Biological Control of Nursery Diseases of Forest Crops : An Ecofriendly Arsenal for Plant Health Management

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Abstract

Forest diseases can cause great damage worldwide especially in case of loss of vulnerable forest ecosystems. In addition to biotic factors, abiotic factors such as climate change also aggravates forest decline syndrome primarily caused by forest diseases. The increased dependence on synthetic chemicals has affected the ecosystem in much drastic way. The indiscriminate use of agrochemicals caused an adverse impact on environment, which takes place due to dumping nearly 2.5 million tons of pesticides every year. Use of beneficial microorganisms or biological control against forest-tree pathogens have not gained full momentum yet there is huge unexplored scope in this direction. The obstruction lies in their application strategy and the fact that biocontrol agent needs to establish prior commensalism interaction with trees in order to prevent pathogens from invading trees. Because of having diverse mode of action of biocontrol agents like disease suppression ability, growth enhance ability and activation of defense mechanism of host plant, pathogen/s get less chance to develop resistance against a particular biocontrol agents. There are different successful example of management of plant diseases by biocontrol agents preferably with the indigenous strains, some of which are cited in this article. As registration process of effective biocontrol agents does not involve strict regulations in the country like India. This article reflects the available information related to important diseases of forest nurseries with some successful example for its management with biocontrol agents, their mode of action, formulation and registration procedure. This article will enable researchers to explore ecofriendly management practices against different diseases of forest nurseries in a better way which are constantly challenged in the natural conditions

Keywords

Biological control, forest crops, plant health management

Introduction:

Forest trees and woodlands as per estimates of FAO (2011), cover nearly about 40% of the total earth's terrestrial surface that forms a major part of global biomass providing habitat for large number of animals and plant species with varying levels of association (FAO, 2011). As per estimates of Food and Agricultural Organization of United Nations in 2010, Indian forest cover was nearly 68 million hectares or 22 per cent of the country's area in 2010 as compared to an increase upto 69.8 million hectares in 2012, marking an increased forest cover of 5,871 sq. km. in 2 years (State Forest Report, 2013). The trend in forest cover has reportedly marked a significant increase of 8,021 sq. km. from 2015 to 2017.

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Nearly 25 per cent (one fourth) of India's total land area is under forest cover accounting for 708,273 sq. km. of total forest cover and 73,815 sq. km. of tree cover, that is 21.54 % and 2.85 % of India's geographical area, ranking 10th in the world in terms of forest cover (India State Forest Report, 2019). In 2018, the total forest and tree cover in India increased to 802,088 sq. km. that is 24.39 per cent of total geographic area. As per latest India State Forest report (ISFR, 2019), the total forest cover and tree cover of the country in 2019 was reported as 807,276 sq. km as compared to 802,276 sq. km in 2017, marking a significant increase of 5,188 sq. km. from 2017 to 2019. Three states reportedly Andhra Pradesh, Karnataka and Kerala have contributed most to increasing forest cover with 2,141 sq. km., 1,101 sq. km and 1,043 sq. km. respectively.

While the overall forest and tree cover marked an increase at national level but the total forest cover in north-eastern region (NER) of India showed decline in forest cover *i.e.* 171,306 sq. km. which is 65.34 per cent of its geographical area. The extent of decline in forest cover in the region showed contraction of approximately 630 sq. km. forest area marking consistent decline from 2013 to 2015. Out of eight NER states, Assam and Manipur marked an increase in forest cover with 567 sq. km. and 263 sq. km. respectively, while the forest cover shrunk in Mizoram (531 sq. km.), Nagaland (450 sq. km.), Arunachal Pradesh (190 sq. km.), Tripura (164 sq. km.) as well as Meghalaya (116 sq. km.).

In terms of forest cover, India is one of the 17 mega diverse regions in the world, including tropical evergreens, tropical deciduous, swamps, mangroves, sub-tropical, montane, scrub, sub-alpine and alpine forests. Forestry in India is a significant rural industry, a major environmental resource which is more than just about wood and fuel. India has a thriving non-wood paper forest products industry, which produces latex, gums, resins, essential oils, handicrafts, incense sticks as well as medicines. About 50 per cent of the total revenue from forest industry in India is from non-wood forestry sector, also providing a significant supplemental income to over 400 million people in India (FAO, 2002). In 2002, forest industry contributed 1.7 per cent to India's GDP but largely dropped to 0.9 per cent in 2010.

The importance of forestry largely accounts for overall socio-economic development of the country through adequate supply of quality planting materials aiming forest rehabilitation and development programme. However, the major problem encountered while implementation of such forest rehabilitation and development programme are the shortage of quality planting materials of commercial importance for e.g. timber. Instead, establishment of forest plantations, reforestation programme, enrichment planting in logged-over forests, planting of open spaces, degraded areas and roadsides are the major operations carried out through such planting materials.

Trees, shrubs, forb and grass seedlings serves as the foundation of healthy forest as well as terrestrial ecosystems playing a major role in mitigating worldwide crisis due to forest land degradation. High-quality, nursery grown plants often plays a critical role in successful implementation of forest and landscape programme in order to produce healthy, functional, sustainable and resilient ecosystems. They provide multiple ecological, social and economic benefits that include sustainable livelihoods, food security, carbon sequestration, shelterbelts, wind breaks, agroforestry production, quality air, fuelwood, medicine, recreation as well as aesthetic values. Development of high-quality nursery

plants relies on target plant concept including key factors *viz.* appropriate genetic seed sources, nursery environment (annual temperature, precipitation pattern, water quantity and quality), supplies, equipment *etc* for successful nursery production.

Owing to its numerous applications, growing forest nurseries and seedlings play an important role in keeping nation's forest lands productive, therefore, the need for more land coverage under forests is steadily increasing. Currently, million acres of commercial forestland under private, state, or federal ownership are either non stocked or poorly stocked, where, most of these non-productive lands are created each year either by fire, pests or timber harvests. In such cases, re-planting of under stocked forestlands can be an advantageous strategy to avoid or reduce timber shortage in nearing future.

The factors that influence quality, quantity as well as timber supply include biotic factors such as seedling diseases caused by fungi, bacteria, and nematodes. These disease incitants may remain active from the time of nursery sowing until entire planting season that may kill seedlings either directly, or by stunting or malformation. Therefore, nursery diseases may not only affect the market acceptability by lowering field survival of planted seedlings but also poses a major threat to our forestlands by accidental incorporation of new disease in previously unaffected areas. The economic losses occurring due to diseases of forest nurseries includes more than just cost of producing dead and culled seedlings but also cost for secondary site preparation of the plantation, resulting from unavailability of enough planting materials.

Forest diseases can cause great damage worldwide especially in case of loss of vulnerable forest ecosystems. Decline in productivity of cork oak *(Quercus suber)* takes place by multiple fungal pathogens *viz. Diplodia corticola* (Fam: Botryosphaeriaceae), *Biscogniauxia atropunctata* (Fam: Xylariaceae)*, Phytophthora cinnamomi* (Fam: Peronosporaceae)*, Armillaria solidipes* (Fam: Physalacriaceae) (Fernandes *et al.,* 2014; Cimmino *et al*., 2016). In addition to biotic factors, abiotic factors such as climate change also aggravates forest decline syndrome primarily caused by forest diseases (Sturrock *et al.,* 2011). The list of common nursery diseases of forest crops along with their causal organisms have been listed in Table: 1.

Table 1: Common nursery diseases of forest crops with their causal organism

Name of the Crops	Botanical Name	Name of the disease	Causal Organism
Neem	*Azadirachta indica*	Damping off	*Pythium, Phytophthora, Fusarium* and *Rhizoctonia*
		Leaf Web Blight	*Rhizoctonia solani*
		Leaf spot and blight:	*Colletotrichum gloeosporioides*
		Alternaria Leaf spot and blight	*Alternaria alternata*
		Leaf spot	*Pseudocercospora subsessilis*
		Powdery Mildew	*Oidium azadirachtae*

Name of the Crops	Botanical Name	Name of the disease	Causal Organism
Neem	*Azadirachta indica*	Bacterial leaf spot	*Xanthomonas azadirachtii* and *Pseudomonas viticola*
		Seedling wilt	*Fusarium solani*
Albizia	*Albizi*a spp.	Leaf spot and blight	*Cercospora albizziae, Colletotrichum* sp., *Alternaria alternata, Camptomeris albizzia, Pleiochaeta setosa* and *Epicoccum* sp.
		Seedling wilt	*Fusarium oxysporum*
		Rhizoctonia Leaf Web Blight	*Rhizoctonia solani*
		Leaf Rust	*Ravenalia clemensiae*
		Little leaf disease	*Phytoplasma*
Gomari	*Gmelina arborea*	Foot rot	*Fusarium oxysporum*
		Poria root-rot	*Poria rhizomorpha*
		Root rot and Collar rot	*Sclerotium rolfsii*
		Leaf spot	*Pseudocercospora ranjita* (Assam), *Deptoshaeria gmelinae, Phoma tropica, Alternaria alternata* and *Macrophomina phaseolina* (Madhya Pradesh), *Corynespora cassicola* is from Kerala
		Leaf and shoot blight	*Glomerella cingulata*
		Powdery Mildew	*Phyllactinia suffulta* var. *gmelina*
		Phoma stem rot	*Phoma nebulosa*
		Canker disease	*Thyronectria pseudotricha* and *Hendersonula toruloidea*
		Phomopsis Die back	*P. gmelinae*
Bhimal	*Grewia optiva*	Leaf spot	*Curvularia lunata*

Name of the Crops	Botanical Name	Name of the disease	Causal Organism
Malabar neemwood	*Melia dubia*	Collar rot and seedling web blight	*Pythium* sp. *Rhizoctonia solani*
		Leaf spots	*Colletotrichum dematium* and *Cylindrocladium ilicicola*
		Die back	*Botryodiplodia theobromae*
		Root rot	*Fusarium*
		Leaf blight	*Helminthosporium, Alternaria*
Chandan	*Santalum album*	Seed rot	*Alternaria, Aspergillus*
		Damping-off	*Phytophthora, Rhizopus*
		Wilt	*Fusarium* sp.
		Canker disease	*F. oxysporum*
		Seedling rot	*F. oxysporum*
Whistling pine	*Casuarina equisetifoloa*	Damping off	*Fusarium lacertarum*
		Root rot and seedling blight	*Rhizoctonia* sp.
		Bacterial wilt	*Ralstonia solanacearum*
Poplar	*Populus ciliata*	Canker disease	*Septoria musiva, Cytospora chrysosperma, Phomopsis macrospora*
		Leaf spot or anthracnose	*Marssonina brunnea*
		White and brown rot	*Junghuniana vincta, Rosellinia necatrix, Ganoderma applanatum, Trametes*
		Crown gall	*Agrobacterium tumefaciens*
Willow	*Salix* spp.	Bacterial twig blight	*Psuedomonas saliciperda*
		Crown gall	*A. tumefaciens*
		Canker disease	*Cytospora* sp.
		Powdery mildew	*Phyllactinia guttata Uncinula salicis*
		Root rot	*Phymatotrichum omnivorum*
		Leaf and twig spot	*Marssonina* sp.
		Leaf spot and blight	*Fusicladium pongamiae*
Indian beech	*Pongamia pinnata*	Leaf Rust	*Ravenelia hobsoni*
		Powdery mildew	*Oidium* sp.

Name of the Crops	Botanical Name	Name of the disease	Causal Organism
Indian cedar	*Toona ciliata*	Leaf blight	*Phytopthora*
		Tar spot	*Rhytisma acerinum*
		Cercospora leaf spot	*Cercospora* cf. *alchemillicola*
Teak	*Tectona grandis*	Leaf blight	*Rhizoctonia solani.*
		Leaf rust	*Olivea tectonae*
		Leaf spots	*Rhizoctonia solani*
		Powdery mildew	*Phyllactinia corylea*
Sal	*Shorea robusta*	Root rot	*Polyporus shoreae*
		Leaf spots	*Cylindrocladium floridanum*
		Leaf blight	*C. scoparium*
Shisham	*Dalbergia sissoo*	Leaf spot	*Cercospora sissoo, Colletogloeum sissoo, Phyllachora dalbergiae, Phyllachora spissa, Phyllosticta sissoo, Mycosphaerella dalbergiae, Myrothesicum roridum* and *Alternaria alternata*
		Leaf Blight	*Rhizoctonia solani.*
		Powdery Mildew	*Phyllactinia dalbergiae*
		Rust disease	*Maravalia achroa*
Australian Wattle	*Acacia* spp.	Leaf blotch	*Alternaria alternata, Curvularia* sp.
		Leaf spot	*Glomerella cingulata, Pestalotiopsis uvicola, Guignardia* sp.
Golden shower	*Cassia* spp.	Leaf blotch	*G. cingulata, Phomopsis* sp., *Curvularia pallescens, Phoma glomerata*
		Leaf spot	*Pestalotiopsis tecomicola*
Arjuna	*Terminali arjuna*	Leaf spot	*Phomopsis* sp., *Guignardia* sp.
		Leaf blotch	*Pestalotiopsis maculans*
Bamboo	*Bambusa* spp.	Damping off	*Pythium, Phytophthora, Fusarium* and *Rhizoctonia*
		Seedling spear rot, Seedling wilt and Web blight	*Rhizoctonia solani*

Name of the Crops	Botanical Name	Name of the disease	Causal Organism
Bamboo	*Bambusa* spp.	Leaf rust	*Dasturella divina*
		Leaf spot	*Colletotrichum gloesporoides*
		Leaf spot	*Curvularia pallescens*
		Leaf tip blight	*Alternaria alternata*
Kadam	*Anthocephalus cadamba*	Damping-off	*Fusarium* and *Pythium*
		Sudden death	*Cylindrocladium parvum*
Pines	*Pinus* spp.	Dieback disease	*Pseudomonas syringae*
		Tip blight	*Diplodia* sp.
		Root rot	*Verticicladiella procera* *Phytophthora* spp.
		Damping-off	*Fusarium oxysporum*
Champak	*Michelia champaca*	Leaf spot	*Colletotrichum fioriniae*
		Leaf spotting and blight	*R. solani*
Wattles	*Acacia* spp.	Powdery mildew	*Oidium* sp.
		Black mildew	*Meliola* sp.
		Leaf spot	*Colletotrichum* sp. *Phaeotrichoconis* sp. *Cercospora* sp.
		Canker	*Nectria* sp.
		Root rot	*Ganoderma* sp.
Hollock	*Terminalia myriocarpa*	Leaf blight	*Fusarium solani*
Olive	*Olea* spp.	Anthracnose	*Colletotrichum acutatum*

a) Damping-off:

The damping-off disease is categorized into pre-emergence and post-emergence damping-off of seedlings. The characteristic symptoms of pre-emergence damping-off includes killing of seedlings before they emerge or germination. Whereas, the classical symptom of post-emergence damping-off includes decay of the hypocotyl at the ground level at their cotyledon stage leading to collapse of seedlings (Plate 1). Species of fungal pathogens *viz. Pythium, Phytophthora, Fusarium, Rhizoctonia* etc. are responsible for causing damping-off diseases of *Azadirachta* sp.*, Santalum* sp.*, Casuarina* sp.*, Bambusa* sp.*, Anthocephalus* sp.*, Pinus* spp. *etc.*

Plate 1: Douglas-fir seedling with damping off symptoms (right) caused by *Pythium irregulare* as compared to control (left) (Courtesy: Weilang *et al*., 2015).

b) Wilt:

The characteristic symptoms of wilt disease started from the tip of the plant and along the margin of the leaves. Wilt is a systemic disease where the entire individual or its parts exhibit wilting symptom of foliage in acropetal succession up to the shoot (Plate 2). The leaves become yellow, lose turgidity and falls off, adversely affecting translocation of water and nutrients. As disease progresses, it advance downward causing defoliation and eventual death of seedlings showing characteristic symptom of vascular discoloration in outer layers of seedlings. Seedling wilt of forest nurseries *viz. Azadirachta* sp., *Albizia* spp., *etc.* is caused by fungal pathogen *viz. Fusarium solani, F. oxysporum* as well as bacterial pathogen.

Plate 2: Wilt of oak *Quercus* spp. caused by *Ceratocystis fagacearum* (Source: drydenwire.com; inspection.gc.ca)

c) Root rot:

The characteristics symptoms of root rot disease appear as gradual death and drying up of leaves. The infection first appear in fine feeder roots, then gradually move

into the main tap root as well as root crown, where the cambium turns brownish to blackish discoloration. Older trees may develop cankers on trunk accompanied by split bark and oozing pitch while lower branches wilt, turn brown and die back (Plate 3). Various fungal pathogens belonging to genera *Sclerotium*, *Fusarium, Rhizoctonia, Phymatotrichum, Polysporus, Phytophthora, Verticicladiella, Ganoderma* are responsible for root rot disease of *Acacia* spp., *Pinus* spp., *Shorea* spp., *Salix* spp., *Melia* spp., *Gmelina* spp. *etc.*

Plate 3: White root rot of coniferous and deciduous trees caused by *Heterobasidion* spp. (Source: first-nature. Com)

d) Canker:

i. Canker of *Platanus* spp.:

Canker disease of *Platanus* spp. is caused by wound colonizing fungus *Ceratocystis platani.* The characteristic symptoms include cankers, xylem staining, bluish-black to reddish-brown discoloration of sap wood and necrosis of inner bark, thus, restricting water flow throughout the tree that eventually leads to death of the tree (Lehtijarvi *et al.,* 2018).

ii. Canker of *Populus* spp.:

Canker of *Populus* spp. is caused by multiple pathogens *viz. Cytospora chrysosperma, Phomopsis macrospors* and *Fusicoccum aesculi.* The characteristics symptoms includes turning of young twigs form brown, sunken, rough circle areas in the bark, which may spread to the larger branches. Large cankers may form on the branches and trunk (Plate 4). Orange/ orange-brown discolouration of bark is often seen exuding orange-brown viscous liquid. Fruiting bodies in the bark make the canker appear pimpled. In the later stages of infection, perithecial stroma form in the dead cankered areas (Ren *et al.,* 2013).

Plate 4: Canker of *Populus* spp. (Source: Commons. Wikimedia.org)

iii. Bleeding canker of horse chestnut:

Bacterium *Psuedomonas syringae* pv. *aesculi* and fungi *viz. Phytophthora cactorum, P. plurivora* etc are responsible for causing bleeding canker of horse chestnut *Aesculus hippocastanum.* The characteristic symptoms include rusty-red/brown/ black gummy ooze found on the bark, while, dead phloem under the bleeds which may appear mottled orange-brown. In extensive cases, affected areas

Plate 5: Bleeding canker of horse Chestnut (Source: wur.nl/en)

encircle the trunk or branch, leaf yellowing and defoliation may occur and eventual crown death (Plate 5). Fungal bodies may also be seen in areas of dead bark (Green *et al.,* 2010).

e) Leaf spot:

i. Cercospora leaf spot:

The disease appear on younger leaves as small to large, round to irregular spots, where, leaves may wither and drop early. Several species of *C. albizziae, C. alchemillicola, C. sisso* are responsible for causing leaf spot disease of *Toona* sp., *Dalbergia* sp., *Acacia* sp. *etc.*

ii. Marssonina leaf spot :

It occurs as small, brownish to black, irregular shaped spots or lesions that develop on leaves and twigs. The characteristics spots consist of white centres bearing acervuli as tiny blisters as well as yellow margin. Under severe conditions, spots may coalesce to form large lesions that can lead to defoliation and reduced growth (Plate 6). Various species of fungus *Marssonina viz. M. rubiginosa, M. kriegeriana* affects *Salix* spp., *Populus* spp. *etc.*

Plate 6: Marssonina leaf spot on white willow, *Salix alba* (Courtesy: Andrej Kunca, National Forest centre-Slovakia, Bugwood.org; forestryimages.org)

iii. Tar spot :

Tar spot usually appear as numerous, very thick, jet black, superficial, discrete spots that look like a drop of tar on upper surface of the leaves (Plate 7). The spots initially appear as yellow green spots that changes into 2-4 cm diam. black coloured, tar spot-like symptoms at later stage. It is caused by fungus *Rhytisma acerinum* on *Toona* spp. (Chandel and Kumar, 2017).

Plate 7: Tar spot of *Toona ciliata* (Chandel and Kumar, 2017)

iv. Anthracnose:

The characteristic symptoms of anthracnose disease initially appear as small necrotic spots, circular to semi-circular shaped, water-soaked that later becoming grayish white in the center with a dark brown margin bordered by tan halo (Plate 8). As the disease progressed, these spots coalesced and cause premature senescence as well as falling of leaves reducing ornamental value of forest

nurseries. Various species of fungal pathogen *Colletotrichum viz. C. gloeosporioides, C. dematium, C. fioriniae etc.* is responsible for causing anthracnose disease. It has been reported *Acacia* spp., *Michelia champaca, Bambusa* spp., *Dalbergia sissoo, Albizia* spp, *Azadirachta indica, Olea* spp. *etc.*

Plate 8: Anthracnose infection of immature berries (left), leaves (centre) and olive flowers (right) caused by *Colletotrichum acutatum* (Courtesy: Sergeeva *et al*., 2008; Sergeeva and Hart, 2010)

f) Sudden death:

The characteristic symptoms of sudden death of *Larix* spp., *Pinus* sp., *Dalbergia* sp., *Eucalyptus* sp., *Salix* sp., *Quercus* spp. *etc.* includes wilted, withered shoot tips with blackened needles. It mainly affects shoots and foliage causing branch dieback bearing numerous canker symptoms on branches as well as trunks (Plate 9). It is caused by an oomycetous fungi *Phytophthora ramorum* (Davidson *et al.,* 2003).

Plate 9: Sudden oak death caused by *Phytophthora ramorum* (Courtesy: Denman *et al*., 2014)

g) Powdery mildew:

Powdery mildew is caused by an ascomycetous fungus *viz. Oidium* sp. in seedlings of *Azadirachta* sp., *Acacia* sp., *etc.* The characteristics symptom includes appearance of white powdery growth on leaf surfaces that may become heavy late in the season, especially on tender leaves of sprouts (Plate 10). These white patches increases and coalesce spreading onto entire leaves causing severe damage to young seedlings.

Plate 10: Powdery mildew of *Acacia magnum* (Source: flickr.com)

h) Crown gall:

The crown gall disease of forest nurseries *viz. Populus ciliata* is caused by gram negative bacterium *Agrobacterium tumefaciens.* The characteristic symptoms include large, rough, woody swellings or galls on the lower part of the stem and crown of the plant (Plate 11). The infected plants may appear as deformed, stunted or even killed.

Plate 11: Crown gall of *Populus angustifolia* (Source: OSU Plant Clinic Image, 2014)

i) Dieback:

i. Chalara ash dieback:

The characteristic symptoms of Chalara dieback of *Fraxinus* spp. caused by *Hymenoscyphus fraxineus* includes dark brown/ orange lesions on leaves, diamond-shaped lesions that may occur on stems, which, if girdled, can cause wilting. The wood beneath lesions usually is strongly stained. Dieback can be seen throughout the crown, with dieback shoots and twigs at the edges of crowns (McMullan *et al.,* 2018).

ii. Chronic oak dieback:

The chronic oak dieback is a complex disorder or syndrome which is also referred as oak decline, dieback-decline affecting *Quercus* spp. particularly *Q. robur.* It results from a combination of abiotic and biotic factors. The characteristic symptoms include early foliage deterioration, gradual branch death and dieback in the crown. Abiotic stressors and weakening of trees allows for opportunistic attack from insects and disease which can result in tree death (Mitchell *et al.,* 2019).

j) Blight:

Dothistroma (red band) needle blight is caused by *Dothistroma septosporum* and *D. pini.* The characteristic symptoms includes development of yellow bands on needles

that later develop into red bands, where small, black fruiting bodies can occur (Plate 12). It can cause needle dieback, defoliation and eventual tree death (Barnes *et al.,* 2004).

Plate 12: Red band needle blight of conifers caused by *Dothistroma septosporum* (Courtesy: Webber, 2008)

k) Sandal spike:

Indian sandalwood is highly valueable hardwood species of Santalaceae, is popular for its fragrant wood and essential oil. Sandalwood Spike disease (SSD) in India is caused by phytoplasmas, *Candidatus* Phytoplasma *asteris*-related strain, an unculturable fastidious vascular bacteria belonging to class *Mollicutes* (Khan *et al.,* 2006). The disease was first reported from Coorg district of Karnataka, South India by Barber in 1899 (Barber, 1903). In India, SSD infestation has resulted an estimation of annual turnover loss amounting to Rs 3 million (Iyengar, 1969). SSD is characterized by distinguishable symptoms such as chlorosis, a reduction in leaf size, shortened internodes causing leaves to become crowded on twigs with a "bushy" appearance (Plate 13) and as stems standout stiffy, they acquire a spike-like appearance (Barber, 1903). SSD can be transmitted through grafting as well as haustorial connection between infected and healthy plants (Coleman, 1923). The disease can also be transmitted through insect vectors *viz. Moona alhimaculata* and *Nephotettix virescence* as well as other hosts such as *Vinca rosea* and *Cuscuta* sp.

Plate 13: Healthy (A) and infected (B) Sandalwood plant with sandal spike disease (Courtsey: Arun Kumar and Joshi, 2012)

l) Ganoderma diseases:

Ganoderma diseases of perennial crops have been reported as one of the serious constraint in decline of productivity as well as death of forest trees. It has been recorded on total of 144 hosts in India and the major species includes *G. lucidum, G. applanatum* that appear to cause widespread diseases of forest nurseries. In perennial deciduous tree, *Dalbergia sisso, G. lucidum* causes root rot and vascular wilt diseases in association with other soil borne pathogens *viz. Fusarium solani.* It infects roots through intact as well as injured surfaces that kills the bark by causing white fibrous root rot in the sapwood. The fungus spreads from diseased to healthy trees *via* root contact and stalked, corky, woody sporophores appear in affected trees (Plate 14). Entire plants show extensive defoliation with the death of branches, while in older trees, crown branches show die-back symptoms exhibiting "stag-headedness" (Bakshi *et al.,* 1972).

Plate 14: Ganoderma of *Acacia magnum* (Eyles et al., 2008)

m) Nematode damage in forest nurseries:

Plant parasitic nematodes (PPN) are microscopic worms that feed on plants by removing the cell contents with a hollow, needle-like mouthpart called as stylet, which functions like-a-straw. Some PPN remain in the soil and feed by repeatedly thrusting their stylets into seedling roots, thus, they are also referred as ectoparasites, while, others PPNs are endoparasites as they invade root systems to feed inside the root tissues. They are reported to be associated with seedling damage and are specialized to attack various types of plant tissues including leaves, flowers, stems and roots, however, most damaging nematodes are soil borne and feed on roots (Shurtleff and Averre, 2000). Nematode

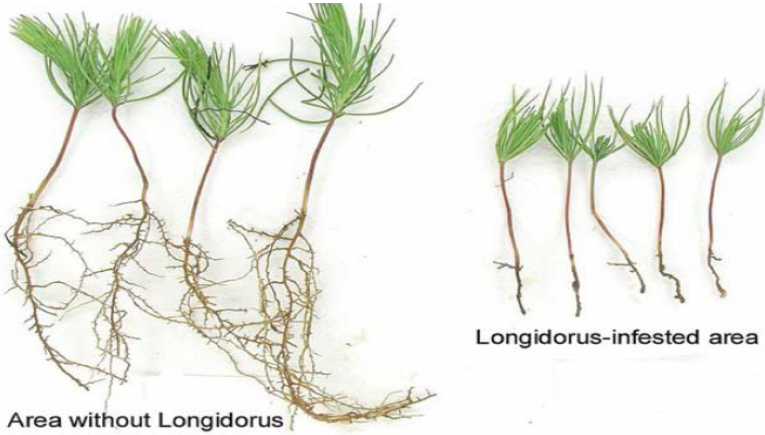

Plate 15: Loblolly pine (*Pinus taeda*) seedlings from nurseries uninfested and infested by *longidorus americanus* (Photo source: Stephen W. Fraedrich, Courtsey: Cram and Fraedrich, 2005)

feeding on roots can develop root diseases from the interaction of the physical damage caused by nematodes as well as soil borne fungal pathogens that colonize the wounded root tissues (Dwinell and Sinclair, 1967).

Nematode damage to forest nurseries either directly or indirectly can affect seedling production in forest nurseries as they cause significant stunting and chlorosis of seedlings (Plate 15). The above ground symptoms include stunting, chlorosis and even wilting of seedlings, while the damage pattern often exist as discrete patches of affected seedlings that expand to larger areas that encompass entire fields (Cram and Fraedrich, 2005). PPN feeding on roots can be associated with the typical damage symptoms observed on roots as enlisted in table 2. Migratory endoparasitic nematodes colonize roots while causing necrotic lesions, while, endoparasitic nemtodes becomes sedentary and stimulate the formation of root galls or swellings.

Table 2: Nematode damage associated with forest nurseries:

Host	Plant Parasitic Nematodes (PPNs) associated	Scientific name of PPNs	Damage symptoms
Pinus taeda	Lance nematode	*Hoplolaimus cronatus*	Patches of severe yellowing and dying of foliage
	Pine cystoid nematode	*Meloidodera floridensis*	Slight swellings on roots that appear as small pearls on root surface
	Needle nematode	*Longidorus americanus*	Feed near root tip, stimulate swellings
	Stunt nematode	*Tylenchorhynchus ewingi*	Underdeveloped and stubby root upon infection
	Spiral nematode	*Helicotylenchus nannus*	Small necrotic lesions on roots, root necrosis and die-back
P. palustris	Root-lesion nematode	*Pratylenchus brachyurus*	Chlorosis, stunting, poorly-developed roots bearing small-brown black lesions
P. elliottii	Root-knot nematode	*Meloidogyne javanica*	Form galls and root swellings
	Stunt nematode	*Tylenchorhynchus claytoni*	Brownish lesion at feeding site, severe stunting and chlorosis
	Stubby-root nematode	*Paratrichodorus minor*	Underdeveloped and stubby root upon infection
Pinus sp.	Sting nematode	*Belonolaimus* sp.	Stunting, wilting, yellowing of foliage

Host	Plant Parasitic Nematodes (PPNs) associated	Scientific name of PPNs	Damage symptoms
Juniperus virginiana	Root-lesion nematode	*Pratylenchus penetrans*	Severe root infection, chlorosis, stunting, poorly-developed roots with brown-black lesions
Cornus florida	Root-knot nematode	*Meloidogyne incognita*	Form galls and root swellings
Quercus spp.	Needle nematode	*Longidorus americanus*	Feed near root tip, stimulate swellings
Picea glauca, P. stchensis	Dagger nematode	*Xiphinema bakeri*	Feed near root tip, stimulate swellings

III. Management of nursery diseases:

Augmenting losses under forest ecosystem due to concomitant increase in proportion of nursery diseases and to meet the increasing demands of nurseries have induced rising use of pesticides in India accounting for 65% of insecticides, 16% of herbicides and 14% of fungicides consumption (Devi, 2011). Uses of agrochemicals are considered as the most reliable and potential arsenal for the management of nursery diseases and in return, increases agricultural productivity manifold. The use of dichloro-diphenyl-trichloroethane (DDT) followed by other organophosphate and carbamate pesticides against pest threatening crops have ruled agrochemical market since 1940s. During fiscal year 2019, India has produced an estimate of 2,17,000 metric tonnes of pesticides annually. The net worth of Indian pesticide market accounts for nearly Rs 19,700 crore in 2018, further aiming for an estimate growth of 8.1 per cent during 2019-24 with Rs 31,600 crore expected net worth by the year 2024.

However, the increased dependence on synthetic chemicals has affected the ecosystem in much drastic way. The indiscriminate use of agrochemicals caused an adverse impact on environment, which takes place due to dumping nearly 2.5 million tons of pesticides every year. Chronic exposure to such chemicals has overwhelmed potential risk to both ecosystem as well as human health as 115 out of 275 pesticides used in Indian agriculture are highly hazardous (Kumar and Reddy, 2017). Such practice, not only affects soil health and water quality but also develops other problems *viz.* insect resistance, genetic variation in plants as well as depositing toxic pesticide residue in food and feed. As per an estimate of World Health Organization (WHO), about 25 million cases of acute occupational pesticide poisoning have been reported from developing countries, whereas, 69,000 cases solely reported from developed countries like USA. Worldwide pesticide poisoning have been estimated to cause 2.2 lakh deaths per year, especially those farmers who are directly exposed to pesticides application are significantly at higher risk of cancer related problems than other farmers. As per estimate, around 14% of all known occupational farm injuries and 10% of all fatal injuries are caused by pesticides application (ILO).

Therefore, seeking an alternative to the use of agrochemicals, for e.g. bio pesticides are the need of an hour owing to growing health related concerns due to use of synthetic (chemical-based) pesticides. Bio pesticides can be defined as the materials that are derived from renewable materials such as plants, animals, bacteria and some minerals or using biological control agents that are defined as the utilization of natural or modified organisms, genes or its product. Also, the general agreement of trade and tariff (T&T) of world trade organization (WTO) has also emphasized to channelize the use of ecofriendly bio pesticides for crop production in view of their least toxic nature, low levels of disease resistance and low residue problems. However, in the recent years growing health concerns regarding the use of synthetic (chemical-based) pesticides has also been contributing to the growth of global bio pesticides market.

a. Biological control of nursery diseases of forest trees

Although, uses of beneficial microorganisms or biological control against forest-tree pathogens have not gained full momentum yet there is huge unexplored scope in this direction. The obstruction lies in their application strategy and the fact that biocontrol agent needs to establish prior commensalism interaction with trees in order to prevent pathogens from invading trees.

i. Definition of Biological control

The term "Biological Control" also acronym as "Biocontrol" has been utilized very well in different domains of life sciences most notably in Entomology and Plant pathology. In entomology, it has been defined in terms of use of entomopathogenic microbial population, entomopathogenic nematodes, predatory insects which aids in suppressing the pest population below the Economic Injury level (ETL). The use of microbial antagonists to suppress the potential plant pathogens causing severe disease of economically important crops has craved overwhelming importance in the domain of plant pathology. However, in both the domains those organisms which suppress the growth and development of pest population are referred to as Biological Control Agents (BCA).

ii. History of Biological control

The history of biological control is chronologically divided into three periods**:**

A. From 200 A.D. to 1887 A.D: During this period, preliminary efforts were made to release living agents rather haphazardly with no scientific approach. Little or precise information existed on success of biocontrol during that time.

B. From 1888 to ca. 1955: The intermediate period discriminating biocontrol had started with the introduction of the Vedalia beetle, *Rodolia cardinalis* Mulsant for the control of cottony cushion scale in 1888.

C. **From 1956 to the present:** The modern period was characterized by more careful planning and precise evaluation of natural enemies. Chinese were the first to use natural enemies to control insect pests. During 3rd century, nests of the ant *Oecophylla smaragdina* were used against citrus insect pests such as *Tesseratoma papillosa* (Lepidoptera) Vallisnieri through the phenomenon of insect parasitism (parasitoid) in 1706 A.D. However, the honor of being first to understand insect parasitism may belong to the microbiologist Antonnio Van Leeuwenhoek who illustrated and discussed a parasite of

a sawfly that feeds on willow in a publication of 1701.

iii. Biological control agents (BCAs) in forest nursery disease management

Biological control agents exhibit an indispensable role in management of several plant diseases by imposing an inhibitory behavior on various plant pathogens.

iv. Reasons for increased inclination towards biological control:

Organic agriculture involves exploring and discovering nature's pathways for crop production contrary to the formulaic approach of chemical farming. However, the concept of organic agriculture remains bitty without the incorporation of biological control approach as a method of controlling pest, weeds and diseases. In spite of the age old practices of biological control in crop production for centuries, their incorporation as an industry is still in its infancy. Biological control has now been incorporated as primary method of pest control for an increasing number of crops and managed ecosystems. The reason for its growing popularity owes towards health safety concerns since past 100 years, thereby, marking the inception of an era of modern biological control.

During the period of green revolution, there were marked uprise in the use of chemical pesticides booming crop production manifold, which in turn ultimately led to the evolution of resistant strains of pest and pathogens. Continuous and extensive use of toxic chemical pesticides upsets natural balance of ecosystem as they persist in soil for long time and enters food chain causing bio magnification as well as deterioration of soil and water quality. However, industrialization of agricultural sector has increased the chemical burden on natural ecosystems, thereby, posing negative impact on health and environment. Prolonged exposure to these harmful pesticides causes serious illness ranging from respiratory problems to cancer in humans and animals. In order to overcome, health and environment related drawbacks of chemical pesticides, farmers are turning their back on chemical pesticides and adopting biological control as a green alternative to these pesticide poisons.

v. Advantages of biological control

Biological control involves the utilization of natural processes, for e.g. natural enemies, microbial agents etc. to control insect pests without harming non- target organisms. They are highly effective in nature due to their high prey searching ability without posing any deterring effect on non-target organisms due to highly specific mode of action. The self-perpetuating and self-sustaining nature of BCAs results in their prolonged activity in a given area for a long period, therefore, recurrent application of BCAs into field may not necessary, thus, making it cost effective in nature. Unlike chemical pesticides, BCAs does not leave behind persisting residues in the environment that neither leach into the groundwater neither nor they harm the soil fertility, thus keeping intact the ecological balance. Therefore, biological Control minimizes the environmental, legal and safety concerns as the use of biological control agents poses no harm to the ecosystem.

BCAs in addition to controlling diseases and pest, also exhibits other beneficial impact on plant system by enhancing physical growth as well as other beneficial metabolic reactions that eventually leads to increase in quality and yield of the crop. It also restricts

the development of resistant strains of pest and pathogen or increased incidence of pest resurgence and outbreak, marking a proof of safety of BCAs. Another major advantage of biological control is that it is compatible with other Integrated Pest Management (IPM) practices. Thus, biological control can be successfully integrated with other management strategies, thereby, increasing the probability of pest and disease control instantly. The list of potential biocontrol agents *viz.* microbial agents, entomogenous nematodes, predators and parasitoids as well as their commercial application have been listed in table 3 and 4.

Table 3: List of potential biological control agents (BCAs) against insect pests

	Potential biological control agents	Target organisms
A. Microbial Biological Control Agents		
1.	*Trichoderma* spp.	Soil borne pathogens
2.	*Beauveria* spp.	Lepidopteran pest
3.	*Metarhizium anisopliae*	Locust, Grasshopper, Cockroach, Termite
4.	*Verticillium lecanii*	Cyst and Root Knot nematode
5.	*Nomuraea* spp.	Lepidopteran pest
6.	*Paecilomyces* spp.	Root knot and cyst nematode
7.	*Pochonia chlamydosporia*	Root knot and cyst nematode
8.	*Bacillus subtillis*	*Rhizoctonia solani*, *Fusarium* sp.
9.	*Pseudomonas* spp.	*Pythium* spp, *Rhizoctonia solani*
10.	*Streptomyces* spp.	Soil borne pathogens
B. Entomogeneous Nematodes		
1.	*Steinernema* sp.	Insect pest
2.	*Heterorhabditis* sp.	Insect pest
C. Predators and Parasitoids		
1.	Spined soldier bug	Insect pest
2.	*Podisus maculiventris*	Insect pest
3.	Minute pirate bug	Insect pest
4.	*Orius tristicolor*	Insect pest
5.	*Hippodamia convergens*	Insect pest
6.	*Trichogramma* spp.	Eggs of Lepidopteran pest
7.	*Encarsia* spp.	Larval and pupal stages of pest
8.	Aphid	Adult pest

vi. Mechanisms of Biological Control

Biological control agents interact with the components of disease triangle to suppress plant diseases. To understand the mode of action of biological control agents, it is essential to get optimum results of disease control .Various mechanisms of Biological

Table 4: List of commercially important biological control agents (BCAs)

Biological Control Agents	Target organism
A. Bacteria	
1. *Agrobacterium radiobacter* K 84	*Agrobacterium tumefaciens*
2. *Bacillus thuringiensis*	Larvae of Lepidopteran pest
3. *Bacillus subtillus*	*Rhizoctonia, Fusarium*
4. *Bacillus popilliae*	Grub of Japanese beetle
5. *Pseudomonas fluorescence*	Soil borne pathogens
6. *Streptomycin griseoviridis*	Soil borne pathogens
B. Fungi	
1. *Trichoderma harzianum*	Soil borne pathogens
2. *Ampelomyces quisquallis*	Powdery mildew pathogen
3. *Aspergillus flavus* AF 36	*Aspergillus flavus*
4. *Gliocladium virens* GL- 21	Parasitic nematodes
5. *Beauveria bassiana*	Lepidopteran pest
C. Protozoa	
1. *Nosema locustae*	European cornborer, ormon crickets
2. *Gypsy moth Nuclear Polyhedrosis Virus*	Gypsy moth caterpillars
3. *Codling moth granulosis virus*	Codling moth
E. Entomogenous nematodes	
1. *Steinernema feltiae*	Larvae of soil borne pathogens
2. *Heterorhabditis heliothidis*	Larvae of soil borne pathogens
F. Parasitoid	
1. *Trichogramma* sp.	Lepidopteran pest
2. *Encarsia farmosa*	Whitefly
G. Predator	
1. *Cryptolaemus montrouzieri*	Whitefly
2. *Chrysoperla carnea*	Mealy bugs
3. *Mite midge*	Pest mite

control that are involved in controlling plant diseases are - Direct antagonism and Indirect antagonism.

a. Direct antagonism:

Direct antagonism refers to the action of any organism that suppress or interfere with the growth of plant pathogens such as bacteria, fungi etc. Direct antagonism or lysis of the pathogen by other microorganism is called as hyper-parasitism and fungi that are

parasitic on other fungi are known as mycoparasites. Direct antagonism results from the physical contact of the pathogen and the antagonist.

b. Hyperparasitism:

It is considered as the most direct form of antagonism mostly involves tropic growth of biological control agents towards the target pathogen, Coil, finally attack and dissolution of target pathogens cell wall or membrane by activity of enzymes. It is one of the main mechanism of antagonism observed in the genus *Trichoderma*. *Trichoderma harzianum* exhibits mycoparasitic activity against hyphae of *Rhizoctonia solani* (Dutta *et al.,* 2013). Mycoparasitism is under the control of enzymes secreted by the antagonists such as Chitinases, Cellulases, Glucanases and other lytic enzymes.

c. Competition

Competition broadly means active demand is excess of immediate supply of material or condition on the part of two or more organisms. Main concept of competition is that pathogen must be deprived of nutrients essential for pathogenesis. Bio control agents and the pathogens compete with one another for nutrients and space to get established in the environment. This process of competition is considered to be an indirect interaction between the pathogen and the bio control agent. Microorganism competes for space, minerals and organic nutrients to proliferate and survive in their natural habitats .Generally, nutrient competition has played important role in disease suppression, although it is extremely difficult to obtain conclusive evidence. Biological control by nutrient competition can occur when the biocontrol agent decreases the availability of a particular substance thereby limiting the growth of the pathogen. Particularly, the biocontrol agents have a more efficient uptake or utilizing system for the substance than do the pathogens. This has been reported in both rhizosphere as well as phyllo sphere. Competition for substrates is the most important factor for heterotrophic soil fungi. Success in saprophytic ability (CSA) and inoculums potential of that species. Those fungi with highest number of propagules or the greatest mass of mycelia growth have the greatest competitive advantage. Competitive saprophytic ability is the summation of physiological characteristics that make for success in competitive colonization of dead organic substrates.

d. Antibiosis:

Antibiosis refers to the production of low molecular weight compounds or an antibiotic by microorganisms that have a direct effect on the growth of plant pathogens. Antibiosis is the procedure of secretion of antimicrobial compounds by antagonist fungi to suppress or kill pathogenic fungi in the vicinity of its growth area. Most fungi are capable of secreting one or more compounds and secondary metabolites with the antibiotic activity, often correlated to specific stages of morphological differentiation, and are associated to the phase of active growth. Fascinatingly, some fungal secondary metabolites can modify the growth and the metabolism of plants, while others seem to target specific fungal processes such as sporulation and hyphal elongation. Thus, the expression of secondary metabolites may occur at a predictable point during the normal life cycle of some fungi, including those used for agriculture applications .Bio-control study of *Trichodema harzianum* against *Sclerotinia sclerotiorum*—a soil-borne plant

pathogen attacking many economically important crops, such as, soybean and also studied about antibiosis of *T. harzianum* against the plant pathogen, assuming that the beneficial effect was due to concurrent mycoparasitism and competition.

e. List of antibiotics produced by some biocontrol agents:

Bio agents are known to produce different types of antibiotics which act in different ways to suppress the diseases or plant pathogens. Bio agents are known to produce three types of antibiotics viz., nonpolar/volatile, polar/ non-volatile and water soluble. Among all of these the volatile antibiotics are more effective as they can act at the sites away from the site of production. A list of antibiotics produced by the bio agents in suppressing the activity if the plant pathogen is given below in table 5.

Table 5: List of antibiotics produced by the bio agents in suppressing the activity if the plant pathogen

Sl. No.	Antibiotic	Source	Target pathogen	Disease
1.	2,4 - Diacetyl-pholoroglucinol	*Pseudomonas fluorescence* F113	*Pythium*	Damping off
2.	Agrocin 84	*Agrobacterium radiobacter*	*Agrobacterium tumefaciens*	Crown gall
3.	Bacillomycin D	*Bacillus subtillus* AU195	*Aspergillus flavus*	Aflatoxin contamination
4.	Bacillomycin D	*Bacillus amylo liquefaciens* strain FZB42	*Fussorium oxysporium*	Wilt
5.	Xanthobacin A	*Lycobacter* sp. Strain K88	*Aphanomyces cochlioides*	Damping off
6.	Gliotoxin	*Trichoderma virens*	*Rhizoctonia solani*	Root rot
7.	Zwitermycin A	*Bascillus cereus* UW85	*Pythium aphanidermatum*	Damping off
8.	Mycostubilin	*Bascillus* BBG100	*Pythium aphanidermatum*	Damping off
9.	Herbicolin	*Pantoea agglomerans* C91	*Erwinia amylovora*	Fire blight

f. Plant growth promotion:

Biocontrol agents also produce growth hormones like Auxin, Cytokinin, Gibberellins etc. These hormones suppress the deleterious pathogens and promote the growth of plants and simultaneously increase the yield. The studies on mechanism of growth promotion indicated that PGPR promotes plant growth directly by production of plant growth regulators or indirectly by stimulating nutrient uptake, by producing siderophores or antibiotics to protect plant from soil borne pathogens or deleterious rhizosphere organisms. *Pseudomonas* spp. may increase plant growth by producing

substances like Gibberellins- like substances, mineralizing phosphates.

g. Endophytism

Forest trees harbour a plethora of endophytes that live within the tree system for at least a part of their life cycle without causing any apparent symptoms (Sun *et al.,* 2014). Endophytes are those microorganisms that occur ubiquitously in natural forest ecosystems inhabiting leaves of forest trees are usually more diverse than leaf epiphytes. They are abundant colonizers of roots, leaves or needles, shoots, adapted within bark as well as the internal tissues of forest trees including intercellular spaces, tissue cavities, or vascular bundles (Kaushik and Dutta, 2016). Depending on the host species, interaction, transmission, fitness benefits, tissues colonized, *in planta* colonization and biodiversity, these endophytes are classified into four types *viz.* (Rodriguez *et al.,* 2009).

Penetration and occurrence of endotrophic fungus came in focus for the first time in the living bud cells of *Picea canadensis* and *Larix laricina* belonging to *Coniferae* family by Lewis as early as 1924 (Lewis, 1924). Later, Carroll and Carroll (1978) studied the occurrence of FEFs in coniferous trees from Pacific Northwest and since then more attentions have been paid on foliar endophytes of forest trees. Based on host species, forest trees are generally rich in endophytic fungi belonging to *Ascomycetes* and *Deuteromycetes* family.

vii. Occurrence of endophytes in forest nursery seedlings

Endophytes, in addition to harbouring different locations of forest trees, also inhabits tree capsules, flowers and seeds. For example, *Diaporthe phaseolorum*, a fungus survives as an endophyte in the seeds of *Paullinia cupana* (Silva *et al.*, 2018). While, the seeds of *Eucalyptus globulus* carried wide range of endophytic fungi such as *Acremonium strictum, Alternaria citri, Aureobasidium pullulans* var. *pullulans, Botrytis cinerea, Chaetomium funicola, Cytospora chrysosperma, Epicoccum purpurascens, Nigrospora oryzae, Xylaria sp.* and *Nigrospora sphaerica* (Lupo *et al.,* 2001). Similarly, filamentous fungi occur endophytically in the seeds of *Cinchona ledgeriana* (Maehara *et al.,* 2016).

The frequency of FEFs occurrence in forest tree leaves varies with host species, age as well as composition. Leaves of *Carissa caranda* from central region of Madhya Pradesh, India showed to harbour a total 126 endophytic fungi belonging to 12 genera amongst, the most dominant endophytes were *Aspergillus flavus* (12.69%), *Trichoderma* spp. (11.11%) and *Pestalotiopsis* spp. (10.31%) (Tenguria *et al.,* 2012). The frequency of occurrence of endophytic fungi, *Fusicladium betulae* in the leaves of silver birch (*Betula pendula)* varies from 70% in sapling stands, 31% in managed forest and 21% in natural forest respectively. Similarly, occurrence of the most frequently isolated endophyte *Gnomonia setacea* was 30% in the natural forests, while in the sapling stands it was only 4% (Helander *et al.,* 2006).

In addition to that, biodiversity of FEFs shows great difference between seedlings and adult trees as the distribution of endophytic community and their species richness varies with the leaf age (Espinosa-garcia and Langenheim, 1990). Host age correlated with selective enrichment for specific endophytes might relate to the shift in endophyte species dominance as indicative of a functional change between these fungi and their

hosts. The community biodiversity of FFEs between seedling and adult loblolly pines (*Pinus taeda*) showed significant variation in adult communities than in seedlings (Oono *et al.,* 2015). However, the basal sprouts and 1-12 year old coastal redwood tree (*Sequoia sempervirens*) from Central California showed the common occurrence of most frequent endophytic species *viz. Pleuroplaconema* sp. and *Cryptosporiopsis abietina.*

Multifaceted interactions occur between endophytic fungi and plants positively affecting plant developments and physiological prospects (Khare *et al.*, 2018). Endophytic fungi inhibiting plant roots play important roles in their host plants, such as acquisition of water and nutrition, improvement of growth and development, resistance to abiotic and biotic stresses, allelopathic resistance, secondary metabolism and carbon sequestration. In trees, endophytes are likely to be among the first potential decomposers of dying and dead leaves and wood. Endophytes may therefore significantly affect the dynamics of the decomposition process by affecting the flow of carbon, nitrogen and other nutrients in forest ecosystems. Recent studies indicate that endophytes may associate with tree's resistance and tolerance properties against several plant diseases, thus, could be utilized as potential bio-agents in sustainable forest protection and management.

a. Endophytes as BCAs in forest nursery

A great number of foliar endophytic fungi (FEFs) have been isolated from leaves of forest trees and as biocontrol agents (BCAs) endophytes in forest trees (Table 6) possess dynamic ability to inhibit disease-causing pathogens either through direct, indirect or ecological mechanisms (Gao *et al.,* 2013). A recent study (Hulme and Shields, 1970) showed that the immediate injection of *Trichoderma viride* into birch logs is able to control decay fungi biologically, by competition. The artificially colonized fungus acts first and removes non-structural carbohydrates from wood without substantially altering its mechanical properties. Thus, no other fungi can grow in the wood.

Foliar endophytic fungi synthesize many anti-microbial compounds that possess strong ability to inhibit or kill pathogenic microbes. Many bioactive metabolites isolated and identified from FEFs are terpenoids, alkaloids, phenylpropanoids, aliphatic compounds, polyketides, and peptides (Mousa and Raizada, 2013; Masi *et al.,* 2018). The cell-free crude extracts of five foliar fungal endophytes isolated from *Pinus strobus* showed antifungal activity in disc diffusion assays.

Volatile compounds obtained from the extract included an aliphatic polyketide, sesquiterpenes and macrolides (pyrenophorol, dihydropyrenophorin, and pyrenophorin) (Sumarah *et al*., 2011). These metabolites showed the antifungal potential against rust causing pathogen *Microbotryum violaceum*. Mcmllin *et al*. (2018) detected antifungal compounds griseofulvin and pyrenophorol (Fig. 1) from FEFs *Xylaria* sp. and *Lophodermium nitens* occurring in the needles of *Pinus strobus*. Recently, a terpene synthases were characterized functionally from endophytic Xylariaceae (Wu *et al*., 2016).

Psychrophilic FEFs occurred in the leaves of *Cupressus arizonica, Cupressus sempervirens,* and *Thuja orientalis* such as *Phoma herbarum, Phoma* sp. and *Dothideomycetes* spp., and showed antibacterial activity against the ice-nuleation active bacterium *Pseudomonas syringae* (Moghaddam and Soltani, 2014). The result suggested

that the FEFs can improve freeze resistance of *Cupressaceae* trees. The antibacterial activity helps preventing formation of cavity and embolism caused by freeze-thawing cycles often occurring in high latitude or altitude zones.

Table 6: Endophyte as BCAs against nursery diseases

Endophyte	Tree	Disease	Pathogen	Mechanism utilised	References
Endophytic bacterium					
Bacillus pumilus, JK-SX001	Poplar tree	Canker disease	*Cytospora chrysosperma, Phomopsis macrospora* and *Fusicoccum aesculi*	Extracellular enzymes (celluases, proteases) Secondary metabolites inhibiting mycelial growth	Ren *et al*., 2013
Alcaligenes sp. (EIL-2)	Rubber, *Hevea brasiliensis*	Abnormal leaf fall	*Phytophthora meadii*	Inhibited hyphal growth	Abraham *et al*., 2013
Pseudomonas denitrificans	Oak, *Quercus robur*	Oak wilt	*Ceratocystis fagacearum*	Siderophores and antibiotic compounds	Brooks *et al*., 1994
Psychrophilic FEFs; *Phoma herbarum*, *Phoma* sp. and *Dothideomycetes* spp.	leaves of *Cupressus arizonica*, *Cupressus sempervirens*, and *Thuja orientalis*		*Pseudomonas syringae*	antibacterial activity against the ice-nuleation active bacterium	Moghaddam and Soltani, 2014
Endophytic fungus					
Trichoderma aureoviride UASWS and *T. harzianum* B100	Oak, *Quercus robur*	Necrotic lesions	*Phytophthora plurivora*	Fungal secondary metabolites	Berger *et al*. (2015)
		Necrotic lesions	*Phytophthora plurivora*	Fungal secondary metabolites	Berger *et al*. (2015)
Verticillium strain WCS850	Elm (*Ulmus* spp.)	Dutch Elm Disease,	*Ophiostoma ulmi* and *O. novo ulmi*	induce host resistance	Scheffer *et al*., 2008

Endophyte	Tree	Disease	Pathogen	Mechanism utilised	References
Crude extract from foliar endophytic fungus, family Massarinaceae	needles of *Pinus strobus*	rust	*Microbotryum violaceum* and *Saccharomyces cerevisae*	Biocidal effect	Richardson *et al*., 2015
FEFs	leaves of *Populus trichocarpa*	rust	*Melampsora*	reduction in disease severity	Busby *et al*., 2016b
FEFs	leaves of *Fraxinus excelsior*, *F. ornus*, and *Acer pseudoplatanus*	ash dieback			Schlegel *et al*., 2018
Trichoderma viride	birch logs	decay fungi		competition	Hulme and Shields, 1970

vii. Classical control approaches for forest diseases

Presently, PPPs are generally synthetic chemicals that disrupt the cellular function, or life cycle of the target organism. Other PPPs work on a physical basis e.g. killing insect or acarid targets on contact via suffocation, or abrasion of the exoskeleton and subsequent desiccation. These products are typically those formulated for use in agriculture. Aboveground and external tree pests and diseases are often controlled with aqueous sprays of PPPs to the foliage and bark. Specialized high-pressure spray systems can be used for such applications to large trees (Hirons and Thomas, 2018). Internalized pests and diseases, such as nematodes, are more difficult to reach due to their physical concealment within the host; adjuvants (additives) may improve the penetration of externally applied PPPs for such targets e.g. through bark (Garbelotto *et al.,* 2007),

Control of fully internalized diseases of trees are also a particular issue, for instance, one of the difficulties in controlling *Verticillium dahliae* and *Xylella fastidiosa* in olive (*Olea europaea*) and grapevine (Baccari and Lindow, 2011) is due to the inaccessible location of the pathogen within the vascular system (Cazorla and Mercado-Blanco, 2016). Similar difficulties are faced in the control of Huanglongbing disease, *Candidatus liberibacter* spp., which causes citrus greening and is a phloem-limited phytoplasma spread by insect vectors (Abdullah *et al.,* 2009).

Root and soil-borne pathogens have been treated by injections into the soil of PPPs or sterilizing agents such as phenolic compounds or methyl bromide gas (Martin, 2003; West and Fox, 2002). While many synthetic PPPs break down quickly when exposed on stems or foliage, soil applied compounds may persist for extended periods once bound to soil particles (Edwards, 1975). Stump treatments, e.g. urea, sodium borate or the saprobic fungus *Phlebiopsis gigantean* have also been applied to exclude and reduce the

build-up of fungal pathogens. They can also utilize buried dead wood saprobically, often *Heterobasidion* spp., but may also exclude *Armillaria* spp. and other basidiomycetes, while allowing non-pathogenic species to proliferate (Nicolotti and Gonthier, 2005)

In Europe, and elsewhere, environmental concerns have fueled a movement away from synthetic "chemical" PPPs or those based on toxic heavy metals e.g. copper (Lamichhane *et al.,* 2018). In the absence of other effective controls this reduction in authorized pesticides may conflict with protecting vital resources such as food and timber. An area that is gaining much more attention in recent years is biological control (or biocontrol) – the use of biological agents to counter a pest or disease. The desired outcome of a biological control application is to reduce the pathogen or pest population below a threshold of ecological and economic impact, ideally enabling the host to regain health and eventually restoring the invaded community to the pre-invaded state (Bale *et al*., 2008).

This approach is highly favourable because most BCA source species are already present in the host's environment, and in some cases provide a narrow range of target specificity, so are less likely to be harmful to non-target organisms. BCAs can come in many forms, from viruses or bacteriophage, to bacteria or fungi, and even higher organisms like nematodes, mites or insects (Lenteren *et al*., 2018). Like pesticides, usage of fungicides also causes great cost and often results in environmental pollution, thus biocontrol over forest diseases is an excellent strategy.

viii. Method of application of BCAs in forest nurseries

BCAs are applied in similar ways as in case of synthetic compounds and the selected method typically aimed to maximize contact with the target organism (Table 7). The application of plant protection products (PPPs) for the control of tree diseases is already often limited by ecological concerns and modulated by the particular local context. These BCAs itself does not move far from the sites of application but manages disease *via* plant-mediated effects (Scheffer *et al*., 2008). However, the greatest challenges of using BCAs with forest trees relate to the scales associated with trees, thus restricting access to the whole tree, canopy and woodlands occupying larger areas.

When endophytic bacterium *B. pumilus* (JK-SX001) was applied as a root drench in poplar, the bacterial cells migrated from the roots up to the leaves and were reported to increase host photosynthetic activity. It ultimately increases biomass production in the saplings and reduces infection rate as well as severity of canker diseases by suppressing pathogenic activities of *Cytospora chrysosperma, Phomopsis macrospora* and *Fusicoccum aesculi* in poplar (Ren *et al.,* 2013). Application of endophytic bacterium *Alcaligenes* sp. (EIL-2) as a foliar and soil drench to one-year old greenhouse plants prior to infection by *Phytophthora meadii* leads to 50% reduction in infection rates (Abraham *et al.,* 2013).

Berger *et al.* (2015) compared foliar applications of phosphite, and the endophytes *Trichoderma aureoviride* UASWS and *T. harzianum* B100 on reducing the necrotic area of *Phytophthora plurivora* lesions on oak leaves (*Quercus robur*). Results showed that given the diffusable nature of phosphite it was able to reduce necrosis on both treated

and untreated leaves. However, with UASWS and B100, only untreated leaves showed reduced necrosis suggesting that a number of fungal secondary metabolites affected the interaction. However, when applied via trunk inject ions (endo-therapy) a similar endophyte, *T. atroviride* ITEC was able to significantly reduce the necrosis size, compared to the control and the phosphite treatment, on 30-year-old beech trees (*Fagus sylvatica*) artificially inoculated with *P. plurivora*. It is clear from this example that the effectiveness of an endophytic BCA is likely to be influenced by the mode of application.

Table 7. Method of application of BCAs in forest nursery against plant diseases

BCAs	Pathogen/ Disease	Method of application	References
Pseudomonas denitrificans	Oak wilt fungus, *Ceratocystis fagacearum*	Injecting trees	Brooks *et al*., 1994
B. subtilis QST 713	foliar pathogens-*Botrytis* of fruit or nut trees	aqueous spray	Abbasi and Weselowski, 2014
B. subtilis QST 713	*Phytophthora* root rots	-aqueous drench, e.g. via pressurized soil injection systems or irrigation -physically incorporated into soils	Abbasi and Weselowski, 2015
Bacillus strains	*Phytophthora cinnamomi* infections of Avocado trees, *Persea americana*	trunk injections into vascular system	Darvas and Bezuidenhout, 1987
Bacillus pumilus (JK-SX001)	*Cytospora chrysosperma, Phomopsis macrospora* and *Fusicoccum aesculi* in poplar	root drench	Ren *et al*., 2013
Alcaligenes sp. (EIL-2)	Abnormal leaf fall of rubber trees, *Phytophthora meadii*	foliar and soil drench to one-year old greenhouse plants prior to infection	Abraham *et al*., 2013
Trichoderma strains	treatment of root diseases	grown on a solid food source such as grain, but also as spore powders, are variously mixed into the soil around roots or placed in cores in close proximity to roots	Srivastava *et al*., 2016

BCAs	Pathogen/ Disease	Method of application	References
Trichoderma aureoviride UASWS and *T. harzianum* B100	necrotic area of *Phytophthora plurivora* lesions on oak leaves (*Quercus robur*)	Foliar application	Berger *et al*., 2015
	Phytophthora plurivora of beech trees (*Fagus sylvatica*)	trunk inject ions (endotherapy)	
Verticillium strain WCS850	Dutch Elm (*Ulmus* spp.) Disease, *Ophiostoma ulmi* and *O. novo ulmi*	punctures in the bark of the tree	Scheffer *et al*., 2008
Trichoderma viride	decay fungi of birch logs	Injection	Hulme and Shields, 1970

ix. Mass production of biocontrol agents at farm level:

Mass production of biocontrol agents is necessary for large scale field application. There are mainly two major methods of mass production of *Trichoderma* or other fungal biocontrol agents, a) solid state and b) liquid state fermentation.

a. *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) (Dutta and Das, 1997). Presently FYM and wheat bran-saw dust are extensively used to produce biocontrol agent of *T. harzianum* and *T. viride*. Well decomposed powdered FYM mixed with 1% peptone and half parts water are to be 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 (Dutta and Das, 1999; 2000; 2002).

Different cheap grains (sorghum, ragi), agricultural wastes and byproducts (tapioca refuse, press mud, coffee-berry husk, spent tea leaf waste, coconut coir pith, groundnut shell and poultry manure) etc are also used for mass multiplication of *Trichoderma* spp (Dutta and Das, 1997). The solid state production is highly labour intensive and fit for cottage industry.

Various cheap cereal grains like, sorghum, millets, ragi are used as substrates. The grains are soaked in water overnight and water is decanted in the morning. The soaked grains are taken in heat resistant bags (Polypropylene bags) and sterilized for 30 minutes in an autoclave or pressure cooker. The sterilized grains are inoculated with *Trichoderma*

spore suspension and incubated for 10-15 days. *Trichoderma* produces dark green spore coating on the grains. These grains can be powdered finely and used as seed treatment or the grains can directly be used for enriching FYM for soil application.

b. *Liquid fermentation*

This process involves production of large number of conidia mainly intended for seed treatment, soil application and foliar spray. Two *Trichoderma* based liquid bioformulation developed at Assam Agricultural University, Jorhat, Assam and Central Agricultural University, Umiam, Meghalaya, respectively Org-Trichojal (Plate 16) and UmTricho has been developed. The product Org-Trichojal have been found with shelf life of 16 months with highly effective in farmers field for management of plant pathogens and enhance plant growth parameter and plant defence metabolite. On the other hand study *in vitro* efficacy and on going pilot field trial for the product UmTricho (Plate 17) showed encouraging result for management of soil borne plant pathogen of vegetable crops and paddy.

In liquid fermentation, the fungus is grown in cheap media like potato dextrose broth , molasses yeast broth etc. Based on the available facilities *Trichoderma* can be grown in any one of the mentioned liquid media either under stationary or shaker or fermenting conditions. In stationary condition it takes 10 days for full spore production and in shaker, it takes 7 days and in fermenter peak production occur with 3 days. Fermenter biomass of *Trichoderma* consisted mainly of submerged conidia with some amount of aerial conidia and mycelial fragments. Biomass from liquid fermentation either can be separated from medium and concentrated or entire biomass with medium can be incorporated into dusts, granules, pellets, wettable powders using an inert or food based carrier material or emulsifiable liquids. Talc can be used as carrier material and is mixed with fermenter biomass in 1:2 ratio to prepare talc-based formulation of *Trichoderma*. Talc formulations can be applied to the seed either by dry seed treatment just before sowing or by seed biopriming (incubating the treated seed till radical emergence and then planting) for control of several soil borne diseases. Granular or pellets preparations have been used for soil application directly and have provided effective control of diseases both under green house and field conditions.

Plate 16. Org-Trichojal (A liquid bioformulation of *T. harzianum* of Assam isolate)

Plate 17. Um-Tricho (A liquid bioformulation of *T. harzianum* of Meghalaya isolate)

c. *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.

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

Autoclave both starter and fermented medium for 1 h

▼

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

▼

Inoculate fermented media with starter inoculam and supply regularly at for 10-15 days by shaking/bubbling

▼

Filter through cotton muslin using funnel

▼

Air dry or freeze dry the mat for 3 days find 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 5^0C and 3 months if stored at 20^0 C. Maximum biomass is obtained generally after 15 days of inoculation.

e. 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.

f. *Materials Required for preparation of formulation*

The materials required for preparation of formulations are, a. Freshly isolated native *Trichoderma harzianum* pure culture, b. PDA media, c. substrates , d. distilled water, e. poly propylene bags/ saline bottle, f. racks, g. rubber bands, h. laminar flow cabinet, i. inoculating needle, j. spirit lamp, k. cotton (both absorbent and non absorbent), l. methylated spirit, m. mthyl alcohol, n. petriplates, o. cork borer, p. measuring cylinder, q. muslin cloth, r. plastic Bucket and mugs, s. BOD incubator etc.

In general, product formed from solid or semi solid-state fermentation does not require sophisticated formulation procedures prior to use. For example, grain or other types of organic matter upon which antagonists are grown are simply dried ground and added to the area to be treated. There are several problems with solid state fermentation, which may make the system inappropriate for commercial product development. The preparations are bulky, they may be subject to a greater risk of contamination and they may require extensive space for processing, incubation and storage. The liquid state fermentation is devoid of such problems and large quantitates of biomass can be produced within few days. Biomass either can be separated from medium and concentrated or entire biomass with medium can incorporated into dusts, granules, pellets, wettable powders or emulsifiable liquids. *Trichoderma* spp can be formulated as pellets (Papavizas and Lewis, 1989), dusts and powders (Luchmeah and Cooke, 1985; Nelson and Powelson, 1988; Ricard, 1988) and fluid drill gels (Conway, 1986; Mihuta and Rowe, 1986), encapsulated product (Sharma, 2019), emulsifiable liquids (Bhattacharyya and Dutta, 2020).

x. Management of nematode diseases in forest nurseries:

Crop rotation is a common cultural management practice used to reduce soilborne pests such as nematodes. Most nurseries rotate their production crops with cover crops (e.g., green manure crops) to increase soil organic matter, reduce compaction, and reduce pests.

Tylenchorhynchus spp. such as *T. ewingi* and *T. claytoni* Steiner, that are generally found in some nurseries, generally have wide host ranges that include sorghum-sudan grass, rye, corn (*Zea mays* L.), ryegrass (*Lolium multiflorum* Lam.), oats (*Avena sativa* L.), buckwheat (*Fagopryum esculentum* Moench), and various legumes (Cram and Fraedrich, 2009, Fraedrich *et al*., 2012). The common use of these species and other small-grain hosts for cover crops has probably made these plant-parasitic nematodes more difficult to control in some nurseries. Currently, the best, nonhost grain identified for control of *T. ewingi* and *T. claytoni* are certain varieties of pearl millet (*Pennisetum americanum* [L.] Leeke) (Johnson and Burton, 1973; Cram and Fraedrich, 2009; Fraedrich *et al*., 2012). Pearl millet hybrids have been tested and bred for resistance to nematodes for many decades in the agriculture industry and various pearl millet cultivars have been reported to be resistant to *Paratrichodorus minor* (Colbran) Siddiqi, *Meloidogyne* spp. *Belonolaimus longicaudatus* Rau, and *Pratylenchus brachyurus* (Godfrey) Filip. & Stek. (Johnson and Burton, 1973; Timper *et al*., 2002; Timper and Hanna, 2005). The practice of fallowing fields is an effective cultural practice to control plant-parasitic

nematodes through starvation (Duncan and Noling, 1998; Zasada *et al*., 2010). Several field studies in forest nurseries have shown that fallowed fields kept weed free had significantly reduced nematode population densities. In the South, *L. americanus* and *T. claytoni* were controlled in fallowed fields within 1 year (Fraedrich *et al*., 2005; Cram and Fraedrich, 2009). In the North, Sutherland and Sluggett (1975) reported that corky root disease caused by *X. bakeri* could be controlled with fallow for 1 year and frequent disking during the summer months. Many nurseries can only afford a 1-year rotation with an alternate crop or fallow because of limited land base. The length of time it takes for a nematode population to decline to nondamaging levels in a fallow field or a field with a nonhost crop may determine which rotation option is best suited for the nursery.

xi. Registration of biopesticides in India:

In India the use of pesticides is regulated by the CIB&RC. Any individual or organization inteneding to manufacture, import or export pesticides should register the product with CIB&RC. The CIB&RC then anaylses the product for potential risk to human and animal health, its environmental impact and usefullness in agriculture. The use of pesticides is maninly governed by the Inseticides Act,1968, and insectide rule,1971. The CIB&RC has set guidleines for pesticides sale in the country. Any individual or company, intrending to either manufacture or import any type of pesticides to India, Needs to submit an application for the registration of their product to CIB&RC.

The submission of this application involves several technicalities.

The application is to be done to the registration committee. Details regarding the active ingredents need to be specified. After receiving the application, along with the recommended fee, the committe conducts Physical, Chemical and microscopic tests and enquires to confirm the claims made by the manufacturer or importer as far as the utility to the pesticide is concerned. If the application is found to be valid, a registration no. is allocated and a certificate of registration is then made available within a period of twelve months.

Requirement of the data/information to be submitted by the subsequent applicant for getting registration under section 9(3) /9(3B) of already registered strain:

a) Form-I duly filled in along with requisite registration fee of Rs. 100 as per existing requirement.

b) Already approved Label leaflets of the product/strain

c) Testimonial/documents about the company as per existing requirement.

d) Undertaking about the strain from the inventor of the strain OR first registrant OR subsequent registrant of the strain OR the applicant.

e) One sample (01 kg.) for pre-registration verification (PRV) through Central Insecticides Laboratory as per already approved product specification by RC.

f) Another sample (01 kg) for pre-registration verification (PRV) of Gene code sequencing/16 SR-DNA/finger printing along with a demand draft (as per invoice obtained as testing fee from NBAIM, Mau) in favour of NBAIM, Mau as testing fee for Gene code sequencing/16 SR-DNA/finger printing.

g) Invoice for testing fee for Gene code sequencing/16SR-DNA/finger printing has to be obtained from NBAIM, Mau by the Sectt. of CIB&RC.

a. Registration of new strain of the biopesticides.

It was decided that the applicants for registration of new strain has to submit all the data as per existing guidelines for registration under section 9(3)/9(3B) for all the disciplines. Two samples have to be submitted to the Sectt. of CIB&RC; one for pre-registration verification (PRV) from Central Insecticides Laboratory as per product specification requirement & another sample to be used for pre-registration verification (PRV) of Gene code sequencing/16 SR-DNA/finger printing along with a demand draft (as per invoice obtained as testing fee from NBAIM, Mau) in favour of NBAIM, Mau as testing fee for Gene code sequencing/16 SR-DNA/finger printing.

b. Minimum infrastructure required for production & registration of biopesticides

It was felt that flight-by-night manufacturers are available in the market and other products are sold in name of biopesticides. Many of the manufacturers don't have proper and adequate infrastructure required for quality production of the biopesticides, as many samples of the biopesticides are failing in the test, which is affecting the quality of the produce. Therefore, verification of the infrastructure and technical competency of the applicants already registered under section 9(3B) and applying for registration u/s 9(3) and/9(3B) extension has to be conducted by a team constituted by the Secretary (CIB&RC) for the purpose.

c. Minimum CFU count and nominal concentration strength of the formulation to be continued as per existing guidelines.

d. Verification of Shelf life of strain and verification of product: This aspect needs more conclusive information, hence the existing procedures may be continue.

xii. Guidelines / Data Requirements For Registration of Antagonistic Fungi Under Section 9(3B) And 9(3) Of The Insecticide Act, 1968

I. Standard of Formulations:

1. Colony Forming Unit (CFU) count on selective medium should be minimum of 2x 10^6 per ml or gm for *Trichoderma* spp.

2. Contaminants:

2.1 Biological contaminants:

2.1.1 Pathogenic contaminants such as gram negative bacteria *Salmonella*, *Shigella*, *Vibrio* and such other microbials should not be present.

2.1.2 Other microbial contaminants should not exceed 1 x 10^4 count per ml or per g of formulation.

2.2 Chemical / botanical pesticide contaminants should not be present

1. Stability of CFU counts at 30°C and 65% RH

II. Registration Requiremnets:

A. Biological Characteristics and Chemistry

Sl. No.	Requirements	9(3B)	9(3)
1.	Systematic name (Genus and species)	R	R
1.1	Strain name	R	R
2	Common name, if any	R	R
3.	Source of origin as Annexure-1.1	R	R
4.	Habitat and morphological description	R	R
5.	Composition of the product	R	R
5.1	CFU/g of the product	R	R
5.2	Percent content of the Biocontrol organism in the formulation & nature of biomass.	R	R
5.3	Percentage of carrier/filler, wetting/ dispending agent, stabilizers/ emulsifiers, contaminants/	R	R
5.4	impurities etc. Moisture content	R	R
6.	Specification of the product as per Annexure-I	R	R
7.	Manufacturing process including type of fermentation and biological end products: The microbial cultures are multiplied by liquid solid fermentation. Information pertaining to use of entire mycelial mats with spores separated must be provided in terms of biomass.	R	R
8.	**Test Methods:**		
8.1	Dual culture to attain at least 50% reduction in target organism.	R	R
8.2	Bioassay: based on diseased severity and root colonization as detailed in Appendix-I	R	R
9.	**Qualitative analysis**	R	R
9.1	CFU on selective medium	R	R
9.2	**Contaminants:**		
9.2.1.	Pathogenic contaminants such as Salmonella, Shigella, Vibrio and such other microbials	R	R
9.2.2.	Other microbial contaminants	R	R
9.2.3.	Chemical and botanical pesticide contaminants	R	R
9.3.	Moisture content	R	R
9.4.	Shelf life claims : Not less than 6 months	R	R
9.4.1.	Data on storage stability as per shelf life claims as detailed in **Note-2**	R	R
10.	A sample for verification (100 g)	R	R

B. Bioefficacy:

11.	Field studies: Data from SAU's/ICAR Institute certified by Director Research of SAU or Head of the ICAR Institute	R**	R***
12.	Laboratory studies: The product should be tested at a laboratory under ICAR/ SAU/ CSIR/ICMR.		

C. Toxicity:

13.	**For mother culture**		
13.1	Single Dose Oral (rat and mouse)	R	R
13.2	Single dose pulmonary	R	R
13.3	Single dose Dermal	R	R
13.4	Single dose intraperitoneal	R	R
13.5	Human safety records.	R	R
14	**For formulation**		
14.1	Data on mother culture as in (13) above	R	R
14.2	Single Dose Oral (Rat & Mouse)	R	R
14.3	Single dose pulmonary	R	R
14.4	Primary skin irritation	R	R
14.5	Primary eye irritation	R	R
14.6	Human safety records	R	R
15.	**For formulated product to be directly manufactured:** (Mammalian toxicity testing of formulations)		
15.1	Single dose oral (Rat & Mouse) Toxicity/Infectivity/Pathogenicity	R	R
15.2	Single dose pulmonary Toxicity/Infectivity/Pathogenicity (Intratracheal preferred)	R	R
15.3	Single dose dermal Infectivity	R	R
15.4	Single dose intraperitoneal (Infectivity)	R	R
15.5	Primary skin irritation	R	R
15.6	Primary eye irritation	R	R
15.7	Human safety records (Effect/Lack of effects)	R	R
16.	**Environmental safety testing: Core Information requirements** (For formulation only) **Non-target Vertebrates** Mammals[a] Birds(two species)[b]		
16.1	Fresh water fish[c]	NR	R
16.1.1		NR	R
16.1.2	**Non-target invertebrates**	NR	R
16.1.3	Soil invertebrates[d]	NR	R
16.2			
16.2.1			

D. Packaging & Labelling

	Formulation:		
17.	Manufacturing process/process of formulation		
17.1	Raw material	R	R
17.2	Plant and Machinery	R	R
17.3	Unit Process operation/Unit process	R	R
17.4	Out-put (Finished product and generation of waste)	R	R
18.	Packaging:		
18.1	Classification-solid, liquid or other types of product.	R	R
18.2	Unit pack size – In metric system	R	R
18.3	Specification – Details of primary, secondary and transport pack	R	R
18.4	Compatibility of primary pack with the product (Glass bottles are not recommended).	NR	R
19.	Labels and leaflets: As per Insecticides Rules, 1971/as per existing norms indicating the common name, composition, antidote, storage, statements etc	R	R

Notes:

1. Applicants are required to submit an undertaking that strain is indigenous, naturally occurring, not exotic in origin, and not genetically modified as per.

2. Additional two months data for six months shelf-life claim, three months additional data for one year shelf-life claim at two different agro climatic locations at ambient temperature along with meteorological data should be submitted.

3. Considering the fact that many small entrepreneurs are engaged in the business of cultivation of antagonistic fungi the following simplification has been considered.

3.1 If same microbial strain is used for making formulation by different entrepreneurs then the information submitted once on the said strain will be sufficient. All entrepreneurs need not to generate relevant data.

3.2 If same microbial strain, same method and same adjutants, stabilizers etc. are used for making the given formulation, data once submitted for validating these claims will be sufficient for subsequent registrants, as substantiated by the relevant supportive documents.

4. The packaging material should also be ensured to be free from contamination from handling, storage and transportation and is as per prescribed standards, as the case may be.

Abbreviations:

R = Required

NR = Not Required

R** = Two seasons/years data on bio effectiveness from minimum two agro-climatic conditions

R*** = Two seasons/years data on bio effectiveness from minimum

three agro climatic conditions

a = Information on infection and pathogencity in mammals will be available from mammalian safety testing.

b= Information on infection and pathogenicity: suggested test: single-dose, oral test. suggested test species: pigeon and chicken.

c = Information on infection and pathogenicity: suggested test species: *Tilapia mossambica* or other appropriate spp.

d= Information on mortality effects. It is recommended that test species include an earthworm (*Lumbricus terrestris*) or other appropriate macro invertebrates of ecological significance.

xiii. Indian Standards For Antagonistic Fungi Draft Specifications

1. Form and appearance

2. pH

3. Composition

4. CFU/g of the product

5. Percent content of the Biocontrol organism in the formulation& nature of biomass.

6. Percentage of carrier/filler, wetting/ dispending agent, stabilizers/ emulsifiers,

contaminants/ impurities etc.

1.4 Moisture content

1.CFU counts: *Trichoderma* $2x10^6$ CFU/ml or gm. (Stability at 30^oC and 65%RH).

Contaminants:

2.1 Biological Contaminants:

2.2 Pathogenic Contaminants: such as gram negative bacteria *Salmonella, Shigella, Vibrio* etc.: **absent**

2.3 Other contaminants should not exceed $1x10^4$/ml or g

2.4 Chemical/ botanical pesticides contaminants: **absent.**

xiv. Method of analysis:

1. CFU counts by serial dilution and examination under regular compound research microscope with bright field optics.

2. Plating for contaminants on specific media

3. Antagonistic mycolytic capability on target organism by bioassay on plants (Laboratory test).

4. Bioassay procedure based on diseased severity and root colonization as detailed in

5. An undertaking should be submitted that strain is indigenous, naturally occurring, not

exotic in origin and not genetically modified as per.

xv. Conclusion

Biological control of plant diseases is utmost necessary for not only to save the crops/trees from the different diseases but also to save the environment. Management of plant diseases by biological approaches initially involved more cost as compared to conventional practices. But, once the biological control agents established within a particular environment they may persist for longer duration and there is not question of repeated application and ultimately reduces the management cost. Because of having diverse mode of action of biocontrol agents, pathogen/s get less chance to develop resistance against a particular biocontrol agents. Moreover biocontrol agents have multiple action like disease suppression ability, growth enhance ability and activation of defense mechanism of host plant. There are different successful example of management of plant diseases by biocontrol agents preferably with the indigenous strains, some of which are cited in the article. As registration process of effective biocontrol agents does not involve strict regulations in the country like India. This article reflects the available information related to important diseases of forest nurseries with some successful example for its management with biocontrol agents, their mode of action, formulation and registration procedure. This article will enable researchers to explore ecofriendly management practices against different diseases of forest nurseries in a better way which are constantly challenged in the natural conditions.

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