropics (ICRISAT), Telangana.

FAO established an Emergency Prevention System for Transboundary Animal and Plant Pests and Diseases (EMPRES) in 1994, with a goal of addressing world food security and fighting transboundary animal and plant pests and diseases. The core principles of the EMPRES programme are: i) early warning, detection and reaction, ii) contingency planning, iii) promotion of environmentally sound control technologies, and vi) close collaboration and partnership with affected countries, national and international agricultural research centres and other international institutions.

During the annual Meeting of G20 Agricultural Chief Scientists (MACS-G20) meet in April 2019, three major decisions were taken to monitor and management of TPPs across the world which include i) Designation of National Reference Laboratories (NRLs) for early detection and correct diagnosis of TPPs and the establishment of networks among them, ii) Improvements in Bio-vigilance or global surveillance systems for identification and mitigation of potential threats before they impact the agricultural sector, and iii) The challenges and future directions of research collaborations, and contributions to the International Year of Plant Health 2020.

## **6. Conclusions**

Invasive pests and TPPs are threat to plant health which reflects on food security globally. Thirty-three invasive insect and non-insect pests reported in India so far since 1847 to till date have already entered, occupied new crop habitats and established. The COVID-19 induced lockdowns aggravated the spread and outbreaks of certain invasive pests including fall armyworm, cassava mealybug, root-knot nematode etc., due to restricted movement of field level plant protection workers, non-availability of insect mitigation inputs like pesticides, biopesticides, botanicals etc. The ICAR institutes/State Agricultural Universities of India have developed well equipped Integrated Pest Management (IPM), pest-wise based on pest-biology and -ecology of the invaded pests in the new habitats which are readily available in public domains for the stake-holders to adapt. However, rigorous/rampant monitoring of the entry of new invasive pests through sea- and air-ports should be a continuous exercise of the officers, extension and field level workers etc., as the free trade policies, globalization etc., are favourable for frequent entry of invasive pests.

Biocontrol agents including parasitoids, predators, entomopathogenic fungi, bacteria, virus *etc.*, are reported to have good control potential on the invasive pests. Many such natural enemies which had/have been moving along with host insects in to new ranges/habitats are being identified and utilized for biological control. However the natural enemies of many invasive

pests are not reported to move along with host insects wherein, the importation of potential natural enemies from the native/origin of invasive pests is recommended.

The net working of various EWSs is summarized in a flow chart (Fig. 1).

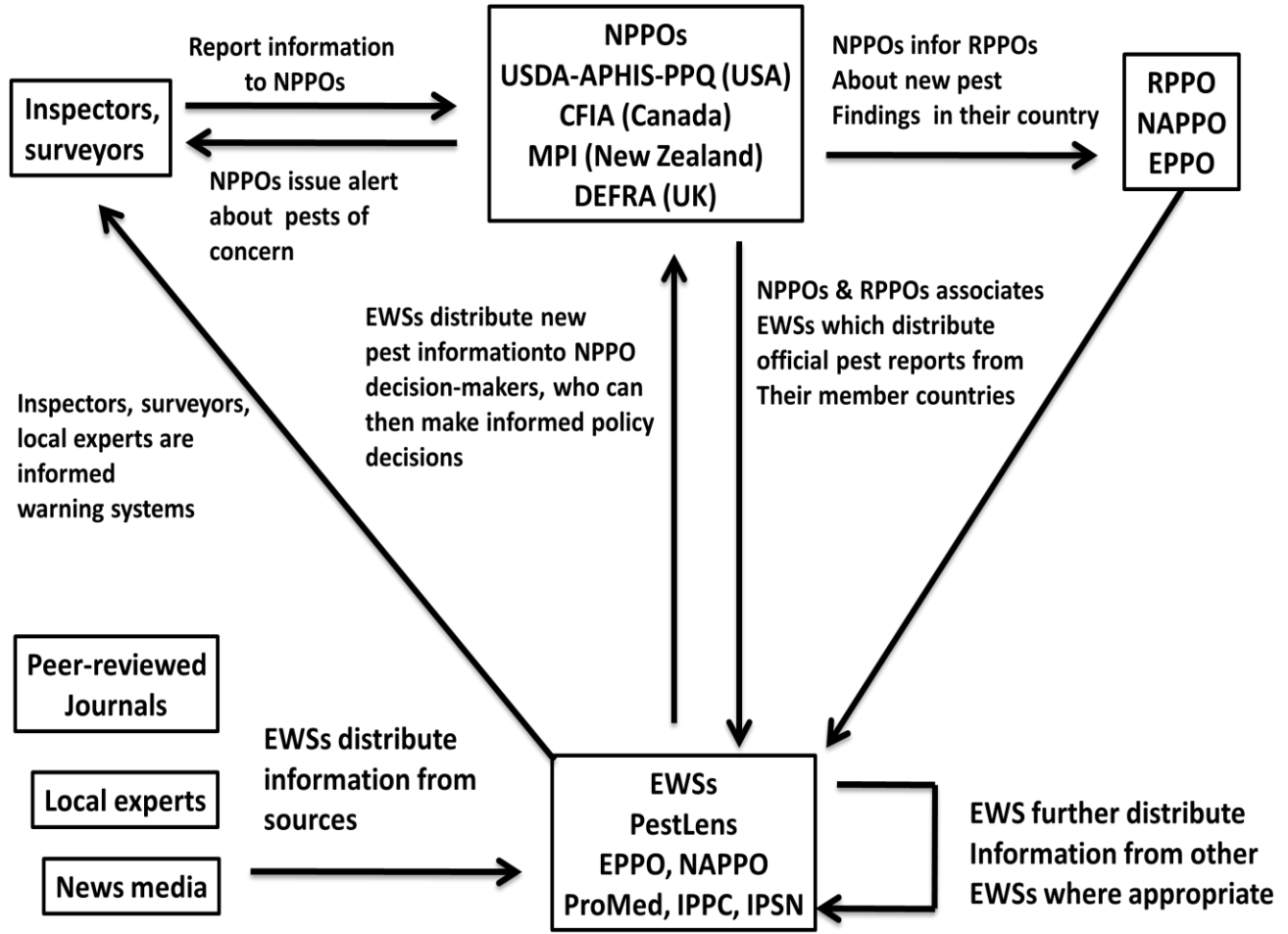


Fig 1. Information flow between early warning systems and plant protection organizations  
Inspired from Noar et al. (2021)

The desert locust and Carambola fruit fly are considered as potential transboundary insect pests to India. The Locust Warning Organization, Faridabad is the premier agency to monitor and forewarn the movement and occurrence of desert locust in India, using the geo-spatial technologies including GPS, GIS, remote sensing etc. The Carambola fruit fly reported to prevail in Bangladesh may be likely to invade India which requires rigorous monitoring of geo-boundaries. In addition, early monitoring, early detection and early/fore warning on the movement of already existing and/or likely to invade in future in India are pre-requisites. Few Early Warning Systems (EWSs) are functioning globally which disseminate the information on emerging, invasive, transboundary pests etc., to the member countries in the form e mail, bulletins, websites, newsletters etc., on subscription/request. In India, Plant Quarantine Organization has been gathering information from International Plant Protection Convention

(IPPC) and alerting stakeholders, government authorities, and policy makers about contemporaneous high priority plant pest news. The successful early warning in bio-security requires close collaboration between developers and end-users to ensure that generated warnings are duly considered by decision makers, reflect best practice, scientific understanding and the working environment facing frontline actors.

The plant stress phenotyping, using Artificial Intelligence (AI), with cascading steps of Deep Learning (DL), Active Learning (AL), Machine Learning (ML) and Transfer Learning (TL) (Singh et al. 2021) and development of electronic-nose with biosensors (Al-Dayyeni et al. 2021), handheld smartphone based sensors, field-portable sensors etc.(Das and Mohar 2021) for *in situ* detection of damages caused by emerging/invasive pests etc., can be the recent developments that need to be explored in developing countries like India.

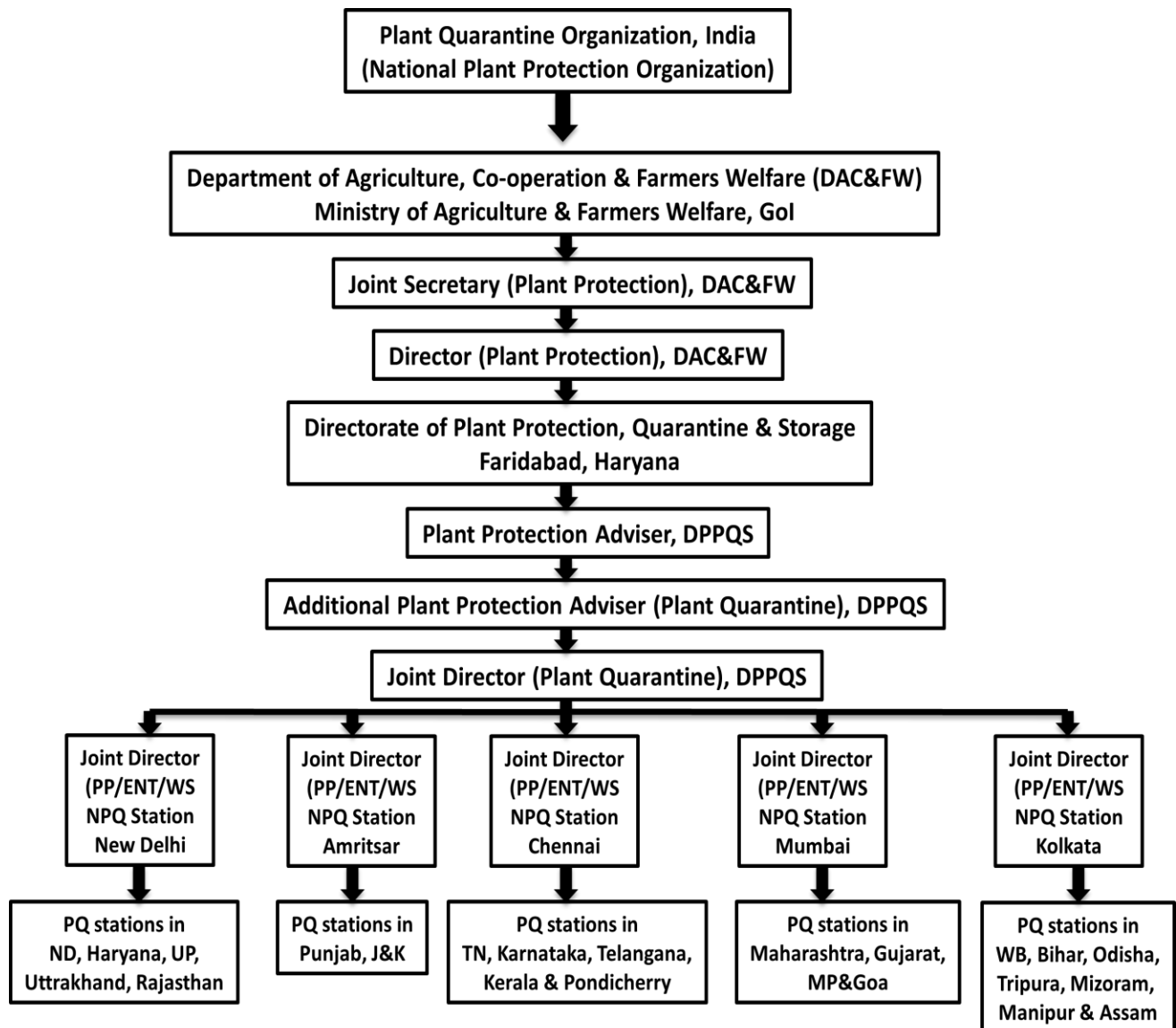


Fig 2. Indian set-up for monitoring and early detection of invasive and transboundary pests

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Host: Coffee  
Problem: Green scale + biological control fungus (Verticillium)

