Compendium of Lectures

ICAR SPONSORED SHORT COURSE ON "Making Greater Use of Biocontrol Agents for Organic Agriculture" 4 - 13 November 2019

COURSE DIRECTOR
Dr. PRANAB DUTTA

COURSE COORDINATORS
Dr. B.C. DAS
Dr. D.K. SARMAH

Organized by
DEPARTMENT OF PLANT PATHOLOGY
ASSAM AGRICULTURAL UNIVERSITY
JORHAT - 785013, ASSAM
ICAR Sponsored Short Course

On

“Making Greater Use of Biocontrol Agents For Organic Agriculture”

Organized by:

Department of Plant Pathology
Assam Agricultural University
Jorhat- 785013, Assam
The publication has been brought out on the occasion of a Short Course on “Making Greater Use of Biocontrol Agents for Organic Agriculture” sponsored by ICAR, New Delhi and organized by Department of Plant Pathology, Assam Agricultural University, Jorhat-13, Assam from 4th to 13th November, 2019

Editorial Board

Dr. Pranab Dutta
Dr. B. C. Das
Dr. D. K. Sarmah

Member

Ms. Arti Kumari

Cover design:

Mr. Gaurav Phookan

©Department of Plant Pathology, Assam Agricultural University, Jorhat-13, Assam
## CONTENT

<table>
<thead>
<tr>
<th>Chapter No.</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>An overview of AAU's achievement on biological control</td>
</tr>
<tr>
<td>2.</td>
<td><em>Trichoderma</em>- the most powerful arsenal for plant disease management</td>
</tr>
<tr>
<td>3.</td>
<td>Exploitation of biological agents for management of viral diseases of agricultural crops</td>
</tr>
<tr>
<td>4.</td>
<td>Biological Control- an ecological perspective</td>
</tr>
<tr>
<td>5.</td>
<td>Mass production technology of <em>Beauveria bassiana</em></td>
</tr>
<tr>
<td>6.</td>
<td>Morphological characterization of fungal bio-control agents</td>
</tr>
<tr>
<td>7.</td>
<td>Mass production technology of <em>Trichoderma harzianum</em> with locally available substrates</td>
</tr>
<tr>
<td>8.</td>
<td>Mass culture technique of Biocontrol agents for management of mites of agricultural crops</td>
</tr>
<tr>
<td>9.</td>
<td>Agro-forestry and Organic Farming</td>
</tr>
<tr>
<td>10.</td>
<td>Disease management of vegetable crops in organic agriculture</td>
</tr>
<tr>
<td>11.</td>
<td>Field use of bioformulations for management of rapeseed- mustard diseases</td>
</tr>
<tr>
<td>12.</td>
<td>Embracing social engineering for innovative pest management</td>
</tr>
<tr>
<td>13.</td>
<td>Biological management of nematode pests of agricultural crops</td>
</tr>
<tr>
<td>14.</td>
<td>Protection of Biopesticides under the IPR regime: an overview</td>
</tr>
<tr>
<td></td>
<td>Topic</td>
</tr>
<tr>
<td>---</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>15.</td>
<td>Exploitation of VAM in the management of plant diseases caused by pathogenic nematode and its mass multiplication</td>
</tr>
<tr>
<td>16.</td>
<td>Isolation of fungal biocontrol agents, endophytes and study on their evaluation</td>
</tr>
<tr>
<td>17.</td>
<td>Phylloplane bacteria and plant disease management</td>
</tr>
<tr>
<td>18.</td>
<td>Preparation of plant extracts, efficiency test and their use for plant disease management</td>
</tr>
<tr>
<td>19.</td>
<td>Biological management of diseases of flowering plants</td>
</tr>
<tr>
<td>20.</td>
<td>Entomopathogenic Nematodes- a promising biocontrol tool against insect pests</td>
</tr>
<tr>
<td>21.</td>
<td>Isolation of biocontrol agents from soil sample</td>
</tr>
<tr>
<td>22.</td>
<td>Purification of fungal/bacterial antagonists</td>
</tr>
</tbody>
</table>
An Overview of AAU’s Achievement on Biological Control

A. Bhattacharyya
Acting Vice Chancellor cum Director of Research (Agri)
Assam Agricultural University, Jorhat-785013, Assam

At Assam Agricultural University, the work on biological control has started during 1970-80’s initially on biological control of Rhizoctonia solani by Trichoderma viride. Since then a large number of biocontrol agents has been explored and developed effective biopesticides for the management of plant pathogens and insect pests. Following are some of the example of biopesticides with the targeted pests and diseases.

<table>
<thead>
<tr>
<th>Name of Bioformulation</th>
<th>Biocontrol agent</th>
<th>Target pests/diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Liquid</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Org-Metajal</td>
<td>Metarhizium anisopliae</td>
<td>Termite, aphid, scale insect Also act as endophyte and give induced resistance</td>
</tr>
<tr>
<td>Org-Trichojal</td>
<td>Trichoderma harzianum</td>
<td>Soil borne plant pathogen like Rhizoconia solani, Sclerotium rolfsii, Sclerotinia sclerotiorum, Fusarium sp., etc. Also act as plant growth enhancer</td>
</tr>
<tr>
<td>Org-Vertijal</td>
<td>Verticillium lecanii</td>
<td>Red spider mite, Scale insect</td>
</tr>
<tr>
<td>Org-Beauverijal</td>
<td>Beauveria bassiana</td>
<td>Rice hispa, Halopeltis, Tea Mosquito bug, Fusarium etc.</td>
</tr>
<tr>
<td>Org-Cillumjal</td>
<td>Purpurocillium lilacinus</td>
<td>Root knot nematode</td>
</tr>
<tr>
<td>Org-Pochojal</td>
<td>Pochoniachlamydsoria</td>
<td>Root Knot nematode</td>
</tr>
<tr>
<td>Org-Metahim</td>
<td>Metarhizumanisopliae</td>
<td>Termite, aphid, scale insect</td>
</tr>
<tr>
<td><strong>Solid</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biofor-PF-2</td>
<td>Pseudomonas fluorescens and Trichoderma harzianum</td>
<td>Fungal and bacterial soil borne plant pathogen</td>
</tr>
<tr>
<td>Biozin-PTB</td>
<td>Pseudomonas fluorescens, Trichoderma viride, Bacillus brevis</td>
<td>Fungal plant pathogen like Pythium, Fusarium, Colletotrichum, Rhizoctonia, Macrophomina, Sclerotinia, Xanthomonas, Ralstonia</td>
</tr>
<tr>
<td>Bio-Llium</td>
<td>Verticillium lecannii</td>
<td>Nematodes, whitefly, thrips, mites, aphids, jassids, ants</td>
</tr>
<tr>
<td>Bio-Meta</td>
<td>Metarhizium anisopliae</td>
<td>Red ants, termites, mosquito larvae, planthoppers, cattle ticks</td>
</tr>
<tr>
<td>Bio-Sona</td>
<td>Beauveria bassiana</td>
<td>Rice hispa, Helicoverpa, white fly, mites, coffee borer</td>
</tr>
<tr>
<td>Bioveer</td>
<td>Trichoderma viride</td>
<td>Bacterial and fungal wilt, anthracnose, root and stem rots</td>
</tr>
<tr>
<td>Biogreen-5</td>
<td><em>Beauveria bassiana, Metarhizium anisopliae, Pseudomonas fluorescens, Trichoderma viride and Bacillus thuringiensis</em></td>
<td>Rhizome rot and wilt of ginger &amp; turmeric, wilt disease of tomato &amp; chilli, Tea mosquito bug, Tea looper, red spider mites, stem borer, rice hispa</td>
</tr>
<tr>
<td>---------------</td>
<td>--------------------------------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Biotime</td>
<td><em>Metarhizium anisopliae, Pseudomonas fluorescens and Trichoderma harzianum</em></td>
<td>Bacterial and fungal wilt, anthracnose, root and stem rots, diseases of tea, Red ants, Termites, coconut leaf beetle, stem borer, leaf folder</td>
</tr>
<tr>
<td>Biosona</td>
<td><em>Beauveria bassiana</em></td>
<td>Rice hispa, aphids, Tea mosquito bug, stem borer, thrips, whitefly, looper, mites</td>
</tr>
<tr>
<td>Biozium</td>
<td><em>Trichoderma harzianum</em></td>
<td>Bacterial and fungal wilt, anthracnose, root and stem rots</td>
</tr>
</tbody>
</table>

MOs of the above mentioned products has already been tested in farmers field and found effective for the management of various listed pests and diseases.

Besides these, Assam Agricultural University, Jorhat also started work on classical biological control of invasive weeds or alien invasive weeds for which the institute has received fund from DFID, CABI, UK etc. After the host specificity test of *Puccinia spegazzinii* - Trinidad Isolate (W1761) to *M. micrantha* which was tested by centrifugal phylogenetic testing sequence in quarantine facility of NBPGR, New Delhi showed none of the 74 economically important plants species were found infected with the fungus. So, a classical biological control (CBC) agents *viz.*, *P. spegazzinii* intentionally released to combat *M. micrantha* in the tea ecosystem of Assam for the first time in India & continental Asia. Thus, India became the eighth country in the world to have released a plant pathogen for CBC of invasive weed. In an another experiment *Puccinia cacao* was identified as potential biocontrol agent from the North East India for the management of *Hygrophia polysperma*.

Similarly, Successful control of water hyacinth, *Eichorniacrassipes* has been achieved by the exotic weevil *Neochetinaeichhorniae, N. bruchi @50,000 per ha and found the dispersal of the weevil in 8 districts of Assam through aerial migration & Brahmaputra river and its tributaries. In Sibsagar district of Assam > 700 ha of water body has been cleared off by the action of this exotic weevil & control achieved is about 90% (Basit, 2002).

On the other hand for the management of many lepidopteran pests like sugarcane borer, *Chilospp*, rice stem borer, *Scirpophaga incertulas; tomato fruit borer, Helicoverpa armigera; cut worms, Agrotis spp; cotton boll worm, *Pectinophora gossypiella and Ear spp*, maize bore, *Chilopartellus* egg parasitoid Trichogramma has been identified as potential bioagents which
suppress various caterpillar pests damaging many agricultural crops. Trichocard that contain the eggs of the parasitoid are released in the field 30-45 days after sowing or transplanting @50,000 to 1,00,000 eggs/ha/release. Totally 3-6 releases are made depending upon the build up of pest population coinciding with egg stage of the target pests.

In the North East India there is huge scope of biopesticides because of congenial environmental condition that favours the BCA i.e., RH (>80%), temperature(12-35 °C), low pH, Rainfall (300-3000 mm) favourable for BCA, wide diversity in terms of species and strains of biopesticidal resources i.e. plants, fungi, bacteria, virus, parasitoids, predators and good potential market for biopesticides for large scale cultivation of tea and rice as organic.

Path ahead:

• Further studies needed to determine the environmental effects on the fate of bio-agents
• New technologies such as micro encapsulation of bio-control agents may be of high priority in enhancing their potential
• Integration of bio-pesticides with botanical pesticides has a lot of potential in pest management
• Integration of bio-pesticides with chemical pesticides as part of Bio-intensive Integrated Pest Management (BIPM)

*********
Chapter-2

Trichoderma- the most powerful Arsenal for plant disease management

A.N. Mukhopadhyay
Former Vice-Chancellor, Assam Agril. University, Jorhat, Director General Tea Research Association and Dean, G.B. Pant University of Agriculture and Technology, Pantnagar.
Address: ‘Sangini’ 151 Akanksha, Udyan II, Raibareilly Road, Lucknow-226025, U.P. INDIA
Email: amar.mukhopadhyay@gmail.com

During last decade, species of *Trichoderma* have emerged as most powerful bioprotectants for management of wide variety of plant diseases. This is more true in the context of the fact that there is considerable public pressure and pressure from environmental scientists to reduce emphasis on chemical protectants and use bioprotectants. The genus *Trichoderma* by virtue of its broad spectrum action against a number of plant diseases caused by fungi, bacteria, viruses and even nematodes has occupied the top position among the bioprotectants developed for plant disease management. *Trichoderma* based biopesticides have been proved successful in a large number of field, vegetable, fruit and flowering crops for the management of diseases. Because of its eco-friendly nature and low cost when compared with chemical protectants, the technology has been very widely adapted all over the world. The literature accumulated on the subject during last decade is quite vast. *Trichoderma* strains exert biocontrol against phytopathogens either indirectly by competing for nutrients and space, modifying the environmental conditions, or promoting plant growth and plant defensive mechanisms and antibiosis or directly by mechanisms such as mycoparasitism. These indirect and direct mechanisms may act coordinately and their importance in the biocontrol process depends on the *Trichoderma* strain, the antagonized fungus, the crop plant, and the environmental conditions, including nutrient availability, pH, temperature, moisture and iron concentrations. *Trichoderma* species are plant symbiont opportunistic virulent organisms, able to colonize plant root by mechanisms similar to those of mycorrhizal fungi. Root colonization by *Trichoderma* species frequently enhances root growth and development, crop productivity, resistance to abiotic stress and the uptake and use of nutrients. Root-fungus association also stimulate plant defensive mechanisms. *Trichoderma* added directly to rhizosphere or as seed treatment protects plant against numerous classes of pathogens, e.g. those that produce aerial infections, including fungal, bacterial, nematodes and viral pathogens. This reveals induction of resistance mechanisms similar to the hypersensitive
response (HR), systemic acquired resistance (SAR) and induced systemic resistance (ISR) in plants. The low cost technology has opened up a new vista for plant disease management and is likely to be a boon for seed industries who would like to provide protection to seeds as well as plants against a large number of seed, soil-borne and foliar diseases.

*******
Chapter-3

Exploitation of biological agents for management of viral diseases of agricultural crops

Kajal Kumar Biswas and P. D Nath*
Principal Scientist, Division of Plant Pathology,
ICAR-Indian Agricultural Research Institute, New Delhi 110012
Email: drkkbiswas@yahoo.co.in

*Professor, Department of Plant Pathology, AAU, Jorhat-785013, Assam

Crop losses due to insects, diseases, and weeds across the world have increased from about 34.9% in 1965 to about 42.1% in the late 1990s worldwide. Annual crop losses due to plant diseases are estimated worldwide at $60 billion. Although losses caused by plant viruses alone are difficult to estimate, viruses are considered to be the second greatest contributor to yield loss. More than 40% of crop loss is due to viral infections in vegetable (Anonymous 2012). Modern practices regarding the control of viral diseases in plants mainly include the use of synthetic chemicals to destroy the vector, manipulation of environmental factors, incorporation of resistance against the virus by genetic engineering and biocontrol measures. Plant virus diseases have always been of great concern to farmers, researchers and policy makers because they cause enormous yield loss in many cereal, vegetable, fruit, legume and cash crops like cotton. Plant virus diseases are very difficult to control and are much problematic than commonly occurring fungal diseases.

Virus infection causes loss by reducing the productive life of the crop, by adverse effect on vegetative propagation, on germination of seeds and growth of seedlings, by reduction in quality of fruits, by loss of vigour and by number of other usual and unusual ways (Baranwal and Verma, 2000; Biswas, 2017 & 2019). The recent outbreak of cotton leaf curl virus disease in cotton in northern cotton growing region of India has led to huge yield loss of cotton fibre. Approximately 12,000 ha of area under cotton were affected by leaf curl virus disease during 1996 in Rajasthan alone. An annual loss of US $ 300 million is caused by MYMV by reducing the yield of black gram, mung bean and soybean. It is therefore, important to develop management strategy for virus diseases of important crops so that the losses can be minimized. Viral parasitism is unique, in contrast to fungi and bacteria. Viruses do not attack the structural integrity of their host tissues, but instead they subvert the synthetic machinery of the host cell, acting as molecular pirates. Therefore, the management of virus diseases is a difficult task.
Control strategies are mainly aimed at reducing or eliminating existing sources of infection, prevention of virus transmission, etc. The development of methods of virus disease control, continue to be vital elements in the drive to improve crop productivity. The use of molecular genetic techniques has provided new insights into how plants defend themselves against pest attack.

Biological control is the control of disease by the application of biological agents to a host plant that prevents the development of disease by a pathogen. With regard to plant diseases the biocontrol agents are usually bacterial or fungal strains isolated from the endosphere or rhizosphere. Biological vector control has not yet deserved good attention but still some studies pin-pointed the parasites like Coccinellid grubs, Epilachna beetle and Trioxys spp for the control of aphids like Dactynotus ambrosiae, Melanaphis sacchari and Rophalosipham maidis those transmit SCMV. Ladybird bettle is a predator of aphids, spidermites, scale insects and whitefly; parasite wasp attacks aphids and Aphidius colemani attacks Myzus persicae. Modern practices regarding the control of viral diseases in plants mainly include the use of synthetic chemicals to destroy the vector, manipulation of environmental factors, incorporation of resistance against the virus by genetic engineering and biocontrol measures. Management of diseases by biological agents occurs at the microbial level, typically in biological microcosms (leaf surfaces, fruit surfaces, etc.) and includes competitors as well as parasites. There are no cures for viral diseases such as mosaic once a plant is infected. Fungicides will not treat this viral disease.

Cross protection is a phenomenon in which a prior infection with one virus prevents or interferes with subsequent infection by another isolate of the same or a closely related virus; suggesting that cross protection using attenuated viruses appears to offer a promising strategy for biological control of plants viral diseases. Some attenuated viruses have been commercially used in some countries like Japan. Because cross protection is effective in general, it is necessary to increase good attenuated strains against many severe viruses. The classical cross protection to control the diseases caused by the viruses, Citrus tristeza virus, Papaya ring spot virus, Zucchini yellow mosaic virus, Cucumber mosaic virus-satellite RNA, Tomato mosaic virus and Cocoa swollen shoot virus were applied in field level and greenhouse condition in many countries. Cross protection using mild strain has been in experiments against viruses belonging to different groups where mild strains are occurring naturally. The important groups where mild strain have
been obtained and used are badna-, clostro-, nepo-, poty- and tobamo-viruses. Cross protection has also been observed to occur between viroids and plant virus satellites (Biswa,

Secondary metabolites from Bacillus *Bacillus amyloliquefaciens* (VB7) was probed to have antiviral activity against GBNV after spraying on cowpea plants (Vanthana et al., 2018). The bacterial isolates, *Pseudomonas aeruginosa* (S1HL3), *Burkholderia* sp. (S1HL4) and *Bacillus* spp. (JS2HR4 & JS3HR2), collected from rhizosphere and phyllosphere of healthy cotton plants were used as a source of as potential biocontrol agents, to control Cotton leaf curl virus (CLCuV) in GH conditions (Ramzan et al., 2016). These isolates are also promoting plant growth, but also provide resistance against diseases. An antiviral producing *Streptomyces* species were isolated from soil rhizosphere in Egypt. The isolates of *Streptomyces* were *S. calvus*, *S. canarius*, *S. vinaceusdrappus*, *S. nogalater* and *S. viridosporus*. The *Streptomyces* spp. grown in glycerol asparagine broth medium and the culture supernatants can control Cumber mosaic virus (CMV) in greenhouse condition (El-Dougdo et al., 2012).

**References:**


Vanthana, M., S. Nakkeeran, V.G. Malathi, P. Renukadeviand S.Vinodkumar. 2019. Induction of in planta resistance by flagellin (Flg) and elongation factor-TU (EF-Tu) of Bacillus
amyloliquefaciens (VB7) against groundnut bud necrosis virus in tomato. *Microbial Pathogenesis*, 137; doi.org/10.1016/j.micpath.2019.103757


********
Chapter-4

Biological Control – An Ecological Perspective

D. K. Sarmah
AICRP on Mushroom, Department of Plant Pathology,
Assam Agricultural University, Jorhat- 785013, Assam

Biological control of plant diseases involves the use of an organism or organisms to inhibit the pathogen and reduce disease (Chaur, 1998). In variously defined biological control processes the basic idea is to evolve a strategy for reducing disease incidence or severity by direct or indirect manipulation of microorganisms (Shurtleff and Averre, 1997). A clear understanding of the mechanisms of biological control of plant diseases through the interactions between biocontrol agent and pathogen may allow us to manipulate the soil environment to create conditions conducive for successful bio-control or to improve biocontrol strategies (Chaur, 1998). Biocontrol agents are widely regarded in general as natural and therefore non-threatening products, although risk assessments must clearly be carried out on their effects on non-target organisms and plants. Moreover, knowledge concerning the behaviour of such antagonists is essential for their effective use (Monte and Liobell, 2003).

Biological disease control is an attractive alternative strategy for the control of plant diseases. Many factors have to be considered in deciding whether a biological system is feasible for the control of a particular pathogen. The availability of a suitable antagonist capable of maintaining itself on the host plant is of prime importance. The environment under which the crop is grown will play a significant part in determining whether effective population levels of an antagonist can be established in competition with the existing microflora. Environment may also govern the choice of antagonist. Bio agents themselves being non-pathogenic to plants need to be formulated in a way that favours the activity and survival of the microbe it contains. Over the past few years, the novel applications of molecular techniques have broadened our insight into the basis of biological control of plant diseases. New molecular approaches have been available for assessment of interaction between the antagonist and pathogen, ecological traits of antagonists in rhizosphere and improving the efficacy of bacterial, fungal and viral biocontrol agent. Thus, biological control will be a viable alternative strategy for the control of plant diseases given the history of fungicides, in the near future.
In spite of decades of research on the biological control of soil-borne plant diseases, there remain few commercially successful examples of biological control using introduced microbial inoculants. There are a number of reasons for the lack of development and grower adoption. Among the more important are problems in formulation and delivery, variability in performance, and problems with poor efficacy under optimum conditions for disease development. There are countless examples of biological control organisms that perform quite effectively under defined laboratory conditions but fail miserably when introduced on different crops under varying conditions in the field. Still others might perform effectively in the field, but exhibit strong year-to-year or site-to-site variability. Unpredictable performance coupled with this extreme variability represents one of the greatest obstacles to the implementation of biological disease control practices in agriculture. Our inability to predict the behaviour of microorganisms introduced for biological control purposes stems from a lack of sustained and broad research on the mechanisms regulating biological control processes in plant-associated microorganisms. The emphasis in past studies of biological control mechanisms has been on the attributes of the biocontrol organism and the role of specific microbial properties in pathogen suppression (Martin and Loper, 1999; Whipps, 2001).

As a result, our understanding of how various microbial traits influence biological control processes is fairly well understood. However, the important role of the host plant in defining and regulating biological control processes is often overlooked. An increasing number of studies indicate the importance of the host plant in influencing microbial interactions in the spermosphere and rhizosphere (Chanway, Holl et al., 1988; Chanway, Nelson et al., 1988; Mavingui et al., 1992; Lemanceau et al., 1995; Hervas et al., 1997; Koch, 1997; Bensalim et al., 1998). The host plant also has an important role in supporting biological control interactions, which has been indicated in an increasing number of studies (Atkinson and Neal, 1975; Azad et al., 1987; Koch, 1997; Grayston et al., 1998).

The study of interactions among plant pathogens and other microorganisms is a fascinating but challenging area of scientific investigation that has potential applications for biological control of plant pathogens. In pioneering studies as early as 1920, antagonistic fungi were introduced to forest nursery soils to reduce damping off of pine seedlings (Hartley, 1921).
Reduction in disease occurred in some treatments and Hartley concluded that “competition of different fungi is a factor to be considered”. This potential for biological control has continued to be a well established objective of plant pathologist for many decades. If the study of interactions of plant pathogens and other microorganisms is to be applied to the management of plant diseases, factors that contribute to the lack of available systems must be identified and effective strategies developed for the application of biological controls to disease management. There are several areas where the development of such systems can be encouraged: the ecological selection and evaluation of potential agents, the environmental enhancement of biocontrol efficacy, the genetic enhancement of efficacy, the commercial production and development of biocontrol agents and the registration of biocontrol products.

After its establishment in the soil, the BCA will interact not only with the pathogen to be controlled but also with all the biotic components of the soil. There is a fear that a successful BCA might displace the microbial balance of the soil and have some unexpected effects on the non target organisms. Therefore, there is a need to study the side effects of an introduced antagonist on the native microbial communities. In Europe, application of BCA is subjected to the Directive 91/414-ECC, which imposes this type of study. Until recently, there were no practical methods available to detect the impacts of an introduced BCA on the whole soil microbial community. With the development of molecular approaches based on extraction of total DNA from the soil, it is now possible to overcome this limitation today. Several methods are available to assess microbial community structures by molecular fingerprinting. Among them, terminal restriction fragment length polymorphism (T-RFLP) has already been used to address the impact of cultural practices on the structure of bacterial and fungal communities (Edel-Hermann et al., 2004; Pérez-Piqueres et al., 2006).

Ecological selection

A growing plant contains several ecological microhabitats that represent unique microclimatic and nutritional conditions. Terms such as rhizoplane, rhizosphere, phylloplane, spermosphere, gemmisphere, cauliplane, palynosphere and anthoplane are used to emphasize the uniqueness of these habitats and their influence on the growth and survival of pathogenic and saprophytic microorganisms. The ecological competence of biological control agents within
individual habitats is a primary determinant of potential efficacy. Furthermore, the study of biological control must be placed within the context of the ecological requirements of the pathogen and the biological control agent. Potential agents can be selected from indigenous populations collected from the target habitat or from non indigenous population in other habitats. Classical approaches have selected from indigenous populations, with the assumption that such microorganisms are ecologically competent within that habitat. However, studies on biocontrol of insect and weed pests have suggested that there is up to a 75% greater chance of success if the parasite and the host represent a new biological association instead of an old association (Hokanen and Pimental, 1984; Waage and Greathead, 1988). These considerations are based on the principle that interactions between two organisms that have coevolved may be less disruptive than interactions that have not coevolved. Little information is available on the influence of population origin on probability of selecting effective biological controls for plant diseases but promising agents have been identified using both approaches (Cook and Baker, 1983).

**Environmental enhancement of biocontrol efficacy**

The influence of environment on biological control can be subdivided into physical environment and the influence of the chemical or nutritional environment on the growth and survival of agents. Manipulation of chemical and nutritional environments of plant surfaces has potential for the enhancement of biological control. Like the addition of adjuvants in case of fungicides to enhance their efficacy, the efficacy of biocontrol agents can be improved by adjuvants that modify the environmental, physical, or nutritional conditions in the target microhabitat. Many organic substrates influence the biological activity of pathogens and biocontrol agents (Cook and Baker, 1983).

**Selection criteria**

Programmes for screening antagonists for disease control of plant pathogens are often focused on testing antagonistic properties in vitro, in bioassays and subsequently in crops. For commercial use, however, antagonists must fulfil many more criteria. Besides the toxicological profile of an antagonist, industries will consider technologies for production and formulation and
their costs, genetic stability of the antagonist, market size for the biocontrol product and the possibilities of patent protection for the application (Whitesides et al., 1994; Köhl, 2010).

Microbial antagonists occupy the same ecological niche as the target plant pathogen and interact directly with it. The mechanisms of interaction include parasitism, competition for space, water or food, or ‘chemical warfare’ using antibiotics or other secondary metabolites that harm the target pathogen. The second class involves an indirect effect in which the control agent induces a resistance response in the plant that gives it protection against virulent plant pathogens. The strain of biocontrol agent which possesses most of these criteria is supposed to be the most effective one.

Suppressive soils

Several soil-borne pathogens, such as *Fusarium oxysporum* (the cause of vascular wilts), *Gaeumannomyces graminis* (the cause of take-all of wheat), *Phytophthora cinnamomi* (the cause of root rots of many fruit and forest trees), *Pythium* spp. (a cause of damping-off), and *Heterodera avenae* (the oat cyst nematode), develop well and cause severe diseases in some soils, known as conducive soils, whereas they develop much less and cause much milder diseases in other soils, known as suppressive soils. The mechanisms by which soils are suppressive to different pathogens are not always clear but may involve biotic and/or abiotic factors and may vary with the pathogen. In most cases, however, it appears that they operate primarily by the presence in such soils of one or several microorganisms antagonistic to the pathogen. Such antagonists, through the antibiotics they produce, through lytic enzymes, through competition for food, or through direct parasitizing of the pathogen, do not allow the pathogen to reach high enough populations to cause severe disease (Agrios, 2005).

Numerous kinds of antagonistic microorganisms have been found to increase in suppressive soils; most commonly, however, pathogen and disease suppression has been shown to be caused by fungi, such as *Trichoderma*, *Penicillium*, and *Sporidesmium*, or by bacteria of the genera *Pseudomonas*, *Bacillus*, and *Streptomyces*. However, in several diseases, continuous cultivation (monoculture) of the same crop in a conducive soil, after some years of severe disease, eventually leads to reduction in disease through increased populations of
microorganisms antagonistic to the pathogen. This effect, which is selective for certain pathogens and not for others, is an area of in depth investigation.

**Durability**

The durability of a control method for plant protection is defined as the persistence of its efficacy in space and time. Erosion of effectiveness of conventional plant protection methods has been widely studied in the past. The durability of chemical control has for instance been studied because of the frequent and recurrent apparition of resistance to fungicides in major plant pathogenic fungal populations (Brent and Hollomon, 2007). The breakdown of varietal resistance, especially that conferred by major resistance genes, has also been widely studied for plant pathogens (McDonald and Linde, 2002). In contrast, the durability of biological control has long been assumed to be higher than that of chemical control (Holt and Hochberg, 1997). However, recent results concerning pest management in agricultural systems have shown that this assumption may not always be justified.

The most striking example may be the development of resistance to the most widely used bio-insecticide in the world. Resistance to one or several toxins produced by the bacterium *Bacillus thuringiensis* (Bt) has been described shortly after the market approval of products based on various strains of this bacterium.

In contrast with the situation for pests, the durability of biological control of plant diseases has hardly been studied. This may be related to the limited use of biological control against plant diseases in practice until recently. A bibliographical study conducted in the framework of the European project ENDURE (European Network for Durable Exploitation of Crop Protection Strategies) established that despite the large amount of microorganisms as potential candidates for biological control (Nicot et al., 2011), there are still few biocontrol agents registered against plant diseases in the European Union (Heilig et al., 2011).

However, several studies reported the inconsistency of efficacy of various biocontrol agents when introduced under commercial field conditions-being less effective or completely ineffective even though their efficacy was very good in controlled conditions (Shtienberg and Elad, 1997; Guetsky et al., 2001; Mark et al., 2006; Nicot et al., 2011).
This variability of efficacy is generally attributed to climatic variations (temperature, humidity, radiation) encountered in field conditions, a lack of ecological competence (survival, colonization ability) of the biocontrol agent, intrinsic traits of the antagonistic microbe (variable production of required metabolites or enzymes) and/or an unstable quality of the formulated product (Elad and Stewart, 2004; Mark et al., 2006; Ruocco et al., 2011). However, reduction of efficacy in the field may also result from the diversity of sensitivity of plant pathogens to biocontrol agents, with the existence of less sensitive isolates in natural populations of plant pathogens. The durability of biological control against plant pathogens may be related to specific traits of the plant pathogen such as genetic diversity and ability to evolve in response to a selection pressure. This is affected by population genetic processes including mutation, population size, recombination, gene flow and selection. This point was extensively studied to achieve durable plant disease resistance in agriculture (McDonald and Linde, 2002; McDonald, 2014). Thus, McDonald and Linde (2002) have hypothesized that populations of plant pathogens with high evolutionary potential are more likely to overcome a varietal resistance.

The same assumption can be proposed for the development of resistance to biocontrol agents. The durability of biological control against plant pathogens may also be related to the selection pressure exerted by the biocontrol agent. This selection pressure clearly depends on the extent of use of biocontrol agents in practice (surfaces treated, doses of application etc.). It may also depend on the specific mode of action of biocontrol agents. Various modes of action are involved in the protective effect of biocontrol agents against plant pathogens. Although the number of studies done on this subject is important, knowledge of the precise mode of action of biocontrol agents is still partial. However, it is generally considered that there are three main ways for a biocontrol agent to control a plant pathogen (Jacobsen, 2006; Alabouvette et al., 2009): first, by acting directly on the plant pathogen, through antibiosis, competition for nutrient or space, or parasitism; secondly by interfering with the mechanisms of pathogenesis of the plant pathogen, and thirdly by modifying the interaction of the plant pathogen with its plant host for instance, through the induction of local or systemic acquired resistance. These modes of action are not incompatible, they can instead be complementary and a single species or a single strain of a biocontrol agent may act with several of these modes of action (Janisiewicz and Korsten, 2002). A given biocontrol agent may therefore operate through several mechanisms potentially
expressed successively, simultaneously or synergistically and possibly depending on the environmental conditions encountered. Nevertheless, it is not yet clear if biocontrol agents have a dominant mode of action and under what conditions they switch from a mode of action to another. Even though all biocontrol agents should create selection pressure on target populations of plant pathogens once treatments are applied in the field, some modes of action may present a clear opportunity for pathogens to evolve resistance.

**Capacity of plant pathogens to adapt to biological control**

Besides existing diversity in susceptibility of plant pathogens to biocontrol agents, another concern could be that resistance would develop through adaptation under selection pressure, following the generalized use of a biological control method in the field, as has already occurred for various pathogens with certain fungicides. The estimation of this potential risk can be achieved through experimental evolution studies with the production of successive generations of the pathogens under selection pressure, as commonly carried out to evaluate the durability of efficacy of antimicrobial compounds in human pathology (Cowen et al., 2002), or to assess the capacity of plant pathogens to adapt to fungicides (Brent and Hollomon, 1998). The experimental evolution studies illustrate the potential of plant pathogens to adapt to the effect of biocontrol agents. Studies also suggest that the use of chemical methods in parallel or in combination with biological control may have an impact on the durability of the efficacy of certain biocontrol agents.

**Conclusion**

Ecological factors play very important roles in the performance and activity of biocontrol-active microorganisms. Biological control agents against plant pathogens, especially those in soil, operate within physically, biologically, and spatially complex systems by means of a variety of trophic and nontrophic interspecific interactions. The biocontrol agents themselves are also subject to the same types of interactions, which may reduce or in some cases enhance their efficacy against target plant pathogens (Knudsen and Dandurand, 2014). Characterization of these ecologically complex systems is challenging, but a number of tools are available to help unravel this complexity. New molecular approaches have been available for assessment of interaction between the antagonist and pathogen, ecological traits of antagonists in rhizosphere
and improving the efficacy of bacterial, fungal and viral biocontrol agent. However, other methods in IPM for crop disease control are still necessary in various environmental conditions, because an agro-ecosystem is a variable and functioning system that includes several factors that influence disease and crop development.

References


********
Chapter-5

Mass Production Technology of *Beauveria bassiana*

**K. C. Puzari and Pranab Dutta,**
Department of Plant Pathology
*Assam Agricultural University, Jorhat-785013, Assam*

**Introduction:**

The search for an alternative to chemical control for insect pest management resulted in the identification of a mycoinsecticides, an indigenous strain of white muscardine fungus, *Beauveria bassiana*. The pathogen has long association with the insect complex of north east India. During the few decades, the fungus has been thoroughly studied in the laboratory of Mycology Research Section and Laboratory of Department of Entomology, Assam Agricultural University, Jorhat with an aim of utilizing it as an efficient biocontrol agent for the management of insect pest under study. *Beauveria bassiana*, can infect more than 700 species of insects. It has an extensive host list that includes important pests like cotton whiteflies, *Bemisia tabaci*; grasshoppers, *Heiroglyphus banian*; termites, *Odontotermus* sp.; Colorado potato beetle, *Leptinotarsa decemlineata*; Mexican bean beetle, Japanese beetle, *Popillia japonica*; lygus bugs, chinch bug, *Blissus leucopterus*; European corn borer, *Ostrinia nubilalis*; codling moth, *Cydia Pomonella* and cabbage caterpillar, *Pieris brassicae* and *Dicladispa armigera* (Hazari et al., 2005 b). Unfortunately, natural enemies, such as lady bird beetles, are susceptible too. The much studied and discussed fungi *B. bassiana* was found to be effective against *Helicoverpa armigera*, *Spodoptora litura* and stored product pest, *Sitophilus oryzae*, mole cricket, *Gryllotelpa africana*. Scientist of AAU, Jorhat reported that *B. bassiana*@ 10\(^7\) conidia/ml infected and killed adults, larvae and eggs of rice hispa, *Dicladispa armigerain* field. *B. bassiana* was also found to be effective against different rice pests like *Scripophaga incertula*, *Chilo suppressalis*etc. The fungus has been found to have virulence of 78-87% with LC 50 value of 90.16. During the last few years, methods and technologies have been generated for its mass production (which can yield 39.33 X 10\(^7\) spore/ml) and field application. The pathogen has the capacity to spread widely and cause epizootic in a very short period.
Mode of action:

After landing on the host surface the spore germinate, and penetrate through cuticle and then haemocoel. Having proved in the body the conidia begin to circulate in haemolymph. They first attack fat bodies as it act as a source of glucose/ sucrose, and then try to attack the food tract of epithelial cell wall. Epithelial cell wall produces some enzyme for self protection. Therefore, if the epithelial wall get damaged/infected, their protection ability get reduced and the insect become weak. The fungus grow profusely by utilizing the haemolymph and remify the host cell and cause death. In later part the fungus burst out from the insect body as spore.

Symptom on host:

Infected insects become unrest and matting tendency increased. The affected insect is covered with white crust of fungal growth/ wadded coating (conidiophores).

Dispersal:

As infected insects become unrest and matting tendency increased, the infected insect flies to a long distance and thus the entomopathogen spread from one place to another. The entomopathogen is also spread by wind, rain or contact with other insects can spread infection.

Criteria for Choosing Microbial Insecticides:

The groups of criteria that concern pest and ecosystem characteristics are:

- **Direct or Indirect Pest.** Microbial control generally has a higher success rate against indirect pests i.e. ones that damage parts with no inherent commercial value) than direct pests.
- **Extent of Damage.** A large potential market is preferable over a small one for a commercial microbial insecticide product.
- **Pest Feeding and Behavior.** Insects that infest large vegetative areas or that live in soil usually offer more microbial control opportunities than aquatic insects or those with sucking mouthparts.
- **Member of a Pest Complex.** Even the more generalist pathogens usually are not effective against a wide range of pests and users prefer a control agent that will suppress all pests in a complex.
Economic Injury Level (EIL). Due to the relatively slow speed or necessity for ingestion of virtually all entomopathogens, crops with a low EIL are less likely to be conducive to microbial control than those with a moderate or high.

Criteria for selecting an organism as entomopathogen:

The following set of criteria address entomopathogen characteristics,

- **Host Range.** An entomopathogen with a relatively broad effective host range is better for commercial development over one with a narrow host range, however, in rare cases where a beneficial or endangered insect population might be affected.

- **Virulence.** For the short-term microbial insecticide approach, a high degree of virulence is virtually essential.

- **Cost of Production.** A major reason for failure in the market has been their cost-competitiveness with other pesticides. Obligate pathogens which must be produced in living insects are more costly to produce than facultative pathogens.

- **Suppresses Pest Population Below EIL.** For its success, it must possess characteristics that enable it to reliably suppress the pest population below the EIL.

- **Environmental Safety.** Environmental safety is a prerequisite.

- **Advantage over Competing Controls.** The entomopathogen must have a relatively good degree of cost effectiveness compared with other controls.

- **Registration.** Majority of entomopathogens present no particular difficulty in meeting government guidelines for registration as a pesticide. Although costs are low compared with those for chemicals, they are still prohibitive for a small company developing a microbial insecticide.

- **Compatible.** Another strength of entomopathogens is their compatibility with other insect controls and with agricultural and resource management practices in general.

Production technology:

Materials Required:

1. Freshly isolated entomopathogen.
2. PDA media
3. Substrates (Rich husk, Saw Dust, Rice Bran)
4. Chitin  
5. Distilled water  
6. Poly propylene bags  
7. Racks  
8. Rubber bands  
9. Laminar flow cabinet  
10. Inoculating needle  
11. Spirit lamp  
12. Cotton (both absorbent and non absorbent)  
13. Spirit lamp  
14. Methylated spirit  
15. Ethyl alcohol  
16. Petriplates  
17. Cork borer  
18. Measuring cylinder  
19. Muslin cloth  
20. Plastic Bucket and mugs  
21. BOD incubator

**Fungus:**

Freshly isolated *Beauveria bassiana* infected dead cadavers are to be isolated in potato dextrose agar medium.

**Cultural and microscopical characteristics of the fungus:**

**Cultural Characteristics:**

Colonies are milky white and moderately rapid growing, spreading woolly, powdery or mealy in texture, white to yellowish white or occasionally pinkish in colour.

**Microscopical characteristic:**

The entomopathogenic fungus *Beauveria bassiana* produces at least three distinct single-cell propagules, aerial conidia, vegetative cells termed blastospores, and submerged conidia,
which can be isolated from agar plates, from rich broth liquid cultures, and under nutrient limitation conditions in submerged cultures, respectively.

Conidiogenous cells are hyaline, flask shaped with long zig-zag appearing rachis beraing lateral conidia. Conidia are hyaline, 1-celled and globose to ovoid with a length about 3.5 µm. Clusters of conidiogenous cells appear as small powdery balls in the aerial hyphae when viewed through a dissecting microscope.

**Mass culture of *B. bassiana***:

- To harvest large quantity of propagules, the isolate are to be mass cultured in polypropylene bags containing sterilized (autoclaved at 121 °C for 20 min. repeated for 2 successive days) in rice hull : saw dust: rice bran medium @ 25 : 25 : 100 respectively supplemented with 2 % dextrose.
- Polypropylene bags each having capacity of 150 g containing 50 g medium are to be inoculated with 1ml of 1 X 10⁷ conidia /ml suspension and incubated at 24 ± 1°C for 20 days.
- To obtain uniform growth of the fungus, the bags were mechanically shaked thoroughly after 5 days.
- After 21 days of incubation the bags were kept in racks at room temperature and ready for application.

**Preparation of spore/ conidial suspension of *B. bassiana***:

- The conidial density as obtained from the mass cultured medium (RH: SD: RB) is 39.33 x 10⁷ conidia/ml of water.
- A conidial concentration 1 x 10⁷ conidia/ml of water have to be used for spraying by suspending 1000g of mass cultured substrate with homogenous fungal growth in 5000 ml of water.
- Prior to spray the mixture has to be filtered through single layered muslin cloth to prevent the nozzle block.
Method of application:

The prepared spore/conidial suspension (as per the method mentioned above) of *B. bassiana* can be applied with the help of Knapsack sprayer in the morning hours at least 2 hrs prior to sun rise or in the evening at least 1 hrs after the sunset. In rainy days Tween 80 or Teepol or toilet/bath soap @ 0.02% should be mixed in the spore suspension prior to the spraying.

Advantages:

- Microbial insecticides based on *B. bassiana* are non-toxic and non-pathogenic to wild life, humans and other organisms not closely related to the target pest.
- They are specific to a single group or species of insects not affecting the predators and parasites of the pest.
- They are compatible with the chemical insecticides i.e. not deactivated or damaged by chemical insecticides.
- They can be used when the crop is ready to harvest as the residues have no hazard to human or other animals.
- They may establish themselves in a pest population or its habitat and provide controls to subsequent pest generations.
- Resistance development to microbial pathogens is also slow. They can control insect in cavities, where chemical insecticides cannot reach.

Disadvantages:

- Each application of microbial controls only a portion of the pests but the rests continues to damage in the treated areas.
- Heat, desiccation or exposure to UV rays and long storage reduces the effectiveness of microbials. As they are pest specific, their potential sales market is limited.
- Some of the products of microbial are not widely available and are relatively expensive.

Precautions:

- Select freshly isolated virulent pure culture
- *B. bassiana* must be passed through host as much as possible for higher infection percentage.
- Avoid using contaminated culture for mass culturing and field application
- Spraying must done before sunrise or after sun set
- Spraying equipment must be free from any other chemical pesticides
- In rainy days sticker must be used.
- Avoid spraying other pesticides in the *B. bassiana* applied field

******
Chapter-6
Morphological Characterization of Fungal Bio-Control Agents

M.S. Ali
Retd. Professor & Head,
Assam Agricultural University, Jorhat-785013, Assam.

The prevalent conventional techniques for characterization & identification of fungi are not sufficient to provide a complete draft of the subject as these techniques describe only shape, colour, size, host-range, pathogenicity & often with some information of assimilation capacity & bio-chemical characters of narrow range of fungi which are however culturable only. Considerable advancement in the study of microbial taxonomy has taken place, such as chemotaxonomy, DNA-DNA hybridization, DNA amplification and sequencing, whole genomone sequencing etc. Despite of these progress and development of genome based techniques; any individual technique cannot be relied upon solely as a source of taxonomic information. Taxonomic information of an unknown microbe is highly essential to establish its biodiversity, relationship among other organisms in the ecosystem and its functional aspects (Gevers et al, 2005).

Most of the fungal bio-control agents belong to hyphomycetous fungi (Ascomycota) and very often manifest their anamorphic phase rather the teleomorph either on natural substrate or culture. They lack closed fruiting bodies, and formed conidiophores or canidia directly on the hyphae. S.J. Hughes (1953), proposed a scheme of classification for hyphomyctes based on conidial ontogeny. The International Conference on Criteria and Terminology in the classification of fungi imperfecti held at Kananaskis, Canada, in 1969 endorsed that Saccardon Classification must be replaced by a system based on canidial ontogeny.

Three categories of information employed for description of an asexual fungus are: candidomatal types, Saccardo’s groups on conidial shape and septation and candiogenous events. Identification of hyphomycetes in primarily based on microscopic morphology, cell wall texture, conidium ontogeny &conidogenous cells, borne on sporodochia or synnemata.

Biopesticides can control many different kinds of pests, although each separate active ingredient is relatively specific for its target pest(s). Therefore, proper identification is
very important, else there would be possibility of failure while attempting to manage a target pest. *Beauveria bassiana* can infect more than 700 species of insects like *Bemisiat abaci*, grass hoppers, cabbage caterpillar, *Dicladispa armiger*. The bioagent also found effective against rice pests in Assam viz., *Scripophaga incertula, chilo suppresalis* etc.

On culture, produces white with distinct white spore balls. Rachis shows typical zig-zag extension conidia round, single celled, hydrophobic. The fungus can cause the “white muscardine” disease on insects by penetrating the host cuticle and kill the host. It also shows promise against insects like aphids, white flies, stem borer, rice hispa, termites etc.

Another bio-control agent viz., *Trichoderma spp* (Fig, Keys) are extensively used for control of several soil borne diseases and exploit their growth promotion effect. Besides few potent antagonists are *Metarhizium, Verticillium, Paecilomyces* etc. have been used for management of plant diseases and pests. So there isolation and proper identification via conventional or molecular or both is highly desirable. Ideally, identification should be easy, efficient and user friendly; so that researchers of other discipline may be able to follow the keys for identification. It is seen that in many cases, molecular markers provide reliable and faster identification than identification made by morphological means. But reliable and easy identification of organisms are also possible by conventional means too. However one method of identification might supplement the other method for quick and obviously proper identification. Therefore every effort should be made to characterize the target pathogens/microbes which can work to focus for achieving the desired goal. Moreover, a draft based system of identification can only work if all the species have the ‘diagnostic’ sequences present in the data base (Goswami, 2018). If a new organism/microbe has to be identified, there will be no option left to study by scientist/s except morphotaxonomist.

*Metarhizium anisopliae* (Fig, Key) causes green muscardine disease on arthropods, has been exploited to manage insect pest like termites, diamond black broth, mosquito larvae etc. Growth on culture media appears green & characteristic orange color on lower side with concentric rings or radiating streaks. The fungus also produces droplets of “destruxins” on the surface of ceiling.

Another very interesting bio-agent is *Verticillium lecanii*, which can be used for the bio-control of plant parasitic nematodes, insects and also fungal pathogens. The fungus has
typically verticellate growth, conidia ovoid, produced in chains. Conidiophore dilutely pigmented. The colony bluish green & underside distinct purple. It exudes pink & purple coloration to the culture media. Previously known as *Verticillium lecanii* Zimm. , the fungus is today referred to as *Lecanicillium lecanii*. Few genera having biocontrol potential viz., *Paecilomyces, Nomuraea, Akanthomyces, Hirsutella, Gliocladium* are to be exploited for the management against maximum possible pests and diseases.

*Trichoderma*:

In bio-control of plant diseases, fungal antagonist like *Trichoderma* spp are extensively used for control of soil borne diseases or growth promotion of host plants. *Trichoderma* has become a model bio-control agent in the management of plant diseases and play a vital role of IDM. Therefore characterization of the genus is prerequisite to manage target diseases and pests. Taxonomy of *Trichoderma* still needs a comprehensive means to delineate the different species whether morphological, biochemical, physiological, microscopy and molecular. However, presently much stress has been given on polyphasic taxonomy where each of the method might have contributory sharing of information to identify its taxonomic position and identification. Another problem associated with microbial taxonomic analysis is the existence of some common taxa that cannot be cultured, & have to resort for culture independent approaches. Therefore, the use of a polyphasic approach involving a combination of other available conventional techniques and molecular microbiological methods is vital to obtain better understanding of the referred microbes.

*Trichoderma*:

Taxonomy of *Trichoderma* has gone through a remarkable transformation, and many more new species have been recognized within the last 40 yrs. Besides morphological means to characterize *Trichoderma*spp, several researchers also tried to identify the species by means of biochemical, and molecular study. Some of them are described here under, which are found to have antagonistic potential or found relevance to the topic being selected for discussion.

Key to the species (*Trichoderma* Pers. Ex. Fr.)

(Girisham et al, 2016)

*Trichoderma* has five sections – (Bissett, 1991)

- Pachybassium
- Longibrachiatum
- Trichoderma
- Saturnisporium
- Hypocreanum

With the advent of molecular study from 1995 onwards, Bissett's scheme was largely confined but saturnisporium and Hypocreanum appeared monophyletic. Pachybasium was determined to be paraphyletic, many of its species clustering with *Trichoderma*.

**Salient phenotypic characters of *T. viride* (Tv)&*T.asperellum* (Ta)**

<table>
<thead>
<tr>
<th>Bioagent</th>
<th>Conidial ornamentation</th>
<th>Canidiophore</th>
<th>Phialide</th>
<th>Clamydospores</th>
<th>Temp. optimum (PDA)</th>
<th>Colony radius (PDA) at opt. temp (mm) after 48 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tv</td>
<td>Conspicuous</td>
<td>Irregularly branched, branches often not paired</td>
<td>Often sigmoidal or hooked L/W=3.3</td>
<td>Typically absent</td>
<td>25</td>
<td>11 – 33</td>
</tr>
<tr>
<td>Ta</td>
<td>Inconspicuous</td>
<td>Regularly branched, branch typically paired</td>
<td>Straight, L/W=2.4</td>
<td>Typically present</td>
<td>30</td>
<td>31 – 47</td>
</tr>
</tbody>
</table>

**Key to Trichoderma with roughened, warded conidia**

1. Conidia hyaline (white in mass), roughened when young; sterile, sinuous, spinulose conidiophore extensions projecting from conidial tufts...........*H. Pachybasioides*.  
   1. Conidia in mass green........................................................................................................2  
   2. Conidia conspicuously tuberculate, appearing as blisters, many phialides arising singly from the main axis. – *T. saturnisporium*  
   2. Wart on conidia smaller or inconspicuous; many or most conidia appearing smooth..................................................................................................................3  
   3. L/W ratio of conidia measured from CMD>1.8; conidia obscurely tuberculate or smooth.........................................................................................................................................................4  
   3. L/W ratio of conidia measured from CMD<1.5; conidia cast conspicuously or obscurely tuberculate.................................................................................................5  
   4. Most conidia measured from CMD 4-5x1.7 – 2.5μm – *H. andinensis*
4. Most conidia measured from CMD 4.5-6.2x2.2-3um – *T. ghanense*

5. Most conidia conspicuously roughened or warded; conidia typically globose to sub-globre (L/W=1.1) but ellipsoid in some collection; phialides cylindrical, often sinuous or hooked.................................................................*T. viride*

5. Conidia finely warted, many appearing smooth, globose, sub-globose or ovoidal, L/W averaging 1.2; conidiophores regularly branched with branches conspicuously paired; phialides ampullii form, arranged in cruciate whorls of 3 or more, rarely solitary – *T. asperellum*

Keys to *Paecilomyces, Trichoderma and Verticillium* have been annotated here under *(Girisham et al., 2016)*

**Paecilomyces Bainer**

**Colony**: Growing fast

**Hyphae**: Funicoluse, olivaceous to brown.

**Conidiophore**: Mononematous, macronematous, simple or branched, septate, hyaline to pigmented, arise directly on aerial hyphae.

**Conidiogenous cells**: Phialides, borne singly in pairs or in verticals or on the penicellate heads, sometimes irregular arranged.

**Phialides**: Awl shaped, divergent, base swollen with slender long neck.

**Conidia**: Catenate tending to slip or slime down, hyaline or darkly pigmented smooth or reoughened, sometimes spherical ovoid to fusiform, sometimes spherical.

**Chlamydospores**: Aleuro spores or chlamydospores are produced sometimes Aleuro spores.

**Teleomorph**: Byssochlamys

**Key to species of *Paecilomyces***

1. Colonies yellow-brown; odour sweet aromatic; usually thermotolerant or thermophilic (Sect. Paeciomyces) Ascomycota absent: Conidiophores repeatedly branched; conidia of different sizes; more or less ellipsoidal; chlamydospores present.
   1a. Colonies of other bright colors; odour not sweet aromatic; mesophillic....................2

2. Colonies vinaceous to violet.........................................................................................4

2a. Colonies other colors..................................................................................................3

3. Conidiophores stalks pigmented, rough-walled; reverse uncoloured or purple; aleurioconidia absent............................................................................................................ *P. lilacians*
3a. Conidiophores stalks hyaline, smooth-walled; reverse yellow; aleurioconidia usually present........................................................................................................P. marquandii

4. Conidia finely echinulate; colonies white to pink, thin, powdery, synnemata absent; reverse usually dark green.................................................................................P. carneus

4a. Conidia smooth-walled; colonies usually deep and woolly, forming synnemata on insects and rarely in cultures; colony reverse not green.....................................................5

5. Colonies usually pink; conidia fusiform to cylindrical.
   3-4x1-2μm................................................................................................................P. fumosoroseus

5a. Colonies white to luteous; conidia ellipsoidal to fusiform, less than
   3.5μm................................................................................................................................P. farinosus

5b. Colonies yellowish-brown, conidiophores repeatedly verticillate, phialides
   Distributed irregularly along the fertile hyphae, conidia strongly ellipsoidal, smooth walled, size variable..............................................................P. varioti

Trichoderma Pers. Ex. Fr

Synonyms = Aleurisma
= Pachyingbarium

Colonies spread, repeated, branched, hyphae hyaline, septate, conidiophore in tufts with divergent often irregularly branched and flask shaped phialides. Conidiophores may end in a sterile appendage with phialides borne on lateral branches in some species. Conidia hyaline or usually green, non-septate, globose to spherical. Hyaline chlamydospores are usually present in the mycelium of older cultures. Hypocrea is the teleomorph of this genus.

Ishikawa et al. (1973) have reported production of trichodermin and trichoderminol by T. hamatum, T. harzianum. Hypocrea is the perfect stage of some species belonging to Trichoderma and Trichotheciumroseum.

Key to the species

1. Conidiophore and branches short and stout often with sterile hyphal elongation bearing crowded phialides colonies white, whitish-green or green..............................................2

1a. Conidiophore and its branches strong and slender without sterile hyphal elongation, phialides not crowded. Colonies yellowish, bright-green to dark-green...............................5

2. Sterile hyphal elongation absent, conidia globose, hyaline.....................T. piluliferum(1)
2b. Sterile hyphal elongation present or modified or rarely absent, conidia not globose..................................................................................................................3

3. Conidia green, short, ellipsoidal, surrounded by a wide irregular
   veil....................................................................................................................................T. saturnisporum (2)
3a Conidia smooth walled or finely punctuated.................................................................4

4. Conidia hyaline, small, 2.4-3.8x1.8-2.2 μm..........................................................T. polysporum (3)
4b. Conidia green, small to large, 3.8-6.0x2.2-2.8 μm.................................................T. hamatum (4)

5. Conidia rough, 3.6-4.8x3.5-4.5 μm.................................................................T. viride (6)
5a. Conidia smooth walled.................................................................................................6

6. Conidiophore with complicated dendroid branching, phialides regularly
   disposed in no. 3 or more................................................................................................7
6a. Conidiophore with simple branching system, phialides irregularly laterally
   disposed often arising singly..........................................................................................9

7. Conidia ellipsoidal or oblong often appearing angular
   3.0-4.8x1.9-2.8 μm........................................................................................................T. koningii (6)
7a. Conidia shorter with a length: width ratio of less than 1.5 μm..............................8

8. Conidia obovoid with truncate.......................................................................................T. aureoviride (7)
8a. Conidia shorter with a length: width ratio of less than 1.5 short
    obovoid..................................................................................................................................T. harzianum (8)

9. Conidia subglobose to ovoid.....................................................................................T. reesei (9)
9a. Conidia ellipsoidal..........................................................................................................10

10. Phialides usually distinctly attenuate at the base, conidia smaller, pale
    green 2.8-4.8 μm, mostly oblong ellipsoidal..................................................T. pseudokoningii(10)
10a. Phialides usually only slightly attenuate at the base, conidia large, partly
    dark-green up to 7.0 μm long mostly ellipsoidal........................................T. longibrachiatum (11)

T. harzianum Rifai

**Synonyms:** = Spororichum narcissii Tochinai and Shimala
= Trichoderma numbergii
= T. narcissi

**Teleomorph:** Hypocerealixic
Colony: Growing rapidly, smooth surfaced, watery white with sparse mycelial mat but soon develop aerial hyphae appearing granular or powdery with the formation of conidia rapidly turning to yellowish-green to dark-green producing tufts or pustules finzed by sterile white mycelium.

Reverse: Colourless to dull-yellowish, buff or drab

Exudates: Colourless or amber or greenish-yellow, abundant in some isolates

Odour: Distinct or fairly earthy

Chlamydospores: Fairly abundant, intercalary or terminal on short branches, solitary contents subhyaline to pale-yellowish to brownish with age sub-globose to ellipsoidal or pyriform, hyaline.

Verticillium Nees ex. Link

Synonyms = Acrostalggmus
= Ascocylindrium
= Diheterosporaphoconia

Colony: Floccose, white, yellow, orange, orange-brown, dark

Sterile hyphae: Creeping, septate, branched, hyaline or slightly coloured

Reverse of colony: Similarly coloured becoming black with the formation of dark resting mycelium and microsclerotia.

Conidiophore: Erect, septate, branched, branches of first order whorled, opposite or alternate, branches of second order whorled dichotomous or trichotomous on the branches of first order further branching is similar to terminal branchlets usually flask shaped distinctly pointed at the apex.

Conidia: Borne singly on branchlets, produced in mucous and sliming down to form balls at the apex of phialides, round, ellipsoidal, ovate, inverted egg shaped, short-spindle-shaped, hyaline or slightly coloured.

Diagnostic features: Conidiophore verticellate, pink spore balls appear on phialides, phialides single spores in large number.

Key to the species

1. Colonies intensely orange to orange-brown due to the pigmented conidiophores and conidia; conidia ellipsoidal, 3.5-5x2.0x5.5..............Nectriainventa

1a. Colonies white, yellowish or darkening...............................................................2
2. Colonies darkening in reverse..................................................................................................................3
2a. Colonies remaining entirely white or yellowish.......................................................................................7
3. Colonies producing discrete micro sclerotia but no chlamydospores:
   conidiophores hyaline; conidia 2.5-6(-8)x1.4-3.2...............................................................................V. dahlia
3a. Colonies usually not producing discrete micro sclerotia, but in some species
   Dark resting mycelium and/or chlamydospores occur.............................................................................4
4. Only chlamydospores present....................................................................................................................5
4a. Dark torulose resting mycelium present.....................................................................................................6
5. Chlamydospores 5.5-8(-10) μm diam, usually produced singly; conidia
   Mostly 1-celled, 4-8.5x1.5-2.5 μm...........................................................................................................V. nigrescens
5a. Chlamydospores 8.5-17 μmdia, commonly in chains; conidia often
   2-celled, 4-10(-12.5)x2.5-3.5 μm..................................................................................................................V. nubilum
6. Chlamydospores present, 7.5-11 μmdia, in addition resting mycelium and
   some microsclerotia; reverse golden yellow in young cultures; conidia
   3.5-10x1.5-3.5 μm..................................................................................................................................(V. tricorpus)
6a. Chlamydospores and microsclerotia absent; reverse white to cream in young
   Cultures; conidia 3.5-10.5(-12.5)x2-5 μm...............................................................................................V. albo-atrum
7. Dictyochlamydospores present at least on SEA.....................................................................................8
7a. Dictyochlamydospores always absent......................................................................................................9
8. Dictyochlamydospores predominant; conidia short-cylindrical, 3-4x1.5-2.0μm
   Cohering in chains.................................................................................................................................V. chlamydosporium
8a. Dictyochlamydospores scanty; conidia subglobose, 2.9-3.3x1.9-2.2μm, cohering in
   chains.........................................................................................................................................................V. catenulatum
9. Well-differentiated, thick-walled conidiophores present with globose
   Conidial heads; conidia slightly fusiform, straight or curved
   3.8-7.2x1.2-2.4 μm....................................................................................................................................V. fungicola
9a. Phialides on little-differentiated, often prostrate conidiosphores; conidia
   Commonly transversely attached to the phialides tip..............................................................................10
10. Conidia cylindrical with rounded tips.....................................................................................................V. lecanii
10a. Conidia with pointed tips.......................................................................................................................11
11. Conidia straight, 3-10(-12)x0.8-1.2 μm..................................................................................................V. lamellicola
11a. Conidia ± curved, 5.8-8.0x1.5-1.8 μm............................ *V. psallioa*
Akanthomyces

Hirsutella

Reference:


********
Chapter-7
Mass Production Technology of Trichoderma harzianum with Locally Available Substrates

B.C. Das, Pranab Dutta, Arti Kumari, Gaurav Phookan and Bhuvaneswari V.
Department of Plant Pathology,
Assam Agricultural University, Jorhat785013, Assam

Introduction:

Biocontrol offers safe, cheap and environmental friendly management of plant diseases and avoids or minimizes the usage of harmful fungicides. In biocontrol of plant diseases fungal antagonist like Trichoderma spp are extensively used for control of several soil borne diseases and also have additional advantage of growth promoting effect. Many commercial formulation in solid form based on these antagonists have been developed worldwide.

Trichoderma is a genus of asexually reproducing fungi present in nearly all temperate and tropical soils. The genus is a strong saprophyte, fast growing, prolific producer of spores and powerful antibiotic producer. These properties make this fungi ecologically diverse and can be used to produce a wide range of product of commercial and ecological interest. Trichoderma has become a model biocontrol agent in the management of Plant diseases as a part of IDM because of following characteristics:

- Produces high yield and high quality conidial biomass.
- Possess rhizosphere competence.
- Ability to thrive over a wide range of stress condition.
- Having mycoparasitic ability against large number of soil borne pathogens.
- Stimulate plant resistance & defence mechanism
- Compatible with large numbers of pest
- Easy to mass multiply.
- The final product have shelf life more than 6 months.

Mode of action:

- Mycoparasitism through production of enzymes & coiling.
Antibiosis through production of antibiotics.

- Competition for food & space.

**Selection of Trichoderma strain:**

During last 15 years, we have searched for a suitable biocontrol agent to be used as seed treating & soil treating agent. we succeeded in identifying a strain of *T. harzianum* (DPP-105). With this a formulation “Biogaurd” has been formulated to fulfill the demand of farmers.

The different process involved in the production of “Biogaurd” are

- Inoculum production
- Selection of culture medium
- Growth and mass multiplication
- Harvesting and drying
- Scaling up and
- Formulations

Three methods viz., solid fermentation, liquid fermentation and deep tank fermentation technology are followed for multiplication of *Trichoderma*. Out of these solid fermentation is easy, popular and widely used by the farmers.

**Solid fermentation Process:**

Improved selective medias have been developed for cultivation of *Trichoderma* spp. Solid media are prepared comprising easily available organics such as wheat bran, saw dust, grain bran, wheat straw, rice straw, sorghum grain, banana leaves, banana peeled skin, farmyard manure (FYM). Presently FYM and wheat bran-saw dust is extensively used to produce biocontrol agent of *T. harzainum* and *T. viride*. Well decomposed powdered FYM mixed with 1% peptone and half parts water are autoclaved at 30 lbs pressure for 1 hour intermittently for 2 times and heaped in a clean shady place. The stock culture of *Trichoderma* spp. multiplied on potato dextrose agar (PDA) medium for 15 days inoculated under aseptic conditions, 500 ml of homogenous spore suspension prepared in a mixer is used for 10 kg of FYM. Heaps are then covered with polythene sheets to prevent loss of moisture) for 30 days. Heaps are mixed thoroughly at an interval of 7 days. After 30 days of incubation this preparation is ready for
application.

**Liquid fermentation**

This process involves production of large number of conidia mainly intended for seed treatment but is again for limited extent. The balance nutrients are used.

**Deep tank liquid fermentation technology**

This involves mass production of fungus. In this technique *Trichoderma* spp. are mass cultured in commercially available inexpensive ingredients (molasses and brewers yeast). The rapid mass production of antagonists for seed treatment formulation, the deep tank liquid fermentation mostly used.

**Growth and mass multiplication:**

The fermenter medium used to grow and mass multiply *Trichoderma* spp consists of Molasses (30 kg) + Brewers yeast (5g)+ 1 litre water (fermenter medium).

Starter medium on which *Trichoderma* spp. is usually a selective medium of Fermenter medium of above concentrations is used. Procedure for mass production of *Trichoderma* spp. is given below:

1. Autoclave both starter and fermented medium for 1 h

2. Inoculate starter medium with efficient strain of *Trichoderma* and shake on rotary shaker

3. Inoculate fermented media with starter inoculum and supply regularly for 10-15 days by shaking/bubbling

4. Filter through cotton muslin using funnel

5. Air dry or freeze dry the mat for 3 days fine grind into powder this contain mycelium and
chlamydospores

Powder is diluted with different diluents and used as dusts, granules, pellets, wettable powders, emulsifiable concentration

Produce is viable for 6 months, if stored at 5°C and 3 months if stored at 20°C. Maximum biomass is obtained generally after 15 days of inoculation.

Formulations

Different formulations are available and prepared for the usage as given below-

1. Powder formulation: It is done by mixing fermenter biomass with talcum powder as carrier.
2. Encapsulation in organic polymer like sodium alginate.
3. Pelleting biomass and bran with sodium alginate.
4. FYM, banana leaves, banana peeled skin, wheat bran for soil application.
5. Molasses enriched charcoal powder granules.
6. Liquid coating formulations bio-protectant as powder on which suspension of aqueous binder is sprayed on seeds to form 0.1mm thick layer.
7. As spray form emulsifiable concentrations with 10 spores in all of the above formulations. It is necessary to maintain level of 1-10 cfu/ml or g.

Materials Required for preparation of formulation

1. Freshly isolated native Trichoderma harzianum pure culture
2. PDA media
3. Substrates
4. Distilled water
5. Poly propylene bags/ saline bottle
6. Racks
7. Rubber bands
8. Laminar flow cabinet
9. Inoculating needle
10. Spirit lamp
11. Cotton (both absorbent and non absorbent)
12. Spirit lamp
13. Methylated spirit
14. Ethyl alcohol
15. Petriplates
16. Cork borer
17. Measuring cylinder
18. Muslin cloth
19. Plastic Bucket and mugs
20. BOD incubator

Methods of application

The methods presently used for effective management of diseases includes the application of *Trichoderma* by any of these methods viz., broadcast application, furrow application, root zone application, seed coating or seed treatment, wound application.

*a. Broadcasting*:

Broadcasting is used for soil application. The quantity required is high and varies from 125-250 kg/ha. It is mixed with FYM and broadcasted on the soil and then incorporated into the soil through harrowing. It is used to control Sclerotinia rot of vegetables, damping off of vegetables, stem rot of soybean. *Trichoderma* is applied by this method at least 2 weeks before sowing of crops.

*b. Furrow application*:

It is comparatively economical to broadcasting and is followed in nurseries. The quantity required 130-160 kg/ha. It should be applied in the open furrows covered with the soil. *Trichoderma* is applied by this method 15 days before sowing of the seeds.

*c. Root zone application*:

This is used in wide spaces crop such as plantation and fruit crops. It is done in peach and plum against silver leaf diseases.
d. **Seed treatment:**

In this method of application, the seeds are first mixed with water 5-7 ml/kg, so that the seed surface becomes moist. Take known quantity of seed formulation (Powder form) 4 g/kg seed, mix thoroughly by agitating the container so that the powder adheres to the seed surface. Then treated seeds should be dried in shade for two hours and sown immediately after treatment. This formulation can used to apply on and around the root zone. This helps good seed germination, seedling emergence and finally good plant stands. It also gives protection in the rhizosphere against root rots, stem rot, seedling blight, damping off, collar rot, white mold, rhizome rot of various crops such as chick pea, cabbage, cauliflower, french bean, brinjal, chilli, cucumber, potato, ginger, rice, sunflower caused by *Rhizoctonia solani, Pythium aphanidermatum, Sclerotiniasclerotiorum, Fusarium oxysporum, F. moniliforme, Sclerotium rolfsii* etc.

**Economics:**

| Cost of production (Excluding laboratory equipment) | Rs. 30/kg |
| Selling price | Rs. 60/kg |

**Advantages:**

- Parasital activity takes place on different stages of insect ontogenesis;
- The fungus possesses the high speed of growth, huge reproductive ability and high specificity;
- It is able to remain for a long time in nature without the decline of infectivity;
- The effectiveness of this product depends very much on climatic condition (temp 25–28°C and relative air humidity of 80–90%), the methods of application and doses.
- Easy to deliver
- Improve plant growth, flowering and increases yield
- Safe to the environment, human beings, animals
- The no risk of development of resistance in pathogen
- Compatible with biofertilizer like rhizobium, azospirillium etc.
Precautions:

- Formulation must be free of contamination
- Store in cool and dark place, avoid direct sunlight
- If use for seed treatment, the treated seeds must be dried in shade at least for 6 hrs prior to sowing
- Application must be done in cooler hrs of the day
- Culture must be periodically re-isolated from the crop rhizosphere or phyllosphere
- Avoid direct contact on skin
Chapter-8

Mass Culture Technique of Biocontrol Agents for Management of Mites of Agricultural Crops

Sahidur Rahman
Principal Scientist,

Department of Entomology, Assam Agricultural University,

Jorhat-785013, Assam

Mites are the microscopic arthropods under the class Arachnida and subclass Acari. They are extremely diverse group of arachnids, closely related to spiders and scorpions. As a whole mite are categorised as phytophagous and predatory mites. Phytophagous mites are serious pests of crop plants and are generally controlled by synthetic pesticides. Pesticides adversely affect natural enemies and lead to environmental pollution, human health problems, harmful side effects, and risk of pest resistance. Therefore, demand for organic and pesticide-free crops is increasing due to public awareness of pesticides and food security. In recent decades, the focus on crop production has moved from yield to quality, safety, and sustainability. To achieve this goal, Integrated Pest Management (IPM) strategies are being employed. Though several tools are enlisted under IPM, biocontrol of pests has gained importance. Biocontrol has been achieved through various biocontrol agents like insects, mites, spiders, fungi, bacteria, viruses etc.

Mite predators are important in integrated management of mites, particularly in complex crop systems where they may remove the need for any chemical intervention. Several kinds of mite predators occur in nature that can regulate mite populations. The fundamentals of a biocontrol program are to precisely recognize the pest and to find efficient predators using their life table parameters and trophic interactions. Using mite predators is a commercial venture because of the need of mass production and their large-scale releases to control phytophagous mites. Two important challenges in commercial biocontrol strategies are problems associated with production and marketing of biocontrol agents. Governments and international organizations have important roles in marketing biocontrol agents by making strict rules benefitting biocontrol programs. To achieve this goal and to approach safety and security in crop production, biocontrol practices should be globally encouraged. Based on the high demand for
safe food, a future high demand for using biocontrol agents is predicted. Therefore, more research and activity on different aspects of biocontrol as an ecofriendly practice is inevitable.

**Brief history of biological control of mites in India:**

The history of biological control dates back to the seventeenth century and since then a great deal of success has been achieved in biological methods of pest control. In India, organized and systematic biological control research began with the establishment of the Indian station of Commonwealth Institute of Biological Control (CIBC) at Bangalore in 1957 with need based substations at various locations in the country. The All-India Co-ordinated Research Project on Biological Control of Crop Pests and Weeds (AICRP) was established in 1977 with 10 centres under the aegis of the Indian Council of Agricultural Research (ICAR) for carrying out biological control research in different parts of the country.

**Biological Control Agents for Mite Pests**

- Predators: They are mainly free-living species that consume preys in large number during their lifespan. Predators against mite pests include insects like Lacewings, Beetles, and dragonflies and predatory mite of various families.

- Pathogens: Virus, Bacteria, and fungi are relatively pathogenic micro-organisms that are host specifics to kill their host. Some of the microbial diseases occur naturally which may be used as biological pesticides.

**Importance of predators in biological control of insect and mite pests:**

Within recent years as a result of the widespread use of inorganic insecticides, especially chlorinated hydrocarbons, it is observed a tremendous increase in the population of phytophagous mites that infest agricultural crops and orchard trees, as the population of the natural enemies have decreased. In this context, the role of predators either insects or mites assumes particular significance. Alternative to insecticides and acaricides biological control has been attempted on a variety of crops against mites and some other pests. Biological control provides an environmentally safe, cost-effective and energy efficient means of pest control, either alone or as a component of IPM. The objective of using biological control agent is to
restore and or to enhance the relationship between pests and their natural enemies. As a control tactic, biological control is most suited pest species with a relatively high economic injury level. This is because the minimum prey density will usually be required to support a permanent predatory population.

Application of predatory mites against phytophagous mites is a recent addition to non-chemical pest management strategy. The most important predatory mite species explored in this regard includes members of phytoseiidae, cunaxidae, laelapidae, tydeidae, ascidae, stigmaeidae, anystidae, erythraeidae, and gamasidae. Few predatory mite species are commercially available to control tetranychids and thrips.

**Major Groups of Predatory Mites**

**Phytoseiid mites**

Phytoseiid mites belong to the family Phytoseiidae of the order Mesostigmata. Phytoseiid mites are predators of spider mites and other small insects and mites on plants. Some species feed on nematodes, fungal spores, pollen and exudates from plants. The phytoseiid is a large family of worldwide distribution. Several members of this family are of great importance in the biological control of spider mites and thrips in green house production.

The family consist of three sub-families – Amblyseiinae, Phytoseiinae and Typhlodrominae. Effective biocontrol agents occur in all these three sub families. Phytoseiidmites ability to prosper on non-animal food items like pollen, honey and nectar is another factor behind their success as a biocontrol agent. Examples of phytoseiid mites are- *Phytoseiulus persimilis* (Athias-Henriot), *Euseius* (Amblyseias) *finlandicus* (Oudemans), *Amblyseiusandersoni, Neoseiuluscucumeris* (Oudermans), *Amblyseiusswirskii* (Athias-Henriot), *Neosciuluscalifornious* (McGregor), *Neoseiuluslongispinosus* (Evans), *Neoseiulus fallacies* (Garman), *NeaseiuluswomersleyiSchicha* etc.

**Laelapid mites**

The family Laelapidae is a member of the super family Dermanyssoidea in the order Mesostigmata. Mites of this family include many vertebrate parasites, some of which attack domestic animals and are of veterinary importance. Laelapids are well sclerolized mites of
medium to large sized. The deutasternum has five to seven transverse rows of denticles. The dorsal shield is entire. The presternal area is often reticulate. The sternal shield of the female has three (rarely four) pairs of setae and two pairs of pores. Examples *Hypoaspis aculeifer* (Canestrini) and *Hypoaspis miles* (Berlese).

**Ascidae**: The Ascidae is family of the super family Ascoidea. They are predatory mites in soil on plants. They are small to medium in size and often pale yellow to brown in colour. The palps have six setae on the genu and a two tined apotele on the tarsus. The idiosoma has one single, shield or two shields, with 25-45 pairs of setae. The seternal shield usually bears two or three pairs of setae; the fourth pair of sternal setae are sometimes on a pair of metasternal shields. The genital shield is usually trapezoidal to subrectangular. Several species of Ascid are biocontrol agent of soil inhabiting pests in greenhouses.

**Parasitidae**: The parasitidae is the only family of the super family parasitoidea. They are common predators of the soil fauna and are distributed world wide. The parasitids are medium to large predatory mites, often yellowish to dark brown in colour the chelicerae are strong and dentate. The hypostome bears four pair of subcapitular setae and ten or more rows of denticles. The sternal shield in the female bears three pairs of setae. The fourth pair of sternal setae are on a pair of large metasternal shield, which flanks the anterolateral margins of a triangular genital shield. The ventrianalshield is often fused with the podal, peritrematic and more rarely dorsal shield.

**Stigmaeidae**: It is a family of super family Raphignathoidea. This is a cosmopolitan family and consists of nearly 400 species in 25 genera. The stigmaeids are small to medium sized mites, with most species measuring 200-500 µm. They are ovoid or round in shape, and yellow, orange or red in colour. The chelicerae are separated or fused together, with styliform movable digits which is not recurved basally as in spider mites. The palps are five segmented, the palpal tibia bears a strong tibial claw. The degree and extent of sclerotization of shields on dorsal idiosoma vary greatly and have been used in generic classification. The prodorsum has three or four pairs of setae, a pair of eyes and postocular bodies are present in some species. The dorsal hysterosoma bears five rows of up to 22 setae. Ventral opisthosaoma bears one to five pairs of genital setae. The genital and anal openings are fused or adjacent, bearing one to three pairs of
genital setae and three pairs of pseudonal setae. The legs are five segmented, terminating in a pair of true claws and an empodium with paired tenent hairs arising from a median shaft. The adult males have slightly tapered idiosoma and an aedeagus as in spider mites.

**Anystidae**: The Anystidae is a family of the Anystoidea. It is a cosmopolitan family of generalist predators found on a variety of habitats. The Anystidae are medium to large, red or orange mites with radiating long legs. The chelicerae are independent and each bears two setae. The palps are five segmented with one to three species distally on the inner face of the tibia. The stigmata are located near the cheliceral bases, with short emergent peritremes. There are one or two pairs of eyes on the prodosum. The genital and anal values are separated in both sexes. Legs terminate in a pair of true claws and a claw like or cup like empodium. Some species have mobile prelarvae. Sperm transfer is by deposited in spermatophores.

**Cumaxidae**: The cunaxidae is a family of the Bdelloidea. They are cosmopolitan and occurs in soil, leaf litter, compost, plants and stored products. Cunaxids are small to medium sized mites and often red or brown in colour. The chelicerae are independent and elongated, each bearing one seta, the movable digits are short and hooked, whereas the fixed digits are reduced. The palps are 3 to 5 segmented, raptorial often armed with long spines on the internal margin. The stigmata are located at the base of cheliceral without peritremes. The prodorsum has two pairs of prominent trichobothria and one or two pairs of ordinary setae. The genital pore is terminoventral in both sexes with a maximum of two pairs of genital acetabulae. Legs are five segmented, terminating in a pair of true claws and a rayed empodium. A trichobothrium is present on tibia IV. Adults build silk webbing for eggs and their development. Cunaxids are generalist predators of small arthropods and nematodes.

**Erythraeidae**: It is a cosmopolitan family of the super family Erythraioidea. The larvae are parasites of arthropods but deutonymphy and adult are free-living predators of small arthropods. Most species is are orange, red and brown in colour and the body is covered with a coat of setae, giving a veluety appearance. The characteristics chelicerae are elongated. styliform and retractile. The palps have a strong tibial claw. A pair of stigmata open near the base of the cheliceral body. Larvae are heteromorphic. A prodorsal shield is present on the prodorsum, bearing two pairs of trichobothria and two to several pairs of normal setae. One or two pairs of
eyes are located lateral to the prodorsal shield. The coxae of leg I and leg II are well separated. There is no anal opening in the ventral opisthosoma. The legs are very long and terminate in a pair of lateral claws and a median claw like empodium.

**Tydeidae** : Some members of this family have been recorded as causing significant damage by feeding on leaves. However, some members of this family are also predators of pest mites. *Homeopronematus anconai* (Baker) attacks *Aculopslyco persici* a pest of tomato in greenhouses and significantly reduce its density. *Saniosulus nudus* is usually found is association with scale insects. Mites feeds on eggs of the scale insects *Lepidosaphes pollida, L. beckii, L. ulmi, Chrysomphalusaonidium* and *C. dictyospermi* and on crawlers of the *Aonidiellaaurantii, L. pallida* was the most preferred prey, and its consumption was associated with an increase of predatory fecundity. *Coccipolipus epilachnae* and *C. benciti* has possible importance in the biological control of phytophagous coccinellid beetles.

**Exotic Predatory mites introduced in India**

1. *Orthogalumnaterebrantis* (Orthogalumnidae) was brought from Argentina via USA, in 1986 and released for the control of water hyacinth at Bangalore (Karnataka) and later in Kerala. Its establishment was obtained within 6 months in all the tanks. In Kerala, field releases of *O. terebrantis* commenced during 1990 in different water bodies. *O. terebrantis* has established all over the release sites and is spreading on its own. The mite was more efficient in water bodies where weevils, *Neochetina spp.* have established. *O. terebrantis* has established in Kerala and Karnataka and it complements the two exotic weevils in hastening the collapse of water hyacinth.

2. *Amblyseius chinensis* (Phytoseiidae) was imported from USA (1984), released and recovered from spider mites, *Tetranychus* spp. on various crops such as beans, okra and strawberry.

3. *Phytoseiulus persimilis* (Phytoseiidae) was brought from Chile via Switzerland (1984), and was released and recovered from spider mites, *Tetranychus* spp. on various crops.
Mass production of phytoseiid mite predator, *Neoseiulus longispinosus*

The phytoseiid predator, *Neoseiulus longispinosus* has been more popular for mass production and control of two spotted spider mites. This is an obligatory predator of spider mites, cannot be reared on alternate foods like pollen.

Hence this involves two stages

1. Production of spider mite for prey
2. Production of the predator

**Mass production of predatory mites**

1. Sowing bean seeds in earthen pots
2. Shifted to polyhouse in 3 leaves stage
3. Plants are infested in 9 leaves stage with 200 two spotted spider mite per plants
4. Released predatory mites, *N. longispinosus* @1:100 ratio 12 days after releasing two spotted spider
5. Predator harvested from 75% plants for field release of phytoseiids
6. Predators from 25% plants keep for further multiplication re infestation
7. Retain predator free culture

After about twenty days when there are no eggs of the spider mites, but few nymphs and adults of spider mites are present the predator can be harvested.

**Insect predators:**

Though huge numbers of predatory insects are available naturally feeding on insects or mites, a few of them only have been reported to multiply under controlled conditions. Mostly exploited
insect as predator is members of coccinellidae. Different species of coccinellid can be reared on different diet. *Cheilomenes sexmaculata* can be reared on *Aphis craccivora* as prey insects. Likewise *Stethorus* spp. can be reared on tetranychid mites.

**Mass Rearing of Coccinellid Under Laboratory Conditions:**

Ladybird beetles (Coleoptera: Coccinellidae) are predatory insects with diverse food habits and live in a variety of habitats. Both adults and larvae feed on small soft bodied insects and mites. For mass rearing, coccinella beetles should be collected from field and allowed to stay in pairs of both the sexes in different cages following the method described by Priyadarshani *et al.*, 2017. For egg laying Polymate Plastic boxes or glass cages may be used as egg laying cages. Previously sprouted bean seeds may be placed in the boxes providing a sand-soil layer for germination. Ventilation pores are made on the lid of plastic box/cage. After 4 days of germination, aphids or mites were introduced into the box, then mated pairs of beetles are introduced into individual box for egg laying. The numbers of eggs laid were counted daily. After the eggs hatched, the young larvae that emerged individually are transferred into the Petri dishes (15 cm diameter). Larvae to be fed only with *Aphis craccivora* or *Tetranychus urticae*. Pupal durations need to be recorded. The newly emerged adults should be provided with sufficient preys for development of further generations. This way coccinella beetles may be mass multiplied. In absence of natural food of the beetles, chicken liver can also be used as artificial diet of coccinella adult and grubs.

**References:**


*******
Chapter-9

Agro-Forestry and Organic Farming

K.K. Sharma
Chief Agronomist
AICRP on IFS, Assam Agricultural University
Jorhat -785013, Assam

Agroforestry is a land use systems and technology where woody perennial plants (tree, shrubs, herbs etc.) are deliberately introduced in the same land management practices, along with the agricultural crops and/or livestock, in a spatial/temporal sequence (ICRAF). Again, organic agriculture is an ecological production management system that promotes and enhances biodiversity, biological cycles and soil biological activity (NOSB, 1995). It is based on minimal use of off farm inputs (artificial chemicals including fertilizers, pesticides and herbicides) and on management practices to restore, maintain, and enhance ecological harmony. Organic agriculture practices cannot ensure that products are completely free of residues; however, methods are used to minimize pollution to air, soil and water. Organic food handlers, processors and retailers adhere to standards that maintain the integrity of organic agricultural products. The primary goal of organic agriculture is to “optimize the health and productivity of interdependent communities of soil life, plants, animals and people.”

Need for organic nutrient supply

The negative effect of modern agriculture with synthetic chemicals leads to deficiency of soil organic carbon and micro-nutrients. This ultimately resulted into:

- Decline or plateau in productivity with the existing use of synthetic fertilizers and low use efficiency of applied fertilizers.
- Problem of water logging, salinity and acidity i.e. deterioration of soil quality with existing practice.
- Poor quality and self life of the products.
- Widening gap between demand and supply of the nutrient use.
- Harmful effect of agricultural chemicals on soil micro flora and fauna.
- Leaching of nitrate and phosphatic fertilizers leads to soil and water pollution.
• Non availability and high cost of fertilizers.
• Concern for health and sustainability of production systems.

Scope of agro-forestry in organic agriculture

We have to judge the suitability of agroforestry system as a better system to be included as a component of organic farming in the light of following aspects like increase in soil organic matter, carbon sequestration, improvement of water quality, more biodiversity, conservation of energy. Looking at the advantages of agroforestry system there is no doubt about inclusion of agroforestry as a integral system for organic agriculture in India.

Agroforestry Practices

Different agroforestry practices followed commonly in India are

• Alley Cropping: Alley Cropping is planting rows of trees at wide spacings with a companion crop grown in the alleyways between the rows.
• Silvo-pasture: It is the practice of combining forestry and grazing of domesticated animals in a mutually beneficial way. Advantages of a properly managed silvopasture operation are enhanced soil protection and increased long-term income due to the simultaneous production of trees and grazing animals.
• Forest Farming: It is the cultivation of high-value specialty crops under the protection of a forest canopy that has been modified to provide the correct shade level. Crops like ginseng, shiitake mushrooms, and decorative ferns are sold for medicinal, culinary, and ornamental uses.
• Riparian Forest Buffers: A riparian forest buffer is a planned combination of trees, shrubs, grasses and forbs planted along a stream or river. It can include many different species and perform several different functions.
• Windbreaks: A windbreak (shelterbelt) is a plantation usually made up of one or more rows of trees or shrubs planted in such a manner as to provide shelter from the wind and to protect soil from erosion.
• Multi storied Farming: Multistoried cropping are multi-layer cropping and multi-tire cropping. It is one kind of intercropping. Growing plants of different height in the same
field at the same time is termed as multistoried cropping. It is mostly practiced in orchards and plantation crops for maximum use of solar energy.

**Improved agro forestry systems followed in different regions of India are:-**

i) Agri-silviculture: Agri-silviculture is a production technique which combines the growing of agricultural crops with simultaneously raised and protected forest crops. A similar practice involving forest villagers and tribemen is known as the "taungya system" in Asia.

ii) Silvipastoral system: (Tree+ pasture/animal) The production of woody plants combined with pasture is referred to as Silvipastoral system. The trees and shrubs used primarily to produce fodder for livestock. This system is needed in dry area to meet the fodder demand throughout the year.

iii) Agrisilvipastoral system: (Tree Crops + Grain crops + animals) This is the system in which the forest tree crops for fodder like Anjan, Subabul, Babhul, Tamrind, Hadga and Khejedi etc. are taken with intercrops of grasses like Stylo, Burssem, Haemeto are taken for fodder purpose as well as the food grain crop like Wheat, Rice, Jowar etc. are taken in between the strips of forest tree species. The forest tree species are planted at 10 to 12 m distance and in the lines the grasses and food grains are cultivated as intercrop.

iv) Horti-silviculture system: This system is defined as growing of trees and fruit trees or ornamental trees or vegetables/flower together in same lands at the same time. This system is common in home gardens.

v) Agri-horticulture system: It is a land management system in which agricultural crops are grown on space between two rows of fruit tree species. Integration of fruit crops in croplands is referred to as agri-horticultural landuse system of Agroforestry.

vi) Agrihortisilviculturesystem: In this system, in addition to arable crops, MPTS (Multi-Purpose Tree Species) like Subabul, are grown along with fruit trees like ber and aonla. The MPTS , besides providing green fodder and fuelwood annually, also protect the fruit trees from hot winds in the summer and cold winds in the winter and improve the soil by
virtue of their nitrogen-fixing abilities. It is possible to grow 100 to 400 fruit trees per hectare along with arable and also with MPTS like Subabul (200 to 400 per hectare) which are planted in between the fruit trees.

vii) Multipurpose forest tree production (other specialized agroforestry systems): *Multipurpose trees are trees* that are deliberately grown and managed for more than one output. They may supply food in the form of fruit, nuts, or leaves that can be used as a vegetable; while at the same time supplying firewood, add nitrogen to the soil, or supply some other combination of multiple outputs. "Multipurpose tree" is a term common to agroforestry, particularly when speaking of tropical agroforestry where the tree owner is a subsistence farmer.

viii) Apiculture with trees: In this system various honey (nectar) producing trees frequently visited by honeybees are planted on the boundary of the agricultural fields.

ix) Aquasilviculture or Aquaforestry: It is a management strategy that combines and harmonizes fish production and mangrove development. In this system various trees and shrubs preferred by fish are planted on the boundary and around fish ponds.

x) Agrisilviaquaculture: In paddy field, fish can easily be reared by planting trees on field bunds or boundary. A land management system followed in high rainfall areas which involve rearing of fish in fields and planting of trees on bunds or boundary.

**Complementary effect of Agroforestry in view of organic agriculture:**

Agroforestry- Maximizes production through, Efficient utilization of solar energy, Efficient utilization of nutrients and Sustains the scarce resources for future generations. For example silvipasture system is beneficial from the point of Income from Annual fodder, Long-Term Timber Income, Lower Animal Stress, Reduce Wildfire Risk, Wildlife Benefits, Visually Pleasing, Carbon Sequestration: *It describes long-term storage of carbon dioxide or other forms of carbon to either mitigate or defer global warming and avoid dangerous climate change.* It has been proposed as a way to slow the atmospheric and marine accumulation of greenhouse gases, which are released by burning fossil fuels.
Two types of tree – crop interactions in agroforestry systems are noticed on the basis of experimental evidences. These are:

A. Complementary Effect
   i) Increased productivity
   ii) Improved soil fertility (Organic matter addition)
   iii) Soil conservation
   iv) Nutrient cycling
   v) Micronutrient improvement
   vi) Sustainability

B. Competitive Effect
   i) Above and below ground competition.

**Increased productivity**

There are ample evidences to show that the overall productivity of an agroforestry system is generally greater than that of an annual system although not necessarily greater than that of a forestry or grassland system. Poplar (Populasdeltoides) has become a popular species in agroforestry system. Deceduous nature of poplar allows agricultural crops to grow under it without adversely affecting the yield. Upto first 2 years the returns obtained from sugarcane+poplar combination are higher than any other crop, as the preparatory and cultural operation for both of these are complementary. However, third year onwards shade loving crop like turmeric and ginger can be grown under it. During winter season wheat can be grown till its harvest (Sharma et al, 2001). Poplar tree matures for harvest after 7-8 years having a girth of 90-100cm.

Dry foliage yield of different trees combination are given. Highest dry foliage yield of 10 q/ha has been found in case of shisam sole which has been due to the less competition as compared to the other trees combination(Gill, 2003). Effect of fast growing species on fruit yield of mandarin orange has been given. Highest yield of mandarin has been found in case of casuarina of 10.9kg/ha due to its fast growing ability and synergism conditions (Debroy, 1989). Performance of fruit tree based agrihorti systems in the NEH region has been shown. Good performance and higher net returns has been observed in case of khasi mandarin grown tree crop.
with ginger due to their compatible growth between the two (Mohapatra et. al 2009). Income from various crops grown with poplar has been shown. Highest net income of Rs 7500 has been found in case of onion grown with poplar due to the higher yield of onion due to the favourable growth conditions (Chandar,1998).

Biomass production from trees adequately compensated the crop production. Land equivalent ratio of agroforestry land use was comparable or even better than monocropping systems indicating suitability of this systems for the western Himalayan valley region (Pratap Narayan,1998)

Soil productivity improvement is possible through

The primary objectives of soil conservation is improved/maintains soil fertility. To achieve this, control of erosion, maintenance of organic matter, maintenance of physical properties, addition of organic matter, maintenance of nutrient is necessary. In this way agroforestry system constitute sustainable land use and help to improve soils in a number of ways.

An experimental evidence under agrisilvicultural system (Aonla+leucaena+blackgram) in rain fed conditions showed that after nine years 65 to 109.4% organic carbon was increased under below canopy and 28.1 to 62.5 % under open canopy compared to initial value (0.32%). The organic carbon percent under the canopy of aonla is higher under the canopy than open canopy owing to complete falling of aonla leaves mostly limited to below tree canopy (Ram Newaj et al, 1999).

The influence of Eucalyptus citriodora plantation on the physical properties of soil was observed due to the differential organic matter addition, root density and root length and soil faunal activity. Contribution of 12 years old E. Citrodora plantation with tree density of 1600 tree /ha towards in situ litter accumulation and nutrient concentration of leaves (Table-2) was carried out by Balamurugan et al (2000). In Table-2 In situ litter accumulation and percentage contribution of different vegetative components of total in situ litter accumulation and nutrient concentration in leaf litter.
Component wise in situ litter accumulation (t/ha) | Nutrient conc. Of leaves (%)
---|---|---
Leaves | 3.25 | N | 0.58
Large twigs | 0.75 | P | 0.04
Small twigs | 1.25 | K | 0.41
Bark | 0.50 | Ca | 1.55
Total | 5.75 | Mg | 0.59

Source: Balamurugan et al. 2000

Addition of carbon in soil, Release and recycling of nutrients, The rate of infiltration of soil water is 3 to 5 times more in forest soil, Reduction of loss of soil (erosion) through root binding, Improves physical condition of soil, Nitrogen fixation, More microbial associations, Moderating the effect of extreme acidity and alkalinity, Utilize waste and degraded land, improve environment, Provide employment opportunities, Increase farm income.

**Soil conservation**

In agroforestry multistrata practices simulate the protective nature of natural forest. Purposefully planted cover can take several forms that can differ in their ability to counter soil and water loss. Vegetative cover is the key to arrest runoff and soil loss. It has been estimated that soil erosion is accelerated to more than 900 times when tree plantation were clean weeded or the litter was burnt. In a semi-arid alfisols degraded land established pastures reduced the runoff to 26% and soil loss to 8% (Pathak, 2000). In another study agroforestry system recorded runoff 2.7%, soil loss 0.54 tonnes/ha/year, N loss 2.1 kg/ha/year and K loss 0.7 kg/ha/year with mean monsoon rain of 899 mm compared with runoff 25.4%, soil loss 3.21 tonnes/ha/year, N loss 12.5 kg/ha/year and K loss 4.3 kg/ha/year from the field crops raised under the same condition (Grewal et al., 1995).

By alternate land use systems like silvipasture, agri- silviculture, the productive and protective benefit from watershade management are considerably higher than the investment, benefit-cost ratios ranging from 1.92:1 to 7.10:1 with 48 to 99% reduction in runoff and 81 to 98% soil loss besides the check of out migration of population from 26.6% before the implementation of WSM programme to 9.3% during the project period (Samra et al., 1999).
On sloppy land contour hedge rows have consistently been demonstrated to be highly effective in controlling soil erosion even in as little as 18 months (Lal, 1989). The woody hedge rows provide a semi permeable barrier to surface movement of water, while mulch from the trees reduces the impact of raindrops on the soil and minimize splash and sheet erosion (Young, 1989).

Quantities of N fixed by different tree species has been shown. Highest N is fixed by *L. leucocephala* tree species which fixed nitrogen of 224-274 kg/ha/year as compared to other tree species mentioned in the table which is due to the fact that it has higher nodular activity in the roots which fixed the atmospheric nitrogen (Mac dicken, 1994). Total C storage under agro-forestry systems in different regions of the country has been shown. Highest C storage has been found in case of Block plantations in Central India as compared to others which is due to higher C absorption by the plants (Dhanyiet al. 2008).

**Nutrient cycle**

This is one of the most important hypotheses. It is based on the capacity of the tree root systems to trap nutrients int he soil solution that would otherwise be lost by leaching nd to recycle them through litter to the soil surface. For low input systems, the hypothesis states that nutrient cycling can become highly efficient, so that fallowing will only be necessary after long period. Alternatively, under condition of weak leaching, continuous cultivation might be possible, with nutrient removal in crop harvest compensated by natural inputs. Under high input systems, the hypothesis states that the ratio of nutrient recycling to nutrient losses will be greater for agroforestry than the agricultural systems, with consequent economic benefit through more efficient fertilizer use.

Poplar based agroforestry for an alluvial soil under irrigated condition in western UP revealed that the total amount of 913.8 kg/ha and 1291.8 kg/ha leaf litter was added in the soil through 3 and 4 year old plantation. Considerable amount of nutrients was recycled to the soil through leaf fall. The analytical results of leaf litter showed that the quantity of nutrients added into the soil deceased in the order of Ca>N>Mg>K>P. The return to the soil through leaf fall at different distances.
Microclimate improvement

The use of trees as shelterbelt in areas that experience high wind or sand movement is well established example of microclimate that resulted in improved yields. Increased agricultural due to wind break and shelterbelt in India has also been reported (Rao and Sitaram, 1980). Establishment of micro-shelterbelts in arable lands, by planting tall and fast growing plant species such as castor bean on the windward side, and shorter crop such as vegetables in the leeward side of tall plant helped to increase the yield of lady’s finger by 41% and cowpea by 21% over the control (Venkateshawrelu, 1993).

Sustainability

The sustainable land use is that which maintain an acceptable level of production and at the same time conserve the basic resources on which production depends, so enabling production to be maintained. The utility of agroforestry practices and their symbiotic effects on better growth and yield of crops is widely recognized. Agroforestry as subject of scientific investigation has assumed wider recognition in view of the need to maximise production on the basis of sustainable land management (Singh et al., 1998).

Negative interaction

a. Competition for light
b. Competition for moisture and nutrients
c. Allelopathy effect
d. Others, like soil disturbance etc.

Tree Species To Be Chosen For Agroforestry:

a) They should be amenable to early wide spacement.
b) They should tolerate relatively high incidence of pruning, i.e., their photosynthetic efficiency should not decrease with heavy pruning.
c) They should be light branching in their habit.
d) They should be tolerant to side shades.
e) Their phenology, particularly with reference to leaf flushing and leaf fall, should be advantageous to the growth of the annual crop in conjunction with which they are being raised.

f) The rate of litter fall and litter decomposition should have positive effects on the soil.

g) Their root systems and root growth characteristics should ideally resulting exploration of soil layers that are different to those being tapped by agricultural crops. (Source: Gill and Roy, 2012)

**Useful Tree Species**

Based on the research findings and field observations, the following tree species have been recommended for agro-forestry, under Indian conditions including North-eastern region are:

**Fodder cum Fuel wood species**

1. Amara (*Albiziaamara*), Corol tree (*Erythrina sp.*), Gliricidia (*Gliriciadiasepium*), Anjan (Hardwickiabinata), Subabul (*Leucaena leucocephala*), Madras thorn (*Pithecellobium dulce*), Shevari (*Sesbaniasesan*)

**Fuel Wood And Timber Species**

Babul (*Acacia nilotica*), Siris (*Albizia lebbeck*), White Siris (*Albizia procera*), Neem (*Azadiracta indica*), Casuarina (*Casuarina equisetifolia*), Shishum (*Dalbergia sissoo*), Bamboo (*Dendrocalamus strictus*), Pongamia (*Derris indica*), Horse bean (*Parkinsonia aculeata*), Portia (*Thespesia populnea*)

**Softwood And Pulpwood Species**

Tree of Heaven (*Ailanthus excelsa*), Dhup (*Ailanthus tryphysa*), Silk cotton (*Bombax ceiba*), White Albizia (*Paraserianthes falcataria*), Poplar (*Populus deltoides*)

**Fruit and Vegetable Species**

Ramphal (*Annona reticulata*), Custard apple (*Annona squamosa*), Jack fruit (*Artocarpus heterophylus*), Amala (*Emblica officinalis*), Drum stick (*Moringa oleifera*), Ber (*Zizyphus mauritiana*)
Heavy Rainfall And High Water Table:

Bamboo (Dendrocalamus strictus), Arjun (Terminalia arjuna), Eucalyptus (Eucalyptus grandis), Porita (Thespesia populnea), Pongamia (Derris indica), Sesbania (Sesbania sesban), White siris (Albizia procera), Casuarina (Casuarina equisetifolia), Silk cotton (Bombax ceiba)

Dry Areas:

Babul (Acacia nilotica), Amara (Albizia amara), Custard apple (Annona squamosa), Neem (Azadiracta indica), Dassod (Cassia siamea), Horse bean (Parkinsonia aculeata), Khejdi (Prosopis cineraria), Ber (Zizyphus mauritiana).

Well Managed Irrigated Areas

White siris (Albizia procera), Casuarina (Casuarina equisetifolia), Shishum (Dalbergia sissoo), Gliricidia (Gliricidia sepium), Anjan (Hardoickia binata)

Constraints and problems of Agroforestry Systems…

- Scarcity of saplings of suitable tree species
- Seasonal occurrence of plant and animal diseases
- Inadequate compensation for destroyed crops
- Lack of credit facilities
- Inadequate education on tree tenure
- Crop destruction by felling timber species on farms.
- Poor marketing system
- Lack of knowledge on logging regulations/procedures
- Inadequate harvesting & processing techniques
- Lack of knowledge regarding value added product
- Laws restricting harvesting, transporting & sale of trees
- Lack of assured financial support for popularizing agro-forestry
- Lack of proper transfer of technology, trained manpower, infrastructure & funds
- Identification of suitable species
Prospects of Agroforestry…

- Practice of mixed cropping
- Knowledge on tree importance
- Presence of nurseries
- NG0s involvement in promoting agroforestry
- Plantation of Fruit trees for subsistence and income
- Reducing the cost of farm inputs
- Increasing homestead garden through proper management
- Inclusion of apiculture, sericulture in the agroforestry system

Future thrust:

- Farmers participation and adoptive research need to be increased
- Integrating Agroforestry with sericulture
- Appropriate feed systems with tree fodder for livestock develop for different ecological seasons
- More thrust be given to agroforestry research on dry land farming

Conclusion:

Agroforestry can support food production, increase the total efficiency and stability of the system in North Eastern region. It holds promise to satisfy all human needs (food, fuel, fodder, timber etc.) and it also can act as an insurance against drought, flood and natural calamities those are familiar to north-east region of India. With inclusion of agroforestry with different crop components and livestocks one can be benefitted in terms of monetary as well as ecological sustainability. Thus, area based effective research strategies in North Eastern region is required for meeting the diversified needs of the people as well as for increasing the food production.
References:


********
Chapter-10

Disease Management of Vegetable Crops in Organic Agriculture

G.K. Upamanya
Assistant Professor, Department of Plant Pathology,
SCS College of Agriculture, AAU, Dhubri (Assam)
E-mail: gku_2003@yahoo.com

1. Introduction

Organic Farming is recognized globally as a priority area in view of the growing concerns on environmental pollution due to increased awareness about the fallouts of the indiscriminate use of agro-chemicals. Demand for safe and healthy food has been increasing with every passing day. The ill effects of plant protection chemicals on the flora, fauna, humans and environment as a whole are the major concerns. The disease management in organic farming is primarily dependent on preventive measures rather than curative practices which are based on the ecologically safer management methods.

The general principles of plant disease management which can be summarized by the acronym REPEAT: Resistance, Eradication; Protection, Exclusion, Avoidance, and Therapy should also be used in organic disease management. The health of the ecosystem must be the major emphasis of the disease management in organic agriculture which enables the plant to become resistance to attack by diseases. Modification in the cultural practices such as crop rotation, soil quality management through the addition of organic amendments constitute the preliminary defence against the attack of diseases followed by use of the curative methods like use of predators, parasitoids, plant products and ecologically safer chemicals forms the next alternatives of defence against the diseases. Apart from conventional fungicides and microbial biocontrol agents, plant products or extracts have been found detrimental against a wide range of pathogens (Amadioha, 2003) Chemicals like salicylic acid, 2, 6-dichloroisonicotinic acid and benzothiadiazole induce systemic acquired resistance in plants (Guleria et al., 2005). Execution of systemic acquired resistance is manifested by the expression of genes coding for pathogenesis related (PR) proteins increase in activity of enzymes such as phenylalanine ammonia lyase and peroxidase and level of fungitoxic phenols (Kagale et al., 2004). A number of studies on direct
effect of neem leaf and fruit extracts on target, pathogens have been reported (Amadioha, 2000). Chemicals applied in the control of disease pollute the atmosphere and affect the properties of medicinal plants. To avoid the hazardous effects of chemicals, natural products of some plant have been found effective to control the disease (Bhatia and Awasthi, 2007). A number of reports are available showing the efficacy of plant extracts especially neem (A. indica and Ocimum sanctum) showing the antifungal properties (Mesta et al, 2009).

2. Principles of plant disease management used in organic agriculture:

2.1 Resistance

Use of plant varieties that are genetically resistant or tolerant to a specific pathogen is the most economic and easiest method of disease control. For example, many vegetable varieties have resistance to species of Verticillium and Fusarium, common soilborne pathogens that cause vascular wilts. However, the pathogen population can change with time, leading to a breakdown of plant resistance to the pathogen. Additionally, new virulent races of the pathogens also break the resistance of the existing varieties of the crops. Therefore, other disease control methods are necessary to manage the pathogen population so as to minimize selection pressure and extend the usefulness of the resistant variety once it is used.

2.2 Eradication:

It is the method of controlling disease by eliminating or reducing disease causing pathogens in the environment. Eradication can include rotation with non-host crops, rogueing of infected plants, elimination of infested crop residues, use of biocontrol agents, and any other physical/chemical method to destroy or reduce inoculum in the field.

Crop rotation is an effective means to control diseases since many pathogens have a strong crop preference. Rotation with a non-host crop breaks the disease cycle and over time reduces the inoculum in the soil. However, in case of the pathogens having broad host range, proper identification of non hostcrop is important to fit in the rotation effectively. Removing infected plants and debris can also help break the disease cycle and reduce the quantity of pathogens, thus slowing disease progress or eliminating the pathogen source for subsequent plantings. Biorationals, including biological agents or secondary compounds extracted from plants, provide another means of eliminating pathogens. Some commercially available biological agents will parasitize or inhibit the growth of the pathogen, reducing the viability of soil
propagules or decreasing the overall aggressiveness of the pathogen. The use of cover crops can also reduce inoculum by acting as a trap-crop to stimulate the germination of pathogen propagules in the soil in the absence of an appropriate host. Some cover crops release toxic secondary compounds into the soil during growth or decomposition. These compounds can be toxic to plant pathogens and, thus, help control plant disease. For example, many plants in the Brassicaceae (Mustard) family accumulate glucosinolates that breakdown during decomposition, releasing doses of isothiocyanates lethal to many soilborne pathogens.

Various hygienic approaches can eliminate the pathogen and exclude it from new environments. Like application of surface disinfestants—such as sodium hypochlorite solutions (household bleach)—so as to clean work surfaces, propagation tools, gloves, boots, and equipment, thereby eliminating pathogens and preventing or limiting their spread in greenhouse, shade house, and field operations. Cleaning soil and plant debris from tractors and implements between fields, especially in fields with a disease history, can also greatly reduce the chance of spreading the pathogen to new areas. The use of a surface disinfestant or steam can aid in cleaning equipment. However, most of the detergents are not allowed in certified-organic vegetable production because they include a synthetic surfactant. Soaps generally are permitted. Manipulation of the physical environment—such as increasing the air or soil temperature or limiting oxygen exchange—is another means of eradicating soilborne pathogens. Soil solarization uses a plastic mulch to trap solar energy, thereby raising soil temperature to levels lethal for many soilborne pathogens. Soil steaming, long used to sterilize potting mixes for greenhouse production, is another use of heat to eradicate soilborne pathogens.

### 2.3 Protection:

The bio control agents or secondary compounds extracted from plants interfere with a pathogen’s ability to infect susceptible plants. This approach requires the application of materials prior to the rise of conditions favorable for disease development or immediately following the appearance of the first symptoms of the disease. In foliar applications, these materials provide a protective barrier on plant surfaces against the plant pathogens. But repeated applications are required with subsequent rain/irrigation that wash materials from foliage and fruit or with the emergence of new foliage. Similar strategies are possible for protective agents applied to seeds, roots, or soil. These materials can provide temporary protection to roots, but results can vary.
depending on the crop, the pathogen and the environment. Subsequent root growth or rain/irrigation can wash materials away from the root zone. Some biological agents can grow within the root zone and persist longer than chemicals. However, because these biological agents must compete with native soil microorganisms, they tend to persist at levels ineffective for control. Biological agents are generally most effective in the control of root rots associated with seed germination and seedling/transplant establishment.

2.4 Exclusion:

This is the method of controlling disease by preventing the introduction or establishment of a pathogen in an environment. In theory, this is the best management strategy. But in practice, it is often the most difficult. For practical purposes, preventing the pathogen from entering the production system begins with the use of disease-free seed, transplant, or propagation material. Many diseases are seedborne, so the certified and pathogen free should be used. In addition to careful selection of seed, exclude disease from the field by screening seedlings for any symptoms of disease and removing these sources before the disease spreads. However, these methods do not provide any guarantee against subsequent disease since some pathogens can remain in a dormant state on the plant until specific conditions occur. However, the use of disease-free planting materials and good production hygiene will limit the amount of pathogen introduced into the environment and, thereby, control the incidence and severity of a disease outbreak. Limiting the size of an initial disease outbreak provides an opportunity to manage the disease successfully.

2.5 Avoidance:

Avoidance is the controlling plant disease by maintaining healthy growing conditions for the plant and growing the plant under environmental conditions not favorable for the pathogen or for the development of disease. Poor soil structure lead to poor drainage, which not only stresses the plant, but also creates conditions favorable for many soilborne pathogens. Poor water quality and nutrient management may also responsible for disease susceptibility. Soil amendment with biocontrol agent enriched farmyard manures or vermicompost not only improve soil structure but also create a nutrient source that supports the activity of soil microorganisms and promote crop
health. The date of planting of the crop can be manipulated to favor times when the pathogen is either inactive or at a lower level in the environment.

2.6 Therapy:

Therapy is controlling disease by treating infected plants through chemical or biological agents or by manipulating the environment to eliminate or limit pathogen growth and subsequent disease. While various choices are available for the therapeutic treatment of foliar diseases, there are few therapeutic treatments for root and crown diseases. Once a pathogen has colonized tissues of the root or crown, it is nearly impossible to treat. Few chemicals or biorationals approved for organic production are systemic. However, reducing irrigation or fertilization will often slow the progress of the disease.

3. Disease management strategies for organic farming:

The following strategies are generally used for disease management in organic agriculture which interferes with the micro-environmental conditions to make them uncongenial for pathogen propagation, multiplication and initiating infection. Further majority of these strategies are specific to particular disease in a crop and hence a combination of strategies based on the crop growth stages and disease cycle need to be integrated as a module for a crop in a particular agro-climatic region.

- Modification of cultural practices
- Use of biological control agents
- Use of botanicals
- Risikrishi
- Restricted use of pesticides

3.1 Modification of cultural practices

Modification in the cultural practices leads to increase in the agricultural biodiversity and thus have a greater impact in the management of pathogens. However, there are certain limitations in this method like these are preventive in nature rather than curative. The method of modifications of cultural practices are depicted below.

(a) Use of resistant cultivars: Plant breeders traditionally have focused on creating disease-resistant varieties. As genetically modified crops (GMOs, transgenic crops) are not
permitted in organic production systems, so it is important to find out about the mechanism of disease resistance with the breeding methods such as introduction and selection, hybridization and selection, pedigree method, backcross method, composite cross, recurrent selection and mutation breeding are followed for development of resistant varieties. Some of the disease resistant hybrids of vegetable crops are given in the following table.

Table 1: Disease resistant hybrids

<table>
<thead>
<tr>
<th>Crop</th>
<th>Resistant to</th>
<th>Hybrids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cabbage</td>
<td>Black rot</td>
<td>Harirani, Kranti, Geetanjali, Bahar, Pragati</td>
</tr>
<tr>
<td>Cauliflower</td>
<td>Black rot</td>
<td>Early Himlata No.7, No.8, No.10, Panchalik,</td>
</tr>
<tr>
<td>Okra</td>
<td>YVMV</td>
<td>Adhunik, Tara, Supriya, Uphar, Varsha, Vijay, Vishal</td>
</tr>
<tr>
<td>Ridge gourd</td>
<td>Mildews</td>
<td>Surekha</td>
</tr>
<tr>
<td>Watermelon</td>
<td>Fusarium wilt, TMV</td>
<td>Amrit, MHW-6, Green Gold, MCPH-II</td>
</tr>
<tr>
<td>Capsicum</td>
<td>TMV &amp; PVY, Fusarium &amp; Verticillium wilt, YVMV</td>
<td>Indra, Ratna, Rishi, Maitri, Manmohan, Lerica Meenakshi, Menka, Mohini</td>
</tr>
<tr>
<td>Tomato</td>
<td>Bacterial wilt, TYLCV, Foliar diseases</td>
<td>Suraksha, Avinash II, Akash, Utpan, Ruchi</td>
</tr>
<tr>
<td>Chilli</td>
<td>Anthracnose, TMV</td>
<td>Vardhan, Kranti, Krishna, Vijli, Agni</td>
</tr>
</tbody>
</table>

Courtesy: De and De (2019)

(b) Crop rotation: The disease causing pathogen particularly the soil borne pathogens are killed by starvation or releasing toxic root exudates with the method of crop rotation. To be most effective, rotations between susceptible crops should be 3-7 years. A number of soil borne pathogens like *Fusarium* spp., *Verticillium* spp. and *Ralstonia* spp causing
wilts can effectively managed by crop rotation. In fields, where rice-solanaceous crop rotation is followed the severity of bacterial wilt is reduced. In sorghum and pigeon pea mixed cropping helps in reducing *Fusarium* wilt because of toxic exudates of sorghum plant parts which eventually reduces the inoculum load and hence the disease (Satish Chandra and Baiswar, 2007).

(c) **Planting time**: Adjustment of sowing time can also be considered as an effective strategy against disease. It helps in managing disease by avoiding the concurrence of susceptible host and favourable environment. For example, Pea and Gram planted soon after rain, when soil temp and moisture level are high, shows high incidence of root rot, blight and wilt. So late sowing (Nov.-Dec.) reduce the incidence of the diseases. Late sown winter vegetables escape incidence of root rot and wilt favoured by high temperature and moisture usually occur after summer rainy season.

(d) **Plant density**: Generally as the density of a crop increases, the incidence of disease also increases. Increased the density of seedlings in their nursery beds lead to serious epidemics of damping-off caused by fungi such as *Pythium*, *Fusarium* and *Rhizoctonia*. There are a number of reasons of increasing disease incidence in densely populated crops. Most are associated with the ease of transferring inoculum from one plant to another when the plants are close together. There is also increased contact between the leaves or roots of neighbouring plants. In addition, with denser plantings, more plants may be wounded during cultivation creating more opportunities for weak pathogens to infect. Finally, the micro-environment within the crop is altered. Temperatures are more uniform, the relative humidity increases and leaves stay wet longer after rain or dew. All these conditions favour the development of disease.

(e) **Fertility management**: Organic production does not allow synthetic fertilizers or sewage sludge. Over fertilized plants may subject to more diseases and become targets of attack. The organic manures create a partial nitrogen stress up to certain period without any negative effects on crop growth and thus induce resistance through intrinsic production of defence compound such as phenols, tannins and lignins that make the leaf toughness and production of more cell wall related structural compounds (Surekha and Rao, 2000). Antifungal activity among various organic composts has been reported by various workers against soil borne and foliar pathogens. Aqueous extracts of vermicompost and
organic compost inhibited the mycelial growth of *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Sclerotium rolfsii*, *R. solani* and *Fusarium oxysporum f. sp. lycopersici* in vitro (Nakasone et al., 1999). Deficiency of certain elements can increase susceptibility of host plants, for example wilt of tomato is due to Ca deficiency (Singh, 1998). Early blight and charcoal rot of potato can be checked by furrow irrigation. Bioorganic fertilizer containing the biofertilizer controls the plant pathogenic fungi directly as well as indirectly. Directly they parasitize the pathogens; application of *Rhizobium* culture on the legume seeds controls seed borne fungi such as *Colletotrichum*, *Ascochyta*, *Helminthosporium*, etc. The rhizobia produce a toxic substance when they multiply on the seed and rhizosphere. Phosphate solubilizing fungi such as *Aspergillus niger* and other *Penicillia* produce antibiotic substances and thus kill the pathogenic fungi. Indirect killing of the plant pathogens is achieved by producing healthy seedlings. Application of mycorrhizae produce better root systems which over come the attack of root rotting and soil borne pathogens.

(f) **Water management**: Irrigation has both direct and indirect effects on population of pathogen. Plants under stress due to drought can be more attractive to pathogen. The need for irrigation is influenced by crop growth and weather rather than the need for pathogen control. In case of potato scab maintaining soil moisture near field capacity during tuber formation protects the crop from scab because of favourable effect of irrigation on bacterial microflora antagonists to *Streptomycyes scabies* (Weinbold and Bowman, 1968). Charcoal rot fungus *Macrophomina* species and *Fusarium* spp. cause wilt diseases to those crops which are grown under moisture stress conditions. Irrigation can be used as a tool to reduce the level of inoculum and to retard disease development. Drying and rewetting soil encourages the activity of microorganisms that destroy sclerotia. Overhead irrigation can reduce or inactivate airborne inoculum by washing it out of the atmosphere. Short daily watering encourage the germination of powdery mildew spores but the plants do not stay wet enough for long enough for the fungus to penetrate. On the other hand, flood, furrow and overhead (spray, sprinkler) irrigation can facilitate the spread of pathogens. Flood irrigation can spread soil-borne inoculum all over an area while furrow irrigation disperses inoculum along rows. The action of overhead irrigation systems
washes inoculum out of the air and facilitates the spread of pathogens that rely on water splash for dispersal. Many important foliage and fruit pathogens such as *Phytophthora infestans* and *Alternaria solani* form their spores at night and release them during the day. Overhead irrigation in the early part of the day washes these spores from the air and splashes them about. If overhead irrigation is delayed until the evening or early night, spores of *P. infestans* dry out on the plant and cannot infect. Trickle or drip irrigation, developed in response to the need to conserve water, supplies water directly to the root zones of individual plants and the rate of application is insufficient to disperse pathogens. Moreover drip irrigation produces a mosaic of soil moisture conditions, rather than uniformly moist conditions, which probably inhibits the spread of root pathogens.

**Tillage:** Organic producers usually depend on tillage to control weeds and to prepare the soil for planting. Some practices to reduce tillage in organic systems include Zero tillage are the ridge tillage are some of the important practices used in organic production. Tillage incorporates various types of organic matter including crop residues, manure, green manure, volunteer crop plants and weeds into the soil. Tillage practices tend to have indirect effects on the spread of plant pathogens, although some forms of inoculum can be widely dispersed by implements. Tillage reduces populations of weeds and volunteer crop plants that harbour pathogens between crops. It also buries plant pathogens from the top soil into deeper layers of the soil where they cause less or no disease. Tillage may also influence nutrient release mechanisms and the total effect is often expressed as increased crop vigour. Modern agriculture has moved away from regular cultivation of soil between crops towards a system of minimum tillage, or even no-tillage. Minimum tillage is a method of planting crops that involves no seed bed preparation other than opening the soil to place the seed at the intended depth. Minimum tillage reduces injury to the roots of crop plants caused by mechanical tillage or hand weeding, reducing the opportunities for opportunistic pathogens to infect. It also reduces the spread of pathogens by tillage practices. Minimum tillage practices are thought to promote greater microbial antagonism in the vicinity of crop roots than normal cultivation practices. However, little is known about the influence of cultivation on these activities although incorporation of organic matter (e.g. green manure crops) is known to
reduce the incidence of some diseases. Plant residues intensify the microbial activity of the soil which may result in the formation of fungitoxic or even phytotoxic compounds.

**Mulches:** Mulching, the application of a covering layer of material to the soil surface, is a commonly used cultural practice, especially in horticulture. Mulching systems include plastic and natural materials. Although, the use of plastic mulch is frequently not allowed by organic certification agencies because it relies on a non-renewable resource. Natural materials used for mulching include cereal straw and stalks, crop debris, sawdust, leaves, grass, manure, weeds, reeds, Spanish moss and various aquatic plants. In fact, almost any readily available, preferably cheap, organic material is used. When crop residues are used as mulch they provide many pathogens with a food source as well as an environment for reproduction, therefore, influence disease incidence. On the other hand, crop residues generally enhance competition among soil micro-organisms for nitrogen, carbon or both resulting in fewer problems with soil-borne pathogens and encourage the growth of antagonist which in turn reduce the amount of inoculums.

**Sanitation:** Sanitation involves destruction of crop debris, weeds, diseased plant parts which eventually reduces the inoculums load and subsequent the disease. Sanitation practices such as ploughing under soil, or destruction of diseased leaves and other infected plant parts help in reducing the primary sources of inoculums of pathogen causing the diseases of crops.

### 3.2 Use of biological control agents:
A wide variety of soil microorganisms, including bacteria, actinomycetes, fungi, viruses and protozoa have been shown to have biocontrol activity against various fungal pathogens or disease they cause, and have been studied as biological control agents in several pathosystems. Some of the best known examples are included in the following table. There are undoubtedly many more types of organisms that may have potential for biological control but have not yet been isolated, screened or tested. There still remains an enormous, essentially untapped pool of potential biocontrol organisms in natural ecosystem. To date primarily only organism that are easily isolated, readily identifiable and abundantly produced in culture have been tested extensively.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Target pathogen/disease</th>
</tr>
</thead>
</table>

**Table 2: Organisms as potential biological control agents of fungal disease**
### Bacteria

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Diseases/Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis</td>
<td>Rhizoctonia, Fusarium, Alternaria, Phytophthora</td>
</tr>
<tr>
<td>B. cereus</td>
<td></td>
</tr>
<tr>
<td>Burkholderiacepsacina</td>
<td>Rhizoctonia, Fusarium, Pythium</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>Pythium damping off, seedling rot</td>
</tr>
<tr>
<td>Erwinia herbicola</td>
<td>Sclerotinia, Pythium white mold rot</td>
</tr>
<tr>
<td>Pseudomonas fluorescens</td>
<td>Pythium, Rhizoctonia, Gaeumannomyces/damping off, take all</td>
</tr>
<tr>
<td>P. putida</td>
<td>Penicillium, Botryts/post harvest decay</td>
</tr>
<tr>
<td>P. syringae</td>
<td></td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>Magnaporthe/Summer patch disease</td>
</tr>
<tr>
<td>Streptomyces sp</td>
<td>Streptomyces, Fusarium, Alternaria, Botryts, Pythium</td>
</tr>
<tr>
<td>Xanthomonas maltophilia</td>
<td>Botryts, Magnaporthe</td>
</tr>
</tbody>
</table>

### Fungi

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Diseases/Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampelomyces quisqualis</td>
<td>Powdery mildew</td>
</tr>
<tr>
<td>Candida oleophila</td>
<td>Botryts, Penicillium/post harvest decay</td>
</tr>
<tr>
<td>Chaetomium globosum</td>
<td>Fusarium, Botryts</td>
</tr>
<tr>
<td>Cladorrhinum foecundissium</td>
<td>Botryts, Sclerotinia</td>
</tr>
<tr>
<td>Coniothyrium minitans</td>
<td>Botryts, Sclerotinia</td>
</tr>
<tr>
<td>Fusarium oxysporum F. solani</td>
<td>Fusarium/wilt</td>
</tr>
<tr>
<td>Gliocladium roseum</td>
<td>Rhizoctonia, Pythium damping off. Rots</td>
</tr>
<tr>
<td>G. vires</td>
<td></td>
</tr>
<tr>
<td>Glomus sp</td>
<td>Fusarium</td>
</tr>
<tr>
<td>Laetisaria arvalis</td>
<td>Rhizoctonia, Pythium</td>
</tr>
<tr>
<td>Myrothecium verrucaria</td>
<td>Rhizoctonia, Botryts</td>
</tr>
<tr>
<td>Paecilomyces lilacinus</td>
<td>Rhizoctonia</td>
</tr>
<tr>
<td>Penicillium spp</td>
<td>Rhizoctonia</td>
</tr>
<tr>
<td>Phlebiagiganteum</td>
<td>Heterobasidion/woody decay</td>
</tr>
<tr>
<td>Pythium oligandrum</td>
<td>Pythium</td>
</tr>
<tr>
<td>Stilbella aciculosa</td>
<td>Rhizoctonia</td>
</tr>
<tr>
<td>Sporodesmium sclerotivorum</td>
<td>Sclerotinia</td>
</tr>
<tr>
<td>Talaromyces flavus</td>
<td>Verticillium wilt</td>
</tr>
<tr>
<td>Trichoderma harzianum</td>
<td>Botryts, Fusarium, Rhizoctonia, Pythium, Sclerotinia, seedling disease/ wilt/ rot</td>
</tr>
<tr>
<td>T. hamatum</td>
<td></td>
</tr>
<tr>
<td>T. asperillum</td>
<td></td>
</tr>
<tr>
<td>T. viride</td>
<td></td>
</tr>
<tr>
<td>Verticillium bigutatum</td>
<td>Verticillium, Rhizoctonia</td>
</tr>
</tbody>
</table>

### Others

<table>
<thead>
<tr>
<th>Others</th>
<th>Diseases/Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viruses (dsRNA)</td>
<td>Cryphonectria, Monosporascus, Rhizoctonia</td>
</tr>
<tr>
<td>Protozoa: Amoeba</td>
<td>Pythium/damping off</td>
</tr>
<tr>
<td>Earthworm</td>
<td>Rhizoctonia</td>
</tr>
</tbody>
</table>

#### 3.3 Use of botanicals and their mixtures:

The use of botanicals for the control of diseases are last options in the organic agriculture, if all the earlier methods are failed. The crude
extracts as well as commercial formulations made from plants like neem, pongamia, derris, citrus grass and tobacco that showed efficacy in conventional agriculture for the management of diseases are allowed in organic farming because of their less residual action and ecological safety. A broad array of pest-repellent products, including homemade herbal teas, plant extracts, and fermentation products, and industrial clay and rock powder products (e.g., kaolin) are authorized for use in organic agriculture.

3.4 Rishi krishi: India had ancient wisdom of farming since beginning of human civilization. It came from enlightened Rishis who lived in forests and understood all the rhythms of nature. One of these great rishis was Parashara but there were many others who taught the art and science of cultivation as per Vedic science. In modern times Mohan Deshpande has propagated this method which is called ‘zero budget farming’ as the raw materials to produce amazing crops, amazing soil fertility and no trouble from pests and diseases. Vrikshayurveda is a treasure trove of information on agriculture and as such could lend support to organic agriculture. Panchagavya has been one such piece of wisdom, meant to safeguard all the human beings, animals, plants and microrganisms dwells on earth surface. Panchagavya is amixture of five products from cows viz cowdung, cow urine, milk, curd and ghee. The recepie of the product is modified by different organization for making more suitable for agriculture. The modified version of panchgavya by Tamilnadu Agricultural University are given below.

**Composition of modified version of Panchagavya:**

- Cow dung - 7 kg
- Cow ghee - 1 kg

Mix the above two ingredients thoroughly both in morning and evening hours and keep it for 3 days

- Cow Urine - 10 liters
- Water - 10 liters
After 3 days mix cow urine and water and keep it for 15 days with regular mixing both in morning and evening hours. The panchagavya will be ready after 30 days.

- Cow milk - 3 liters
- Cow curd - 2 liters
- Tender coconut water - 3 liters
- Jaggery - 3 kg
- Well ripened poovan banana – 12 nos.

All the above items can be added to a wide mouthed mud pot, concrete tank or plastic can as per the above order. The container should be kept open under shade. The content is to be stirred twice a day both in morning and evening. The Panchagavya stock solution will be ready after 30 days. (Care should be taken not to mix buffalo products. The products of local breeds of cow is said to have potency than exotic breeds). It should be kept in the shade and covered with a wire mesh or plastic mosquito net to prevent houseflies from laying eggs and the formation of maggots in the solution.

3.5 Restricted use of pesticides:

Curative control techniques involve application of measures after a pathogen has established itself in the crop. There are limited options for curative control allowed in organic disease management. In principle, the use of synthetic pesticides is prohibited in organic farming. If there are exceptions for restricted use, these pesticides are specifically listed. Pesticides from natural sources such as plant extracts or toxins produced by bacteria are often allowed after a thorough case-by-case evaluation, provided that no synthetic materials are used in their formulation. Mined products are usually also allowed, e.g. silicate from diatomaceous earth. In most countries, copper fungicides are considered to use use against bacterial and fungal diseases, but the number of countries with restricted use of copper fungicides is increasing, especially in Northern Europe. Table 1 provides a representative list of fungicides and insecticides, plant products, microbial agents and other naturally available materials typically approved under organic standards.

Table 3: Frequency in organic in comparison with conventional crop production
<table>
<thead>
<tr>
<th>Group</th>
<th>Pesticides</th>
<th>Conditions for use in organic farming</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthetic pesticides</td>
<td>Various systemic and contact insecticides and fungicides; synthetic pyrethroids</td>
<td>Prohibited</td>
</tr>
<tr>
<td>Organics</td>
<td>Soaps, oils, compost teas, acetic acid</td>
<td>More common</td>
</tr>
<tr>
<td>Inorganics</td>
<td>Sulfur dust and sprays, diatomaceous earth, micronutrients (Si or Zn); copper sulfate, copper hydroxide, bordeaux mixture, potassium phosphite, potassium bicarbonate, potassium silicate</td>
<td>More common; in some countries</td>
</tr>
<tr>
<td>Botanicals</td>
<td>Plant extracts without petroleum-based synergists (pyrethrum, nicotine, neem, horsetail, seaweed, yucca)</td>
<td>Rare or common</td>
</tr>
</tbody>
</table>

Courtesy: Bruggen and Finckh (2015)

4. Organic management practices of some vegetable diseases:

The organic management practices of important diseases of vegetable crops are given in the following table.

**Table 4: Organic management practices of important diseases of vegetable crops**

<table>
<thead>
<tr>
<th>Crop</th>
<th>Name of the diseases</th>
<th>Organic management</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transplanted Vegetables</td>
<td>Damping off</td>
<td>• Use certified seeds.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Partial sterilization of the soil by surface burning of a thick stack of farm trash; solarisation by covering the nursery bed with alkathene.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Formation of raised beds with better drainage facilities. Application of 400 g of neem cake</td>
</tr>
</tbody>
</table>
per sq. m. of nursery bed 15 days before sowing, and watering at 3–5 days interval.
- Use of light soil for nursery beds, thin planting, light and frequent irrigation and application of well decomposed manure.
- Seed coating with the spores of *Trichoderma harzianum*, *Penicillium oxalicum* or *Pseudomonas fluorescens*

<table>
<thead>
<tr>
<th>Plant</th>
<th>Disease</th>
<th>Prevention Strategies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomato</td>
<td><em>Fusarium wilt</em></td>
<td>- Crop rotation with non-solanaceous crops reduces inoculums in the soil.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Seedling root dip in a solution containing ten grams each of turmeric and asafetida dissolved in a litre of water is preferred before transplanting.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Keep the fruits away from the soil by proper training and pruning.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Pull out the affected plants and destroy them.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Use varieties like Mar globe, Kanora, Sioux and Roma which are resistant.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Spray fifteen days' old panchagavya, diluted with ten parts of water.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Seed treatment, root dip treatment and soil application of antagonist like <em>Trichoderma harzianum</em>, <em>Pseudomonas fluorescens</em> or the consortia of two</td>
</tr>
<tr>
<td>Potato</td>
<td><em>Early Blight</em></td>
<td>- Use of disease free tuber, removal of diseased crop debris, crop rotation and spraying of copper sprays can prevent further development of the fungus.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Selection of healthy tuber, use of resistant variety, high ridging, crop rotation, delayed</td>
</tr>
</tbody>
</table>
### Brinjal

<table>
<thead>
<tr>
<th>Alternaria Leaf Spot</th>
<th>Pant samrat variety is tolerant.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial Wilt</td>
<td>Crop rotation with cruciferous vegetables such as cauliflower help in reducing the disease incidence.</td>
</tr>
<tr>
<td>Cercospora Leaf Spot</td>
<td>Fields should be kept clean and effected parts are to be collected and burnt.</td>
</tr>
<tr>
<td>Collar Rot</td>
<td>The diseases are more prevalent in the presence of root knot Nematodes, so control of these nematodes will suppress the disease spread.</td>
</tr>
<tr>
<td></td>
<td>Seed treatment with 4 g of <em>Trichoderma viride</em> formulation per kg seed will help in reducing the diseases.</td>
</tr>
<tr>
<td></td>
<td>Collection and destruction of diseased parts and portions of the plant.</td>
</tr>
<tr>
<td></td>
<td>Spray Copper fungicides to control the diseases</td>
</tr>
</tbody>
</table>

### Cercospora Leaf Spots

<table>
<thead>
<tr>
<th>Fusarium Wilt</th>
<th>Once the disease becomes destructive, it is advisable to find clean fields even if such a plan involves renting additional land.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Powdery Mildew</td>
<td>A better plan is to use a 6-year rotation before the fungus is destructive; this permit many years of okra growing without too much loss.</td>
</tr>
<tr>
<td>Vein-Clearing/Yellow Vein Mosaic</td>
<td>For sowing during the summer season, when the whitefly activity is high, the susceptible varieties should be avoided.</td>
</tr>
<tr>
<td></td>
<td>By selecting varieties resistant to yellow vein mosaic like Parbhani Kranti, Arka Abhay,</td>
</tr>
</tbody>
</table>
Arka Anamika, Co3, and Varsha Upkar, the incidence of the disease can be minimised.

- Even in these varieties, when a plant starts exhibiting symptom of the disease, it should be pulled out immediately and burnt by which the spread of the diseases can be prevented.

5. Conclusion:

Disease management of vegetable crops in conventional systems is typically maintained by continuous external inputs. Organic farmers strive for a healthy ecosystem with high biological diversity, minimal nutrient losses and natural buffering capacity against diseases and pests. However, it takes many years for new microbial and chemical equilibria with relative stability to become established after the conversion from conventional to organic system of cultivation and during the transition period, several pest and disease outbreaks may occur. Nevertheless, epidemic spread of many plant diseases can be curbed as a result of greater crop diversity in time and space and the use of natural vegetation, barrier and cover crops. After a transition period of about 5 years, soil-borne diseases are commonly suppressed in organic farming, including fungus- and nematode-transmitted virus diseases, provided crop rotation is sufficiently long. Diseases that are promoted by high nitrogen contents in plant tissues, such as some rusts and powdery mildews, are usually not problematic in organic farming. However, foliar pathogens that survive in crop residues or on weeds can be enhanced depending on the level of natural control achieved. Insect-vector-transmitted virus diseases can also be more problematic in organic system owing to the smaller scale and thus the proximity of field margins should contain alternative hosts. In addition, diseases caused by multiple-cycle pathogens for which no adequate resistance is available, such as late blight of potatoes, can constitute a severe problem for organic farmers in humid areas, as effective control measures do not exist. Lack of resistances represents a major problem in many minor crops. To overcome this, concerted breeding efforts for organic farming will be required, with the involvement and participation of organic farmers.

********
Chapter-11

Field Use of Bioformulations for Management of Rapeseed-Mustard Diseases

Ranjana Chakrabarty
Jr. Scientist (Plant Pathology)
Regional Agricultural Research Station, AAU, Shillongani, Nagaon

India is the third largest rapeseed-mustard producer in the world after China and Canada with 12 per cent of world’s total production (2016-17). This crop accounts for nearly one-third of the oil produced in India, making it the country’s key edible oilseed crop. Indian mustard (Brassica juncea) is predominantly cultivated in the states of Rajasthan, Uttar Pradesh, Haryana, Madhya Pradesh, and Gujarat which contribute 81.5% area and 87.5% production, out of which more than 47.0% is contributed by Rajasthan state alone. The crop takes 135-150 days to mature. Some early varieties maturing in 110-120 days are also available. The cultivation of brown sarson which once dominated the entire rapeseed-mustard growing region is now shadowed by Indian mustard. There are two different ecotypes of brown sarson (Brassica rapa var brown sarson): lotni (self-compatible) and toria (self-incompatible). The lotni is predominantly cultivated in colder regions of the country particularly in Kashmir and Himachal valley. The toria, on the other hand is cultivated in limited areas of eastern Uttar Pradesh, Assam and other NE states. Yellow sarson (Brassica rapa var. yellow sarson) is now mainly grown in Assam, Bihar, North-eastern States, Orissa, eastern Uttar Pradesh and West Bengal. Toria (Brassica rapa var. toria) is a short duration crop cultivated largely in the eastern states of Assam, Bihar, Odisha and West Bengal, mainly as a winter crop. In Haryana, Himachal crop during September-December; but the area has declined due to shift in the cropping pattern. Taramira (Eruca sativa) is grown in the drier parts of North West India comprising the states of Rajasthan, Haryana and Uttar Pradesh. Gobhisarson (Brassica napus) and karan rai (Brassica carinata) are the new emerging oilseed crops having limited area of cultivation. Gobhisarson is a long duration crop (more than 155 days) confined to Punjab, Himachal Pradesh and Haryana.

Due to the gap between domestic availability and actual consumption of edible oils, India has to resort to import of edible oils. By increasing the domestic production substantial import substitution can be achieved. Rapeseed-mustard is the major source of income especially for the marginal- and small-farmers in rainfed areas which are about 25% of the total cultivated area.
Due to low water requirement (80-240 mm), rapeseed-mustard crops fit well in the rainfed cropping system and, it suits and adapts well in different cropping systems. It is cultivated in 26 states in the northern and eastern plains of the country and, about 6.4 m ha is occupied under these crops (2016-17). It is cultivated across the country mainly in Rajasthan, Madhya Pradesh, Uttar Pradesh, Haryana, West Bengal, Assam and Gujarat which contribute maximum to its production (>93%) and acreage (>91%) and now its cultivation is also being extended to non-traditional areas of Karnataka, Tamil Nadu, Telangana and Andhra Pradesh.

Despite the high quality of oil and meal and also its wide adaptability for varied agro-climatic conditions, the area, production and yield of rapeseed-mustard in India have been fluctuating due to various biotic and abiotic stresses coupled with India's domestic price support programme. Biotic stresses caused by insects (painted bug, aphids), fungal (Sclerotinia stem rot, white rust, downy mildew and Alternaria blight), bacterial and viral pathogens, parasitic weeds (Orobanche) and other weeds collectively result in approximately 45% yield loss annually. Nevertheless, the crop has potential to ensure the nutritional security and contribute to livelihood security.

**Concept of Bioformulation**

Typically a formulation is a mixture of an active ingredient in a formulated product with inert (inactive) substances. However, regarding bioformulation we see that there is no uniform definition available and various authors define it in their own way. Burges and Jones (1998) stated that bioformulation comprised aids to preserve organisms, to deliver them to their targets, and once there to improve their activities, whereas Arora et al. (2010) defined the term bioformulation to preparations of microorganism(s) that may be partial or complete substitute for chemical fertilization/pesticides. Bioformulation are best defined as biologically active products containing one or more beneficial strains in easy to use and economical carrier materials. A good carrier should have the capacity to deliver the right number of viable cells in good physiological conditions in right time (Arora, et al. 2008). Carrier can be divided into three basic categories: (1) soil (peat, coal, clays and inorganic soil) (2) plant waste materials (3) inert materials viz., vermiculite, ground rock phosphate, polyacrylamide gels and alginate beads (Bashan, 1998).

According to Jeyarajan and Nakkeeran (2000), characteristics of an ideal formulation are,
i. Should have increased shelf life.
ii. Should not be phytotoxic to the crop plants.
iii. Should dissolve well in water and should release the biological agents.
iv. Should tolerate adverse environmental conditions.
v. Should be cost effective and should give reliable control of plant disease.
vii. Should be compatible with other agrochemicals.
vii. Carrier must be cheap and readily available for formulation development.

Types of Formulation

Broadly two types of bioformulations are available, liquids and solids (Burges and Jones 1998)

Solid Formulations

Solid formulations include granules (GR), microgranules (MG), wettable powders (WP), wettable/water-dispersible granules (WG, WDG), and dusts (Guijarro et al. 2007). They are produced by adding binder, dispersant, wetting agents, etc. (Brar et al. 2006).

Granules (GR): Granules are dry particles and contain active ingredient, binder, and carrier. Concentration of active ingredients in granules is 5–20 % (Brar et al. 2006). On the basis of particle size, they are classified as coarse particles (size range 100–1000 μm) and microgranules (size range 100–600 μm). The granules should be non-caking, non-dusty, and free flowing and should disintegrate in the soil to release the active ingredient. They are usually safer having no risk of inhalation and mostly used in soil treatment. Granular formulations are more concerned with storage and increased shelf life (Callaghan and Gerard 2005). Most commonly used granules are wheat meal granules, corn meal baits, granules formed with gelatinized corn starch or flour, gluten, cotton seed flour and sugars, gelatin or acacia gum, sodium alginate, diatomaceous earth and semolina (durum) wheat flour. Although granular formulations are very effective, their application is also limited due to inactivation of active ingredient in ultraviolet (UV) light.

Wettable Powders (WPs): Wettable powders (WPs) are one of the oldest types of formulations. They consist of 50–80 % technical powder, 15–45 % filler, 1–10 % dispersant, and 3–5 %
surfactant by weight to achieve a desired potency formulation (measured in international units) (Brar et al. 2006). These dry formulations are of much interest as they are readily miscible with water and can be easily added to a liquid carrier, normally water, just before its application. WPs have a longer shelf life and by controlling moisture content, their shelf life may exceed 18 months. Agricultural materials and industrial waste by-products such as wheat bran–sand mixture, sawdust–sand–molasses mixture, corn cob–sand–molasses mixture, bagasse–sand–molasses mixture, organic cakes, cow dung–sand mixture, compost/farm manure, inert charcoal, diatomaceous earth, and fly ash can also be used to prepare powder formulations. It was found that 55.3 % of Trichoderma formulations are commercialized as WPs.

**Wettable/Water-Dispersible Granules (WG, WDG):** Wettable/water-dispersible granules (WG, WDG) are also known as dry flowables. They have been designed to make WPs more user and environmental friendly, non-dusty, free-flowing granules quickly dissolving in water. They contain wetting agents and dispersing agents similar to those used in WPs, but the dispersing agent is usually at a higher concentration. Like WPs, WDG also show excellent shelf life. WDG formulations have wider role in nematode control and capture 90 % of the total market available for nematode-based products.

**Dusts:** Dusts are also one of the oldest formulation types and contain very finely ground mixture of the active ingredient (usually 10 %) with particle size ranging from 50 to 100 μm. Although they have been used since a long time and in some instances more effective in killing (Ifoulis and Savopoulou-Soultani 2004), there have always been handling and application problems associated with dusts (Harris and Dent 2000).

**Liquid Formulations**

Liquid formulations are also known as flowable or aqueous suspensions and consist of biomass suspensions in water, oils, or combinations of both (emulsions). A typical liquid formulation contains 10–40 % microorganisms, 1–3 % suspender ingredient, 1–5 % dispersant, 3–8 % surfactant, and 35–65 % carrier liquid (oil or water) (Brar et al. 2006). Liquid formulation may be of the following types.
Suspension Concentrates (SCs): SCs are produced by adding solid active ingredient(s) with poor solubility in water and satisfactory stability to hydrolysis. SCs are diluted in water before use. Farmers generally prefer suspension concentrates to wettable powders because they are non-dusty and easy to measure and pour into the spray tank.

Oil-Miscible Flowable Concentrate (OF): OF is stable suspension of active ingredient(s) in a fluid intended for dilution in an organic liquid before use.

Ultralow Volume (ULV) Suspension (SU): They are suspension ready for use through ULV equipment. ULV are aerial or ground spray equipment and generate extremely fine spray.

Oil Dispersion (OD): OD is a stable suspension of active ingredient(s) in water-immiscible solvent or oil. Recently Mbarga et al. (2014) developed a soybean oil based formulation and found that *Trichoderma asperellum* containing OD had great potential for the control of cacao black pod disease with increased half-life of the conidia in comparison to aqueous suspension. Some of the *Trichoderma* containing liquid formulations used in biocontrol are Trichojet, Enpro-Derma, and Trichorich-L.

Encapsulation

Encapsulation involves coating or entrapping microbial cells within a polymeric material to produce beads which are permeable to nutrients, gases, and metabolites for maintaining cell viability within the beads (John et al. 2011). Encapsulation provides good protection to active ingredient from harsh environmental factors. Currently, gelatin, starch, cellulose, and several other polymers are used for encapsulation of active ingredients.

Although both liquid and solid formulations have been extensively used in agrosystems, dry formulations are generally preferred over wet formulations because they provide extended shelf life and are easier to store and transport (Burges and Jones 1998).
Method of application of Bioformulation

1. **Slurry seed treatment:** Take 5-6 g/10g of bioformulation per kg of seed. Mix well with 10 ml of water. Air-dry the seeds for 30 minutes to 1 hour before sowing.

2. **Wet seed treatment/Dip method:** Dissolve 20-25 g of bioformulation in 1 litre of water. Dip 1 kg seed or appropriate nos. of seedlings/sets/rhizomes in the solution for 1 hour. Dry under shade for 1 hour before sowing/planting.

3. **Soil drenching:** Dissolve 20-25 gm of bioformulation in 1 litre of water for spot application by jerry cane or sprayer whenever felt necessary.

4. **Soil application:**
   - Mix 100 kg of dried cow dung with 10 kg of Mustard Oil Cake. Moisten by sprinkling water.
   - Add 1 kg commercial formulation of bioagent, mix thoroughly and cover with news paper or banana leaf for 3 days.
   - On 4th day open the cover, mix thoroughly, sprinkle water and cover it again.
   - On 7th day, mix thoroughly the final product and apply to soil before planting. For 1 hectare of land, the requirement is approximately 500 kg of dried cow dung, 50 kg of Mustard oil cake and 5 kg of bioformulation.

5. **Foliar spray:** Foliar application is done at the rate of 5ml/litre of water.

List of Commercial bioformulations

<table>
<thead>
<tr>
<th>S.N</th>
<th>Biocontrol agents</th>
<th>Trade name</th>
<th>Pathogen controlled</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Trichoderma viride</em></td>
<td>Ecofit</td>
<td>Soil borne pathogens such as <em>Pythium, Macrophomina, Phytophthora, Sclerotium</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Basderma</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Biderma</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td><em>T. harzianum</em></td>
<td>F-Stop</td>
<td>Soil borne pathogens such as <em>Pythium, Macrophomina,</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
|---|-----|-----|-----------------------------------------------------------------
| 3 | Gliocladium virens | Gliocard | Phytophthora, Sclerotium, Seedling diseases of ornamental plants |
| 4 | T. polysporum     | Binab-T  | Wood decay fungi                                                 |
| 5 | Bacillus subtilis | Kodiak   | Post harvest diseases of fruits and brown rot of fruits          |
| 6 | Pseudomonas fluorescens | Dagger G | Rhizoctonia and Pythium                                          |
|   | Pseudomonas fluorescens | Bio-Save, Blight Ban, Cedomon, Biocoat, and Victus |                                                   |
| 7 | Agrobacterium radiobacter(K-84) | Gallex, Galltrol | Agrobacterium tumifaciens                                        |
| 8 | Streptomyces lydicus | Actinovate | Root and seed rot in peas; lower disease in spinach caused by Pythium and Fusarium (soil-borne fungi). |
| 9 | Six species of Bacillus, Streptomyces griseoviridis, Trichoderma harzianum plus organic nutrients | Compete Plus | Black Scurf potato (Rhizoctonia solani) and common scab of potato (Streptomyces scabies). |
| 10 | PGPR               | BioYield | root rot (Pythium, Rhizoctonia) and wirestem (Rhizoctonia) in broccoli. |
| 11 | Coniothyriumminitans | Contans | lettuce drop caused by Sclerotinia species                      |
| 12 | Bacillus subtilis | Kodiak   | Black Scurf and stem canker on potato.                           |
| 13 | *Muscadoralbus* | Muscador | Root, hypocotyls rot and *Phytophthora* fruit rot on pepper. |
| 14 | *Trichoderma harzianum* | Plant Shield | *Rhizoctonia* rot of bean and potato, Common Scab on potato, *Botrytis* on tomato, or Early Blight on tomato. |
| 15 | *Bacillus subtilis* | Serenade | Root rot caused by *Rhizoctonia* on both beans and radish. |
| 16 | *Trichoderma virens* | Soil Gard | Black Scurf and Common Scab in potato. |
| 17 | Nonpathogenic *F. oxysporum* | Biofox C | Used in basil, cyclamen, tomato and carnation. |

**Bioformulation developed at Assam Agricultural University (AAU)**

<p>| 18 | <em>Pseudomonas fluorescens</em> | Biofor PF-2, Pseudocon Biozin-PTB, Bioveer and Biozium | Diseases of vegetable, field and plantation crops |
| 19 | <em>Trichoderma spp.</em> | Bicure F, Trich-X-P, Viricon-L, Bioderma, Trichostar | Diseases of vegetable, field and plantation crops |
| 20 | Plant Growth Promoting Microbe (PGPM) and entomopathogens viz <em>Bacillus thuringiensis M. anisopliae, B. bassiana, V. lecanii</em> | Biogreen 5, Biogreen L and Biotime | Pest and diseases of Agricultural, Horticultural and plantation crops |
| 21 | <em>Metarhiziumanisopliae</em> | Org-Metajal | Termite, aphid, scale insect |
| 22 | <em>Trichoderma harzianum</em> | Org-Trichoal | Soil borne plant pathogen and plant growth enhancer |
| 23 | <em>Verticillium lecanii</em> | Org-Vertijal | Red spider mite |</p>
<table>
<thead>
<tr>
<th></th>
<th><strong>Species</strong></th>
<th><strong>Organism</strong></th>
<th><strong>Use</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td><em>Beauveria bassiana</em></td>
<td>Org-Beauverijal</td>
<td>Rice hispa, Halopeltis</td>
</tr>
<tr>
<td>25</td>
<td><em>Purpurocillium lilacinus</em></td>
<td>Org-Cilliumjal</td>
<td>Root knot nematode</td>
</tr>
<tr>
<td>26</td>
<td><em>Pochoniaclamydomospora</em></td>
<td>Org-Pochojal</td>
<td>Root knot nematode</td>
</tr>
<tr>
<td>27</td>
<td><em>Metarhiziumanisopliae</em></td>
<td>Org-Metahim</td>
<td>Termite, aphid, scale insect</td>
</tr>
<tr>
<td>28</td>
<td>Nano bioformulation of <em>Trichoderma</em></td>
<td>Trichosan N</td>
<td>Seed treating agent (3 ml/l) to curb soil borne diseases</td>
</tr>
</tbody>
</table>

**Biological control of Rapeseed-mustard diseases**

Till date chemical management was the only option against the problem of rapeseed-mustard diseases. In many European countries, organic agriculture has rapidly been transformed from a farmers’ movement to an institutionalized part of agricultural policy. In certification, compliance with published organic standards is verified through annual inspections on farms (Laura and Juha, 2004). However, some reports indicate possibility of biological management of the disease. Phyllosphere residents *Aureoba sidium pullulans* and *Epicoccum nigrum* reduced the infection by *A. brassicicola*, especially when they were inoculated 14 h before the pathogen (Pace and Campbell, 1974).

Spray of soil isolates of *Trichoderma viride* at 45 and 75 days after sowing could manage *Alternaria blight* of Indian mustard (*Brassica juncea*) as effectively as mancozeb (Meena et al., 2004). Botanicals viz., bulb extract of *Allium sativum* has been reported to effectively manage *Alternaria* blight of Indian mustard (Meena et al., 2004; Patni and Kolte, 2006).

Seed treatment with biocontrol agents viz., *T. viride*, *G. virens* or botanicals like *Allium sativum* bulb extract (1 % w/v) or carbendazim @ 0.1% a.i. or mixture of carbendazim with Apron 35 SD (6 g/kg) was found effective against some diseases. There is a need for mixture of fungicides for avoiding resistance development in pathogens to fungicides. Use of biocontrol agents is advantageous as they are often effective against a wide range of soil-borne pathogens.
Moreover, they are ecofriendly, cost effective and their use avoids the risk of development of resistance in the pathogen towards the control agent.

Seed treatment with *Trichoderma harzianum* @ 10 g/kg seed followed by foliar spray of Ridomil MZ 72 WP @ 2 g/ l water after 50-60 days of sowing, significantly reduced the *Alternaria* leaf and pod blight up to 43.6 and 30.8 per cent, respectively and white rust and stagheads up to 39.5 and 23.3 per cent, respectively (Rathi and Singh, 2010)

Among the bio-control measures, seed treatment by *Trichoderma harzianum* @ 10g/ kg seed + foliar spray of *Pseudomonas fluorescens* (oil based) @10 ml /l water after 50 days sowing reduced *Alternaria* blight up to 28.0 per cent followed by seed treatment with *Trichoderma harzianum* @ 10g kg seed + foliar spray with *Trichdermaharzianum* @ 10ml/l water. Both the biocontrol measures were found at par with fungicidal seed treatment alone, reducing *Alternaria* leaf blight severity as well as yield respectively (Rai et al. 2014). Success of the bioagent against *Alternaria* blight in Indian mustard has been also reported by Meena et al. (2004). Reshu and Khan (2012) tested biocontrol agents against *A. brassicae* in mustard in field found that *T.viride* proved to most effective in the reduction of disease intensity on leaves and pods

Biological control is a promising method of control of *Sclerotinia* rot diseases (Bardin and Huang, 2001). Highest reduction of *Sclerotinia* rot (69.0%) was achieved by foliar spray of *T. harzianum* isolate GR @ (1:9) 5 g/l over control followed by soil application of *T. harzianum* isolate SI-02 with FYM (60.8%) @ (1:9) 2 g/kg and foliar spray of garlic bulb extract (w/v) 2% (60.8%). Highest *Trichoderma* spp. population count was observed at 60 days after sowing in soil of *T. harzianum*-GR isolate applied in soil with FYM (Meena et al., 2014).

*Coniothyrium minitans* occurs naturally in soil as a mycoparasite of *S. sclerotiorum*. It was involved in the decline of viable sclerotia of *S. sclerotiorum* during crop growth and thereby suppresses the ascospores release (Whipps and Gerlagh, 1992; Sandy-winschet al., 1993).
Trials conducted at RARS, AAU, Shillongani, Nagaon, Assam


Treatment: 4  
Replication: 6  
Design: RBD

Module I

- Soil application of enriched *Trichoderma viride* @ 2.5 kg/ha +
- FYM 2 t/ha + Rock phosphate 30 kg/ha +
- Biofertilizer (*Azotobacter* & PSB each @ 50 g/kg seed) +
- Spray with *Bacillus megeterium* @ 5 ml/l of water (30 & 45 DAS) + 2-4 foliar sprays of NSKE 5% (need based)
- Installation of Bird perch @ 40 nos./ha
- Installation of yellow sticky trap (0.5 m x 0.5 m) @ 20 nos./ha

Module II

- Seed inoculation with *Trichoderma viride* @ 10g/kg seed + Biofertilizer (*Azotobacter* & PSB each @ 50g/kg seed) +
- Soil application with vermicompost @ 1t/ha + Rock phosphate @ 30 kg/ha +
- Foliar spray with *Bacillus megeterium* @ 5ml/l of water (30 & 45 DAS) + 2-4 foliar sprays of NSKE 5% (need based)
- Installation of Bird perch @ 40 nos./ha
- Installation of yellow sticky trap (0.5 m x 0.5 m) @ 20 nos./ha

Conclusion and Future Steps

A bioformulation is not effective until it does not have an impact in field conditions, market existence and reliability and cost-effectiveness (Brar *et al.* 2006). Social and public interactions toward Bt-based biopesticides are given by Navon (2000). He concluded that the toxicity of protein as an oral insecticide and environmental conditions reduces the efficacy of the product. Production of bioformulation is not only dependent on the detailed knowledge of microbial as well as plant physiology, but a number of technological challenges are also involved such as fermentation process, formulation type, population of microbe, and delivery systems (Malusaet *et al.* 2012).
Although the research is ongoing, we have not succeeded in producing such an elite formulation which not only has broad spectrum activity but also fulfills economic challenges. Although many microbial inoculants have been developed, very few products are found to be promising. At present development of bioformulation involves collective effort of both microbiological and technological aspects, and vigorous research efforts are required for technological part (Arora, 2015). Besides this there is urgent requirement in the field of bioformulation technology to reevaluate the whole process and plugging of loop holes. We have to consider every step such as selection of organism, production method, delivery system, application technology, factors affecting development, persistence in the environment, and ultimately market availability of product.

References


Ifoulis, A.A. and Savopoulou-Soultani, M. (2004). Biological control of *Lobesia botrana* (Lepidoptera: Tortricidae) larvae by using different formulations of *Bacillus thuringiensis* in 11 vine cultivars under field conditions. *J Econ Entomol.* **97**:340–343.


********
Chapter-12
Embracing Social Engineering for Innovative Pest Management

Badal Bhattacharyya, ElangbamBidyarani Devi, Nang SenaManpoong, Peter Shyam and ParthaPratimGyanudoy Das
All India Network Project on Soil Arthropod Pests,
Department of Entomology, Assam Agricultural University, Jorhat 785013, Assam
email: ainpsapaaau.gmail.com

Social engineering is a discipline in social science that refers to efforts to influence particular attitudes and social behaviors on a large scale, whether by governments, media or private groups in order to produce desired characteristics in a target population. Adaptive research and development methods, participatory technology development and community involvement are important elements for the desired outcomes. Social engineering which means a balance between the competing interests in society, in which applied science are used for resolving individual and social problems. Social engineering is a data-based scientific system used to develop a sustainable design so as to achieve the intelligent management of resources and capital with the highest levels of freedom, prosperity and happiness within a population. A participatory approach, tends to focus initially on small numbers of clients participatory and is location specific in nature. Rather than "passive participation," it is aimed to inspire "self-mobilization", where communities organize and take initiative independently to solve their problems/issues. Community mobilization is the process of engaging communities to identify community priorities, resources, needs and solutions in such a way as to promote representative participation, good governance, accountability, peaceful change and achieving the objectives. Carrying out a participatory technology development programme with farmers can be done in collaboration with NGOs and extension staff, albeit with training and adjustments to present methods of operation. In case of group approach the main challenge is sustainability of groups. This concept heavily relies on all the members coming together to achieve a common goal, finding technical solutions and building capacities in the extension system and bridge the gaps in knowledge and technology dissemination.

The underlying principles of social engineering/participatory approaches/community mobilization can effectively and intelligently be explored in solving some crucial constraints.
related to agriculture and allied sciences. Such type of approaches not only improves crop productivity and livelihood but also tremendously improves overall knowledge contents and capacity building of the farming community. Most of such approaches are ecofriendly, economical and sustainable. The visibility of extension programmes as well as accountability are also become more vibrant.

Under the aegis of All India Network Project on Soil Arthropod Pests, a group based research and extension programme to tackle a highly endemic and severe key pest species of white grub (*Lepidiotamansueta*) in Majuli river island of Assam was carried out from 2005 onwards and the brief achievements are highlighted below:

**Social engineering for the management of white grub in Majuli river island of Assam: A case study**

i. **How the research/extension work was conceptualized?**

The research/extension works were carried out on *Lepidiotamansueta* Burmeister (Coleoptera: Melolonthinae), an atypical species of white grub endemic to Majuli river island of Assam as well as sugarcane growing areas of Haridwar and the adjacent areas of Ganga River of Uttarakhand. Majuli is the largest fresh water mid-river deltaic island in the world which is situated between 26°45 N to 27°12N latitude and 93°39E to 94° 35E longitudes. It is situated in the upper reaches of the Brahmaputra, 630 km upstream of the Indo-Bangladesh border and 100 km from its mouth and the elevation from the mean sea level is 84.50 meters. Majuli falls under the tropical climate zone; however, the numerous wetlands, streams, etc. endow Majuli with a sub-tropical climate. The average annual temperature is 22.50°C. The maximum summer temperature varies from 30-35°C with a minimum temperature of 12°C. The average annual rainfall ranges from 200-250 cm with 80 per cent relative humidity. The island is a “Bio-diversity hotspot” and has rich ecology with rare breeds of flora and fauna and is a part of a major path for many species of migratory birds. The government of Assam has also proposed that the island be included in the UNESCO’s “World Heritage Site” list because of its unique historic importance, rich biodiversity and co-existence of various cultures.
Majuli is a sub district in the Jorhat district of Assam and the total population in Majuli river island is 1,67,304 as per the survey of census during 2011 by Indian Government. Out of the total population, tribal population is about 70 per cent and hence Majuli Legislative Assembly Constituency (LAC) is reserved for Scheduled Tribes. The tribes inhabiting the island are Mising, Deori and Sonowal-Kachari, of which the Mising constitute the largest ethnic tribe in the island. The tribal people usually live in huts raised on bamboo and wooden posts, settle themselves on the river side particularly along the river Brahmaputra and its tributaries. They have been living with the nature and since ages are primarily engaged in seasonal cultivation and fishing. The island had a total area of 1250 sq. km, but having lost significantly to erosion it has an area of only 421.65 sq. km in 2001. Of the total land area of Majuli, the area suitable for cultivation is only 32,237.16 hectares. Another 14,834.66 hectares remains always under water whereas a total land area of 7,671.23 hectares is not suitable for productive purposes. A total of 22 numbers of 'Sand bars (Char areas)' have covered 5,939.01 hectare. In addition to this, 61,153.09 hectares have been reserved as Government reserved land. In Majuli River Island, total of 243 small and large villages exist. Of these 210 are Cadastral Villages (revenues generated by the administration and supported with revenue maps) and 33 are Non cadastral village (these are villages with no revenue maps, mostly resettled or rehabilitated villages shifted due to flood and erosion). The cultivable land is highly fertile and suitable for production of different crops. The pristine tribal population of Majuli has a rich and diverse agricultural tradition, growing as many as 100 varieties of rice, with almost zero use of chemical fertilizers or pesticides. Among the interesting rice varieties are komolchaol, the ready to eat soft rice and bao dhan, the deep water rice. Besides rice, rapeseed-mustard, potato, sweet potato, pulses, sugarcane, wheat, various seasonal vegetables and fruits like orange, banana, pineapple, jackfruit, etc. are also grown in abundant quantity. Other important economic activities of the tribal populace of Majuli are fishing, dairying, piggery, goatery, poultry, weaving, pottery and boat-making. Agriculture, the basic means of survival for the tribal community of Majuli since time immemorial has been suffering due to recurrent flood waves as well as erosion during the monsoon (June-August/Sept) and post monsoon (Sept-Oct) seasons. The crops grown during the flood free winter months (rabi season) suffer due to ravages of insect pests and diseases. The insect pests attacking aerial plant parts and acting as major yield reducing factors in various crops include army worm, case worm, gundhi bug, swarming caterpillar and stem borer in rice,
mustard aphid and sawfly in rapeseed-mustard, pod borers in pulses and early shoot borer and plassey borer in sugarcane; fruit borer in tomato, shoot and fruit borer in brinjal, trunk borer and leaf miners in citrus and pseudostem borer in banana. While the major subterranean pests inflicting severe damage to crops are cut worms and white grubs in various crops; both of which are moisture loving insects and soil moisture is always sufficiently available for them in flood prone Majuli river island.

The white grub *Lepidiotamansueta* was first detected in October 2005 in the farmers’ field of Majuli river island. Field surveys conducted during 2005-2009 revealed that *L. mansueta* had appeared as an extremely severe key pest in Majuli river island and the most severely affected crops were potato, sugarcane, Colocasia and green gram, and the extent of damage varied from 42-48, 15-20, 35-40 and 30-35 per cent, respectively (Bhattacharyya et al., 2013).

![Biennial life cycle of L. mansueta](image)

**Fig. 1: Biennial life cycle of *L. mansueta***
Realizing the seriousness of the problem, the seasonal life cycle and biology of the white grub beetle, \textit{Lepidiotamansueta} were studied in crop fields of Majuli river island and in the laboratory of All India Network Project on White grubs and Other Soil Arthropods (funded by ICAR, New Delhi), Assam Agricultural University, Jorhat Centre, respectively during 2005-2009. \textit{L. mansueta} has a biennial life cycle (Fig.1), which is the first of its kind from North East India. It is a unique biennial species, spending its entire life cycle under the ground except for a very short period during which adults come out of the ground for mating. Grubs are voracious feeders. However, there is no evidence showing that the adults fed on any plants either in the field or laboratory and hence this species has the unique distinction as the first Indian phytophagous white grub species with nonfeeding adults (Bhattacharyya \textit{et al.}, 2015). The probable reason of endemicism/outbreak due to the high organic carbon content of the soil (0.75-1.00\%) and presence of abundant thatch zone (dead grass, stems and other organic debris) in the endemic pockets. Other reasons may be nonarrival for last several years, of the migratory bird Siberian crane (\textit{Grus leucogeranus}), a seasonal predator of the grubs in Majuli probably because of the changing climate with erratic rainfall and early onset of summer in the riverine island. Moreover, conversion of virgin low grass lands (sand bars) by the flood and erosion affected people into agricultural farm lands—a potential problem inviting future outbreaks of the species in massive proportions in Majuli. Even though this species has not been reported from anywhere else from the main land of Assam or the nearby north eastern states, there is every possibility of the species to cross the geographical barrier of the mighty Brahmaputra and spread to other areas.

After unravelling the seasonal cycle and biology, the investigators learned few vital tips off worthy of managing the beetles as mentioned below:

- Rush of adult emergence took place for a short period of time in the evening during April-May. Except for this short aerial life form nuptial activity, the species lives a subterranean life.
- Both sexes of the beetle were positively phototactic.
- Beetles emerged from soil forming during evening hours and spend almost one hour (6.15 pm-7.15 pm) for premating flight. Beetles could be collected in huge numbers by operating light traps in endemic pockets during 6.30 pm-7 pm.
• Scouting for handcollectionisalso effectiveincethemated pairsare found abundantly onselected sheltering plants in field during 7pm-8.30 pm.
• Beetles canalsobeusedas animal feed forpoultry,pigs, dogs, cats etc.
• Some indigenous tribes also consume the beetles as their food.
• Concept of SocialEngineering/Farmers’ participatory approach could be encouragedfor the masscollectionand destructionofbeetles during the period after premonsoon showers in the endemic areas of Majuli river island. In white grub endemic areas adult collection campaigns are being resuscitated, as farmers do not appear to realise their potential impact on reducing damage; this is because they are not conversant with the life cycle of the white grub. In this regard, the community action programmes aimed at collecting adult beetles as they emerge offer a practical and cost-effective method of management, and should be pursued.

ii. Salient features of the approach:

Field and laboratory studies on seasonal cycle and bio-ecology of *L. mansueta* conducted during 2005-2009 in Majuli and adjacent endemic villages located in different sandbarsrevealed that the mass collection and destruction of adult beetles by mass campaigning during the period after premonsoon showers became inevitable in the endemic areas. During that period, farmers of Majuli did not realised the potential impact of this pest since they were not at all conversant with the biennial life cycle of *L. mansueta*with nonfeeding adults but voracious grubs. Majority of the farmers believed that the grubs of *L. mansueta* were not pests but helpful in increasing the fertility of soil, just like earthworms. Considering farmers wrong perceptions about this notorious pest, a parallel planning was done to carry out both basic research as well as community action programmes/social engineering/farmers participatory approaches aimed at collecting adult beetles during evening hours (6pm-9pm during April-May) as a practical and cost-effective method of management. These extension activities were initiated from 2010 onwards in collaboration with different stakeholders under the theme “Mass campaigning against *Lepidiotamansueta* in Majuli river island and adjacent sandbars through social engineering”.

iii. Villages / farmers targeted:

From the very beginning of mass campaigning against *Lepidiota* beetles, group based approach for the mass collection and destruction of beetles was given the top most priority. Each tribal village was selected based on the population and extent of damages caused by the grubs, presence of functional farm management committee/self-help groups/gram panchayats and a “Lepidiota Management Group” was formed in each tribal endemic village consisting of 25 active farmers.

iv. How the research/Extension work was organized?

The grubs of *L. mansueta* were first detected in October 2005 in the farmers’ field of Majuli river island and adjacent villages/sandbars located on the bank of mighty Brahmaputra river. Field surveys conducted during 2005-2006 revealed that the bioecology and damage potential of this species of white grub was not identical with other predominant species of white grubs, in this island and appeared as a severe key pest of potato, sugarcane, colocasia and green gram and the extent of damage varied from 42-48, 15-20, 35-40 and 30-35 per cent, respectively. It is worthy of mention that organic agro-farming was the mainstay of the island’s economy and primary occupation of most of the islanders, mostly of the *Mising, Deori* and *Sonowal Kachari* tribes. This island is located in the middle of the Brahmaputra river and hence experiences flood every year. The river bank erosion caused by the spate of Brahmaputra and Subansiri River has forced the affected farmers of this region to search for new unutilised potential areas of sandbars for cultivation. However, *L. mansueta* infestation was a potential problem in low grasslands (sand bars) which were brought into cultivation for the first time mostly by the flood and erosion affected people.

As majority of farmers were not conversant with the life cycle of the white grub, they mostly tried to suppress the infestation with insecticides. However, selection of inappropriate insecticides, faulty method and untimely application not based on proper knowledge about biology of the pest further aggravated the problem. The investigators were also given to know about the superstitious belief among the tribal farmers that the grubs damage their crops because of the sins committed by their forefathers. Farmers were exceedingly worried because their livelihood security was affected. Even, in some endemic pockets farmers discarded growing
colocasia, which was otherwise considered as the most preferred vegetable among the tribal communities due to its high nutritive value, deliciousness and flavour. The report of *L. mansueta* from Majuli river island also served as eye-opener to the other white grub entomologists of India to concede the fact that white grubs can also be a serious problem in a river island like Majuli, where flood is a common feature. Considering the above facts, concerted efforts were made from 2007 onwards to investigate the seasonal cycle and bio-ecology of this pest in the field as well as in the White grub laboratory of Assam Agricultural University, Jorhat.

After the unravelling of seasonal cycle and bioecology of this insect pest, investigators came to the conclusion that there were lots of “worrying factors” as far as management of this atypical species of white grub was concerned as mentioned below:

a) *L. mansueta* has biennial life cycle which is first of its kind from North East India. Third instar grubs were found to be voracious feeders of roots/tubers/corm of crops and they remained active in the field up to 18 months. As it spends its entire life cycle under the ground except for a short period during which adults come out of the ground for mating, the species was thought to be irregular in occurrence or even migratory; whereas it was very much resident species of Majuli.

b) Application of soil insecticide was effective only against the short-lived 1st and 2nd instar grubs but not against the 3rd instar grubs. Third instar grubs showed typical downward vertical migration into the deeper layer of soil and remained inaccessible to the insecticidal treatments and hence unaffected.

c) It was very difficult to detect the presence of white grubs in both cultivated and noncultivated fields though the grubs were abundantly available in endemic areas (10-15 grubs/sq. m) without showing any detectable above ground symptoms of infestation on the plants.

d) One of the major tactics for managing the adult scarab beetle population that congregated on some preferred host plants during pre-monsoon or monsoon by spraying recommended insecticides during daytime was found to be absolutely ineffective in case of *L. mansueta*. Because, the adults of both sexes were observed not to feed on any plants in the field and hence this species has got the unique distinction of being the “First record of Indian phytophagous scarab beetle with
non-feeding adults”. Moreover, the adult beetles became over ground during evening hours only for 2-3 weeks in April-May and hence, managing the huge population adults within a very short period in the evening over a large area was utterly difficult.

e) Majuli river island is an aspirant for getting the tag of “World Heritage site” from UNESCO. Therefore, there was also an urgent need to ponder about nonchemical approaches of managing this insect pest since the application of chemical pesticides is not allowed in such sites.

Considering all the above facts, efforts were made towards a participatory approaches for beetle management as a way ahead.

Towards participatory approaches for beetle management: the way ahead:

Parallel planning was done to carry out both basic research as well as community action programmes/social engineering/farmers participatory approaches aimed at collecting and destructing the adult beetles during April-May with the help of tribal and other farmers in Majuli from 2010 to 2014. Besides involving farmers, collaboration in this regard was sought from farm management committee, self-help groups, KVK Jorhat, state extension staff, gram panchayat, NGOs and district administration, Majuli. To sensitize farmers the following tools of social engineering were used:

a) Smart SMSing to farmers through www.way2sms.com
b) Video-conferencing
c) Use of social networking site
d) Use of print and electronic media
e) Extension trainings
f) Farmer-scientist interaction
g) Field day
h) Exhibition
i) Awareness meeting
j) Documentary shows
k) Posters and banners
l) Distribution of photographs/leaflets
m) Exposure visit
n) Documentary show
o) Use of public address system
p) Conducting field experiments in endemic areas
q) Visit of Entomologists from other institutes/university
r) Telephonic discussion
s) Demonstration on collection of beetles using Solar LED light traps
t) Demonstration on using *Lepidiota* beetles as human food/animal feed

This mass campaigning programme received overwhelming response and was exceedingly successful leading to massive collection and killing of about 11.33 Lakhs *L. mansueta* beetles in Majuli river island during 2010-2019. The major advantages of such approach are -(i) the gravid females are killed before egg laying, (ii) capacity building amongst the farmers in white grub endemic areas and the management approach is ecofriendly and cost effective. It is worth mentioning that some of the local tribal people relished the cooked/fried adults of *L. mansueta* as protein rich food which opens up an avenue of further research on its nutritive/ nutraceutical value.

v. Principal milestones reached:

A. Principal milestones achieved in research:

a) Identified and reported a key species of white grub (*Lepidiotamansueta*) from Majuli river island, Jorhat, Assam. *L. mansueta* has biennial life cycle which is first of its kind from North East India. The duration of egg, grub and pupal stages varied from 12-17, 635-671 and 28-35 days, respectively. Third instar grubs caused heavy damage to the crops and showed a prolonged developmental period ranging from 545 to 563 days. *L. mansueta* is a rarely observed species, because it spends its almost entire life cycle concealed under the ground except for a short period during which the adults come out of the ground for mating.
b) Extent of damage, bioecology, behaviour and integrated management approaches of this species have been studied in detail.
c) Adults of both sexes were observed not to feed on any plants in the field and hence this species has got the unique distinction of being reported as the “First record of Indian phytophagous scarab beetle with non-feeding adults” (worldwide rank: 2nd).

d) Unraveled the mechanism of survival of *L. mansueta* grubs in water logged conditions of Majuli river island. The stereo zoom and Scanning Electron Microscopy (SEM) of spiracular system of *L. mansueta* grubs indicated that they possessed peripneustic tracheal arrangement. It showed absence of a conventional spiracular opening, but presence of a convex and projecting central bulla in place of the opening and a sclerotized and smooth sieve plate with ultramicroscopic (<3 micron wide) aeropyles, which provided protection against entry of water into the tracheoles but allowing only gaseous exchange for respiration. This study was undertaken to investigate the survival of grubs of *L. mansueta* under flooded condition since Majuli, being a river island is inundated by flood every year during rainy season. This research finding received accolade at both national and international levels among the white grub entomologists and the long-standing concept that “White grubs are the pests of only dry land areas in India” has thus got redefined.

e) The key morphological character of 3rd instar grub of *L. mansueta* for field recognition of the stage was identified i.e., the presence of distichous palidium in raster, which will be of great help in identifying the most damaging stage at farmers’ level.

f) While studying the sexual dimorphism of *L. mansueta* based on pupal characters, it was observed that the pupae which had a prominent protrusion on the ventral surface towards the posterior of the abdominal segment were males and those with flat abdominal segments were females. This character became useful in segregating the sexes of the virgin adults required for pheromonal studies on *L. mansueta*.

g) Scanning electron microscopy (SEM) study of lamellate antennal lobes of both the sexes of *L. mansueta* adults exhibited a wide variation in respect of distribution of sensillae. The females showed seven types of chemoreceptors whereas the males exhibited only three types, which clearly indicated the role of pheromonal compounds in chemical communication between the two sexes.

h) Pheromonal compounds of *L. mansueta* were investigated in collaboration with National Institute of Agro Biological Sciences (NIAS), Japan and National Bureau of Agricultural Insect Resources (NBAIR), Bangalore. GCMS-EAD analysis revealed that both male and
female adult antennae showed significant responses in FID-GC peaks which clearly indicated the presence of a probable aggregation pheromone in males while female abdominal extract elicited good responses from the male antenna indicating the presence of a sex pheromone. Recently, the compounds responsible as female sex pheromone (4 compounds) and male aggregation pheromone (4 compounds) have been identified. Efforts are already on to synthesize the pheromones artificially and to further test their performance in field.

B. Principal milestones achieved in extension:

- Educated, trained and mobilized the farmers about all aspects of the white grub beetle, *L. mansueta* by using different tools of social engineering ahead of taking up the mass campaigning programme.
- Encouraged the farmers for group formation for community based participatory programme for the management of adult *L. mansueta* through light trap technology and scouting.
- Organized massive “Mass campaigning against *L. mansueta*” for the collection & destruction of *L.mansueta* beetles in Majuli, Jorhat by using the concept of Social Engineering/Farmers Participatory Approach.

vi. Impact of the work done in area on

-Technology dissemination and adoption: Majority of the farmers from group opined that mass campaigning could bring about reduction in by white grub infestation. They also mentioned that there were less emergence of beetles from soil and low population of grubs in both cultivated and non-cultivated fields in areas where the mass collection and destruction of adults by light traps and scouting were undertaken in the previous years.

-Productivity enhancement: The crop productivity had also increased in different crops, according to the respondents of group and nongroup members. In case of potato, 89.00 per cent of the respondents perceived that productivity was increased. Likewise, in case of sugarcane 87.00 per cent of the respondents mentioned that the productivity was increased after formation of groups and group based activities. Similarly, 77.00 and 62.00 per cent of the respondents perceived that *Colocasia* and green gram productivity respectively also increased.
Readopting the crops that were discontinued due to white grub infestation: It was observed that some of the respondents of group members again started cultivation of the crops that had been previously discontinued due to severe infestation of white grub.

Expansion of area under crop cultivation after reduction of pest population: Farmers who had the capacity to increase their area under cultivation had started to expand the crops due to reduction of white grub infestation but majority of the respondents could not be able to expand their area because of small land holdings or no scope for further expansion.

Income enhancement: Majority of the respondents of group informed that their farm income was increased after involving in group activity.

Nutritional improvement: Colocasia is a starch-rich, globular fleshy taproot of aroid family plants and its crunchy, underground root, known as corm, is one of the popular edible root vegetables among the tribal people of Majuli. Because of the heavy infestation by the white grubs, some tribal farmers had discarded Colocasia cultivation in Majuli. However, after mass campaigning, many farmers have again taken to Colocasia cultivation that had been previously discontinued. Like many other ethnic tribes, the Mising has magico-spiritual and religious traditional beliefs on growing this crop for their ethno medicinal claims to cure different diseases. From nutritional point of view, Colocasia has more calories than potatoes (100 g provides 112 calories), free from gluten, carry high-quality phyto-nutrition profile comprising of dietary fiber, and antioxidants in addition to moderate proportions of minerals, and vitamins. Together with slow digesting complex carbohydrates, moderate amounts of fiber in the food help gradual rise in blood sugar levels. Root has very good amounts of potassium, which is considered as an important component of cell and body fluids that help regulate heart rate and blood pressure. Taro leaves as well as yellow-fleshed roots have significant levels of phenolic flavonoid pigment antioxidants such as ß-carotenes, and cryptoxanthin along with vitamin and these compounds are required for maintaining healthy mucus membranes, skin and vision. Therefore, the readoption of Colocasia cultivation by the farmers has restored their nutritional security.
**-L. mansueta** beetles as human food and animal feed:

It is worth mentioning that some of the tribal people, mostly the “Mising” tribe of Majuli relished the cooked/fried adults of *L. mansueta* as protein rich food which opens up an avenue of further research on its nutritive/nutraceutical value. Since, the traditional method of preparation of the beetles was somewhat crude, attempts were made to float up a concept of **“Beetle Fry”** dish. In certain areas of Majuli, **“Roasted Beetles”** were also consumed by the tribal people. The beetles were also used in bulk quantities as feed for pig, dog and poultry. Besides, the farmers were also encouraged to explore the grubs of *L. mansueta* as bait for fishing purpose (Bhattacharyya et al., 2018)

**VII. Technology dissemination:**

The technology dissemination was sustainable because of the following reasons:

**a)** The Massive Mass campaigning against *L. mansueta* was carried out by sensitizing and mobilizing the farmers by following the concept of Social Engineering/Farmers Participatory Approach in Majuli river island of Assam. The farmers were facilitated to get organised into groups; oriented, guided and trained properly, so that excellent capacity building is acquired on this notorious pest, which could eventually make the group self-reliant to solved their problem by helping each other. Group based approach was implemented meticulously and successfully and it proved to be highly effective because different tools of social engineering were explored in such a way that farmers could realize the immediate outcomes like decrease in population of white grub, decrease in pest infestation/damage, increase in crop production and productivity, increase in farm income, better adoption of recommended practices for management of white grub and increase in the extent of people participation. Even, the famers who were not included in groups showed their eagerness to form groups for the task due to spreading effect of group approach in a passive way. This approach left a far-spreading impact among the farmers of the island, which proved to be the beauty of the project (Deka et al., 2018).

**b)** The mass collection and destruction of beetles were carried out during the evening hours (6-9 pm) during the months of heavy emergence of beetles i.e. April-May. Therefore, the farmers virtually did not lose any effective working hours/man-days.
c) Majuli river island is organic by default. Therefore, the farmers have shown preference to as well as adopted the technology because without applying insecticides this dreaded pest could be managed. Farmers were convinced and specially delighted when they could kill the gravid females before egg laying in their field.

d) Others stakeholders associated with this mass campaigning have also endorsed this technology because a non-chemical approach of management strategy which was primarily based on the concept of the beetle population regulation was successfully implemented. The people of Majuli river island who have been continuously active as crusaders for getting the “World Heritage Site” tag from UNESCO also backed this ecofriendly technology on the ground that pesticide application is completely banned in heritage site.

References:


********
Chapter-13

Biological Management of Nematode Pests of Agricultural Crops

Bhabesh Bhagawati
Professor, Department of Nematology,
Assam Agricultural University, Jorhat-785013, Assam

1. Introduction

Around 4100 species of PPN have been described (Decraemer and Hunt, 2006) and, collectively, they impose an important restriction on the delivery of global food security. The plant parasitic nematodes cause serious damage to agricultural crops. On a worldwide basis, the ten most important genera of plant parasitic nematodes were reported to be (i) RKNs (Meloidogyne spp.); (ii) cyst nematodes (Heterodera and Globoderaspp.); (iii) root lesion nematodes (Pratylenchusspp.); (iv) burrowing nematode (Radopholussimilis); (v) stem and bulb nematode (Ditylenchus dipsaci); (vi) pine wilt nematode (B. xylophilus); (vii) reniform nematode (R. reniformis); (viii) dagger nematode (Xiphinema index); (ix) false root-knot nematode (Nacobbus aberrans); and (x) rice white tip nematode (Aphelenchoidesbesseyi) (Jones et al., 2013). Timely management of phytonematodes is of utmost importance to protect the crops. Use of biopesticides/bioagents has gained more importance in recent years due to its advantages like maintenance of ecological balance, identification of indigenous/native isolates of bioagents that would be less expensive compared to chemical, problems of resurgence by the nematodes can be minimized, eco-friendly, help to achieve pollution free environment, reduces residues and health hazards, easy marketability and once established they remain effective over long periods especially for perennial crops. Biomanagement of nematodes is considered to encompass control that result from the action of soil microorganism, which is mediated through mechanism such as parasitism, predation, competition and antibiosis.
1. **Nematode pests of important agricultural crops in Assam:**

<table>
<thead>
<tr>
<th>Causal Nematode</th>
<th>Crop</th>
<th>Pathway</th>
</tr>
</thead>
</table>
| Root knot disease: *Meloidogyne incognita* | Vegetables: Tomato, Brinjal, Chilli, Potato etc
Pulses: Greengram, Blackgram, Lentil, Cowpea, Chickpea etc.
Fiber crops: Jute
Horticultural crops: Citrus, Mango, Gladiolus, Carnation etc | Soil or Plant Parts Corms, Rhizomes, Tubers roots Rootstock, swept Plant debris |
| Citrus nematode: *Tylenchulus semipenetrans* | Citrus                         | Soil, Roots, Nursery Stock, Rootstock             |
| Rice root-knot nematode: *Meloidogyne graminicola* | Rice                          | Seedlings root & Nursery Stock                    |
| Rice root nematode: *Hirschmanniella oryzae* | Rice                          | Seedlings root and Nursery Stock                  |
| Ufra Disease: *Ditylenchus angustus.*      | Rice                          | Stubbles/left over plant parts after harvest      |
| Lesion nematode: *Pratylenchus penetrans*  | Coffee                        | Soil, Roots                                       |

2. **Biological control of plant parasitic nematodes:**

The term "biological control" in the classical sense is defined by De Bach (1964) as "the action of parasites, predators or pathogens in maintaining another organism's population density at a lower average than would occur in their absence." Commonly more than one microorganism occurs with plant parasitic or saprozoic nematodes in a particular rhizosphere. Constant association of these organisms in a given ecological niche undoubtedly has a greater impact on the establishment of such nematodes than would be caused by each microorganism alone. Such association results in a biological balance that may manifest itself in the form of direct parasitism by attachment and penetration by one or more pathogenic microorganisms in the eggs, juveniles, or adult nematodes, causing death and possibly allowing subsequent invasion by many or selected saprophytic microrganisms. Egg masses, sedentary females, or cysts may be directly invaded by pathogenic or some opportunistic organisms that draw their nutrients from the mucilaginous compounds present in the invaded body or indirectly invaded by the action of toxic, diffusible metabolites produced by one or more organisms on various developmental stages of nematodes. These toxins often render nematodes (particularly eggs and juveniles) more...
vulnerable to infection or to the activities of organisms that are either nonvirulent, slightly pathogenic, or basically saprophytic in nature (Jatala, 1986).

3. **Biocontrol agents against plant parasitic nematodes:**

<table>
<thead>
<tr>
<th>Categories</th>
<th>Bioagents</th>
<th>Nematodes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fungi</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predacious fungi/Trapping fungi</td>
<td><em>Dactylellalobata</em>, <em>D. ellipsospora</em>, <em>Arthrobotrys oligospora</em>, <em>Dactylaria candida</em>, <em>D. bembicoides</em></td>
<td>Plant parasitic nematodes and other nemaotdes</td>
</tr>
<tr>
<td>Endozoic fungi</td>
<td><em>Harposporium anguillulae</em>, <em>Meriaconiospora</em>, <em>Nematocotonusleiosporus</em></td>
<td></td>
</tr>
<tr>
<td>Fungal parasites of eggs/cysts</td>
<td><em>Paecilomyces</em>, <em>Verticillium</em>, <em>Fusarium</em>, <em>Trichoderma</em>, <em>Cephalosporium</em></td>
<td><em>Root knot nematodes, cyst nematodes, lesion nematodes, Burrowing nematodes</em></td>
</tr>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obligate bacterial parasite</td>
<td><em>Pasteuriapenetrans</em></td>
<td><em>Root knot nematodes, cyst nematodes</em></td>
</tr>
<tr>
<td>Toxin producing bacteria</td>
<td><em>Agrobacterium radiobacteri</em>, <em>Bacillus thuringensis</em>, <em>B. subtilis</em>, <em>Pseudomonas fluorescens</em>, <em>P. chitinoliticae</em></td>
<td><em>Root knot nematodes, cyst nematodes, lesion nematodes, Burrowing nematodes</em></td>
</tr>
<tr>
<td>Plant growth promoting rhizobacteria (PGPR)</td>
<td><em>Pseudomonasspp.</em>, <em>Rhizobacterspp.</em>, <em>Azotobacterspp.</em></td>
<td><em>Root knot nematodes, cyst nematodes</em></td>
</tr>
<tr>
<td><strong>Mycorrhiza</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Arbuscular mycorrhizae</em></td>
<td><em>Glomus fasciculatum</em>, <em>G. mosseae</em>, <em>G. epigaes</em>, <em>Aucalosporalaevis</em></td>
<td><em>Root knot nematodes, cyst nematodes, lesion and Burrowing nematodes</em></td>
</tr>
</tbody>
</table>

4. **The advantages and limitations of potential biological control agents with different modes of action against plant-parasitic nematodes**

<table>
<thead>
<tr>
<th>Type of agent</th>
<th>Mode of action</th>
<th>Advantages / limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trapping fungi</td>
<td>Traps produced on modified mycelium give rise to infective trophic hyphae</td>
<td><em>Advantages</em>: easily produced <em>in vitro</em>; some species rhizosphere competent; wide host range. Limitations: difficult to ensure trapping activity coincides with activity of infective stages of cyst and root-knot nematodes; often do not produce resting structures and so may be difficult to formulate.</td>
</tr>
<tr>
<td>Purpuriocilliium lilacinum</td>
<td>Hyphal penetration</td>
<td><em>Advantages</em>: easily produced <em>in vitro</em>; rhizosphere competent; attacks the eggs of several nematode species; treatment of planting material (e.g. seed tubers) can be effective. <em>Limitations</em>: requires high soil temperatures; has given variable control in</td>
</tr>
<tr>
<td>Organism Type</td>
<td>Biocontrol Method</td>
<td>Advantages</td>
</tr>
<tr>
<td>---------------</td>
<td>------------------</td>
<td>------------</td>
</tr>
<tr>
<td><em>Pochonia chlamydosporium</em></td>
<td><em>Hyphal penetration</em></td>
<td>Easily produced in vitro; some isolates rhizosphere competent, and virulent; resistant resting spores produced; survives throughout growing season in soil.</td>
</tr>
<tr>
<td>Obligate parasites <em>Pasteuria</em> spp.</td>
<td>Adhesive spores</td>
<td>Most isolates highly virulent; infective spores resistant to drying; good shelf-life; reduce infectivity of nematodes as well as fecundity.</td>
</tr>
<tr>
<td><em>Trichoderma</em> spp.</td>
<td><em>Hyphal penetration</em></td>
<td>Relatively easy to culture in vitro; attack infective nematodes in soil.</td>
</tr>
<tr>
<td>Rhizosphere bacteria</td>
<td>Toxins or modification of root exudates</td>
<td>Easy to culture in vitro; can be applied as seed treatments; reduce plant damage.</td>
</tr>
<tr>
<td>Endophytic fungi (non-pathogenic root-infecting fungi and mycorrhizae)</td>
<td>Competition in roots and modification of root exudates</td>
<td>Include agents with potential to control migratory endoparasitic nematodes in roots; may improve plant growth even in absence of nematodes; reduce damage caused by wide range of nematodes and limit their multiplication; can be mass produced and formulated; could be applied to seeds or transplant material; may reduce fungal root rots.</td>
</tr>
</tbody>
</table>

*Sources:* Kerry, 1987; Stirling, 1991; Sikora, 1992.

5. **Methods of delivery of bio control agents for nematode management**

1. **Soil bed application:** Biocontrol agents are multiplied on suitable substrates and incorporated into beds at the time of sowing.

2. **Soil drench:** The biocontrol agents like *P. fluorescens* and *P. lilacinum* are applied at the time of planting as soil drench for the management of root knot nematode, *M. incognita* and burrowing nematodes, *Radopholus similis.*
3. **Nursery bed treatment:** Application of *P. fluorescens* as nursery bed treatment has been found to be effective against *M. graminicola*.

4. **Seed treatment/priming:** Seeds are mixed with an organic carrier and then moisture content is brought to a level just below that required for seed treatment which has been used to deliver *T. harzianum* for the control *M. incognita*.

6. **Some of the recommendations made for biomanagement of plant parasitic nematodes in Assam**

   I. Application of *Pseudomonas fluorescens* @ 20 g/m² at sowing for the management of rice root-knot nematode, *Meloidogyne graminicola* in rice (Direct seeded)

   II. Seed treatment with NSKP @ 5 g/kg seed + *T. viride* @ 5 g/kg for the management of root-knot nematode, *Meloidogyne incognita* in mung

   III. Application of Carbofuran @ 1 kg a.i./ha + Bioforpf @ 2.50 kg/ha (with vermicompost at 1:10) one day before sowing of seeds for the management of root-knot nematode, *Meloidogyne incognita* and *Ralstonia solanacearum* in jute.

7. **Future prospectus of successful biomanagement:**

   1. Interaction with other microflora, fauna and pathogen
   2. Isolation and identification of indigenous population
   3. Adaptability to local environment
   4. Mass multiplication on commercial scale
   5. Demonstration on the efficacy of bioagents/biocomponents to the farming community.
   6. The recommended technology should be feasible and be a part of package of practices with easy availability and acceptability by the farming community
   7. Publication of extension bulletines/popular article/mass media with long-term advantages in relation to the use of chemicals.
   8. Co-ordination of scientist, organization and commercial agencies to create impact on identification, development and promotion of bio-friendly agents in the management of nematodes and other pathogens in order to protect the environmental pollution.
References:


*********
Chapter-14

Use of Bio-Control Agents in Seed Invigoration for Organic Agriculture

Sharmila Dutta Deka
Principal Scientist, National Seed Project
Assam Agricultural University, Jorhat-785013, Assam

Organic agriculture is a specialized form of diversified agriculture that aims at reducing ecological threat by agrochemicals and produce nutritious quality food. Growing awareness of health and environmental issues associated with the intensive use of chemical inputs has led to interest in alternate forms of agriculture in the world. Organic agriculture is a holistic production management system which promotes and enhances agro-ecosystem, including biodiversity, biological cycles and soil biological activity. Organic farming is gaining gradual momentum across the world; which is now practiced in more than 130 countries. The organic chain is incomplete without organic propagation material; this led to emergence of new division in seed industry: Organic seed. Organic seed is a planting material produced by a crop that is planted and raised organically for at least one generation in the case of annual crops, and two generation in the case of biennial and perennial crops (Lammerts2002). The criteria for production of organic seeds are; initially the mother seed is obtained by purchasing conventional un-treated seeds, which are grown organically for one season and then sold on to organic farmers as organic seeds. The organic seed also mean a cultivar that bred to perform optimally in organic systems. Organic seed production and distribution in European countries is presently a multibillion dollar business and at a great momentum with creation of organic seed producers and distributors database for European Union (EU). Presently organic seed production in India is mainly done by private company which is highly valuable in global market. Comprehensive nutrient management must be followed which includes, organic manures, vermin-compost, bio-fertilizers, biological control agents, bio plant growth promoters were used to meet nutrient requirement of crop. Livestock keeping and dairying in particular have helped number of small and marginal farmers to improve their income; field survey revealed that marginal and small farmers have helped to raise farm profitability as well as availability of cattle dung in sufficient amounts. A large number of fast growing nitrogen fixing crops like daincha (Sesbaniaspp.), sunhemp and cowpea may be used as green manure that can fix nitrogen to the extent of 60—100 kg/ha. Bio fertilizers are very
important components in organic agriculture which convert and fix unavailable source of elemental N, bound phosphate and decomposed plant residues into available forms. The legume—*Rhizobium* association can fix nitrogen ranging from 40—120 kg/ha under optimum conditions. In general, application of 10 t/ha of any locally available organic source like compost, FYM, biogas slurry, press mud, carpet waste, vermin-compost coupled with inoculation of bio-fertilizers like *rhizobium, azatobacter, azospirillum* and green manuring alternate years can take care of the nutrient requirement for most of the crops.

**Organic Seed Production**

Seed production packages for organic seed deviates totally from conventional seed production practices involving usage of agrochemicals. The major inputs which can be used for organic seed production: Organic manures-FYM, sheep manure, crop residues, poultry manure, oil cakes and other farm wastes, compost-coir pith compost. Green manure—sunhemp, daincha, legumes are used for green manures. Biologically degradable and decomposable organic wastes are used as earthworm feed. Bio-fertilizers—natural fertilizers containing carrier based microorganism viz., *rhizobium, azatobacter, azospirillum, BGA, azolla, micorrhizae and phospho-bacteria*. Biological agents for controlling insects—Protozoa are available as biological agents controlling the population of insects. The natural enemies like spiders, insects, mites, nematodes, birds, fungi, bacteria, and virus may be the biological agents.

**Challenges of organic seed production**

Seed quality is issue of high importance. Organic crop production may demand even higher quality propagation material compared to conventional farming. Farmers should rely on the quality of the seeds they are using. Preventing measures using chemicals are prohibited and competition with weeds requires high vigor planting material. Moreover, for organic farmers, seed health and the absence of genetically modified contamination is also very important. Strong organic plant breeding programmes is essential for development of varieties in various crops which must suit local conditions. Use of organics like FYM, bio-fertilizers, bio-control agents is essential for meeting nutrient requirement of crop and disease and pest management which is critical. Further, an appropriate seed certification standard is needed for organic seed compared conventional seed crop. Problem is also with absence of quality standards along disease and pest
management, and weed control. Early seedling vigour is of utmost important area that’s needs proper investigation and research.

**Seed invigouration**

Seed invigouration is a pre sowing and post harvest seed treatments which improves germination and seedling growth or help in providing the seed with other agro-chemicals or nutrients required at the time of sowing and at plant growth. There are many different kinds of seed invigouration techniques using different chemical, hormones and more recently many biological agents. Seed priming is widely used in the cultivation of plants to improve germination efficiency and field emergence under adverse environmental conditions (Jisha et al., 2013). Seed osmo-priming is a pre-sowing treatment that exposes seeds to a low external water potential that allows partial hydration but prevents radical protrusion through the seed coat (Bradford, 1986). Osmo-priming with polyethylene glycol (PEG) has been described as a good technique for improving seed germination of different. Several reports have demonstrated priming-improved germination performance, but the underlying mechanisms of priming-mediated stress tolerance are still poorly understood. It has been suggested that stress tolerance acquired by osmo-priming treatment may be associated with the accumulation of dehydrins (DHNs) and a more robust antioxidant system in relation to activation of pre-germinative metabolism might imprint in seeds a sort of ‘stress memory’ or ‘priming memory’ (Chen and Arora, 2011, 2013). The seedlings emerging from primed seeds showed early and uniform germination. Moreover, the overall growth of plants is enhanced due to the seed-priming treatments. The main objective of this review is to provide an overview of various crops in which seed priming is practiced and about various seed-priming methods and its effects.

**Organic Seed invigouration /treatments**

Soil microbes since their discovery in late 18th century have been used extensively in crop production. Kloepper and Schroth (1978) first used the term plant growth promoting *rhizobacteria*. Functions and mechanisms of growth promotion by these microbes have been discussed and micro-organisms have been categorised in different classes, Hayat *et al.*, (2010). Several studies documented beneficial effects certain microbial strains with improving growth and development of plants. The advent of seed priming a lot of work has been done on seed
treatment and is now common to obtain uniform crop stand. Among different priming techniques hydration using any biological compound it termed as bio priming. Seed priming creates ideal condition for bacterial inoculation and colonization in seed McQuilken et al., (1998). Research shows that plants derived from primed seeds show a faster initial growth and an earlier ground cover (Groot et al 2005). Seed priming with organic formulations like neem seed kernel extracts, garlic extracts, vermin wash, compost, ultimately enhances germination, seed and seedling vigor and ultimately uniform seedling emergence and plant stand by altering physiological state of seed.

**Seed treatments for Pest and disease Management**

Pest management strategies inorganic seed production is highly important for production of disease free seeds. Biological pest control is an important component of integrated pest management strategy. It assumes importance in sustainable agriculture and organic farming and production with reduced cost without chemical residues. It include agents such as *Trichogramma* spp., *Bracon* spp., *Chrysoperla* spp. and bio-pesticides such as Nuclear Polyhedrosis Virus (NPV) for control of important pest like *Helicoverpa armigera* and *Bacillus thurengensis, Trichoderma harzianum* etc care used. Use of Botanical Products for Pest Control—Plant products such as neem seed kernel extract, neem or margosa, pongamia powder and cakes which have been shown to be effective. Some of the organics used for management of seed borne pathogen were neem seed kernel extract, garlic extracts, vermin wash, compost T, for eg., late blight of potato and tomato caused by *Phytophthora infestans* effectively controlled by seed treatment with horse compost extract (Weltziien 1991). The oils of cassia and clove inhibited growth of established seed borne infection of *Aspergillus flavus, Curvularia pallescens* and *Chaetomium indicum* in maize (Chatterjee 1990). Aqueous extracts of *Strychnos nux-vomica*, ginger rhizomes, basil leaves and fruits of neem were used to control *Alternaria padwickii* in rice seeds and beyond these physical treatments like water treatments, solar treatments, hot air treatments can be effectively used for the management of seed pathogens.
Conclusion

Bio-priming plays a significant role in organic crop production. Organic seed and crop production have various limitations besides its low productivity and challenges of field establishment under direct-seeded condition. However, the limitations are compensated with higher market price along with various health benefits. There is an emerging need for development of techniques for better crop establishment and crop protection in organic crop and seed production.

References


********
Chapter-15

Protection of Biopesticides Under the IPR regime: An Overview

Gargi Sharma
Assistant Professor, Dept. of Plant Breeding and Genetics and Member-Secretary, IPR cell, Assam Agricultural University, Jorhat-785013
E-mail: gargi@aau.ac.in

Introduction:

Biopesticides are the natural alternative sources of pesticides. In recent decades, biopesticides have been promoted as prospective alternatives to synthetic pesticides in the pest management system. With the increase in concern about the hazards caused by synthetic chemical pesticides, the demand for biopesticides and similar products to control pests, diseases and weeds are also increasing to a substantial extent. The expansion of organic farming further raises the demand of biopesticides across the world. The global market of biopesticides is projected to reach USD 8.82 billion by 2022. Unfortunately, production and marketing of spurious biopesticides pose a serious threat for the high quality biopesticides. Therefore, to encourage the research and development of biopesticides, Intellectual Property (IP) protection becomes very important especially for a country like India which holds promising opportunities for biopesticides production and marketing. Intellectual Property when efficiently used becomes an important tool in positioning the business in the market. IP rights combined with other marketing tools are crucial for differentiation, promotion, diversification and marketing of biopesticides. In this era of knowledge based economy, protection of intellectual properties of biopesticides is important for innovators as well as marketers. There are many categories of IPRs viz., patent, copyright, trade mark, industrial design, trade secret, geographical indication, lay of integrated circuits etc. Some of the relevant IPRs associated with biopesticides are discussed below:

Biopesticides and patents in India:

TRIPS agreement in 1995, initiated the process to strengthen patents and other forms of IPRs among all member nations of the World Trade Organization. To comply with the provisions of TRIPS, India enacted the Patent Act in 1970, to grant patents “not merely to enable patentees to
enjoy a monopoly on a patented article” but “to secure that the inventions are worked in India on a commercial scale and to the fullest extent that is reasonably practicable without undue delay”. This cast a duty on the patentee to make efforts for commercial working of the patented invention at the earliest.

The concept of patenting “life forms and living matters” have always been a challenging issue in India. Since, one of the major constituents of biopesticides is the microorganisms and as such patent protection of biopesticides is also a complex one. The chief patentability criteria for an invention are novelty, inventiveness and industrial applications. There have always been controversies as to whether substances isolated or derived from naturally occurring living organisms are "inventions" or "discoveries”. A product of nature is not considered as a patentable subject matter because it is indistinguishable from something that occurs in nature and as such it lacks novelty.

The history of the product of nature doctrine dates back to 1889, when Ex Parte Latimer, the Commissioner of Patents rejected a claim on a new article of manufacture, consisting of the cellular tissues of the Southern pine (*Pinus australis*). The inventor was asked to identify of the claimed substance from its natural counterpart as the claim and description did not set forth any physical characteristics by which the fibre could be distinguished from other vegetable fibres. Afterward, as the fibre claimed could not be distinguished from other fibres by any physical characteristic, the claim, therefore, was refused. It was concluded that a product whose physical characteristics are indistinguishable from those of its naturally occurring counterpart does not constitute patentable subject matter because it lacks novelty.

Again, Section 3 (c) of the Patent Act, 1970 provides that the mere discovery of a scientific principle or the formulation of an abstract theory or discovery of any living thing or non-living substances occurring in nature is not patentable. Section 3(j) of the Act excludes from patentability “plants and animals in whole or any part thereof other than micro-organisms but including seeds, varieties and species and essentially biological processes for production or propagation of plants and animals”. Furthermore, as per the article 27(3)(b) of TRIPS Agreement member states should not grant patents for “plants and animals, other than microorganisms, and essentially biological processes for the production of plants or animals other than non-biological
and microbiological processes.” It is obligatory for all its signatories to extend patents for microorganisms, non-biological, and microbiological processes. India in compliance with TRIPS amended the Patents Act in June 2002, by giving patent rights for new microorganisms. In 2002, amendment of Indian Patent Act added explanation to chemical process which states; chemical processes include biochemical, biotechnological and microbiological process. In our county other patentable areas involving microorganisms are synergistic composition containing the microorganism, which is either new or known, a process using microorganisms to produce a substance, the process of biosynthesis of a new microorganism etc. Any invention resulting from human intervention, where living things have been used initially for conducting experimentation will be patentable in India.

It is pertinent to mention herewith that in the year 2002, the Calcutta High Court delivered a landmark judgment in Dimminaco AG vs Controller of Patent and Designs and others on an issue whether a process involving microorganisms that are living as an end product can be patented or not. Dimminaco AG filed a patent application for the process of creating a vaccine to protect poultry from infectious bursitis. The Controller of Patents determined that the process was not patentable because the end product contained a living organism. Thereafter the applicant appealed the Controller’s decision to the Calcutta High Court. The Controller opined that a patent is given only for a process that results either in an article, substance, or manufacture and a vaccine with a living organism is not to an article, substance or manufacture. As the meaning of manufacture was not defined in the Patents Act, therefore, the court used the normal dictionary meaning of manufacture as “the material in question after going through the process of manufacture has undergone any change by the inventive process and it becomes a material which is different from the starting material.” The Court determined that this meaning does not exclude the process of preparing a product that contains a living substance from patentability. The court decided that “since the claim process for patent leads to a vendible product, it is certainly a substance after going through the process of manufacture.” The court ultimately concluded that “a new and useful art or process is an invention,” and because the process is new and useful, it “is apparently patentable under section 5 read with section 2(j)(i)” of the Indian Patent Act. The court determined that “where the end product is a new article, the process
leading to its manufacture is an invention.” However, later on the definition of invention was amended. The new definition merely calls for a new, non-obvious and useful product or process.

Another landmark judgment was passed the US Supreme Court on patentability of microorganism. Ananda Mohan Chakrabarty, a genetic engineer by profession developed a bacterium *Pseudomonas putida* capable of breaking down crude oil, which he proposed to use in treating oil spills. There were three parts to the patent viz., method of producing the bacteria, composition of a slurry of the bacteria and the bacteria themselves. The United States Patent and Trademark Office allowed the first two claim but rejected the third one because under patent law at that time, living things were generally understood not to be patentable subject matter under section 101 of title 35 U. S. C. Chakrabarty appealed before the US Supreme Court stating that the genetic engineering he did on the bacteria was a form of manufacture, therefore, it met the standard. Finally the US Supreme Court ruled in favour of Chakrabarty, holding that: “A live, human-made microorganism is patentable subject matter under 35 U. S. C. 101. Respondent’s microorganism constitutes a “manufacture” or “composition of matter” within the statute.” Basically “products of nature” are not patentable because the discoverer isn’t really an “inventor” they did not do anything themselves, they just found something that pre existed. However, that argument does not apply to Chakrabarty because he did not work to create the new bacteria.

**Microorganism and Budapest Treaty:**

Budapest Treaty, is an international treaty signed in Budapest, Hungary, on April 28, 1977. Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure entered into force on August 9, 1980, and is administered by the World Intellectual Property Organization (WIPO). An important aspect of the grants of patent in respect of microorganisms is the regulations concerning the requirements for the deposition of microorganisms and accessibility of that microorganism from the depositories as par the provisions of Budapest Treaty in which India is also a member. The microorganism if not being described fully and particularly and is not available to public, the said microorganism is to be deposited before the International Depository Authority under the Budapest Treaty within 3 months of making application in India. All details, available characteristics of the
microorganisms and details of depositary institutions shall be mentioned in specification for correctly identifying the same. Furthermore access to the same is required to be made available only after date of application in India or date of priority. For the purpose of microorganisms and other biological materials Microbial Type Culture Collection and Gene Bank (MTCC) is an internationally recognized depository institution. In India, two microbial repositories viz. Microbial Type Culture Collection (MTCC) and Microbial Culture Collection (MCC) have acquired the status of IDA.

**Trademarks: a marketing tool of biopesticides:**

In India, Trade Marks Act, 1999 and the rules there under are used for registration of trade mark, protection of trademarks for goods and services and to prevent fraudulent use of the mark. Intellectual property more specifically trademark is of primary importance in the marketing process. Biopesticides products also face competition with the products that are often similar, identical and good substitute. To distinguish a particular product from those of its competitors and to maintain the goodwill and trust of the consumers, trademark plays a vital role. Trademark helps to create a brand name in the market and many times consumers are willing to pay even higher price for these branded products. However, use of trademark for effective marketing of products needs expertise in the field of trademark law and practice.

In 2018, Delhi High Court gave verdict on a case filed by Insecticides (India) Limited (IIL) against Parijat Industries (India) Pvt. Ltd (Parijat), where the IIL alleged passing off the marks VICTOR and VICTOR formative marks in respect of insecticides by Parijat and sought restraint order against Parijat from using the mark VICTOR 80 in their products. Finally the Court decreed the suit permanently restraining Parijat from using the mark 'VICTOR 80' or any other mark deceptively similar to IIL’s mark 'VICTOR'.

**Trade Secret and biopesticides formulations:**

A trade secret can be defined as a practice, process, design, instrument or a compilation of data or information relating to the business which is not generally known to the public and which the owner reasonably attempts to keep secret and confidential. Article 39 of agreement on
TRIPS provides protection of undisclosed information, sometimes also referred to as trade secrets.

There are numerous cases across the world where the inert ingredients of pesticides were strongly kept as secrets and pesticide manufacturers claimed that they were trade secrets and it was almost impossible for the users to find out about them.

Trade secrets can be a valuable asset to a company’s growth. Considering the multiple objectives of biopesticide formulations, wide range of materials used in the formulations, formulation processes, microbial formulations are often held by many companies as trade secrets and confidential know how (Behle and Birthisel, 2014). Although, there is specific legislation in India and is enforced contractually or under common law. Indian courts uphold trade secret protection on the basis of principles of equity, breach of confidence, breach of contractual obligations etc.

Conclusion:

Biopesticides have been studied, researched, promoted and marketed as replacement of synthetic pesticides in the recent years. This lead to development of big business in the world of biopesticides. The rich biodiversity of India coupled with its traditional knowledge provide tremendous scope for development of a wide range of newer and effective biopesticides in the future days.Although the prime objective of biopesticides research is to make these biopesticide products available at farmers’ level to be used in the integrated pest management strategies, but commercialization is the final and most difficult step in the development of a microbial product. The protection of biopesticides starting from the developmental stage to the final stage of commercialization of the product under the IPR regime will not only give legal protection and boost to the scientific community but put the business of biopesticides in a safe and respectable position too in the days to come.

References:


Dimminaco AG vs Controller of Patent and Designs and others, [2001] AID No.1


The Patents Act, 1970 as amended by The Patents (Amendment) Act, 2005 along with the Patents Rules, 2003 as amended by the Patent (Amendment) rules, 2006

The Trademarks Act, 1999.


********

********
Chapter-16

Exploitation of VAM in the Management of Plant Diseases Caused by Pathogenic Nematode and its Mass Multiplication

A. Borah and B. Mahanta, Professor, Department of Nematology, Assam Agricultural University, Jorhat-785013, Assam

Vesicular Arbuscular Mycorrhiza plays an important role in nematode management. VAM fungi are important for their potential application as biofertilizer in commercial agriculture, horticulture and forestry. VAM fungi produce characteristic structures vesicles and arbuscules inside the cortical tissue of the host plant. These two structures are used to distinguish VAM fungi from other fungi present in the rhizosphere. They are obligately depend on host plant. VAM fungi and plant parasitic nematode are commonly found inhabiting the rhizosphere and colonizing the roots of their host plants. They may limit the nematode activity and improve plant growth on a wide range of host plant (Hussey and Roncadori, 1982).

The word mycorrhiza is derived from two Greek words- (Mykes= fungus, Rhiza= root). So it is called fungus root. Franc (1885) noted existence of such mutualistic symbiotic association between fungal hyphae and roots of plants. On the basis of hyphal structures formed in the roots of host plants, the mycorrhizae have been classified into two major groups viz Ectomycorrhiza or ectotrophicmycorrhiza and Endomycorrhiza or endotrophicmycorrhiza. Ectomycorrhizae are common on many forest trees belonging to families pinaceae, fagaceae and betulaceae. Endotrophic mycorrhizae are most ubiquitous soil fungi are popularly called vesicular arbuscular mycorrhhizae (VAM). VAM fungi, the fungal hyphae develop some special organs called vesicles and arbuscles on cortical cells. Vesicles are thin or thick walled, spherical to oval in shape, borne at the tip of the hyphae growing intercellularly in the cortical region to serve as food storage organ as well as reproductive structure of the fungus. Arbuscules are dichotomously branched hyphae much like haustoria which grow intercellularly in the cortical cell. Arbuscules are considered to be as major site for bidirectional transfer of nutrients between the two symbionts. According to Morton and Benney (1990) have kept VAM fungi in two orders – Endogonales and Glomales.
Of the total 150 species of VAM fungi known in the world, 102 have been reported from India i.e from Delhi, Tamil Nadu and Andhra Pradesh (Bagyaraj and Padmavati Ravindra, 1995; Raghupathy and Mahadevan, 1993). These VAM fungi in India are represented by 60 species of *Glomus*, ten species of *Gigaspora*, fourteen species of *Acaulospora*, twelve species of *Scutellospora*, three species of *Entrophosphora*, two species of Endogone and one species of *Sclerocystis*. The numbers of *Glomus* are widespread in their occurrence and distribution in mostly cultivated soil. Under natural conditions, about 80% of plant species are mycorrhizal (Vierheiling et al. 1995).

**Beneficial effects of VAM in nematode management**

- VAM helps absorption of N, P, K, Ca, Mg, S, Zn and Cu. VAM mobilizes increase uptake of P in P deficient soil saving phosphatic fertilizer up to 25-30%.

- Extensive network of external hyphae increase absorptive surface area of the root system for nutrient (Hussey and Roncadori, 1982) and water transport (Safiret et al. 1971).

- Helps the plants to withstand high temperature.
- Decreases transplant injuries of seedling.
- Better equipped to withstand stress like drought, heavy metal pollution in the industrial sectors and toxicity of Al, Mn or salt in soil.
- Helps in improvement of soil structure and stabilization of organic status of soil.
- Promotes growth of nitrogen fixing bacteria, improvement in root nodulation and nitrogen fixation in legumes in presence of VAM twin symbiotic association with a legume plant from *Rhizobium* as well as VAM. VAM hold promise in integrated nutrient management system (Goswami and Mishra, 1980).
- Promotes production of plant hormones.
- Plays major role in reducing the severity of disease complex (Devi and Goswami, 1992).
- Increases host tolerance or resistance suppressing establishment and development of soil borne pathogens in plants.

VAM plays key role to healthy plant on the earth and sustainable system of agriculture and forest production.

**Interaction of VAM with plant parasitic nematode**

Plant parasitic nematode and VAM fungi often occur together in the same root of the plant, having a characteristic opposite effect on plant vigor. VAM fungi stimulate plant growth acting as symbiont but plant parasitic nematodes suppress plant growth acting as pathogen. Antagonistic behavior exhibited between the two organisms has been reported on various observations on nematode and VAM fungi in different crop lands while going through several reviews of work done in greenhouse, micro plot and field conditions.

Baltruschat et al. (1973) first showed that tobacco plants preinoculated with VAM (*Glomus mosseae*) were less susceptible to root knot infection. They reported 75% reduction in the number of *Meloidogyne incognita* larvae that develop into adult on tobacco plants. Preinoculated with *G. mosseae* compared with uninoculated control. Saleh and Sikora (1974) observed that number of eggs of *M. incognita* were reduced at 55% or greater colonization by *G. fasciculatum*. 
Schonbeek (1975) observed that *G. mosseae* did not penetrate giant cells produced by *M. incognita* on tomato but presence of VAM in the root retards giant cell formation. The giant cells produced on mycorrhizal tomato plants are smaller, fewer in number and contain less nuclei than giant cells of same age produced on non mycorrhizal plants. Hussey and Roncadori (1978) reported that cotton roots colonized by *Gigasopra margarita* were found with significantly less *Pratylenchus brachyurus* population per gram root than non mycorrhizal plants. Lower nematode reproduction was caused either by the symbionts altering the cortex to make it an unfavorable food source for the nematode or by the symbionts competing with the nematode for space in the cortex. O’ Bannon *et al.* (1979) observed that *Tylenchulus semipenetrans* was less severe on rough lemon in the presence of *G. mosseae*, seedlings inoculated with *G. mosseae* had significantly more growth than seedling inoculated with first generation cyst nematodes (*Heterodera glycine*) were significantly fewer on soybeans colonized by an isolate of *G. fasciculatum*. Kellam and Schenck (1980) reported that VAM decrease giant cell development. Smith *et al.* (1986) observed 50% colonization significantly inhibited nematode development. Jain and Sethi (1988) reported that *G. fasciculatum* decrease cyst production and reproduction of *H. cajani*. Umesh *et al.* (1988) observed that number of *Radopholus similis* in banana roots were significantly lower when VAM (*G. fasciculatum*) was applied simultaneously or seven days prior to nematode inoculation. Increased phenol, lignin, total amino acids, and total sugar and higher N, P, K, Ca and Mg in mycorrhizal inoculated banana roots were associated with reduced reproduction of *R. similis*. Siva Prasad *et al.* (1990) observed that there was remarkable reduction in root knot index and nematode count in the root tissue and rhizosphere soil of pepper plants pre-inoculated with VAM fungi. They also observed that plants inoculated with VAM fungi were taller, more shoot and root weights and phosphorus content. In a micro plot study, Tylka *et al.* (1991) reported that number of soybean cyst nematode *H. glycine* in roots and soil were decreased by VAM (*G. fasciculatum*) up to 73% at the highest inoculum level. Sharma *et al.* (1994) observed that VAM colonization reduced the root-knot nematode infestation in tomato. Jain and Hassan (1994) reported that VAM may be useful as a biofertilizer for its ability to increase host nutrition, particularly phosphorus. Chahal and Chahal (1995) observed that VAM enhance growth characters of pea and N, P content of shoot. Mycorrhizal roots showed fewer galls. Pre and Simultaneous inoculation of *G. fasciculatum* in cotton reduced *Rotylenchulus reniformis* juvenile penetration, egg mass production and population build up on
mycorrhiza plants than control. Sundarababu et al. (1998) observed that prior inoculation of *G. fasciculatum* acted as a barrier for nematode entry, thus successfully managing population of *P. zeae* in maize.

**Role of AM fungi on biological control**

Evidences indicate that plants previously inoculated with AM fungi exhibit increased resistance to fungal root diseases like root rots. Significantly reduced the severity and incidence of *Fusarium* root rot of infected bean plants. Mycorrhizal colonization also significantly increased the tested growth parameters and mineral nutrient concentration, phenolic content and the activities of the investigated defense related enzymes (Phenylalanine ammonia-lyase, Polyphenol oxidase and peroxidase enzymes). They also observed application of AM fungi as a biocontrol agent played an important role in plant resistance and exhibit greater potential to protect bean plants against the infection of *Fusarium solani*. The severity of nematodes disease is generally reduced in mycorrhizal plants. Growth responses due to AM fungi may other disease resistance is shoot and leaf. Several mechanisms were proposed for the suppression of the pathogens as physical barrier, improved P nutrition of the host plant, stimulation of natural microbials, competition, induction of flavanoids, chitinan and proxidans and tissue lignifications. Studies indicated that mycorrhizal seedlings of tomato, when transplanted in neem cake-amended soil were infected by *Meloidogyne incognita*. An increase in plant growth and decrease in root-knot index and final population of *M. incognita* were observed in the treatments where mycorrhizal seedlings were transplanted in neem cake-amended soil. In addition, increases the colonization of *Glomus mosseae* on roots of tomato and enhanced chlamydospore densities *G. mosseae* in there treatments indicated favourable effects of neem cake amendment on the growth of *G. mosseae*.

**TABLE: Am Fungi and their effect on different Fungal diseases**

<table>
<thead>
<tr>
<th>AM fungi</th>
<th>Pathogenic fungus</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Glomus intraradices</em></td>
<td><em>Fusarium oxysporum</em> f. sp. radicis-lycopersici</td>
<td>AM fungus significantly reduced <em>Fusarium</em> root rot on tomato</td>
<td>Caron et al. (1985)</td>
</tr>
<tr>
<td><em>G. mosseae</em></td>
<td><em>F. oxysporum</em></td>
<td>Significantly reduced <em>Fusarium</em> wilt on tomato and pepper</td>
<td>Al-Momany and Al-Raddad(1985)</td>
</tr>
<tr>
<td>AM Fungus</td>
<td>Other Pathogen</td>
<td>Effect</td>
<td>Reference</td>
</tr>
<tr>
<td>-----------</td>
<td>----------------</td>
<td>--------</td>
<td>-----------</td>
</tr>
<tr>
<td><em>G. intraradices</em></td>
<td><em>Pythium ultimum</em></td>
<td>Reduced populations of <em>P. ultimum</em> on <em>Tagetes Patula</em></td>
<td>St-Arnaud <em>et al.</em> (1994)</td>
</tr>
<tr>
<td><em>G. mosseae</em></td>
<td><em>Verticillium dahliae</em></td>
<td>Inoculation of AM fungi reduced disease indices in cotton</td>
<td>Liu (1995)</td>
</tr>
<tr>
<td><em>G. fasciculatum</em></td>
<td><em>F. oxysporum</em></td>
<td>Reduced wilt indices in chickpea</td>
<td>Rao and Krishnappa (1995)</td>
</tr>
<tr>
<td><em>G. margarita</em></td>
<td><em>F. udum</em></td>
<td>Reduced wilt indices in pigeon pea</td>
<td>Siddiqui and Mahmood (1995c)</td>
</tr>
<tr>
<td><em>G. mosseae</em></td>
<td><em>Phytophthora nicotianaevar. parasitica</em></td>
<td>Reduced root necrosis, and necrotic root apices ranged between 63 and 89%</td>
<td>Trotta <em>et al.</em> (1996)</td>
</tr>
<tr>
<td><em>G. mosseae</em></td>
<td><em>F. solani</em></td>
<td>Significantly reduced disease severity in chickpea</td>
<td>Siddiqui and Mahmood (1997)</td>
</tr>
<tr>
<td><em>G. intraradices</em></td>
<td><em>Rhizoctonia solani</em></td>
<td>Defense response elicited by <em>R. solani</em> significantly suppressed by AM fungus in alfalfa</td>
<td>Guenoune et al. (2001)</td>
</tr>
<tr>
<td><em>G. mosseae</em></td>
<td><em>P. parasitica</em></td>
<td>Most effective in reducing disease symptoms produced by <em>P. parasitica</em> on tomato</td>
<td>Pozo <em>et al.</em> (2002)</td>
</tr>
<tr>
<td><em>G. etunicatum</em></td>
<td><em>R. solani</em></td>
<td>Significantly reduced disease severity in micropropagated banana</td>
<td>Yao <em>et al.</em> (2002)</td>
</tr>
<tr>
<td><em>G. mosseae</em></td>
<td><em>F. chlamydosporium</em></td>
<td>Reduced disease severity but best management was obtained when used with <em>Trichoderma viridae</em></td>
<td>Boby and Bagyaraj (2003)</td>
</tr>
<tr>
<td><em>G. mosseae</em></td>
<td><em>Sclerotinia sclerotiorum</em></td>
<td>Reduced the disease severity upto 10.3% on common bean</td>
<td>Aysan and Demir (2009)</td>
</tr>
<tr>
<td><em>G. mosseae</em></td>
<td><em>Alternaria triticina</em></td>
<td>Reduced percent infected leaf area on wheat</td>
<td>Siddiqui and Singh (2005)</td>
</tr>
<tr>
<td><em>G. etunicatum, G. mosseae, G. clarum, G. caledonium, G. fasciculatum, Gigaspora margarita</em></td>
<td><em>Sclerotium rolfsii</em></td>
<td>Inoculation of all the AM fungi reduced the disease severity (37.8–64.7%) in pot condition while in field condition it varied from 30.6 to 47.2%</td>
<td>Ozgonenet <em>et al.</em> (2010)</td>
</tr>
</tbody>
</table>
Mechanisms for the suppression of pathogen

- **Provide mechanical barrier**—Since AM fungi are soil borne and plant parasitic nematodes occupy root tissues, directed competition for space has been postulated as a mechanism of pathogen exhibition.

- **Confer resistance**—Induces alteration in host cells and makes them resistant to soil and root pathogens.

- **Enhance the uptake of nutrient by host**—Especially P, K, Ca, Cu, Mu, Zn uptake is increased and there is also increased in the uptake of sugar and amino acids like phenylalanine, serine. Indigenous AM fungi are more effective in increasing P uptake than the introduced fungi. Increase in activity of acid and alkaline phosphate is also observed in AM infected plants.

- **Increased root growth and function**—AM fungi, through an increase in phosphorus nutrition, enhance root growth and expand the absorptive capacity of the root system for nutrients and water.

- **Production of nematostatic compounds**—AM fungi may exhibit activities by altering the root physiology in way unrelated to P nutrition and enhanced root growth. These changes would alter chemotactic attraction of roots, affecting those species, which require hatching stimulus or directly retard nematode development within root tissues.

- **Competition for host photosynthesis**—These fungi almost totally depend on the soluble carbohydrates produced by the host for their carbon source. One of the mechanisms affecting nematode activities in a mycorrhizal system is the conversion of carbohydrates received form the host into farms that cannot be used by the nematodes.

**Mass production of AM fungi**

*In-vivo*: The production of commercial mycorrhizal inoculum has evolved considerably in recent years. There are various types of microbial cultures and inoculants available on the market today and these have rapidly increased because of the advances in technology.
Large scale AM fungal inoculum production is precluded due to their obligate biotrophic nature i.e they must grow in symbiosis with living host plant roots in order to complete their life cycle and to produce indefinite propagules. AM fungal inoculums is presently produced in a variety of ways utilizing in vitro, green house or field based methods.

The *in vitro* method comprises of monoxenic culture of sterilized AM fungal spores with Ri T-DNA transformed carrot roots.

Mass production of VAM fungi has been achieved with species such as *Acaulospora laevis*, *Glomus fasciculatum*, *G. etunicatum*, *G. intraradices*, *G. mosseae* *(Chandanie et al. 2006)*. *Gigaspora margarita* and *G. rosea* *(Schwartz et al. 2006)* but *G. intraradices* is the most common inoculum of endomycorrhizae product.

Another method of on-farm AM fungal inoculum production involves preparing raised soil beds (60x60x16 cm$^3$) *(Gaur 1997; Douds et al. 2000)*. After fumigation of the beds, the AM fungi form a starter inoculum; which were later inoculated in to the furrows in the rained beds.

**Method of isolation of VAM spores**

The VAM spores are isolated from soil by wet sieving and decanting technique *(Gerdmann and Nicolson, 1963)*. However, the technique was further modified for convenience. From the soil sample about 300 g soil has to be taken in a bucket of 2 litres capacity and water has to be added upto 2/3 volume of the bucket. Then it has to be shaked vigorously and allowed to stay overnight until the soil particles settled down. The clear spore suspension along with the spores adhering to the side of bucket has to be decanted through a series of sieves of different mesh size (50 µ, 100 µ, 250 µ and 500 µ). Coarse particles and organic debris have to be retained in 500 µ sieve, while the contents of the other mesh sizes (250, 100 and 50 µ) has to be thoroughly washed with jet of tap water and collected in a 250 mil beaker. The beaker is then allowed to stand for few minutes till the heavier and finer clay particles settle down, these has to be later removed through suction with the help of a saline tube. This method has advantage for rapid and quick suction and it creates minimum disturbances while removing the settled particles.
Procedure for VAM spore inoculation

Extracted spores in water has to be transferred to a beaker and number of spores has to be estimated by taking 1 ml of suspension with the help of pipette and placed it on a center of a counting dish. Five repeated readings to be taken and average has to be recorded. For mycorrhizal inoculation, inoculum containing counted number of chlaymadospores have to be placed within the rhizosphere of seedling.

Staining of roots and assessment of endomycorrhizal root colonization

Roots have to be stained by following essentially the procedure of Philps and Hayman (1970). The roots have to be gently washed in tap water. Fine feeder roots have to be selected and boiled for about 30 minutes in 10% KOH solution, followed by repeated washing in tap water. Then the roots have to be acidified with 2 % HCl. One set of roots have to be stained with Lacto-glycerine acid fuschin (Kormanik and McGraw, 1982) and another set with 0.05 % trypan blue in lactophenol (Phillips and Hayman, 1970). However, preference has to be given to the first method to avoid harmful effects of phenol. The roots have to be boiled in stains for 5 minutes, and destained either with lacto-glycerine or lactophenol by keeping overnight.

Assessment of mycorrhizal root colonization has to be made from root segments. Root segments of approximately one centimeter length (20-30 nos.) have to be randomly selected and mounted on clean glass slide and observed under microscope for mycorrhizal colonization (presence of hyphal threads, arbuscularis and vesicles). The endomycorrhizal root colonization is calculated using the following the formula (Giovenneti and Mosse, 1980):

\[
\text{Percent VAM colonization (MCP)} = \frac{\text{No. of VAM positive segments}}{\text{Total no. of segments assayed}} \times 100
\]
**In-vitro**

**Isolation**

Spores have to be isolated by wet sieving and decanting technique.

**Production in liquid media**

Isolated VAM fungal spores have to be pre-germinated. These spores have to be cultivated in Cooke Rose Bengal liquid medium with incubation at 24 ± 2°C in dark conditions for 24-48 hours. Liquid medium has to be harvested at regular intervals for investigating various fungal structures (Cooke, 1954; Singh et al., 2016).

**Production on solid (artificial) media**

Pre germinated spores have to be inoculated on Cooke Rose Bengal agar having formula Per litre, Soy Peptone -5.0 g, Dextrose -10.0 g, Monopotassium Phosphate, 1.0 g, Magnesium Sulfate 0.5 g, Agar, 20.0 g and Rose Bengal 35.0 mg, allowing incubation at 24 ± 2°C in dark conditions for 24-48 hours. Fungal biomass produced has to be studied at regular intervals. (Cooke, 1954; Singh et al., 2016).

**Cultivation on solid (natural) media**

Hydroponic system work on the principle that plants grow in a sterile, soil-less medium that allows delivery of nutrients to the roots directly from nutrient-enriched water solution. Hydroponic system provides nutrients to the plants in the form of thin layer on the roots which results in greater proliferation of roots, production of higher number of spores/cm of infected roots. It facilitates better aeration than soil (Sharma et al., 2000). Pregerminated seeds of Maize, Rice and Wheat seeds were used for hydroponics.

**Mechanism of disease suppression of plant parasitic nematode by vesicular arbuscular mycorrhizae**

- VAM mediated changes in root morphology and root exudates pattern may adversely affect the nematode attraction and penetration. (Sikora, 1981)
- Competition for space and available photosynthesis produced in the plant.
Physiological changes induced by VAM may also make the poorer food source for the nematodes by producing conditions that are inadequate for optimum development and reproduction.

Increased level of amino acids and sugar in mycorrhizal roots are associated with increased plant resistance.

Increased nutritional status of roots mainly phosphorus and other micro elements like N, K, Ca and Mg.

VAM stimulates production of higher concentration of phenylalanine and tyrosine reducing growth and reproduction of nematodes.

VAM colonization of roots mechanically preventing nematode penetration and establishment.

Hardening of root tissue due to increased lignin level in the ends and exodermis of mycorrhizal plants.

Increased phenol and decreased auxin levels in the mycorrhizal infected roots prevent larval growth and giant cell formation.

Accumulation coumestrol in mycorrhizal plants are more resistant to nematode attack.

REFERENCES


*******
Chapter-17

Isolation of Fungal Biocontrol Agents, Endophytes and Study on their Evaluation

Pranab Dutta and Himadri Kaushik,
Department of Plant Pathology
Assam Agricultural University, Jorhat-785013, Assam

Isolation of fungal biocontrol agents from rhizospheric soil

- Suspend one gram of soil sample in 9 ml of sterile water, stir in a vortex for 3 min.
- Dilute the mixture serially achieve dilutions of $10^{-1}$ to $10^{-6}$
- Replicate these dilutions for thrice separately and use for analysis.
- Place 1 ml of aliquot (1 ml) from each dilution in petridishes containing Potato Dextrose Agar.
- medium and incubate at 28±1°C for 5 days. Group the developing colonies with general characteristics of the concerned fungal genus (colour, conidial size, conidial shape, etc).

Isolation of fungal biocontrol agent from infected insect cadavers

- Collect mycosed insects from the field.
- Surface sterilize with 4-5% sodium hypochlorite (NaOCl) followed by rinsing with sterile water for three times.
- Crush the disease specimen and transfer a small portion (size 1-2 mm) of the infected part to the culture plate containing PDA medium supplemented with antibacterial antibiotics under aseptic condition.
- Seal the inoculated plates with parafilm and incubate in BOD incubator, at 25±1°C for 5-7 days.
- Observe the plates constantly for growth and development of the associated microorganisms.
- After 5 days, subculture the microbes for purification by selecting the desired colonies.
- Transfer the pure culture of each isolate to slants following the techniques of single spore isolation or hyphal tip culture.

Isolation of fungal endophytes
➢ Wash the collected plant sample thoroughly with double distilled water.
➢ Dissect and discard the outer edge of the samples Make six sections from the trimmed sample, averaging 6x6 mm for leaves and 6 mm long for stems and roots.
➢ Sterilize the samples by immersing them for two minutes each in 5% sodium hypochlorite (NaOCl) and two minutes in 70% ethanol followed by rinsing for three times in sterile distilled water and allow to dry in sterile paper towel.
➢ Place the sterilized plant samples on PDA medium supplemented with antibiotics tetracycline, streptomycin and penicillin at 2 mg/litre.
➢ Seal the inoculated plates with parafilm and incubate in BOD incubator, at 28±1°C under 12 hr light alternating with 12 hr dark period.
➢ Plate 100 µl of third change of rinsed water in PDA medium supplemented with tetracycline (@ 2mg/litre) and incubate in BOD incubator as mentioned above for 10 days to assess the successful sterilization, if fungal growth appeared, do not consider the corresponding samples for analyses.
➢ Take observations on fungal colonies and those considered as positive results should be randomly selected and transferred to PDA slants. This helps in avoiding contamination of neighboring leaf sections in the original plates.
➢ Allow the transferred colonies to sporulate and based on their morphological characteristics (colour, conidial size, conidial shape, etc.) identified the genus and species.

Methods to test the efficacy of biocontrol antagonist against targeted pathogens.

1. Dual culture technique

Material Required

Sterilized petri dishes, flasks, sterilized PDA medium, laminar air flow, inoculating needle, sprit lamp, culture of fungal antagonist and pathogen, BOD incubator and paraffin.

Protocol
Inoculate sterilized petri dishes (90 mm) containing PDA medium with 5mm diameter mycelia disc of 7 days old culture of pathogen as well as antagonist at equal distance from the periphery.

Incubate the inoculated plates at 28(±1)°C in BOD incubator.

Maintain a plate without antagonist as controls.

Observation

Measure the radial growth of the pathogen at different duration of incubation.

Calculate the per cent inhibition of radial growth of pathogen.

Percentage of inhibition (PI) = (C-T)/C*100

Where, PI=Percentage of growth inhibition, C=Colony diameter/radial growth of pathogen in control and T=Colony diameter/radial growth of pathogen in treatment.

Materials required

Seedlings, targeted insect pest, lantern chimney, muslin cloth, spore suspension of entomopathogenic fungi

Protocol

Raise the seedlings in a plastic cup.

Release the adult targeted insect (20-50 numbers) into the established seedling.

Place lantern chimney over the seedling.

Cover the top of the chimney with muslin cloth.

Spray entomopathogenic fungus at different concentration inside the lantern chimney.

Observed the mortality percentage of the pest after 24 hours.

Convert the mortality percentage to corrected mortality percentage (Abott, 1925)

References

Chapter-18
Phylloplane Bacteria and Plant Disease Management

Pradip Kumar Borah
Department of Plant Pathology,
Assam Agricultural University, Jorhat- 785013, Assam

Beattie and Lindow (1995) considered some of the definitions that have been proposed to describe the epiphytic bacteria. These are bacteria that are capable of living (i.e. multiplying) on plant surfaces. All the bacteria associated with a leaf have been referred to as phyllobacteria.

The terms epiphyte or phylloplane was coined by Aton De Bary. Plants offer a wide range of activities that support the microbial growth. The place in which the microbes survive also varies accordingly, such as if the microbes survive in internal tissues then they are referred as endophytes or if microbes survive in rhizosphere region then we can refer it as rhizosphere microbes.

What is a Phyllosphere?

It includes leaves, stems, blossoms and fruits. Leaves are the dominant tissues in phyllosphere base on surface area available for colonization. But when compared to endophytic and rhizospheric niche the Phyllosphere surface will subject to heavy fluctuations both for abiotic and biotic conditions (Gnanamanickam, 2006).

Phyllosphere bacteria have most diversified plant bacterial associations. These interactions of prokaryotes with host can be classified as commensals, mutualists and plant pathogens. Commensals are the bacteria that are not known to have any negative adverse effect to the plant. Mutualists are the group of bacteria those have the beneficial effect on the host plant. Pathogens are the bacteria those have adverse effect on the host.

Classes of epiphytic bacteria

Equally fascinating are the other major groups of bacterial epiphytes which are either causative agents of major bacterial plant diseases or frost injury to crop plants and are antagonists of such devastating plant pathogens that are beneficial for crop production. These are
- Epiphytic bacteria that are plant pathogens
- Epiphytic bacteria that are biological disease control agents
- Epiphytic bacteria that are ice-nucleation active
- Epiphytic bacteria that form biofilms

**Plant pathogenic phylloplane bacteria**

Bacterial pathogens colonize leaf surfaces of healthy leaves of host plants. This phenomenon was first reported in 1959 by Crosse of Italy by the isolation of large numbers of *Pseudomononassyringaepv. morsprunorum* (bacterial canker of stone fruit trees) from healthy leaves of cherry.

At times of disease onset and development of severe disease, large populations of epiphytic bacterial pathogens have been observed and have been causally linked. Detailed studies on quantitative relationships between foliar disease incidence and population frequencies of bacterial plant pathogens carried out for brown spot disease of bean (Lindeman *et al.*, 1984; Rouse *et al.*, 1985). Disease induction is in turn governed by host genotype and other environmental parameters that prevail. When the bacterial population reaches the threshold size, and if there are changes in the virulence of the pathogen or susceptibility of the host genotype, disease incidence occurs. It has been generally accepted that host genotype more than the environment, determines the outcome of disease or no disease (Gnanamanickam and Patil, 1977; Patil and Gnanamanickam, 1976).
Epiphytic bacteria as biological control agent against diseases

Epiphytic bacteria present in the leaf surfaces or those introduced as foliar sprays do suppress plant pathogenic bacteria (and fungal pathogens) of global importance. *Erwinia herbicola*, the epiphytic bacterium present in the leaf surfaces of rice was known to lower the pH of the rice leaf and thus made it difficult for the bacterial pathogen (*Xanthomonas oryzae*).
oryzae) to grow (Hsieh and Buddenhagen, 1974; Santhi et al., 1987). The diverse kinds of metabolites these epiphytic bacteria produce to suppress rice pathogens or cause enhanced plant growth suggest how well they have evolved in their fitness for performing these vital functions. The mode of action may be varies according to the bacteria it may sometimes positive to Ammonia production, HCN production, Siderophore production, and contributes to the growth promotion activity of the bacteria by secreting Indole Acetic acid in plant system.

**Epiphytic bacteria that are ice-nucleation activity**

Our present understanding of INA bacteria with emphasis on their potential role in precipitation was recently summarized by Morris et al. (2004) who said that the ice-nucleation active (INA) strains of bacteria as certain bacteria that are commonly found on plants and have the capacity to catalyze the freezing of supercooled water at temperatures as warm as -1°C. This capacity is conferred by a protein present in the outer membrane of the bacterial cell. That they participate in a sort of biological cycle of precipitation whereby they are transported into clouds from plant canopies and incite rain thereby causing favorable conditions for their growth on plant surfaces was proposed about 20 years ago. Today, sufficient evidence and meteorological tools have emerged to re-ignite interest in bio-precipitation and in the ways in which plants play a role as cloud seeders.

**Epiphytic bacteria that form biofilms**

Biofilms have been defined as frequently observed assemblages made by plant-associated bacteria that have been referred to as aggregates, microcolonies, and symplasmata. Basically biofilms are assemblages of microorganism adherent to each other and are embedded in a matrix of exopolymers. There are three well known models of hydrated biofilm structures, those are as follows:

- The water-channel model
- The mosaic biofilm model
- The dental plaque biofilm model

This third model of biofilms has high cell densities and arises in a high nutrient environment where they are bathed continuously in a fluid which has a limited fluid flow. While the first three saturated models have been observed in aquatic plants and plants that are raised in
hydroponic systems, the unsaturated biofilms have been observed on roots of terrestrial plants by Auerbach et al. (2000). It is known that the densities of microorganisms occurring in leaf surface biofilms is much lower than those observed in water-unsaturated systems. This is an indication, perhaps for low nutrient availability.

**Microbiology of phylloplane**

Phylloplane is a natural habitat on the leaf surface which supports a heterogeneous population comprising both pathogens and non-pathogens. The phylloplane microbes cover a wide range of organisms including yeasts, filamentous fungi, bacteria, actinomycetes, blue-green algae and even pigmy ferns.

Potter (1910) emphasized the importance of the part played by the epiphytic microorganisms in inciting the disease. Investigations by Last (1955) and Ruinen (1956) emphasized the significance of the leaf surface as an ecological niche for saprophytic and pathogenic microorganisms. Last (1955) termed this niche as phyllosphere (now recognized as phylloplane). The phylloplane microbes are of special interest from various points of view. For instance, some of them have antagonistic action against fungal parasites, degrade plant surface wax and cuticles, produce plant hormones, decompose plant material, activate plants to produce phytoalexins, act as a source of allergic air-borne spores and influence growth behaviour and root exudation of plants.

Recently, there has been a considerable interest to understand the biology of pathogens in relation to leaf surface saprophytes. In nature, intereactions are known to take place between pathogenic and saprophytic microbes as well as within the pathogens themselves. It seems that net effect of phylloplane saprophytes is to reduce the effective inoculum dose of pathogens. However, it is extremely difficult to determine the individual effect of the host, the pathogen or the non-pathogen on leaf surface. The establishment of natural equilibrium in phylloplane is actually a combined effect of all the three components sometimes directly, and at times, indirectly.

Leaves secrete/excrete certain exudates which may directly affect the surface microorganisms, some of which may be pathogenic. The host tissues are also known to exudates phytoncides which are inhibitory to the invading fungi and bacteria. Some of the surface
microorganisms may induce the production of phytoalexins in the host and bring about changes in the host reaction to parasites. The micro-organisms themselves produce self-inhibitory and self-stimulatory substances greatly influencing their own germination. They also interact and this interaction leads to the suppression or stimulation of one of the other bringing in antagonistic or associative effects. This complex problem of the phylloplane has a profound influence on the course of events in host infection and is ultimately related to the formulation of methods of disease control.

Host vs. Phylloplane microflora

Common phylloplane bacteria associated with crop plants are *Erwinia herbicola* (epiphytic), *Aerobacter*, *Corynabacterium*, *Flavobacterium*, *Lactobacillus*, *Pseudomonas*, *Klebsiella aeroginosa*, *Micrococcus*, *Sarcina*, *Achromobacter*, *Bacillus*, *Aeromonas*, *Clavibacter*, *Clostridium*, *Acetobacter*, *Gluconobacter*, *Leuconostoc*, *Arthrobacter*, *Streptococcus*, *Alcaligens*, *Streptomyces*, *Enterobacter* etc.

Epiphytic microbes are influenced by several factors. The indeterminate growth of leaf is the first thing to affect the nature of their substrates and the microbes harboured by them.

The surface microflora is very much under the influence of the host on which it occurs. There is definite exosmosis of materials from the external surface of plants. The moisture film on plant surfaces inevitably carries both organic and inorganic substances. Presence of diffusible inhibitors from healthy leaves of a resistant variety of sugarbeet has been reported by Kovacs (1955). Fawcett (1963) demonstrated the presence of antifungal compounds in certain plants. Some antifungal compounds are slightly inhibitory *in vitro* to a pathogen of a host, but may be strongly inhibitory to other fungi which are unable to attack the host.

The leaf surface substrates have been reported to be comprised of micro- and macro-elements together with organic substances like sugars, pectic substances, alcohols, amino acids, organic acids, growth hormones, vitamins and phenolic substances. Leaves of certain plants secrete phenolic or terpenoid substances which inhibit the microbial growth. Some microbes are reported to be capable of releasing even growth hormone onto the leaf surface.
Certain hosts are known to contain preformed chemical compounds, phytoncides, which are inhibitory to pathogens. Some of these may be volatile and influence the microorganisms present on the leaf surface.

The foliar microbes are likely to induce production of phytoalexins which are fungistatic. Phytoalexins which are produced as a result of interaction between two metabolic systems, inhibit the growth of phytopathogenic microbes. Perhaps Bernard (1909) appears to be the first to point out that local saprophytic association may provide protection against subsequent infections of the host. Later Muller (1956) provided conclusive evidence regarding the formation of phytoalexins. He pointed out that in the course of interaction between the host and avirulent pathogens, a factor is activated in the former which exerts an antibiotic effect on the later, and greater the speed with which the factor appears in the infected host tissue, the earlier is the pathogen checked. The phytoalexin activity is not influenced by chemical constituents of the host tissues. Phytoalexin formation appears to be a general phenomenon. Cruickshank and Perrin (1961) conclude that a host gives a resistant reaction when a given infection stimulates sufficient phytoalexin to inhibit the pathogen and susceptibility may be the result of the tolerance of phytoalexin by the pathogen or its inability to stimulate phytoalexin production.

The morphological features of the leaves also have a significant influence on the microbes. They affect the wet ability of the leaves. The irregular presence of surface moisture on the leaves results in intermittent growth of micro-organisms, particularly bacteria and filamentous fungi. Dense covering of trichomes/crystalline deposits of epicuticular wax increases the water repellency of the surface, restricting microbial growth, spread of pathogen inoculum and leaching of substances from the leaf. The micro-sites (which are present along the veins of leaves) of the leaf surfaces favour the growth of both pathogens and saprophytes (Blakeman et al., 1981).

Leaves at the seedling stage usually harbour minimum number of microbes. With the age of plant, both the quality and quantity of the microbial population changes. The probable reason of variation in the quality and quantity of the microbes on the leaf surface seems to be the types and amounts of substrates at different stages of leaf maturity.

The newly opened leaves are free of any colonization by the microbes. Thus, the earliest colonies do not face the problem of competition and also receive a good supply of nutrients.
With the gradual colonization of the leaf surface by the microbes, the later colonizers have to face stiff competition. Leben (1965) has grouped the colonizers into two groups. The first representing the air-borne spores, called casual inhabitants, is generally present on the leaf surface by chance. The second one, resident inhabitants, is well adapted to the leaf surface and usually outnumbers the casuals. Hudson (1968) says that the forms that succeed in colonizing the leaves at their early stage are the common primary saprophytes, while the forms which are restricted in their host range are the ‘restricted primary saprophytes’.

Physical factors vs. Phylloplane microflora

Once microbes come in contact with the leaf, they are affected not only by the surface substrates but also by a number of other factors. Physical factors like temperature, relative humidity, light and wind velocity also contribute to the population of microbes. Several workers have investigated the microbial population in the phylloplane in relation to meteorological factors. Most of them have reported that, in addition to plant factors, the conditions to which plants are exposed play a major role in the establishment of the microbes on the leaf surface.

Sharma and Gupta (1979) working on the leaf surface microflora of brown sarson found that the climatic conditions played a significant role in the establishment of microbes in the phylloplane. Similar observations have been made by Jensen (1971) on beech leaves, Sharma (1971) on sorghum, leaves and Rajkumar and Gupta (1976) on potato leaves. Dixit and Gupta (1980) analysed the phylloplane microorganism and air spora of barley, and reported that low temperature, low wind velocity and high humidity might have adversely affected both. On the other hand, Narula and Mehrotra (1981) analysed the microbial population on the leaf surface of Colocasia esculenta and observed that temperature and relative humidity did not have significant correlation with the variation in the phylloplane microflora.

Of the several factors which influence the survival of the microbes on the leaf surface, temperature and relative humidity variations during the life of the plants affect the adaptability of the microbes on the leaf surface.

Even natural pollutants like smoke, dust and pollen grains of higher plants contribute appreciably to the leaf surface niche. Fokkema (1971) found that pollen grains of higher plants
alter the microbial population of the phylloplane by enhancing the growth of certain fungal forms.

**Microbes vs. microbes**

The phylloplane microflora is not only under the influence of the host but also subject to its own influence. An important aspect is the production of self-inhibitory and self-stimulatory products by microorganisms present in the phylloplane. Dickinson (1953), working on stem rust of wheat, reported that the germinating rust spores, under certain conditions produced a self inhibiting volatile substance which retarded germ tube elongation.

The microorganisms of the leaf surface have an interacting influence also. The interaction with parasitic forms is of special importance. Sometimes, a fungus or a bacterium present on the leaf surface may be parasitic on the pathogen (hyperparasitism). Causes of hyperparasitism have been reported by many workers including Keener (1954) and Ponet al. (1964).

Non-pathogens on the leaf surface may influence the growth of pathogens and play an important role in reducing the incidence of a disease. Mechanism of antagonism by phylloplane organisms has been discussed by various workers like Blakeman and Brodie (1976), Fokkema (1976). Antagonism includes competition (for food, space and oxygen), antibiosis (production of antibiotics), parasitism and predation. In antibiosis, the metabolites of one or more microorganisms adversely affect other microorganisms. Many phylloplane microbes have been reported to produce antibiotics in vitro. Some of the leaf surface fungal forms inhabiting the leaf have been reported to produce antibiotics, for example, *Alternaria*, *Botrytis* and *Aureobasidium*. Species of *Trichoderma* produce both non-volatile and volatile antibiotics.

There are many reports that saprophytic microorganisms enhance disease resistance in plants. Bamberg (1931) showed that a bacterium isolated from corn, when mixed with smut spores of *Ustilagoeae*, reduced the infection rate of corn and inhibited the germination of chlamydomspores. Newhook (1957) isolated organisms antagonistic to *Botrytis cinerea* from lettuce and tomato. A reduction in the infection of *B. cinerea* from 50 to 20 per cent occurred with the increase in colonization of *Cladosporium herbarum* and *Penicillium*
Resident antagonists vs. biological control

Role of phylloplane bacteria in the interactions between host and pathogen


5. *E. herbicola*, *Flavobacterium* spp, *Serratia marceseens* and *Penicillium oxalicum* (fungi)


7. *Bacillus subtilis*, *Cladosporium*, *Penicillium* and *Aspergillus* : *Helminthosporium oryzae* (Harish et al., 2007)

8. *Bacillus cereus*, *Novosphingobium capsulatum* : *Phytophthora infestans* (Late blight of tomato) (Halfeldet al., 2008)

9. *Ochrobactrum anthropi* : *Pestalotiopsis theae* (Grey blight of tea) and *Exobasidium vexans* (Blister blight of tea) (Sandhararanjanet al., 2012)

10. *Bacillus mojavensis* : *Pseudomonas savastanoi* (olive knot disease) (Ghanneyet al., 2016)
Antagonism by the non-pathogens has opened the possibilities of biological control of foliar diseases. Antagonistic non-pathogens may be employed to control diseases caused by the pathogens. The control may involve application of an effective antagonist on the leaf surface. However, such an antagonist should have the ability to multiply and colonise the leaf surface.

The biological control of foliar pathogens by means of alien saprophytic microorganisms on leaf surfaces has been achieved in a number of cases. Tveit and Wood (1976) reported that Fusarium blight of oat seedlings could be controlled by the antagonistic activity of Chaetomium species. Artificial introduction of Trichoderma and Epicoccum onto the living needles of Pinus trichocarpa was reported to inhibit the development of Melampsora occidentalis. Heuvel (1971) demonstrated that the lesion formation due to Alternaria zinniae (a pathogen) on bean leaves was greatly reduced when the leaves were inoculated with Alternaria tenuissima (a non-pathogenic fungus).

Fokkema and Lorbeer (1974) reported that the infection of onion leaves by Alternaria porri was reduced by Aureobasidium pullulans. Sharma and Gupta (1979) working on the leaf
blight diseases of brown *sarson* caused by *Alternaria brassicae* and *A. brassicicola* reported control by the application of the spore suspension of *Streptomyces rochei* and its diffusate.

Kapooria and Sinha (1969) found that a number of leaf saprophytes of pearl millet (*Pennisetumamericanum*) were antagonistic to *Puciniapeniseti*. The most effective among them was *Chaetomium globosum*. Sinha (1976) obtained considerable suppression of the rust (*Uromycescicerisarietini*) of gram (*Cicer arietinum*) by employing spores of certain leaf saprophytes, viz., *Chaetomium globosum, Trichoderma koningi, Malustela area, Fusarium orthoceras* and *Fusarium oxysporum*.

Dixit and Gupta (1980) were successful in controlling leaf blotch disease of barley caused by *Alternaria alternata* with the help of spores of the antagonist *Streptomyces olivaceous* as well as its diffusates. Sinha (1976) reported the control of leaf blight of wheat (*Alternaria triticina*) by the use of the spore suspension of *Aspergillus nidulans, A. terreus* and *Alternaria alternata*.

Capoor (1985) made qualitative and quantitative surveys of phylloplane microflora of two important cereals (wheat and triticale) and a pulse (mung) in relation to certain foliar diseases, viz., leaf spot of wheat caused by *Alternaria alternata*, leaf blight of triticale caused by *Alternaria triticina* and leaf spot of mung caused by *Alternaria alternata*. The resident antagonists which caused 100 per cent inhibition of spore germination of the pathogens were employed for trials as bioagents for the control of diseases. *Trichoderma harzianum* was used as foliar spray against *Alternaria alternata* on wheat, *Alternaria triticina* on triticale, and *Aspergillus quadrilineatus* against *Alternaria alternata* on mung. Lowest effective concentration of the culture filtrate or spore suspension was used as foliar spray under greenhouse and field conditions. The treatments effectively reduced the disease incidence. Nearly 60 per cent disease control was reported in case of all the three diseases.

**Conclusion**

In spite of reports of successful control of several foliar diseases mentioned by employing antagonists or their diffusates; due emphasis has not been given to the studies on phylloplane microflora especially with a view of evolving biological control for foliar pathogens.

Several methods are known for the control of foliar diseases of plants including the use of fungicides, antibiotics and resistant varieties. However, they are generally losing their relevance
because of certain limitations associated with them. Use of fungicides very often poses the problem of toxicity in the treated plants and pollution hazards in the environment. The persistence of the poisonous chemicals in the plant parts and their passage in the food chain has been the limiting factors for large scale adoption of chemical control. Antibiotics have been brought in use instead of mercurials and copper fungicides with the hope to overcome the problem of toxicity. But these, too, have their own limitations. The high cost of antibiotics prohibits their use on a large scale and in several instances in vitro results do not match with field performance. Resistant varieties serve very well for a few years but fall easy prey to new races of parasites thereafter.

In view of the foregoing observations, it is advisable, for the plant pathologists, to undertake exhaustive investigations on the phylloplane microbiology or important crops known to suffer from foliar diseases so that suitable methods of biological control are developed.

A. Isolation of Phylloplane Bacteria (Wash Method)

(Vozhnakoyskaya&Khudyakov, 1960)

Materials required:

1. Plant leaf (citrus) – 5 g
2. Sterile water (150 ml flask) – 3 nos.
3. Sterile conical flask (150 ml) – 3 nos.
4. Micropipette (1 ml) – 1 no.
5. 9 ml sterile water blank – 10 nos.
6. Glass spatula – 1 no.
7. Cycloheximide – 0.05 g
8. Sterile petridish – 10 nos.
9. NA slants – 5 nos.
10. Inoculation needle – 1 no.
11. Spirit lamp – 1 no.
12. Non absorbant cotton – 1 ball
13. Brown paper – 3 sheets
15. Thread ball – 1 no.
16. Spirit – 50 ml
17. Sterile glass rod – 1 no.
18. Glass marking pen – 1 no.

Procedure:
Take five gram leaf (citrus) in a conical flask with 100 ml sterile water and stir vigorously with sterile glass rod for 10 min.

Prepare sterile dilutions by transferring 1 ml of leaf washing of 9 ml sterile water blanks.

Spread an aliquot of 0.1 ml of each dilution with glass spatula on the surface of nutrient agar containing 0.05 g cycloheximide per litre.

Incubate the plates at 25±1°C for 48 hrs.

Pick up colonies of different shape, size and colour on NA slants.

Further, purify these colonies by streaking on NA agar plates and pick up a single colony and maintain on NA slants.

**B. In vitro Assay of Antagonism Phylloplane Bacteria against X.axonopodispv. citri**

**Materials required:**

<table>
<thead>
<tr>
<th>Material</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Xac slants – 2 nos.</td>
<td></td>
</tr>
<tr>
<td>2. Plb slants – 5 nos.</td>
<td></td>
</tr>
<tr>
<td>3. 150 ml flask with NA media (25 ml) – 5 nos.</td>
<td></td>
</tr>
<tr>
<td>4. Heater/Hot plate – 1 no.</td>
<td></td>
</tr>
<tr>
<td>5. Sterile petriplates – 10 pairs</td>
<td></td>
</tr>
<tr>
<td>6. Inoculation needle – 1 no.</td>
<td></td>
</tr>
<tr>
<td>7. Spirit lamp – 1 no.</td>
<td></td>
</tr>
<tr>
<td>8. Non-absorbant cotton – 1 ball</td>
<td></td>
</tr>
<tr>
<td>9. Sterile water blank (150 ml flask) – 2 nos.</td>
<td></td>
</tr>
<tr>
<td>11. Rubber band – 1 pkt.</td>
<td></td>
</tr>
<tr>
<td>12. Thread ball – 1 no.</td>
<td></td>
</tr>
<tr>
<td>13. Glass marking pen/pencil – 1 no.</td>
<td></td>
</tr>
<tr>
<td>14. Spirit – 25 ml</td>
<td></td>
</tr>
</tbody>
</table>

**Procedure:**

- Prepare bacterial suspension (X. axonopodispv. citri) by mixing sterile water in slant, allow the diffusion of bacteria into water for 10 min and shake it well.
- Mix 1 ml of Xac suspension (0.1 O.D) with 25 ml each melted, cooled NA conical flask and pour into sterile petriplates.
- Spot inoculate the solidified plates with the 24 hr old solid growth of Plb isolates.
- After 3 days of incubation at 26±1°C, examine the plates for antagonistic action indicated by the appearance of inhibition zone at the site of seeding and measure the diameter of clear inhibition zone.
- Plates without Plb will serve as control.

**Diagrammatic Representation of Isolation of PLB from Leaf**
Diagrammatic Representation of In Vitro Assay of Antagonism of PLB against Xaxonopodispv

References:


********
Chapter-19

Preparation of Plant Extracts, Efficacy Test and their Use for Plant Disease Management

Daisy Senapty
Department of Plant Pathology
Assam Agricultural University, Jorhat-785013, Assam

There is a worldwide swing to the use of eco-friendly methods for protecting the crops from pests and diseases. The use of potential harmful chemicals is viewed with dissatisfaction. Recently, in different parts of the world, attention has been paid towards exploitation of plants as novel chemotherapeutants in the plant protection in view of their long term effect on crop disease management, low cost and safety to ecosystem. Plants have been known for their medicinal and antimicrobial properties since ancient times. Ahmed and Grainage (1982) identified many plant products, which were found to be effective for the control of many plant diseases. Among the 5280 plant species tested, 1134, 346, 92 and 90 plant species possessed insecticidal, fungicidal, antibacterial and antiviral properties, respectively. This clearly indicates that the plant kingdom is a vast store house of chemicals that can check many plant pathogens. As many of them have more than one type of activity there is less chance for development of resistance and moreover, the plant products are safer to non-target organisms. We can combine its ability with other bioagents and fungicides as integrated means for disease management.

1. Preparation of plant extracts

Selection/collection of botanicals

Different plant parts like fresh leaves, bulbs and rhizome of selected plants are collected for the preparation of plant extracts. Plants are selected on the basis of their easy availability in a particular locality.

Methods for preparation of plant extracts

Plant extraction involves the separation of medicinally active portions of plant tissues from the inactive or inert components by using selective solvents in standard extraction procedures. Plant extracts are prepared in water or in chemical solvents like alcohol, methanol, acetone, hexane, chloroform, petroleum ether etc. However, the most commonly used solvents
are water and alcohol. Generally water extract shows higher inhibitory activity than alcohol extract. The alcohol extraction is slightly more complex, but still one of the most frequently used methods for extracting botanical compounds. More toxic organic chemicals are also used and can draw out certain compounds that elude water and alcohol, but these harsh chemicals can leave residues that are challenging to remove or minimize.

**a. Preparation of plant extracts in water**

**Cold water extract:** The plant extracts are prepared following Shekhawat and Prasad (1971) with certain modifications. Fresh plant materials (e.g., leaf, rhizomes, bulbs, seeds, shoots, roots etc) of healthy plants are collected and washed thoroughly in tap water followed by sterile distilled water. Hundred grams of washed plant parts are ground in pre-chilled mortar and pestle by adding equal amount (100ml) of sterilized distilled water (1:1 w/v). After grinding, the extract is filtered through muslin cloth and finally the extracts are centrifuged at 10,000 rpm for 20 minutes in centrifuge (Remi C 24) at room temperature. The supernatant is taken as standard plant extract solution (100%). This stock solution can be further diluted to different concentrations with sterile distilled water.

**Hot water extract:** The collected plant materials are washed thoroughly in tap water followed by sterile distilled water, shade dried for 15 days and then powdered. Plant extract (15% w/v) are prepared by brewing in hot water. 15 g dry powder of each plant sample is weighed and put in a cheese cloth bag and suspended in 100 ml of boiling distilled water for 20 minutes. The extract is allowed to stand for some time and decanted off into the flask and supernatant was used to assay the antifungal activity of each plant extract.

**b. Preparation of plant extracts in other solvents**

The collected plant materials are washed thoroughly in tap water followed by sterile distilled water. The plant materials are allowed to air dry and afterwards powdered with the help of hands. The powders are further subjected to extraction protocols. The solvent used for the preparation of extracts are ethanol, methanol, chloroform and petroleum ether etc.

50 grams of plant material are dissolved in 250 ml of solvent (ethanol, methanol, chloroform and petroleum ether) and kept in a mechanical shaker for overnight. The obtained
extracts are filtered with Whatmann No- 42 filter paper. Thus obtained filtrates are concentrated by complete evaporation of solvent at room temperature to yield the pure extract and quantify the yield. The extracts are stored in airtight bottle and kept at 4°C until further use.

2. Efficacy test of plant extracts against pathogens

a. Mycelial growth inhibition test

The efficacy of plant extracts in relation to the growth of fungi is determined by ‘Poisoned food technique’ (Nene and Thapliyal, 2000). The principle involved in this technique is to poison the nutrient media with a fungitoxicant and then inoculate a test fungus on such a media. In this technique, either a solid or liquid medium can be used.

PDA media are prepared in flask and sterilized. To this medium required quantity of plant extract are added in order to get required concentration (say to get 20% concentration, 20ml of 100 per cent aqueous extract is added to 80ml of PDA). The plant extract is thoroughly mixed before the medium is solidified. The medium is then equally distributed into the sterilized petri plates (9 cm diameter) in five replications and allowed to solidify. Mycelial discs prepared using a cork borer (5mm diameter) from the tip of 5 days old cultures of the test pathogen. One disc of the pathogen is placed at the center of a Petri dish after solidification of the medium. The medium without plant extracts serve as control. The plates are incubated at 28±1°C for 7 days.

The diameter of the colony is measured when the mycelium fully covered the petri plates of the control treatments. The percent inhibition of the mycelial growth is calculated by the formula of Vincent (1927).

\[ I = \left( \frac{G-C}{C} \right) \times 100 \]

Where, \( I \) = Inhibition of mycelial growth, \( C \) = Growth in control, \( T \) = Growth in treatment

b. Spore germination inhibition test

The antifungal effect of leaf extracts on conidial germination of the pathogen is tested using different concentrations of aqueous plant extracts by spore germination method using
cavity slides. Spore suspension of the fungus is prepared aseptically from 7 days old pure culture. One drop (50µl) of 20% plant extract is placed in a cavity slide and allowed to air dry. A drop (50µl) of spore suspension (5x10^5 spores/ml) of the test fungus prepared in sterile distilled water is added to the dried plant extract and mixed thoroughly. Three replications are maintained for each treatment. The spore suspension in sterile distilled water served as control. Then the cavity slides are incubated at ambient temperature (25±2°C) in moist chambers (in large Petri dishes containing blotting papers blotted with sterile water) for 48 hrs. After the incubation period, observations are made under microscope to calculate the per cent inhibition (PI) by counting the number of spore germinated and the total number of spores in different microscopic view by using the formula given by Vincent (1947).

\[ I = \frac{G_c - G_t}{G_c} \times 100 \]

Where, \( I = \) Inhibition of spore germination, \( G_c = \) Germination in control, \( G_t = \) Germination in treatment

c. Determination of zone of inhibition:

**Paper disc plate method:** Sterilized petri dishes are poured with PDA medium and allowed to solidify. Sterilized filter/blotting paper disc of 10 mm diameter are dipped in required concentration of plant extract and placed in the centre of plate on the surface of the medium. Then place 5 mm disc of inoculum of the pathogen at three places in the periphery of the plate at equal distance from each other and from centre as well. The plates are incubated at 25°C for 2-6 days and inhibition zone around the paper disc are recorded.

**Agar diffusion assay:** In this test, agar wells are prepared with cork borer and poured with 400µl plant extracts, dried and placed on PDA plates, prior inoculated with fungal spore suspension (1x10^6 spores/ml). Antifungal activity is assessed by measuring the diameter of growth inhibition zone after incubation at 30°C for 48 hrs.

In case of bacterial pathogen, one ml of bacterial suspension are introduced into sterile Petri dishes using a sterile syringe and poured with 20ml of Nutrient Agar medium (NA) and allowed to solidify. After this, wells are made using sterile 5 mm cork borer. Three wells per
plate are prepared and filled with the plant extracts. Three replicates are maintained for each of the different plant extracts used. Antimicrobial activity is assessed by measuring the diameter of growth inhibition zone after incubation at 37°C for 24 hrs.

3. Use of plant extracts in plant disease management

**Preparation of plant extracts:** The plant extracts are prepared as mentioned above in cold water extract, but without centrifugation.

**Different methods of application:**

a) Seed treatment, b) Foliar Spray application, c) Soil application

**a) Seed treatment:** Three ml of extracts are used to treat 10 grams of seeds. Seeds are soaked for 6 hrs and then shade dried for 2 hrs before plating or sowing in pot containing sterile soil.

- Leaf extracts of *Adjantumcandatum* and *Polypodium multilineatum* protect the seeds of wheat, barley, maize, sorghum and rice and the treated seeds were completely free from seed borne fungi (Kanaujia, 1974).
- Garlic extract seed treatment significantly reduced the post emergence seedling mortality in Jute and significant increase in germination (Ahmed and Sultana, 1984)
- Hawlader (2003) reported that seed treatment with allamanda leaf extracts effectively increased germination of brinjal seeds and tremendously decreased nursery diseases.

**b) Foliar spray application:** The aqueous extracts of the botanicals are sprayed at required concentration on the plants with the help of atomizer till the wetting of leaves.

- Spraying with 10% garlic extract controlled *Pseudoperenosporacubensis* causing downy mildew in radish, cucumber and spinach. (Dikshit and Dixit, 1982).
- Siddiquee et al. (2011) evaluated the efficacy of foliar spray with *Allamanda* leaf extract to control scab and die-back of citrus and found it effective to achieve significant reduction in severity of scab and dieback disease and increase fruit yield of lemon.

**c) Soil application:** Application of plant products in soil or soil drenching with plant extractssignificantly reduces many soil borne diseases.
The damping off disease due to *Pythium monosperum* in tomato was significantly reduced by the leaf extracts of *Bouganvillea glabra* or *Piper betle* (Alice, 1984).

Drenching the soil immediately after sowing with the extracts of *Tamarindus indica* and *Leucaena leuephala* at 50% or 20% concentration gave higher percentage of germination and stand of the tomato seedlings against *Pythium indicum* under greenhouse condition (Ravichandar, 1987).

Some commercial formulations are also available: Godrej Achook, Neemark, Neem gold, Neem guard, Nethrin, Nimba (prepared from neem)

**Management of viral diseases by plant extracts**

Plants are known to contain some compounds which are inhibitory to virus. They are called Anti-Viral Principles (AVP) or Anti-Viral Factors (AVF). The presence of AVPs in extracts of several plants has been reported.

**Some plants producing AVPs**

<table>
<thead>
<tr>
<th>Source</th>
<th>Nature of AVPs</th>
<th>Effective against</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Solanum spp</em></td>
<td>Glycoalkaloids</td>
<td>TMV &amp; Sunhemp Rosette virus</td>
<td>Roychaudhury and Basu (1983)</td>
</tr>
<tr>
<td><em>Beta vulgaris</em></td>
<td>Polysaccharides</td>
<td>TMV</td>
<td>Ebrahim-Hesbat &amp; Nienhaus (1972)</td>
</tr>
<tr>
<td><em>Chenopodium album</em></td>
<td>Protein</td>
<td>TMV</td>
<td>Smookler (1971)</td>
</tr>
<tr>
<td><em>Cocos nucifera</em></td>
<td>Protein</td>
<td>Tomato spotted wilt virus</td>
<td>Narayansamy and Ramiah (1983)</td>
</tr>
</tbody>
</table>

**Preparation of AVP extract**

For the preparation of AVP extract, dried leaves are cut and powdered. Twenty kg of leaf powder is mixed with 50 litres of water and heated at 60° C for 1 hr. It is filtered and volume is
made upto 200 litres. This gives 10 per cent extract. Five hundred litres of extract is required to cover 1 ha.

- 10 % AVP extract of dried coconut or sorghum leaves are very effective in controlling Groundnut ring mosaic virus (bud necrosis). Two sprays are to be given at ten and twenty days after sowing. Similarly 10 per cent leaf extracts of *Prospis juliflora* and *Cynodondactylon* effectively reduced the tomato spotted wilt virus in tomato. The leaf extracts are known to contain some proteinaceous substances which induce virus inhibition in the plants.

- Use of leaf extracts of *Vitex negundo* L. as a foliar spray showed high disease suppression efficiency (60%) as an elicitor for inducing resistance in *Carica papaya* against *Papaya ringspot* virus (Kashyap *et al*, 2016).

**References**


*****
Chapter-20

Biological management of diseases of flowering plants

N. Mazumder
Principal Scientist (Pl.Pathol.)
AICRP(Floriculture)
HRS, AAU, Kahikuchi
Guwahati-781017, Assam

Agriculture is the dominant land use category in Assam and is considered as primary sector in the state’s economy of Assam. It account for about 54.11 per cent of total geographical area and about 80 per cent of the total population of Assam is dependent on Agriculture. Assam agricultural sector is dominated by different field crops viz., rice, pulses, oil seed crops and fruits, jute, tea, potatoes etc. But in recent decade, the cultivation of ornamental crops has found an edge over conventional crops in the NE region, including Assam. Among various central aided agri- based schemes under ministry of Agriculture, inclusion of floriculture and ornamentals has paved the ways for increased returns for the farmers. Earlier, flower cultivation was very much localized in certain districts. But presently commercial cultivation of different flowers namely, marigold, tuberose, gerbera, orchid (Dendrobium), chrysanthemum and anthurium is now taken up by the flower growers in the districts of Nalbari, Bongaigaon and Kokrajhar of lower Assam, in addition of Jorhat, Nagaon, Dibrugarh of upper Assam. During 2014-15, Assam recorded loose flower and cut flower of 22.8 thousand tones and 5051.2 lakh number, respectively (Statistical Year Book India, 2018).

In addition, a numbers of annual flowers are cultivated in Assam like other Indian states. They are generally extensively grown for landscape decoration, and or as potting plant, in some cases they are also used in bedding, boarders etc for their attractive variety of bright colours. Considerable economic losses can occur to different types of flowers grown commercially including annuals growers or landscapers because of incidence of various diseases like foot/root rot, powdery mildew, fungal leaf spot/blight, bacterial leaf and flower spot including phytoplasma, viral diseases. An over view on biological control of few important diseases have been elucidated below.
**Emerging diseases of ornamental:** Systematic study on isolation and identification of different frequently occurring as well unknown diseases of ornamentals have been done and the following few are listed below.

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Name of the disease</th>
<th>Causal organism identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Leaf spot/blight of tuberose (both single and double type)</td>
<td><em>Phoma tuberosa</em></td>
</tr>
<tr>
<td>2</td>
<td>Leaf spot/blight of gerbera (var. Red Gem)</td>
<td><em>Botryodiplodia theobromae</em></td>
</tr>
<tr>
<td>3</td>
<td>Leaf spot/blight of gerbera (var. Red Monarch)</td>
<td><em>Phoma glomarata</em> and <em>Aschochyta gerberae</em></td>
</tr>
<tr>
<td>4</td>
<td>Leaf blight of gladiolus</td>
<td><em>Stemphylium botryosum</em></td>
</tr>
<tr>
<td>5</td>
<td>Leaf Blight of Dracaena (Lucky Bamboo)</td>
<td><em>Exserohilum rostratum</em>,<em>, and</em> <em>Fusarium equeseti</em></td>
</tr>
<tr>
<td>6</td>
<td>Root rot of Anthurium</td>
<td><em>Phytophthora parasitica</em>,</td>
</tr>
<tr>
<td>7</td>
<td>Foot /Root rot of Dendrobium orchid</td>
<td><em>Phytophthora parasitica</em>,</td>
</tr>
<tr>
<td>8</td>
<td>Leaf blight of calathea (<em>Calathea</em> spp):</td>
<td><em>Phoma medicaginis</em></td>
</tr>
<tr>
<td>9</td>
<td>Leaf blight of Day lily (<em>Hemerocallis</em> spp)</td>
<td><em>Colletotrichum falcatum</em></td>
</tr>
<tr>
<td>10</td>
<td>Leaf spot of wart fern (<em>Polypodium scolopendrium</em>, <em>P.diversypolium</em>)</td>
<td><em>Phoma exigua</em></td>
</tr>
<tr>
<td>11</td>
<td>Leaf spot of Chinese box/Kamini Kanchan (<em>Murrya koenigi</em>):</td>
<td><em>Hysterium pulicare</em></td>
</tr>
<tr>
<td>12</td>
<td>Leaf Spot of snake plant (<em>Sansevieria trifasciata</em>):</td>
<td><em>Colletotrichum sansavvieriae</em> and <em>Corynespora trifasciata</em>.</td>
</tr>
<tr>
<td>13</td>
<td>Leaf spot of Ixora (<em>Ixora coccinae</em>):</td>
<td><em>Pestalopsis longiappendoculata</em></td>
</tr>
<tr>
<td>14</td>
<td>Leaf spot of Areca palm (<em>Dypsis lutescens</em>):</td>
<td><em>Diplodia phoeniceum</em></td>
</tr>
</tbody>
</table>
Major diseases of commercially grown flower crops in Assam and their management through BCA (biological control agents) are discussed below. Biological control of plant pathogens can be achieved by either promoting the native antagonists to reach a density sufficient to suppress a pathogen (s) or by introducing alien antagonists. Though some earlier works, related to native antagonists was by using organic amendments, the recent trend is to isolate, multiply and introduce the effective antagonists to soil or specific court of infection to achieve a successful biological suppression of a disease. The possible use of fungal (Trichoderma, Metarhizium, Beauveria etc) and bacterial (Bacillus, Pseudomonas etc) antagonists and also their combination have been tried for the control of different flower diseases. The present lecture elucidates some information on the biological control of diseases of ornamentals such as foot/root rot, sclerotial wilt, leaf spot/blight, bacterial blight et

<table>
<thead>
<tr>
<th>Disease</th>
<th>CO</th>
<th>Plant parts infected</th>
<th>Time of Occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Tube rose</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i. Sclerotial wil</td>
<td>Sclerotium rolfsii</td>
<td>Collar region</td>
<td>May-Aug</td>
</tr>
<tr>
<td>ii. Leaf spot/blight</td>
<td>Alternaria polyanthi, Phoma tuberosa</td>
<td>Leaf, stem</td>
<td>April-Oct</td>
</tr>
<tr>
<td><strong>2. Gladiolus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i. Fusarium wilt</td>
<td></td>
<td></td>
<td>Dec-Feb</td>
</tr>
<tr>
<td>ii. Botrytis leaf spot</td>
<td>Botrytis cinerea</td>
<td>Leaf</td>
<td>De-Feb</td>
</tr>
<tr>
<td><strong>3. Gerbera</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i. Foot/root rot</td>
<td>P. cryptogea, Pythium ultimum, R. solani</td>
<td>Foot/root rot</td>
<td>April-Aug</td>
</tr>
<tr>
<td>ii. Leaf spot / blight</td>
<td>Phoma gerberae</td>
<td>Leaf</td>
<td>April-Oct</td>
</tr>
<tr>
<td><strong>4. Marigold</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i. Alternaria LS</td>
<td>Alternaria dianthi</td>
<td>Leaf, flower petals</td>
<td>Dec-Feb</td>
</tr>
<tr>
<td>ii. wilt</td>
<td>P. fluorescens, Fusarium spp, R. solani</td>
<td>Root system, Collar</td>
<td>Dec-April</td>
</tr>
<tr>
<td><strong>5. Anthurium</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i. Anthracnose/ spadix</td>
<td>C. gloeosporioides</td>
<td>Spathe, Leaf</td>
<td>March-Oct</td>
</tr>
<tr>
<td>Rot</td>
<td>Petiole</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----</td>
<td>---------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ii. Bacterial blight</td>
<td>X.axonopodis pv. dieffenbachiae</td>
<td>Spathleaves</td>
<td>Dec-Feb</td>
</tr>
</tbody>
</table>

### 6. Orchid

<table>
<thead>
<tr>
<th>i. Foot or root rot</th>
<th>Phytophthora parasitica</th>
<th>Foot/root</th>
<th>March- Sept</th>
</tr>
</thead>
<tbody>
<tr>
<td>ii. Wilt/Stem rot</td>
<td>Sclerotium rolfsii</td>
<td>Leaf base/pseudobulb</td>
<td>April-Aug</td>
</tr>
</tbody>
</table>

#### 1. Sclerotial wilt or stem rot of tuberose:
The initial symptom of this disease is flaccidity and drooping of outer whorl older leaves followed by yellowing and in this conditions, a characteristic fan shaped white mycelial strand of fungus appear at the base of the infected plant and in the later stage numerous brown mustard like round sclerotia develop on the mycelial growth of causal organism (Sclerotium rolfsii). Removal followed by destruction of all the infected plant along with the sclerotial masses should be done. Pre planting bulb dipping in Trichoderma viride (10 g/l) followed by soil application of T. viride enriched FYM (1:50) @ 100 g/m² at the time of planting were found effective against Sclerotial wilt of tuberose (Mazumder et.al., 2010). Integration of wheat grain colonized Trichoderma sp. (R1) with Vitavex-200 (carboxin + thiram) as soil drenching was found to be most effective (Islam and Bhuyan, 2006). Like wise, pre-planting bulb dipping in spore suspension of T. harzianum and T. viride @ 10 g/kg (10⁷ cfu/ml) or in the solution of metalaxyl (Apron 35 SD) for 15 minutes significantly contolled the disease (Mishra et. al., 2008).

#### 2. Alternaria (A.polyanthi) Leaf spot/blight of tuberose:
Small oval to roundish brownish spots with fiant concentric ring appear on the leaf blades, mostly near the mid rib and flower peduncle. The infected leaves and peduncles become necrotic and dry up with increase of disease pressure. Incidence of the disease is more in ratoon crop and during the winter season. Pre planting bulb dipping in combo BCA formulation consisting of T. viride and P. fluorescens (10⁷ cfu/ml) at 5% for 10 min. followed by 2 sprays with same bioformulation at 2% at 15 day interval also need base spray with Difenoconazole (0.5 ml/l) recorded lowest per cent LS incidence of 8.49, 20.67 and 3.70 at different places (B,lore, Ghy, Lud) (Annon,2019).
3. Fusarium wilt (*F. oxysporum f.sp gladioli*) of gladiolus: Arching, curving of young stalks or premature yellowing of older leaves and faded flower colour are the characteristic symptoms of wilt. Often plants are stunted and fail to bloom. In storage, the pathogen causes corm rot/ dry rot/ core rot of the corms. Corm dressing with *Pseudomonas fluorescens* and *Trichoderma harzianum* (Khan and Mustafa, 2005; Chandel and Tomar, 2007) and soil application of *T. harzianum* alone or in combination with neem cake + carbendazim or *T. virens*, caboxin and combination of both were also found effective (Rukmini and Kapoor, 1997; Mishra *et. al.*, 2000; Mishra *et. al.*, 2004; Sharma *et. al.*, 2004; Tripathi *et.al.*, 2005; Sharma and Chandel, 2006; Nosir *et al.*, 2010). Naqvi and Ahmad (2012) reported higher inhibitory action of *P. fluorescens* (Psl-4) against *F.oxysporum* f.sp gladioli. Like wise, application of *F. moniliforme* could also effectively prevent corm rot infection (Woltz *et al.*, 1978; Magie,1980).

4. Foot rot and root rot (*Phytophthora cryptogea*, *Pythium ultimum*, *R. solani*) of gerbera: Infected plants become stunted, low in vigour and appear as if they were in water stressed. Foliage shows yellowing symptoms followed by wilting and complete die. Root rot and stem girdling by this pathogen at or below the soil line in common resulting in dark brown rot. According to Skrzypczak and Orlikowski (2006) pre-planting application of Chitosan (2% of commercial product of Biochikol 020 Pc) at 500 and 1000 µg/cm³ mixing with *T. viride* (1.5 x10⁶ spore/g) or drenching of Furalaxyl alone (a.i. of Fongard 25 WP) at125 µg/cm³ immediately after planting of gerbera significantly reduced the disease. Similarly, Orlikowski (1995) also reported about the effectiveness of soil application of *Trichoderma hamatum* and *T. viride* (600 g/m³) for the control of foot rot of gerbera. Jamwal and Jamwal(2012) also reported effectiveness of *T. viride* against root rot complex (*F.oxysporum f.sp. gerberae* and *Pythium irregularare*). Similarly, Rajendran *et al.*, (2014) suggested application of talc based formulation of *Pseudomonas florescens* and *T. viride* as root dip, soil application enriched with FYM and foliar spray for the management of Fusarium wilt of gerbera.

5. Bacterial blight (*X.axonopodis pv. dieffenbachiae*) of Anthurium: Necrotic lesions initiating from the margin of the leaf lamina with chlorotic halos were observed. Initial symptoms started as slight yellowish water soaked spots that later became necrotic and subsequently number of spots coalesced to form large dead necrotic blighted areas which gradually covered the entirety of the leaves. *In vitro* study revealed highest (82.63%) per cent
inhibition of X.axonopodis pv. dieffenbachiae by the combination of five bio-agents(talc based bio formulation, BIOGREEN 5) viz., T. viride, B. bassiana, M. anisopliae, P. fluorescens and B. thuringiensis and which was followed by the combination of three bio-agents (BIO-VEER) viz., T. harzianum, M. anisopliae, P.fluorescens (74.50%). In-vivo condition the same BIOGREEN-5 and BIO_VEER recorded lowest per cent blight incidence of 26.13 and 26.75%, respectively(Hazarika, 2019).

References:


Rajendran, L., Raja, P., Jegadeeswari, V., Santhi, V.P. and Selvaraj, N. (2014) *Pseudomonas florescens* and *Trichoderma viride* enriched bioconsotium for the management of *Fusarium* wilt in carnation and gerbera under protected cultivation. *Indian Phytopath.* **67**(1):77-81


Nematodes are defined as triploblastic, pseudocoelomic, unsegmented, bilaterally symmetrical organisms. They are morphologically, genetically and ecologically diverse organisms occupying almost all the niche than any other animals could be except arthropods. Depending on their feeding habit, they may be either **herbivores** (plant parasitic, belonging to order Tylenchida, as well as a few genera in Aphelenchida and Dorylaimida. With the help of a needlelike stylet at its mouth part they puncture the plant cells and feed on the cell sap), **bacteriovore** (mostly lives free in soil and feed only on bacteria. In these nematodes, the "mouth", or stoma, is a hollow tube for ingestion of bacteria. They are helpful in decomposing the soil organic matter), **fungivore** (feeds on fungi and uses a stylet to puncture fungal hyphae. Many members of the order Aphelenchida are in this group. They are also very important in decomposition), **omnivore** (a few kinds of nematodes may feed on more than one type of food material such as fungal spores as well as bacteria. They mostly belong to order Dorylaimida and may feed on fungi, algae, and other animals) or **predators** (they feed on other soil nematodes and on other animals of comparable size. They feed indiscriminately on both plant parasitic and free-living nematodes. Mononchida, is exclusively predacious, although a few predators are also found in the Dorylaimida and some other orders). Herbivores are responsible to cause diseases to the plants, while rest other is basically beneficial to mankind. A group of nematodes, commonly known as entomopathogenic nematodes are also beneficial to mankind as they kill the insect pests of crops.

**What is Entomopathogenic Nematode:** Nematodes, which can cause death of insects, are generally known as entomopathogenic nematode. The term *entomopathogenic* is derived from the Greek word, *entomon*, meaning **insect**, and *pathogenic*, meaning **causing disease**. There are many other **parasitic** nematodes that cause diseases in living organisms, entomopathogenic nematodes, are specific in only infecting insects.
Altogather seven nematode families, viz., Mermithidae, Allantonematidae, Sphaerularidae, Tetradonematidae, Phaneopsitylenchidae, Steinernematidae and Heterorhabditidae are pathogenic to insects. Among these seven families nematodes belonging to the genera *Steinernema* Travassos, 1927, *Heterorhabditis* Poinar, 1976 and *Neosteinernema* Nguyen and Smart, 1994 (Rhabditida : Nematoda) belonging to Steinernematidae and Heterorhabditidae are commonly referred to as entomopathogenic nematodes and most widely used biocontrol agents among nematodes.

Entomopathogenic nematodes (EPNs) live parasitically inside the infected insect host, and so they are termed as *endoparasitic*. They infect many different types of insects living in the soil like the larval forms of moths, butterflies, flies and beetles as well as adult forms of beetles, grasshoppers and crickets.

**A brief History of Entomopathogenic Nematode (EPN):** Nematode parasites of insect are known since 17 century, but serious consideration in management of insect pest of crops received due attention only during 1930. Steiner (1923) described the first EPN as *Aplectana kraussei* (= *Steinernema kraussei*) and at that time was considered no more than a curiosity whose systematic position was problematic. Glasser and Fox in 1929 isolated one nematode infecting grubs of Japanese beetle (*Popillia japonica*) at Haddonfield, New Jersey. Glasser and his colleagues cultured this nematode, produced in sufficient number and then applied in 73 different field plots in New Jersey against Japanese beetle in the year 1930. They recorded that parasitism of this nematode in the grub of Japanese beetle ranged from 0.3 – 81 per cent. They also observed that this nematode had the ability to persist in the field up to 8.5 years. This nematode species was described as *Neoaplectana (= Steinernema) glaseri* (second EPN described) by Steiner (1929). The work of Glasser and his associates open a door to the biological control of insect pests using nematodes. But till 1970, this endeavor did not get much attention from the researchers. During 1960s and 1970s, when US Environmental Protection Agency withdrew many pesticides from the market, and then the interest to work on EPN again received momentum.

In India, much of the earlier work was confined to the use of exotic strains of *S. carpocapsae*, *S. glaseri*, *S. felitae* and *H. bacteriophora*. The work on Steinernematids in India was first started in 1966. The *Heterorhabditid* group has been relatively lately identified from
Tamil Nadu, India. Some efforts were also made to isolate the indigenous populations of EPN, of which have been described as new species viz., *H. indica* Poinar et al. (1992) and *S. thermophilum* Ganguly and Singh (2000). A few strains have been identified as *S. carpocapsae, S. felitae, S. biocornutum, S. glaseri, S. riobrave, S. siamkayai, S. tami, H. bacteriophora* and *H. indica*. The symbiotic bacteria isolated from *S. thermophilum* has also been identified and described as *Xenorhabdous indica*, the first new species from this genus in India. Another bacterium associated with *S. thermophilum*, perhaps of host’s origin, was also described as *Providencia vermicola*.

**Life cycle:** The entomopathogenic nematodes under Steinernematids and Heterorhabditids follow similar pattern of life cycle, but the only difference between the life cycles of *Heterorhabditis* and *Steinernema* is occurred in the first generation. *Steinernema* species are amphimictic and therefore, for successful reproduction they require the presence of males and females. On the other hand, *Heterorhabditis* species are hermaphroditic and able to reproduce in the absence of conspecifics. The free-living, non-feeding developmentally arrested infective juveniles of these nematode species have attributes of both insect parasitoids and predators, they have chemoreceptors and are motile, like pathogens, highly virulent, killing their hosts quickly, and can be cultured easily and have a high reproductive potential. When a host has been located, the nematodes penetrate into the insect body cavity, usually via natural body openings (mouth, anus, spiracles) or areas of thin cuticle. Wang and Gaugler (1999) compared the penetration behavior of *S. glaseri* and *H. bacteriophora* into *Popillia japonica* larvae and found that *S. glaseri* penetrated primarily through the gut. *H. bacteriophora* was not efficient at penetrating the gut, presumably because of the thick peritrophic membrane, but penetration through the intersegmental membranes of the cuticle. Once in the body cavity, a symbiotic bacterium (*Xenorhabdus* for steinernematids, *Photorhabdus* for heterorhabditids, which are motile, Gram-negative, facultatively anaerobic rods in the family Enterobacteriaceae) released from the nematode gut, they multiplies rapidly and causes death to the infected insects due to septicemia. The nematodes feed upon the bacteria and liquefying host, and mature into adults. Steinernematid infective juveniles may become males or females, where as Heterorhabditids develop into self-fertilizing hermaphrodites although subsequent generations within a host
produce males and females as well. The life cycle is completed in a few days, and thousands of new infective juveniles emerge in search of fresh hosts.

**Mode of Action:** EPNs are generally found to be mutually associated with bacteria belonging to the family Enterobacteriaceae. The bacterium carried by Steinernematidae is usually a species of the genus *Xenorhabdus*, and that carried by Heterorhabditidae is a species of *Photorhabdus*. The third juvenile stage of EPNs is referred to as the “infective juvenile” (IJ) or the “dauer” stage. IJs of both genera release their bacterial symbionts in the insect host body and develop into fourth-stage juveniles and adults. The insects die mainly due to a septicemia. Sometimes a bacterial toxaemia precedes the resulting septicemia.

Infective juvenile is the only free-living stage and can survive in soil for several months until susceptible insects are encountered. IJs locate and infect suitable insect hosts by entering the insect host through the mouth, anus, spiracles or thin parts of the host cuticle. After infection, the symbiotic bacteria are released into the insect haemocoel, causing septicaemia and death of the insect. When an insect host is infected in the soil by an EPN, development and reproduction within the cadaver can take 1–3 weeks.

Only the free-living, IJ stage is able to target insect host and the only form found outside of the host. EPNs occur naturally in soil and locate their host in response to carbon dioxide, vibration and other chemical cues, and they react to chemical stimuli or sense the physical structure of insect’s integument.

The insect cadaver becomes red if the insects are killed by Heterorhabditis and brown or tan if killed by Steinernematids. The colour of the insect host body is indicative of the pigments produced by the monoculture of mutualistic bacteria growing in the host insects.

**Host finding mechanism:** Entomopathogenic nematodes have two foraging strategies, *viz.*, ambushers or cruisers. The foraging strategies of EPNs varies between species. For example, *Steinernema carpocapsae* is ambushers. It has an energy-conserving approach and lie on soil in wait to attack mobile insects (nictitating) in the upper layer of the soil. On the other hand, *Steinernema glaseri* and *Heterorhabditis bacteriophora* are examples of cruisers. They are highly active and generally subterranean, moving significant distances using volatile cues and other methods to find their host underground. They are also successful to attack white grubs (Scarab beetles), which are less mobile. Some other species, like *Steinernemafeltiae* and
Steinernema riobrave, use an intermediate foraging strategy (combination of ambush and cruiser type) to find their host.

Advantages of entomopathogenic nematodes:

a) Non polluting and environment friendly

b) No harmful effect to mammals or plants. EPNs and their associated bacterial symbionts are safe to warm-blooded vertebrates, including humans, however detrimental to cold blooded organisms at high doses.

c) Highly effective against insects than other bioagents. Most biological agents require days or weeks to kill the host, while EPN radiply kill their hosts, within 24–72 hours.

d) Compatible with most of the pesticides

e) Can be used with conventional equipments

f) EPN are easy and relatively inexpensive to culture,

g) Can survive from several weeks up to months in the infective stage,

h) They are able to infect numerous insect species, occur in soil. Importantly, most of the species are host specific and therefore, they are likely to cause no indiscriminate mortality.

i) Foliar applications of nematodes have been successfully used to control the quarantine leafeating caterpillars as Tuta absoluta, Spodoptera littoralis, Helicoverpa armigera, Pieris brassicae on several crops and have the potential for controlling various other insect pests. However, they are not well suited for foliar application

j) Application of EPNs does not require masks or other safety equipment like chemicals.

k) EPN usually reproduce within insect hosts and produced thousands of infective juveniles by one infected insect. Thus, a single application may provide continuous control over a long period of time.

Application of Entomopathogenic Nematodes: Entomopathogenic nematodes can be applied with nearly all agronomic or horticultural field equipment including pressurized sprayers, mist blowers, and electrostatic sprayers or as aerial sprays. The application equipment used depends on the cropping system, and in each case there are a variety of handling considerations including volume, agitation, nozzle type, pressure and recycling time, system environmental conditions, and spray distribution pattern. It is important to ensure adequate agitation during application. For small plot applications, hand-held equipment (e.g., water cans) or back-pack sprayers may be
appropriate. When nematodes are applied to larger plots, a suitable spraying apparatus such as a boom sprayer should be considered. Conceivably, applicators could also be using other methods such as through microjet irrigation systems, subsurface injection or baits. Various formulations for entomopathogenic nematodes may be used for applying EPNs in aqueous suspension including activated charcoal, alginate and polyacrylamide gels, clay, peat, polyurethane sponge, vermiculite, and water dispersible granules (WDG).

**Compatibility:** The infective juveniles (IJs) of entomopathogenic nematodes can tolerate short-term exposures (2-24 h) to many chemical and biological insecticides, fungicides, herbicides, fertilizers and growth regulators, and can thus be tank-mixed and applied together. This offers a cost-effective alternative to pest control, and facilitates the use of nematodes in integrated pest management systems. However, the actual concentration of the chemical to which the nematodes will be exposed will vary depending upon the application volume and system used. Compatibility has been tested with so far over 100 different chemical pesticides. In fact, in some cases, combinations of chemical agents with nematodes results in synergistic levels of insect mortality. However, chemicals like aldicarb, carbofuran, diazinon, dodine, methomyl, and various nematicides should be avoided to mix with EPN formulation as they do not have any compatible interaction. Specific interactions can vary based on the nematode and host species and application rates. The organophosphate oxamyl increased *S. carpocapsae* efficacy against *Agrotis segetum* synergistically, but only in fumigated soil, probably by enhancing the nematodes nictation behavior. Furthermore, even when a specific chemical pesticide is not considered compatible, use of both agents (chemical and nematode) can be implemented by maintaining an appropriate interval between applications (e.g., 1 – 2 weeks). Prior to use, compatibility and potential for tank-mixing should be based on manufacturer recommendations. Combination of two nematode species may provide the nematode species may coexist, if the target differs in susceptibility against the search strategies and/or pathogenicity of the two nematodes. Similarly, entomopathogenic nematodes are also compatible with many pathogens like *Bacillus thuringiensis* (Bt) against several lepidopterous pests resulted in additive and antagonistic interactions. Interactions range from antagonism to additivity or synergism depending on the specific combination of control agents, target pest, and rates and timing of application. Synergism between *Heterorhabditis* spp. and *S. glaseri* and neonicotinoid
imidacloprid in scarab larvae has been documented. Imidacloprid reduces the grub’s defensive behavior resulting in increased nematode attachment and penetration. Nematodes are generally compatible with chemical fertilizers as well as composted manure though fresh manure can be detrimental.

**Conclusion:** EPNs are considered as excellent biocontrol agents for insect pests. However, due to host specificity, it is suggested to match the right nematode species against the target pest. Biotic agents including nematode pathogens, predators and other soil organisms, as well as abiotic factors such as ultraviolet radiation, soil moisture/relative humidity, temperature, etc. are reported to affect EPN application efficacy. Recently, improvement of nematode formulation, application equipment or approaches, and strain improvement have been made to enhance EPN application efficacy. More survey work throughout the globe has been adding diversity of EPN (with new species and/or new strains). Region specific species and/or strain of EPN may be more beneficial in controlling the insect pests. Approaches toward lowering product costs, increasing product availability, improving efficacy and carryover effect will stimulate the extensive use of EPNs as biocontrol agent of insect pests, and will serve to reduce chemical insecticide and will contribute to the stabilization of crop yields and the environment.

********
Practical No. 1

Title: Isolation of biocontrol agents from soil sample

Arti Kumari, Gaurav Phookan, Bishal saikia, Sunita Dutta, Pranab Dutta
Department of Plant Pathology, Assam Agricultural University, Jorhat-785013, Assam

Materials required:

- Soil sample
- Sterile water
- Test tubes
- Sterilized Petri plates
- Surface sterilizing agent
- Spirit lamp
- PDA slants and plates
- Inoculating needle/loop

Procedures:

1. Collect 250 g of soil sample from root rhizosphere (15-30 cm depth from the surface).
2. Shade dry the soil sample and sieve.
3. Prepare the stock solution by adding 1 g of soil sample in 10 ml of sterile distilled water.
4. From the stock solution take 1 ml of aliquot and transfer to a test tube containing 9 ml of sterile distilled water and mixed thoroughly.
5. Transfer 1ml of aliquot to another test tube containing 9 ml of sterile distilled water and shake well.
6. Continue upto 7 to 8 fold dilution as required.
7. Finally placed 1 ml of suspension from the final dilution in sterilized petri plates in molten PDA and mixed thoroughly by rotating the petriplate clockwise and anticlock direction for 5-6 times.
8. Incubate the petriplate at 25+1°C for 2-3 days in a BOD incubator.
9. Observe the colony growth at 24 hrs interval for 3-4 days
10. The desired culture should be picked up and sub culturing should be done on fresh medium for further use.

********
Practical No. 2

Title: Purification of fungal/bacterial antagonists

Arti Kumari, Gaurav Phookan, Amal J. Debnath, Partha P. Sarmah, Longkiri Rongpi and Pranab Dutta

Department of Plant Pathology,
Assam Agricultural University, Jorhat-785013, Assam

Materials required

Sterilized Petri plates, Cork borer, PDA media, Dehydrated alcohol, Antibacterial antibiotic (Streptomycin 0.1%), Inoculating needle, Spirit lamp, Absorbent and non absorbent cotton, Brown paper, Rubber band etc.

Procedure

1. Switch on the UV lamp of the laminar air flow desk for 15 minutes.
2. Surface sterilize the working desk as well as hands with alcohol.
3. Light the spirit lamp and switch on the laminar flow.
4. Keep the sterilized Petri plates and/or slants containing nutrient media, alcohol, inoculation needle, absorbent cotton, PDA media etc., on the working bench.
5. Melt the nutrient media prepared in Erlenmeyer’s flasks in micro oven.
6. Add 0.1% of streptomycin solution to the media just before pouring in to petriplates and mix it well.
7. Pour the molten media in petriplates and allow the media to cool down.
8. Place a mycelia disc 5 mm diameter (cut out with cork borer) of fungal antagonist at the centre of the plate containing nutrient media or transfer a fragment of mycelia to the plate by inoculating needle or for bacteria streak a loopful of bacterial colony on the petriplate containing media.
9. Inoculate the inoculated plates at 28± ⁰C for 2-3 days in BOD incubator.

*******
# LIST OF PARTICIPANTS

ICAR Sponsored Short Course on

“Making Greater Use of Bio-control Agents for Organic Agriculture”

Date: 4-13 Nov, 2019

<table>
<thead>
<tr>
<th>SL. NO.</th>
<th>PARTICIPANT'S NAME</th>
<th>ADDRESS AND PHONE NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Dr. DEVENDRAKUMAR RAMANBHAI PATEL Assistant Professor (Agril. Entomology)</td>
<td>College of Agriculture, NAU, Bharuch Ph No.- 09427479514</td>
</tr>
<tr>
<td>2.</td>
<td>Dr. REENA SINHA SCIENTIST (ENTOMOLOGY)</td>
<td>SKUAST-J, Jammu Ph No.- 09419153105</td>
</tr>
<tr>
<td>3.</td>
<td>Dr. RAJESH KUMAR SAHU Associate Professor (Agril. Extension)</td>
<td>I.G.K.V., Raipur Ph No.- 09425222699</td>
</tr>
<tr>
<td>4.</td>
<td>Dr. GIRADHARLAL RATNABHAI BHANDERI Assistant Research Scientist (Agril. Entomology)</td>
<td>M.C.R.S., NAU, Surat Ph No.- 09662530036</td>
</tr>
<tr>
<td>5.</td>
<td>Dr. RAKESHKUMAR RANCHHODBHAI PATEL Assistant Professor (Plant Pathology)</td>
<td>College of Agriculture, NAU, Bharuch Ph No.- 09824243246</td>
</tr>
<tr>
<td>6.</td>
<td>Dr. DIPAKKUMAR MANGANLAL Associate Professor (Plant Pathology)</td>
<td>College of Agriculture, NAU, Bharuch Ph No.- 09909015725</td>
</tr>
<tr>
<td>7.</td>
<td>Dr. SITESH CHATTERJEE Assistant Entomologist</td>
<td>Rice Research Station, Chinsurah, West Bengal Ph No.- 08910542751</td>
</tr>
<tr>
<td>No.</td>
<td>Name</td>
<td>Designation</td>
</tr>
<tr>
<td>-----</td>
<td>-------------------------------------------</td>
<td>--------------------------------------------</td>
</tr>
<tr>
<td>8.</td>
<td><strong>Dr. CHIRASREE GANGOPADHYAY</strong></td>
<td>Assistant Entomologist</td>
</tr>
<tr>
<td>9.</td>
<td><strong>Dr. ROOPA S. PATIL</strong></td>
<td>Scientist (Agril. Entomology)</td>
</tr>
<tr>
<td>10.</td>
<td><strong>Dr. BHAGYASHREE KHAMARI</strong></td>
<td>Assistant Professor (Plant Pathology)</td>
</tr>
<tr>
<td>11.</td>
<td><strong>Dr. NIRMALA MAIBAM</strong></td>
<td>(Plant Pathology)</td>
</tr>
<tr>
<td>12.</td>
<td><strong>Dr. H. KESAVA KUMAR</strong></td>
<td>Scientist (Nematology)</td>
</tr>
<tr>
<td>13.</td>
<td><strong>Ms. CHENGLOUNGANBI THOUDAM</strong></td>
<td></td>
</tr>
<tr>
<td>14.</td>
<td><strong>Ms. JOYARANI PEGU</strong></td>
<td>Assistant Professor (Plant Pathology)</td>
</tr>
<tr>
<td>15.</td>
<td><strong>Mrs. MEGHALI BARUA</strong></td>
<td>Assistant Professor (Plant Breeding and Genetics)</td>
</tr>
<tr>
<td>16.</td>
<td><strong>Dr. KHANIN PATHAK</strong></td>
<td>Assistant Professor (Bio-Chemistry and Agricultural Chemistry)</td>
</tr>
<tr>
<td>17.</td>
<td><strong>Ms. SUKANYA GOGOI</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PhD. Scholar</td>
<td>University, Jorhat University, Jorhat</td>
</tr>
<tr>
<td>----</td>
<td>-------------------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ph No.-08638529846</td>
</tr>
<tr>
<td>18</td>
<td>Dr. SAVANI AJIT KUMAR</td>
<td>Assam Agricultural University, Jorhat University, Jorhat</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ph No.- 09441872003</td>
</tr>
</tbody>
</table>
ICAR SPONSORED 10 DAYS SHORT COURSE

COURSE DIRECTOR
Dr. PRANAB DUTTA
DEPARTMENT OF PLANT PATHOLOGY, ASSAM
ASSAM AGRICULTURAL UNIVERSITY, JORHAT - 785013, ASSAM
EMAIL: pranabdutta74@gmail.com
Web: www.aau.ac.in  Phone: +919678906650