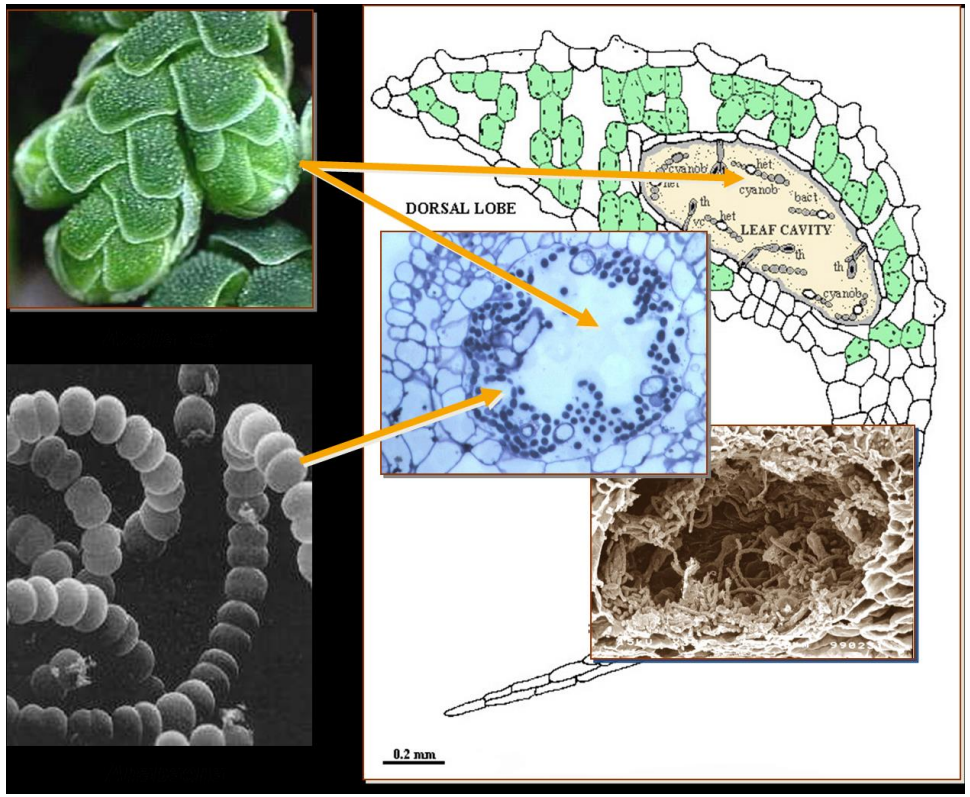
Azolla-Anabaena symbiosis- as biofertilizer



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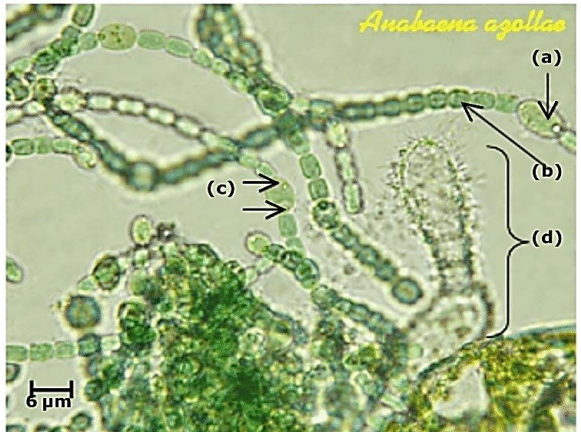
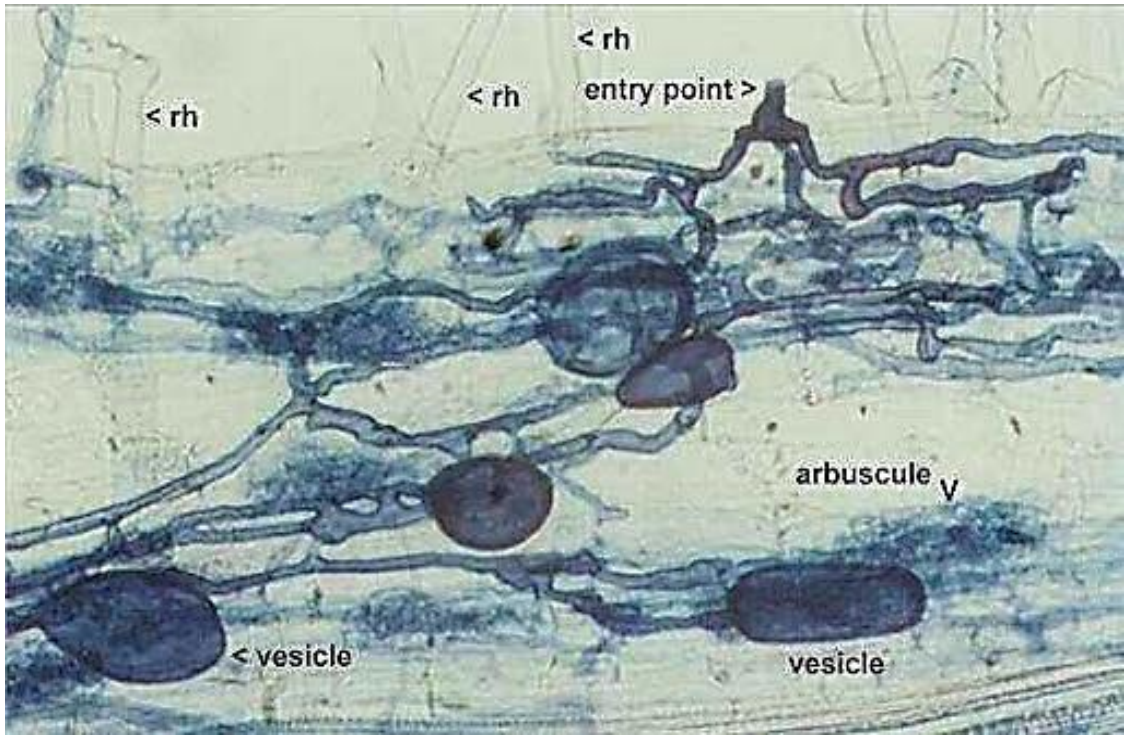
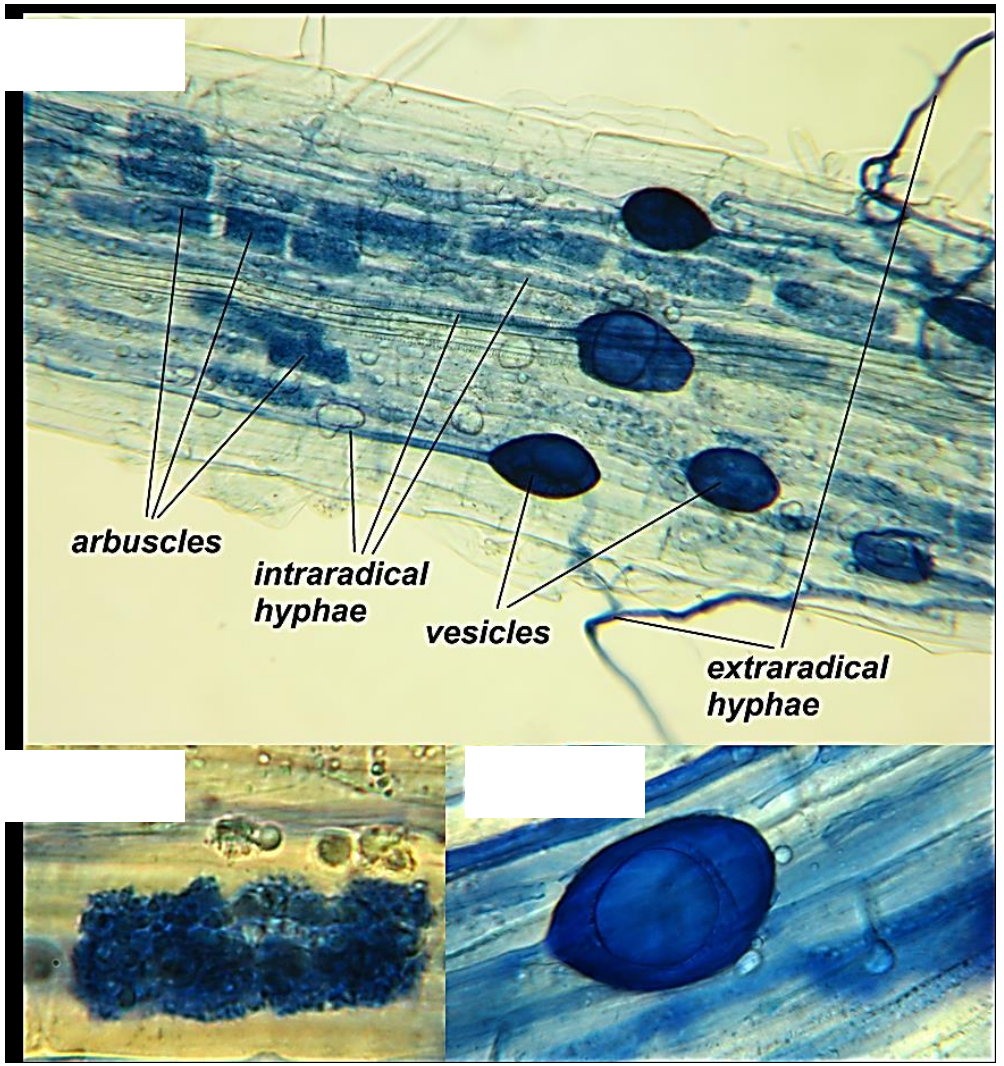
Anabaena azollae: heterocyst (a); vegetative cell (b); polar nodules (c). *Azolla filiculoides*: trichomes (d).



Vesicular arbuscular mycorrhiza



rh- root hair



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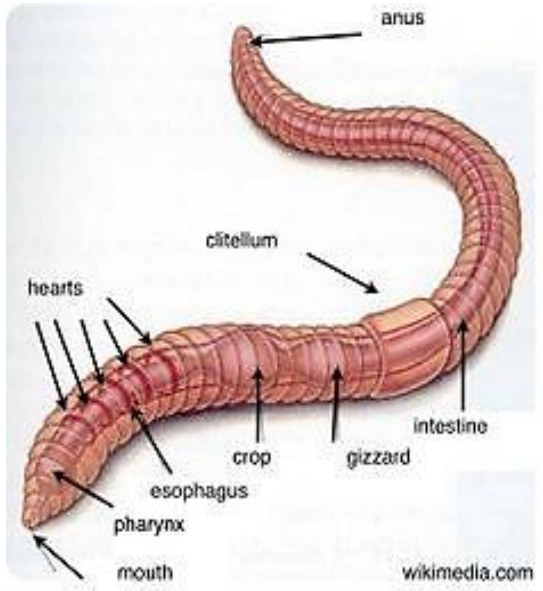
Eisenia fetida in vermicomposting



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Bio-composting

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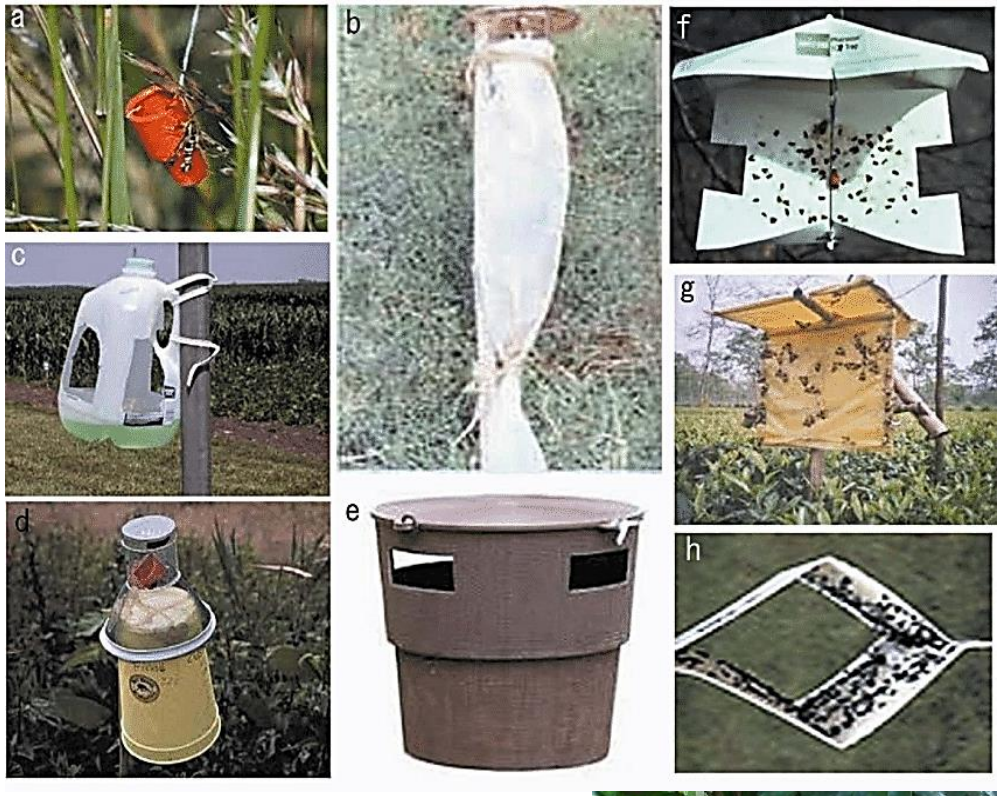
Pheromone traps



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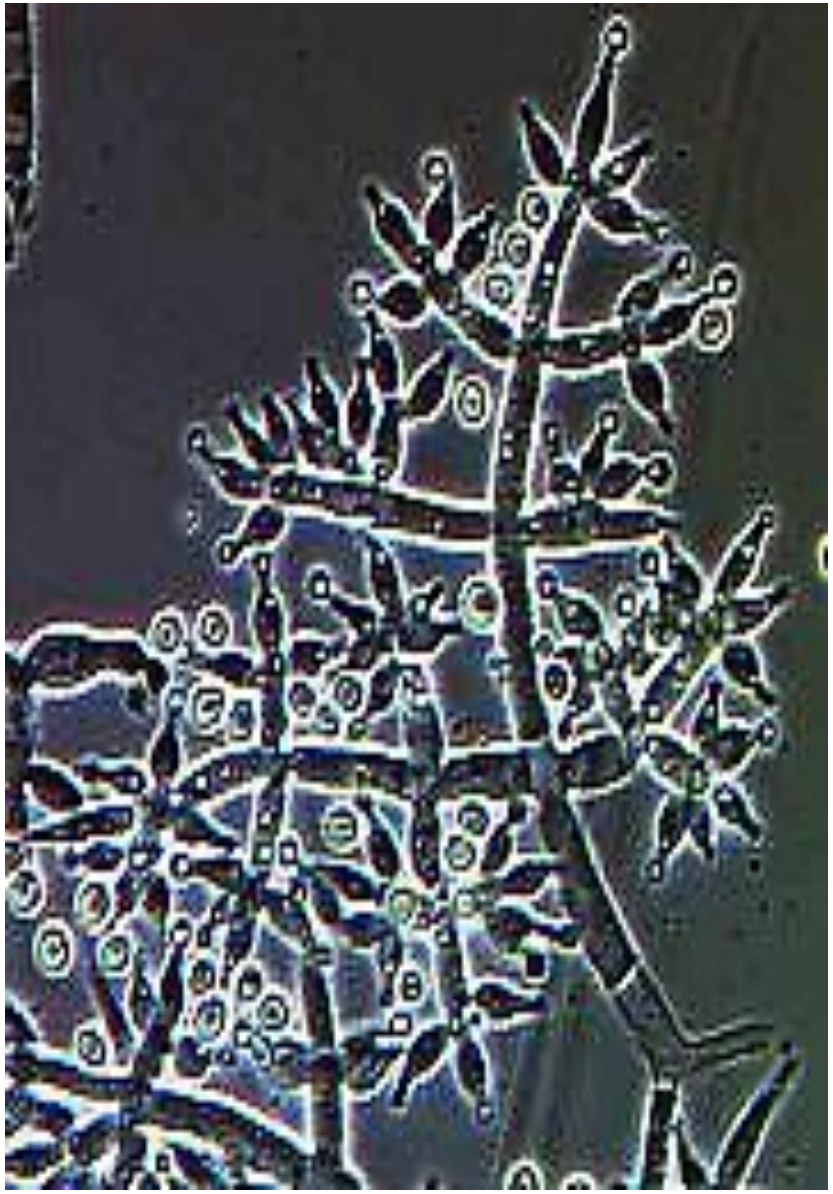
Trichoderma: A bio-control agent



Trichoderma harzianum

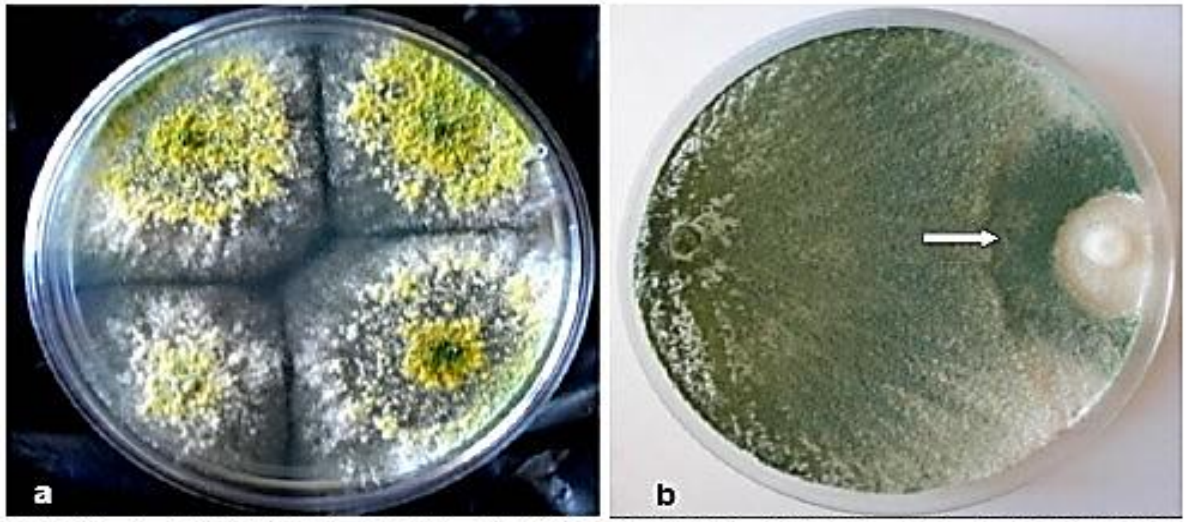
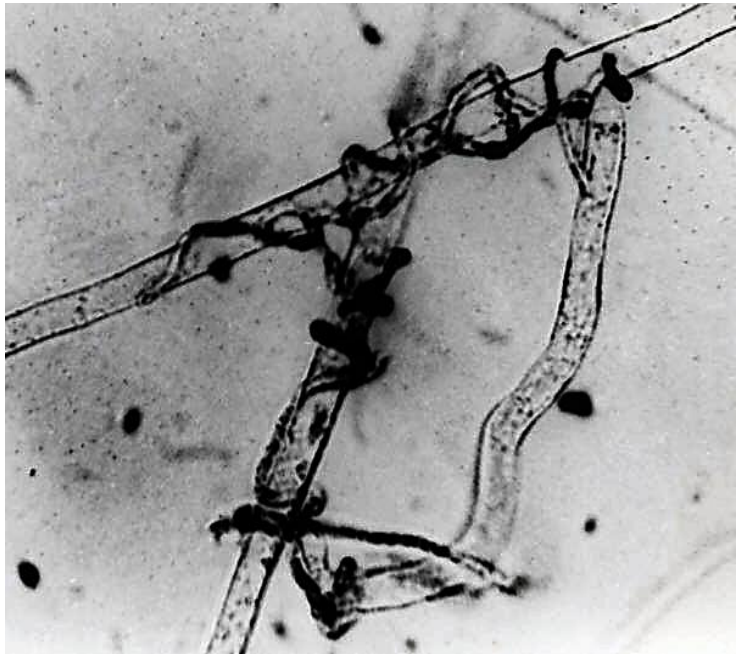
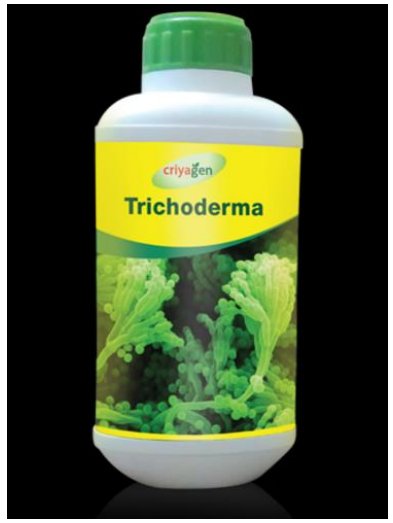


FIGURE 1. *Trichoderma* species growing on potato dextrose agar in Petri dishes (a)*Trichodermaharzianum* growing out of four wood pieces isolated from a grapevine pruning wound. (b) *Trichodermaatroviride* (the green fungus on the left) overgrowing *Eutypalata*(the white fungus on the right) the causal organism of Eutypa dieback.



Trichoderma mycelium parasitizing to mycelium of *Pythium*



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Neem: A bio-control agent

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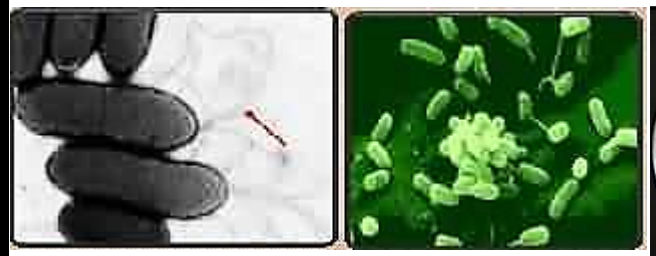
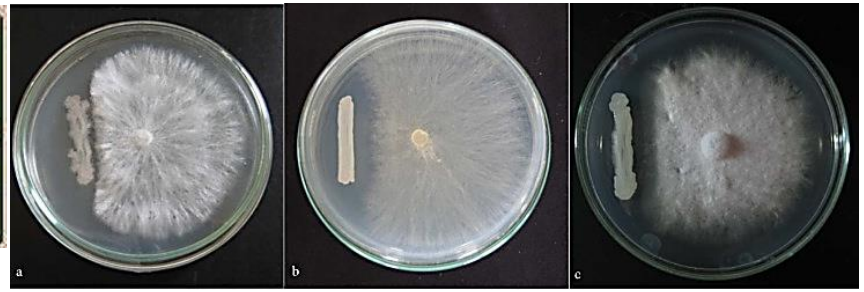
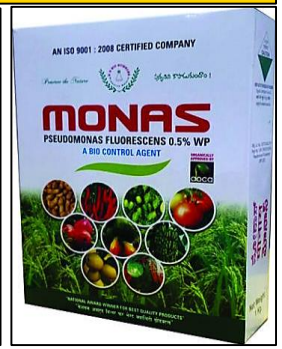
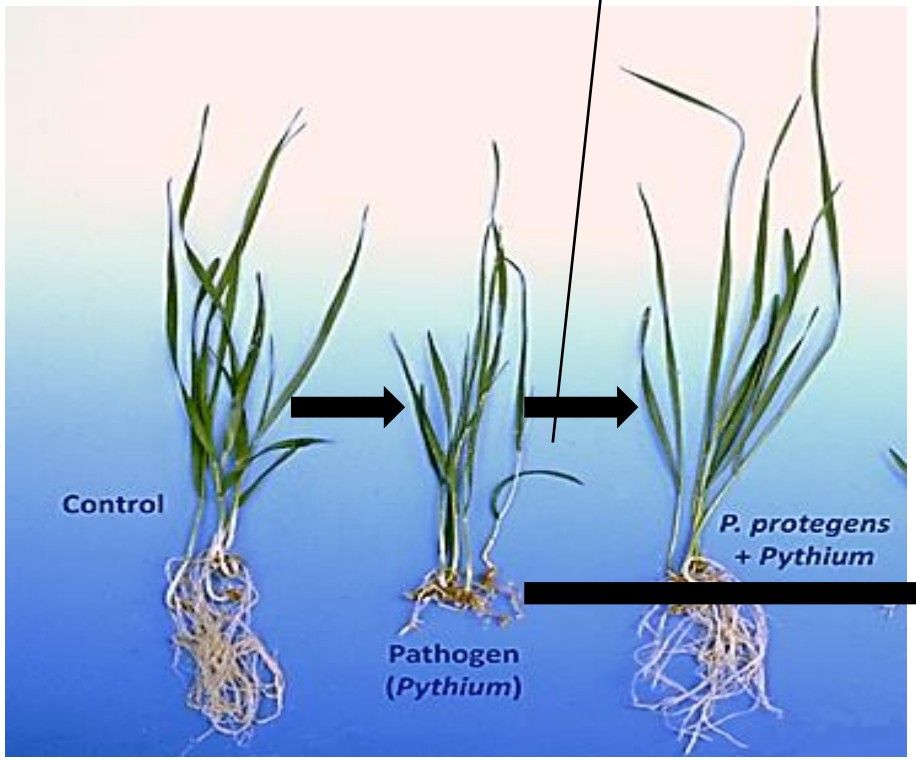
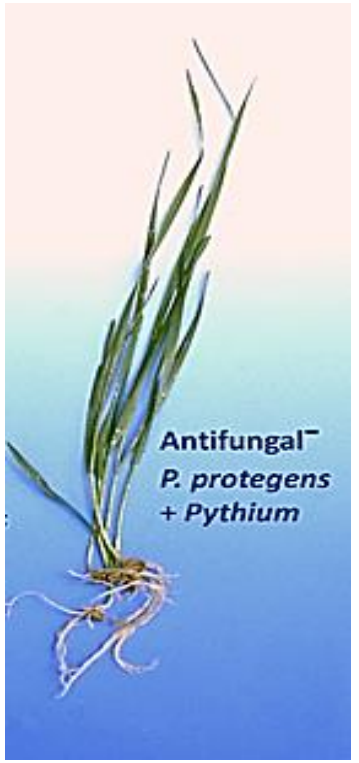
Pseudomonas: A bio-control agent











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WIKIPEDIA

Eisenia fetida

Eisenia fetida (older spelling: foetida), known under various common names such as **redworm**, **brandling worm**, **panfish worm**, **trout worm**, **tiger worm**, **red wiggler worm**, etc., is a species of earthworm adapted to decaying organic material. These worms thrive in rotting vegetation, compost, and manure. They are epigean, rarely found in soil. In this trait, they resemble *Lumbricus rubellus*.

They have groups of bristles (called setae) on each segment that move in and out to grip nearby surfaces as the worms stretch and contract their muscles to push themselves forward or backward.

E. fetida worms are used for vermicomposting of both domestic and industrial organic waste.[2][3][4] They are native to Europe, but have been introduced (both intentionally and unintentionally) to every other continent except Antarctica. Tiger worms are also being tested for use in a flushless toilet, currently being trialled in India, Uganda and Myanmar.[5]

Eisenia fetida	
	
Scientific classification	
Kingdom:	Animalia
Phylum:	Annelida
Class:	Clitellata
Order:	Haplotaxida
Family:	Lumbricidae
Genus:	*Eisenia*
Species:	***E. fetida***
Binomial name	
Eisenia fetida (Savigny, 1826) [1]	

Contents

Odor

When roughly handled, a redworm exudes a pungent liquid, thus the specific name *foetida* meaning "foul-smelling". This is presumably an antipredator adaptation.

Related species

E. fetida is closely related to *E. andrei*, also referred to as *E. f. andrei*. The only simple way of distinguishing the two species is that *E. fetida* is sometimes lighter in colour. Molecular analyses have confirmed their identity as separate species, and breeding experiments have shown that they do produce hybrids. [6]

Reproduction

As with other earthworm species, *E. fetida* is hermaphroditic. However, two worms are still required for reproduction. The two worms join clitella, the large, lighter-colored bands which contain the worms' reproductive organs, and which are only prominent during the reproduction process. The two worms exchange sperm. Both worms then secrete cocoons which contain several eggs each. These cocoons are lemon-shaped and are pale yellow at first, becoming more brownish as the worms inside become mature. These cocoons are clearly visible to the naked eye.

Close-up of *E. fetida* with visible bristles

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External links

- "Friday Fellow: Tiger Worm" (https://earthlingnature.wordpress.com/2017/07/21/friday-fellow-tiger-worm/) at Earthling Nature.

WIKIPEDIA

Vermicompost

Vermicompost (**vermi-compost**, **vermiculture**) is the product of the decomposition process using various species of worms, usually red wigglers, white worms, and other earthworms, to create a mixture of decomposing vegetable or food waste, bedding materials, and vermicast.

Vermicomposting uses worms to decompose waste and make nutrient-rich "worm manure".

Vermicast (also called worm castings, worm humus, worm manure, or worm faeces) is the end-product of the breakdown of organic matter by earthworms.[1] These castings have been shown to contain reduced levels of contaminants and a higher saturation of nutrients than the organic materials before vermicomposting.[2]

Vermicompost contains water-soluble nutrients and is an excellent, nutrient-rich organic fertilizer and soil conditioner.[3] It is used in farming and small scale sustainable, organic farming.

Vermicomposting can also be applied for treatment of sewage. A variation of the process is vermifiltration (or vermidigestion) which is used to remove organic matter, pathogens and oxygen demand from wastewater or directly from blackwater of flush toilets.[4][5]

Contents

Overview

Vermicomposting has gained popularity in both industrial and domestic settings because, as compared with conventional composting, it provides a way to treat organic wastes more quickly. In manure composting, it also generates products that have lower salinity levels.[6]

The earthworm species (or composting worms) most often used are red wigglers (*Eisenia fetida* or *Eisenia andrei*), though European nightcrawlers (*Eisenia hortensis* or *Dendrobaena veneta*) could also be used. Red wigglers are recommended by most vermicomposting experts, as they have some of the best appetites and breed very quickly. Users refer to European nightcrawlers by a variety of other names, including *dendrobaenas*, *dendras*, Dutch nightcrawlers, and Belgian nightcrawlers.

Containing water-soluble nutrients, vermicompost is a nutrient-rich organic fertilizer and soil conditioner in a form that is relatively easy for plants to absorb.[3] Worm castings are sometimes used as an organic fertilizer. Because the earthworms grind and uniformly mix minerals in simple forms, plants need only minimal effort to obtain them. The worms' digestive systems create environments that allow certain species of microbes to thrive to help create a "living" soil environment for plants.[7] The fraction of soil which has gone through the digestive tract of earthworms is called the drilosphere.[8]

Design considerations

Suitable worm species

One of the species most often used for composting is the red wiggler or tiger worm (*Eisenia fetida* or *Eisenia andrei*); *Lumbricus rubellus* (a.k.a. red earthworm or dilong (China)) is another breed of worm that can be used, but it does not adapt as well to the shallow compost bin as does *Eisenia fetida*. European nightcrawlers (*Eisenia hortensis*) may also be used. Users refer to European nightcrawlers by a variety of other names, including dendrobaenas, dendras, and nightcrawlers. African Nightcrawlers (*Eudrilus eugeniae*) are another set of popular composters. *Lumbricus terrestris* (a.k.a. Canadian nightcrawlers (US) or common earthworm (UK)) are not recommended, since they burrow deeper than most compost bins can accommodate.[9]

Blueworms (*Perionyx excavatus*) may be used in the tropics.[10]

These species commonly are found in organic-rich soils throughout Europe and North America and live in rotting vegetation, compost, and manure piles. They may be an invasive species in some areas.[1][11] As they are shallow-dwelling and feed on decomposing plant matter in the soil, they adapt easily to living on food or plant waste in the confines of a worm bin.

Composting worms are available to order online, from nursery mail-order suppliers or angling shops where they are sold as bait. They can also be collected from compost and manure piles. These species are not the same worms that are found in ordinary soil or on pavement when the soil is flooded by water.

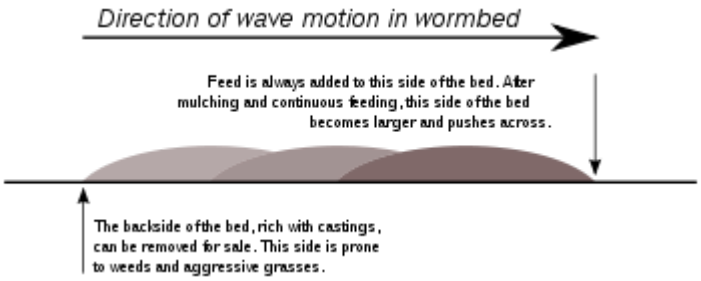
Large scale

Large-scale vermicomposting is practiced in Canada, Italy, Japan, India, Malaysia, the Philippines, and the United States.[12] The vermicompost may be used for farming, landscaping, to create compost tea, or for sale. Some of these operations produce worms for bait and/or home vermicomposting.

There are two main methods of large-scale vermiculture. Some systems use a windrow, which consists of bedding materials for the earthworms to live in and acts as a large bin; organic material is added to it. Although the windrow has no physical barriers to prevent worms from escaping, in theory they should not due to an abundance of organic matter for them to feed on. Often windrows are used on a concrete surface to prevent predators from gaining access to the worm population.

The windrow method and compost windrow turners were developed by Fletcher Sims Jr. of the Compost Corporation in Canyon, Texas. The Windrow Composting system is noted as a sustainable, cost-efficient way for farmers to manage dairy waste.[13]

The second type of large-scale vermicomposting system is the raised bed or flow-through system. Here the worms are fed an inch of "worm chow" across the top of the bed, and an inch of castings are harvested from below by pulling a breaker bar across the large mesh screen which forms the base of the bed.



Movement of castings through a worm bed

Because red worms are surface dwellers constantly moving towards the new food source, the flow-through system eliminates the need to separate worms from the castings before packaging. Flow-through systems are well suited to indoor facilities, making them the preferred choice for operations in colder climates.

Small scale

For vermicomposting at home, a large variety of bins are commercially available, or a variety of adapted containers may be used. They may be made of old plastic containers, wood, Styrofoam, or metal containers. The design of a small bin usually depends on where an individual wishes to store the bin and how they wish to feed the worms.


Demonstration home scale worm bin at a community garden site (painted plywood)

Some materials are less desirable than others in worm bin construction. Metal containers often conduct heat too readily, are prone to rusting, and may release heavy metals into the vermicompost. Styrofoam containers may release chemicals into the organic material.[14] Some cedars, yellow cedar, and redwood

contain resinous oils that may harm worms,[15] although western red cedar has excellent longevity in composting conditions. Hemlock is another inexpensive and fairly rot-resistant wood species that may be used to build worm bins.[16]

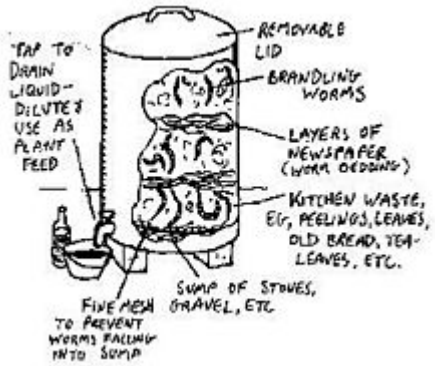


Diagram of a household-scale worm composting bin

Bins need holes or mesh for aeration. Some people add a spout or holes in the bottom for excess liquid to drain into a tray for collection.[17] The most common materials used are plastic: recycled polyethylene and polypropylene and wood.[18] Worm compost bins made from plastic are ideal, but require more drainage than wooden ones because they are non-absorbent. However, wooden bins will eventually decay and need to be replaced.

Small-scale vermicomposting is well-suited to turn kitchen waste into high-quality soil amendments, where space is limited. Worms can decompose organic matter without the additional human physical effort (turning the bin) that bin composting requires.

Composting worms which are detritivorous (eaters of trash), such as the red wiggler *Eisenia fetidae*, are epigeic (surface dwellers) and together with symbiotic associated microbes are the ideal vectors for decomposing food waste. Common earthworms such as *Lumbricus terrestris* are anecic (deep burrowing) species and hence unsuitable for use in a closed system.[19] Other soil species that contribute include insects, other worms and molds.[20]

Climate and temperature

There may be differences in vermicomposting method depending on the climate.[21] It is necessary to monitor the temperatures of large-scale bin systems (which can have high heat-retentive properties), as the raw materials or feedstocks used can compost, heating up the worm bins as they decay and killing the worms.

The most common worms used in composting systems, redworms (*Eisenia foetida, Eisenia andrei,* and *Lumbricus rubellus*) feed most rapidly at temperatures of 15–25 °C (59-77 °F). They can survive at 10 °C (50 °F). Temperatures above 30 °C (86 °F) may harm them.[22] This temperature range means that indoor vermicomposting with redworms is possible in all but tropical climates. Other worms like Perionyx excavatus are suitable for warmer climates.[23] If a worm bin is kept outside, it should be placed in a sheltered position away from direct sunlight and insulated against frost in winter.

Feedstock

There are few food wastes that vermicomposting cannot compost, although meat waste and dairy products are likely to putrefy, and in outdoor bins can attract vermin. Green waste should be added in moderation to avoid heating the bin.

Small-scale or home systems

Such systems usually use kitchen and garden waste, using "earthworms and other microorganisms to digest organic wastes, such as kitchen scraps".[24] This includes:

- All fruits and vegetables (including citrus, in limited quantities)
- Vegetable and fruit peels and ends
- Coffee grounds and filters
- Tea bags (even those with high tannin levels)
-
- Grains such as bread, cracker and cereal (including moldy and stale)
- Eggshells (rinsed off)
- Leaves and grass clippings (not sprayed with pesticides[25])
- Newspapers (most inks used in newspapers are not toxic)
- Paper toweling (which has not been used with cleaners or chemicals)

Large-scale or commercial

Such vermicomposting systems need reliable sources of large quantities of food. Systems presently operating[26] use:

- Dairy cow or pig manure
- Sewage sludge[27][28]
- Brewery waste
- Cotton mill waste
- Agricultural waste
- Food processing and grocery waste
- Cafeteria waste
- Grass clippings and wood chips

Harvesting


Worms in a bin being harvested

Vermicompost is ready for harvest when it contains few-to-no scraps of uneaten food or bedding.[24] There are several methods of harvesting from small-scale systems: "dump and hand sort", "let the worms do the sorting", "alternate containers" and "divide and dump."[29] These differ on the amount of time and labor involved and whether the vermicomposter wants to save as many worms as possible from being trapped in the harvested compost.

The pyramid method of harvesting worm compost is commonly used in small-scale vermiculture, and is considered the simplest method for single layer bins.[30] In this process, compost is separated into large clumps, which is placed back into composting for further breakdown, and lighter compost, with which the rest of the process continues. This lighter mix is placed into small piles on a tarp under the sunlight. The worms instinctively burrow to the bottom of the pile. After a few minutes, the top of the pyramid is removed repeatedly, until the worms are again visible. This repeats until the mound is composed mostly of worms.

When harvesting the compost, it is possible to separate eggs and cocoons and return them to the bin, thereby ensuring new worms are hatched. Cocoons are small, lemon-shaped yellowish objects that can usually be seen with the naked eye.[31] The cocoons can hold up to 20 worms (though 2-3 is most

common). Cocoons can lay dormant for as long as two years if conditions are not conducive for hatching.[32]

Properties

Vermicompost has been shown to be richer in many nutrients than compost produced by other composting methods.[33] It has also outperformed a commercial plant medium with nutrients added, but levels of magnesium required adjustment, as did pH.[34]

However, in one study it has been found that homemade backyard vermicompost was lower in microbial biomass, soil microbial activity, and yield of a species of ryegrass[35] than municipal compost.[35]

It is rich in microbial life which converts nutrients already present in the soil into plant-available forms.

Unlike other compost, worm castings also contain worm mucus which helps prevent nutrients from washing away with the first watering and holds moisture better than plain soil.[36]

Increases in the total nitrogen content in vermicompost, an increase in available nitrogen and phosphorus, as well as the increased removal of heavy metals from sludge and soil have been reported.[37] The reduction in the bioavailability of heavy metals has been observed in a number of studies.[38][39]

Benefits

Soil

- Improves soil aeration
- Enriches soil with micro-organisms (adding enzymes such as phosphatase and cellulase)
- Microbial activity in worm castings is 10 to 20 times higher than in the soil and organic matter that the worm ingests [40]
- Attracts deep-burrowing earthworms already present in the soil
- Improves water holding capacity[41]

Plant growth

- Enhances germination, plant growth, and crop yield
- Improves root growth and structure
- Enriches soil with micro-organisms (adding plant hormones such as auxins and gibberellic acid)

Economic

- Biowastes conversion reduces waste flow to landfills
- Elimination of biowastes from the waste stream reduces contamination of other recyclables collected in a single bin (a common problem in communities practicing single-stream recycling)
- Creates low-skill jobs at local level
- Low capital investment and relatively simple technologies make vermicomposting practical for less-developed agricultural regions

Environmental

- Helps to close the "metabolic gap" through recycling waste on-site
- Large systems often use temperature control and mechanized harvesting, however other equipment is relatively simple and does not wear out quickly
- Production reduces greenhouse gas emissions such as methane and nitric oxide (produced in landfills or incinerators when not composted).

Uses

Soil conditioner

Mid-scale worm bin (1 m X 2.5 m up to 1 m deep), freshly refilled with bedding

Vermicompost can be mixed directly into the soil, or mixed with water to make a liquid fertilizer known as worm tea.

The dark brown waste liquid, or leachate, that drains into the bottom of some vermicomposting systems is not to be confused with worm tea. It is an uncomposted byproduct from when water-rich foods break down and may contain pathogens and toxins. It is best discarded or applied back to the bin when added moisture is needed for further processing.[42][43]

The pH, nutrient, and microbial content of these fertilizers varies upon the inputs fed to worms. Pulverized limestone, or calcium carbonate can be added to the system to raise the pH.

Operation and maintenance

Smells

Worms and fruit fly pupas under the lid of a home worm bin.

When closed, a well-maintained bin is odorless; when opened, it should have little smell—if any smell is present, it is earthy.[44] The smell may also depend on the type of composted material added to the bin. An unhealthy worm bin may smell, potentially due to low oxygen conditions. Worms require gaseous oxygen.[45] Oxygen can be provided by airholes in the bin, occasional stirring of bin contents, and removal of some bin contents if they become too deep or too wet. If decomposition becomes anaerobic from excess wet feedstock added to the bin, or the layers of food waste have become too deep, the bin will begin to smell of ammonia.

Moisture

Moisture must be maintained above 50%, as lower moisture content will not support worm respiration and can increase worm mortality. Operating moisture-content range should be between 70-90%, with a suggested content of 70-80% for vermicomposting-oriented vermiculture operations.[46] If decomposition has become anaerobic, to restore healthy conditions and prevent the worms from dying, excess waste water must be reduced and the bin returned to a normal moisture level. To do this, first reduce addition of food scraps with a high moisture content and second, add fresh, dry bedding such as shredded newspaper to your bin, mixing it in well.[47]

Pest species

Pests such as rodents and flies are attracted by certain materials and odors, usually from large amounts of kitchen waste, particularly meat. Eliminating the use of meat or dairy product in a worm bin decreases the possibility of pests.[48]

Predatory ants can be a problem in African countries.[49]

In warm weather, fruit and vinegar flies breed in the bins if fruit and vegetable waste is not thoroughly covered with bedding. This problem can be avoided by thoroughly covering the waste by at least 5 centimetres (2.0 in) of bedding. Maintaining the correct pH (close to neutral) and water content of the bin (just enough water where squeezed bedding drips a couple of drops) can help avoid these pests as well.

Worms escaping

Worms generally stay in the bin, but may try to leave the bin when first introduced, or often after a rainstorm when outside humidity is high.[50] Maintaining adequate conditions in the worm bin and putting a light over the bin when first introducing worms should eliminate this problem.[51]

Nutrient levels

Commercial vermicomposters test, and may amend their products to produce consistent quality and results. Because the small-scale and home systems use a varied mix of feedstocks, the nitrogen, phosphorus and potassium (NPK) content of the resulting vermicompost will also be inconsistent. NPK testing may be helpful before the vermicompost or tea is applied to the garden.

In order to avoid over-fertilization issues, such as nitrogen burn, vermicompost can be diluted as a tea 50:50 with water, or as a solid can be mixed in 50:50 with potting soil.[52]

Additionally, the mucous layer created by worms which surrounds their castings allows for a "time release" effect, meaning not all nutrients are released at once. This also reduces the risk of burning the plants, as is common with the use and overuse of commercial fertilizers.[53]

Application examples

Vermicomposting (also known as vermiculture) is widely used in North America for on-site institutional processing of food scraps, such as in hospitals, universities, shopping malls, and correctional facilities.[54] Vermicomposting is used for medium-scale on-site institutional organic material recycling, such as for food scraps from universities and shopping malls. It is selected either as a more environmentally friendly choice than conventional disposal, or to reduce the cost of commercial waste removal.

Researchers from the Pondicherry University discovered that worm composts can also be used to clean up heavy metals. The researchers found substantial reductions in heavy metals when the worms were released into the garbage and they are effective at removing lead, zinc, cadmium, copper and manganese.[55]

See also

- Fertilizer
- Home composting
- Maggot farming
- Mary Arlene Appelhof
- Vermifilter
- Vermiponics, use of wormbin leachate in hydroponics
- Waste management

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Pheromone trap

A **pheromone trap** is a type of insect trap that uses pheromones to lure insects. Sex pheromones and aggregating pheromones are the most common types used. A pheromone-impregnated lure, as the red rubber septa in the picture, is encased in a conventional trap such as a bottle trap, Delta trap, water-pan trap, or funnel trap. Pheromone traps are used both to count insect populations by sampling, and to trap pests such as clothes moths to destroy them.

Chamaesphecia empiformis (Sesiidae) on a red rubber septa pheromone lure

Sensitivity

Pheromone traps are very sensitive, meaning they attract insects present at very low densities. They are often used to detect presence of exotic pests, or for sampling, monitoring, or to determine the first appearance of a pest in an area. They can be used for legal control, and are used to monitor the success of the Boll Weevil Eradication Program and the spread of the gypsy moth. The high species-specificity of pheromone traps can also be an advantage, and they tend to be inexpensive and easy to implement.

However, it is impractical in most cases to completely remove or "trap out" pests using a pheromone trap. Some pheromone-based pest control methods have been successful, usually those designed to protect enclosed areas such as households or storage facilities. There has also been some success in mating disruption. In one form of mating disruption, males are attracted to a powder containing female attractant pheromones. The pheromones stick to the males' bodies, and when they fly off, the pheromones make them attractive to other males. It is hoped that if enough males chase other males instead of females, egg-laying will be severely impeded.[1]

Some difficulties surrounding pheromone traps include sensitivity to bad weather, their ability to attract pests from neighboring areas, and that they generally only attract adults, although it is the juveniles in many species that are pests.[2] They are also generally limited to one sex.

Targets

Though certainly not all insect pheromones have been discovered, many are known and many more are discovered every year. Some sites curate large lists of insect pheromones.[3] Pheromones are frequently used to monitor and control lepidopteran and coleopteran species, with many available commercially.[4] Pheromones are available for insects including:

- African bollworm
- African cotton leafworm
- Apple brown tortrix
- Apple clearwing moth
- Apple fruit moth
- Apple maggot
- Artichoke moth
- Asian beetle
- Asian corn borer moth
- Baluchistan fruit fly
- Banana weevil
- Banded elm bark beetle
- Barred fruit-tree tortrix
- Beech tortrix moth
- Beet armyworm
- Bertha armyworm

- Black cutworm
- Blueberry maggot
- Bollworm
- Bright-line brown-eye or tomato moth
- Brown oak tortrix
- Cabbage leaf roller
- Cabbage looper moth
- Cabbage moth
- Carnation tortrix
- Carob moth
- Cherry-bark moth
- Cherry fruit fly
- Citrus cutworm
- Citrus flower moth
- Citrus leafmining moth
- Citrus mealybug
- Codling moth
- Corn earworm
- Corn stalk borer
- Cucumber fruit fly
- Cucumber moth
- Currant clearwing moth
- Cutworm
- Date palm fruit stalk borer
- Diamond back moth
- Douglas-fir tussock moth
- Dubas bug
- Durra stem borer
- Eastern cherry fruit fly
- Eggplant shoot and fruit borer
- Egyptian cotton leaf worm
- Engraver beetle
- European corn borer
- European goat moth
- European pine shoot moth
- European spruce bark beetle
- Eye-spotted bud moth
- Fall armyworm [5]
- False codling moth
- Fruit fly
- Fruit tree leaf roller
- Garden pebble
- Golden leaf roller
- Golden twin moth or groundnut semi-looper moth
- Grape moth or vine moth
- Green oak moth
- Grey tortrix
- Gypsy moth
- Hants moth
- Jasmine moth [6]
- Large fruit tree tortrix
- Leche's twist moth
- Leek moth or onion moth
- Legume pod borer
- Leopard moth
- Lesser peach tree borer
- Longhorn date stem borer
- Marbled orchard tortrix
- Mediterranean fruit fly
- Mediterranean pine engraver beetle
- Melon fly
- Northern bark beetle
- Nun moth
- Olive fruit fly
- Olive moth
- Orange tortrix
- Oriental fruit fly
- Oriental fruit moth
- Pea moth
- Peach fruit fly
- Pear leaf blister moth
- Pear twig borer
- Pine processionary moth
- Pine sawfly
- Pink bollworm
- Plum fruit moth
- Potato moth
- Potato tuber moth
- Queensland fruit fly
- Quince moth
- Red palm weevil
- Rhinoceros beetle
- Rice stem borer
- Rose tortrix
- San Jose scale
- Sesiidae (some)

- Silver Y moth
- Six-spined spruce bark beetle
- Six-toothed bark beetle
- Spiny boll worm
- Spotted bollworm
- Spotted tentiform miner
- Straw coloured tortrix moth
- Sugar beet weevil
- Summer fruit tortrix moth
- Tobacco budworm
- Tomato leaf miner
- Tomato looper
- Turnip moth
- Variegated golden tortrix
- Winter moth
- Xyloterus bark beetle

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Int. J. Agril. Res. Innov. & Tech. 2 (1): 47-55, June, 2012 Available online at http://www.ijarit.webs.com

PHEROMONE USE FOR INSECT CONTROL: PRESENT STATUS AND PROSPECT IN BANGLADESH

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Received 28 February 2012, Revised 10 June 2012, Accepted 15 June 2012, Published online 30 June 2012

Abstract

The insect's world is filled with many odors. Insects use these odors to cue them in a variety of complex social behaviors, including courtship, mating, and egg laying. Scientists and pest control specialists have known about these complex communication systems for decades. The main aim of this study was to visualize the availability, trends and differences in the sources of pheromone control in agricultural growth of Bangladesh. It also concerned on constrains and present use of pheromone and their possible recommendation on behalf of Bangladesh agriculture. It concentrated on the data during last three decades (1980-2010), comprising status of pheromone use in Bangladesh agriculture and its future. Review revealed that Bangladesh has been enormously successful in increasing pheromone use in agricultural production (especially for vegetables). Understanding of the nature of pheromones and their potential for pest control along with the future prospective of pheromone technique in agriculture were stated. Since the pheromone, technologies for control of major crop pests in Bangladesh are still limited. So that this review emphasized on more attention to the authority to increase the research works and project facilities related to develop and promote pheromone techniques. It is highly recommended to increase availability of pheromone in market, more investment in research and development, introduction of newly identified pheromone for specific pest, to assist government and non-government organizations to work with farmers to reduce harmful insecticide use and promote pheromone tactics as one part of integrated crop management (ICM).

Keywords: Sex pheromone, Integrated Crop Management, Monitoring, Trapping, Mating disruption

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Reviewed by Duraikannu Vasanthakumar, UPASI Tea Research Institute, Tamil Nadu, India

Introduction

Bangladesh, one of the smallest countries (area 57 K sq. miles) in South-East Asia, has a predominantly farming-based economy. A delta, historically originated through the sedimentation of the Bay of Bengal, the country is blessed with highly fertile agricultural lands. However, due to very high population, the nation has always been struggling against poverty and starvation. Bangladesh economy draws its main strength from agriculture sector. The sector contributes 19.10% (at current prices) and employs about 51% of the labor force (Mondal, 2010). Despite increase in the shares of fisheries, livestock, and forestry, crop sub-sector alone accounts for 60.83% share of agricultural GDP (BBS, 2011). Agricultural land per capita is decreasing over the years in Bangladesh. Agriculture and environment are closely interlinked. Agricultural production system depends on the environment for utilization of land, rainfall, daylight duration, insect pests and diseases. Pest problem is one of the major constraints for achieving higher production in agriculture crops. Bangladesh loses about 30% of its crops due to pests and diseases each year (BBS, 2011). So how do farmers control their insect pest problems? Farmers in Bangladesh depend on synthetic insecticides because they are readily available, highly promoted, inexpensive, easy to apply and quick acting. However, applied insecticides also kill non-target arthropods, typically insects involved in pollination and predators such as spiders and ground beetles. Insecticide residues find their way into watercourses, particularly in rice cultivation, and affect the water we drink and food we eat (Cork *et al.* 2003, 2005). Furthermore, quite often the indiscriminate and unscientific use of pesticides has led to many problems, such as pests developing resistance, resurgence of once minor pest into a major problem besides environmental and food safety hazards.

There are many alternative approaches to control insect pests. Cultural practices, including crop sanitation, use of resistant varieties and methods to promote the activities of natural enemies and predators all act to reduce the threat from potential insect pests. Thus keeping insect pests below damage thresholds, does not aim reduce populations to a level where genetic change is induced, leading to the development of resistant biotypes. Such biorational pesticides include insecticides such as neem kernel seed extract, different extracts of plant parts, fungi (*Beaveria, Metarhizium*), viruses (nuclear polyhedrosis virus), bacteria (*Bacillus thuringiensis*) and semiochemicals (Akhtar and Mandal, 2008; Islam *et al.*, 2008a; Mamun *et al.,* 2008; Islam, 2009; Islam and Becerra, 2011; Islam and Begum, 2011). Country needs to adopt the total integrated pest management system as the insecticide-based management system has failed to control many pests. The pests are becoming resistant to almost all chemical pesticides as the frequency of spraying is gradually increasing while their efficacy is gradually decreasing. Biological control involves use of a specially chosen organism to control a specific pest. This chosen organism might be a predator or a parasitoid, which attacks harmful insects. Field demonstration results prove that the use of biological agents has no adverse effect on human health and the cost effectiveness of bio-control measures is very attractive, safe and sustainable. Insects communicate by means of scents–pheromones, chemicals used for 'signaling'. With these, they both locate and identify their mates. They are natural chemicals emitted in micro quantities in the form of a vapor by virtually all known insects. Each insect species has its own unique signature scent. In fact, sex provides us with a powerful means of surveillance and control in the insect world. A female insect typically puffs out a thousand millionth of a gram of her signature several times a minute. Males of her species follow this scent to mate with the female. It follows that if you can identify and then duplicate that scent, you have the means of controlling the males of that species. This is the mysterious incidence of pheromone technology.

The existence of pheromones has been known for centuries, apparently originating in observations of mass bee stinging in response to a chemical released by the sting of a single bee. The first isolation and identification of an insect pheromone (silkworm moth) occurred in 1959 by German scientists. Since then, hundreds, perhaps thousands of insect pheromones have been identified by increasingly sophisticated equipment. Today we have a much clearer view of the limitations and possibilities associated with insect pheromones in IPM programs. The two primary uses of insect pheromones are for detection and monitoring of populations and for mating disruption. These uses take advantage of sex pheromones on which a vast majority of insect pests relies to mediate reproduction.

To date, the research works on pheromone in pest management in Bangladesh still limited. There are few scientists involved in trapping of moths using pheromone (Alam *et al.*, 2003; Cork *et al.*, 2001, 2003, 2004a, 2004b, 2005; Uddin, 2008; Mazumder and Khalequzzaman, 2010) in Bangladesh but no one works on the identification of pheromone from Bangladeshi insect species. The survey report on pheromone practice in Bangladesh agriculture is also limited (Islam, 2012). Major objective of this review article is to discuss the pheromone technology in pest management, present status of pheromone use in Bangladesh agriculture and suggest possible opportunities to address the topic that may assist the government and non-government policy makers to develop national economy.

Uses of Pheromones in Pest Management

The use of pheromone for controlling pest insects requires three items: a pheromone chemical, a trap, and a support to hang the trap in the field. Technically sex pheromones can be used in three principal ways:

Detection and Monitoring: The principle use of insect pheromones is to attract insects to traps for detection and determination of temporal distribution. In most instances, the males are responders to female-produced pheromones. Trap baits, therefore, are designed to closely reproduce the ratio of chemical components and emission rate of calling females. Trap baits of many designs have been tested over the years. Trap design is also critical to effective use of traps for monitoring insect populations. Traps vary in design and size dependent on the behavior of the target insects. The information from trap catches can be very useful for decision making on insecticide applications or other control measures. For example, trap catches may indicate a loss of effect of pheromone on mating disruption and the need to reapply a pheromone treatment. Careful monitoring and experience in interpreting collected data are important for success. Traps may also be placed with the objective of destroying males for population control.

Examples of the use of pheromones in pest management programmes for detection, monitoring and timing of pesticide spray programmes are sesiid moth, *Macroscelesia japona* , in orchards of Ibaraki prefecture, Japan (Islam *et al.,* 2007), codling moth, *Cydia pomonella,* in apple and pear orchards in

Australia (Williams, 1989), citrus leaf miner (*Phyllocnistic citrella*) in orchards in Ogasawara (Bonin) Islands and Ehime Prefecture, Japan (Vang *et al.*, 2008), *Heliothis spp.* in USA (Lopez *et al.,* 1990), nettle moth, *Parasa lepida lepida* (Limacodidae) in orchards in Gifu prefecture in Japan (Islam *et al.*, 2009), apple leaf roller, *Bonagota cranaodes* in Brazil (Kovaleski *et al.*, 1998), *Spodoptera litura* in India (Ranga Rao *et al.,* 1991), *Leucinodes orbonalis* in Bangladesh (Alam *et al.*, 2003, Uddin *et al.*, 2008, Mazumder and Khalequzzaman, 2010).

Mass trapping: Sex pheromone baited traps can capture male moths continuously, thus preventing mating and multiplication of the pest. This approach has proven to be particularly efficient and economical. *Rhynchophorus palmarum* is the primary pest of oil, coconut and palm in Central and South America. By 1994 the number of trees needing to be felled was reduced to less than 3,000 per annual demonstrating that mass trapping can be highly effective in controlling palm weevil populations (Alpizar *et al.*, 2002, Hallett *et al.*, 1999, Oehlschlager *et al.*, 2002). Highest mass trapping of males of *Macroscelesia japona* reported by lure baited with *E*2,*Z*13-18: Ald and *E*2,*Z*13-18:OH (Islam *et al.,* 2007). Examples of mass trapping are lures baited by *Z*7,9-10:OH were examined on nettle moth*, P. lepida lepida* (Islam *et al.*, 2009), cotton weevil (*Anthonomus grandis*) successfully baited with its aggregation pheromone (Cork *et al.,* 2003), Japanese strain of *Phyllocnistic citrella* trapped only the lure containing *Z*7,*Z*11-16:Ald (Vang *et al.,* 2008).

Brinjal (*Solanum melongena* L.) is an economically important crop throughout South and South East Asia. Fruit losses in excess of 50% are commonly reported due to the boring activity of larvae of the brinjal shoot and fruit borer, *Leucinodes orbonalis* (Cork *et al.*, 2005). Zhu *et al.* (1987) reported (*E*)-11-hexadecenyl acetate as the pheromone of *L. orbonalis* and traps baited with up to 500 µg attracted more male moths than six virgin females. Subsequently Attygalle *et al.* (1988) identified (*E*)-11-hexadecen-1-ol in addition to the related acetate using insects obtained from Sri Lanka. In field trails conducted in India where blends containing between 1 and 10% *E*11-16: OH caught even more male *L. orbonalis* than *E*11-16: Ac alone. At the 1000 µg dose, addition of 1% *E*11-16: OH to *E*11-16: Ac was found to be significantly more attractive to male *L. orbonalis* than either 0.1 or 10% *E*11-16:OH. Trap catch was found to be positively correlated with pheromone release rate, with the highest dose tested, 3000 µg, catching significantly more male moths than lower doses (Cork *et al.*, 2001). In order to reduce the cost of pheromone based technologies for control of *S. incertulas*, a programme of research to develop an effective mass trapping system. This proved to be highly effective using indigenous traps and lures at a density of 20 traps ha^{-1} (Cork and Krishnaiah, 2000).

Mating disruption: Sex pheromone can be used for disruption of mating, which is achieved by placing high concentrations of pheromone at regular intervals throughout the field. The high concentration of pheromone saturates the area resulting in males failing to find females, which produce very minute quantities of these chemicals, thus preventing mating and multiplication of the pest. The major pest of cotton in Egypt in the early 1980's was the pink bollworm (PBW), *Pectinophora gossypiella*. The female sex pheromone was identified by workers in the USA (Bierl *et al.*, 1974). The diversity of mating communication system in lepidopteran insects was also reported (Islam *et al.,* 2008b). The economic importance of *P. gossypiella* and the fact that its pheromone is relatively cheap and chemically stable, the decision was made to try to control it using mating disruption. It has also been identified as a pest control method in which the insect does not become resistant.

Pheromone traps

Various types of traps are available commercially, while others can be made by farmers inexpensively at home. A pheromone-baited lure inside the trap will bring male moths inside the trap. Proper trap design is critical to kill the pest once it enters the trap. The type of trap to be used depends on the behaviour of the target insect. Various research works showed the most effective traps in pest control are delta traps, winged traps and funnel traps. Different available and relatively low cost traps are shown in Fig. 1**.** Pheromone traps are very sensitive, meaning they attract insects present at very low densities. They are often used to detect presence of exotic pests, or for sampling, monitoring, or to determine the first appearance of a pest in an area.

Present Status on Pheromone use in Bangladesh

There is significant evidence that insecticide use in Bangladesh is increasing dramatically (Fig. 2). The country imports pesticides worth 24000 crore taka every year a huge business. Farmers are applying pesticides where they are not actually necessary, at high cost to themselves and the environment. These pesticides are becoming redundant because of the development of resistance to them and more importantly affect environmental pollution. Governments are becoming aware of the negative environmental and health aspects associated with the use of these compounds, so now there is an urgent need for the development of alternative control technologies.

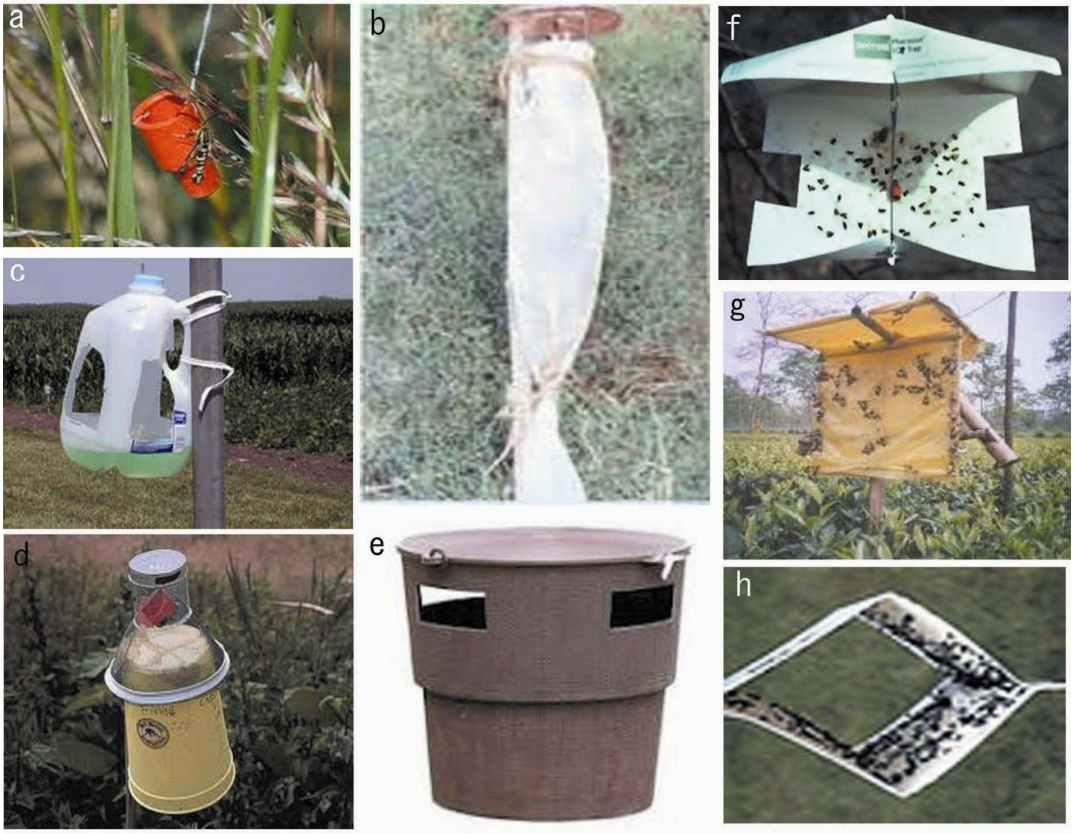

Fig. 1. Different types of pheromone traps used in field; traps used in crop field (a-e), traps with trapped pest insects (f-h). **a.** one lure septum hanged with crop plant, **b.** net trap, **c.** water trap, **d.** bucket trap, **e.** bucket with window trap, **f.** delta trap, **g.** Yellow sticky trap, **h.** wing trap

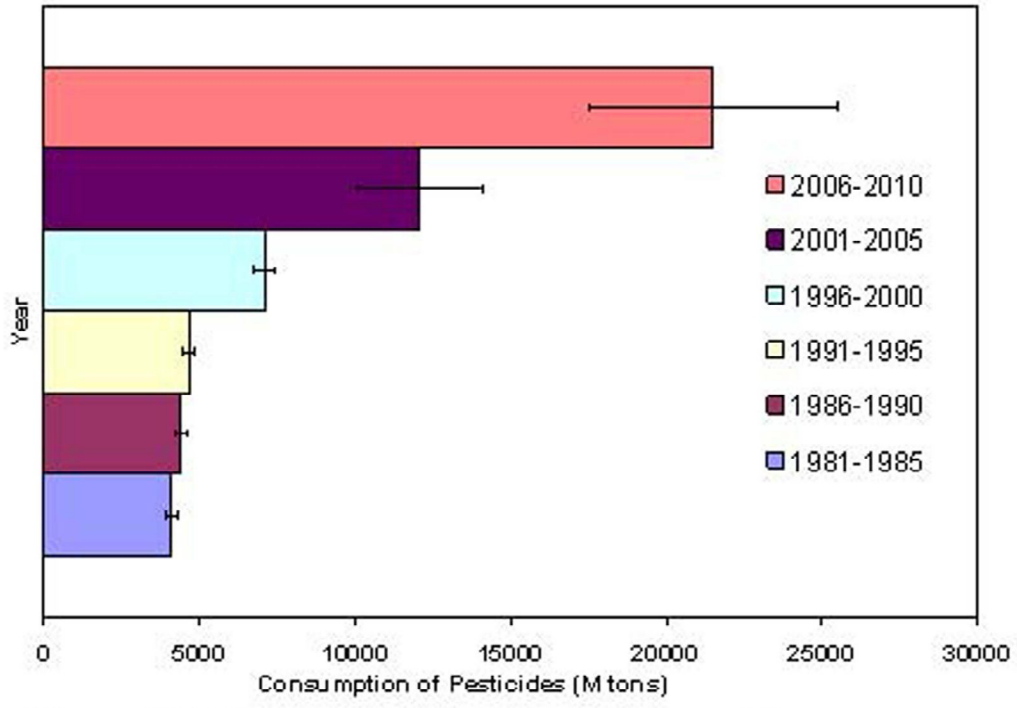

Fig. 2. Pesticide consumption in Bangladesh over 30 years (Source, BBS 2011).

In Bangladesh, about more than 100 major crops are cultivated over a year. There are more than 1000 harmful insects' causes of economic loss on the major cultivated crop. However, the availability of pheromone components of very few insects is reported and available in market. Presently, most of the pheromone components either imported from abroad or synthesized in Bangladesh. However, those chemicals are not confirmed by proper research work for the insects available in Bangladesh. The regional variation in chemical communication reported among the same insect species occurred due to their races differences (Vang *et al.*, 2008). So it should be noted that identified pheromone of an insect from another country may not match with the Bangladeshi strain. This review also emphasized one note that there are extreme limited reports available about the identification of insects' pheromone in Bangladesh.

Bangladesh Agriculture Research Institute (BARI) and Department of Agriculture Extension (DAE) provided synthetic sex pheromones and beneficial insects to a number of farmers in vegetable growing districts like Jessore, Narsingdi, Comilla, Bogra, Pabna and other 244 Upazilas experiment and find out if these inputs could replace harmful pesticides to reduce damage to public health as well as harmful pests can be controlled by using sex pheromone traps, locally known as magic trap. The magic traps are popular among farmers of limited area in Bangladesh that demand for those has increased very quickly. However, ironically the supply is so little that, farmers face problems in expanding this new device for controlling pests in a natural, environment-friendly, safe and secured way to boost crop production. The knowledge and availabilities of pheromone in Bangladesh related similar information reported by Islam (2012).

Limited organizations got permission to import pheromones from abroad. Those organizations got special permission to import several sex pheromones item from plant protection wing of DAE, MOA, GOB. There is a remarkable gap between different research institute and such company or organization engaged in commercialization of pheromones. Limited reports available about the economical benefit achieved after using pheromone technologies in Bangladesh. For example, few farmers in Sikandarpur village in Jessore are being doubly benefited. They are earning more money by producing vegetables at lesser costs while protecting the environment also. They use pheromone trap to kill insects instead of applying insecticides. Alam *et al.* (2003) reported on sex pheromone trap technology for the control of shoot and fruit borer in brinjal and cucurbits in that region of Bangladesh. As above, adult female yellow stem borers (*Scirpophaga incertulas*) attract their mates with a pheromone and this can be exploited in a pest management strategy by developing a synthetic pheromone blend and a lure and trapping system, which attract and trap male moths in Bangladesh. Uddin *et al.* (2008) also reported on such male moth trap, where emphasize on trap types and height to find effective trapping.

Building on work conducted by scientists in India, Syngenta has been collaborating with BRRI and NRI to adapt mass trapping technology for use in Bangladesh (Cork *et al.*, 2004a). According to scientists from three organizations (Natural Resources Institute, NRI; Bangladesh Rice Research Institute, BRRI and the multinational agrochemical company Syngenta) worked in collaboration to find sustainable control method, sleeve traps with a pheromone lure are both effective and cost-efficient in controlling the insect which is responsible for 70-80% of pest damage to rice crops (Anonymous, 2003). Mazumder and Khalequzzaman (2010) reported that the sex pheromones which have been extensively studied and already are in management programmes to improve their efficacy in totality in an Integrated Pest Management Programme on the basis of feedback from the extension workers, and farmers. They studied mainly on the efficiency of male moth catch of eggplant shoot and fruit borer, *Leucinodes orbonalis*.

The previous research reports supported that a wide range of trap designs, pheromone blends and concentrations were tested with farmers in their fields in Comilla and Mymensingh districts in 2001-03, along side a socio-economic study of farmer's resources, constraints and perceptions to ensure the resulting technology was appropriate for adoption (Cork *et al.*, 2004b). On farm, large-scale mass trapping trials demonstrated that 20 traps ha^{-1} were sufficient to reduce male yellow stem borer populations significantly. The trials provided good evidence that mass trapping could significantly reduce the level of mating, with consequent reductions in larval progeny (Cork *et al.*, 2004b).

It is also good news for us that the pheromone of fruit and shoot borer moth is now synthesized and produced in the factory. It is available in the market and to use the sex pheromone in the field, one needs two items: the chemical (or the pheromone) lure and a suitable trap, which are available in Bangladesh with much cost effective price. Thus pheromonal control as an IPM strategy may control eggplant shoot and fruit borer with minimal use of pesticides (Prosad *et al.*, 2005; Uddin et al., 2008; Mazumder and Khalequzzaman, 2010).

Disseminating the idea to farmers is already done in many areas of the country though it is limited. Some of the participating farmers are working in their fields to demonstrate how pheromone/lures worked in their field. It is also important that neighbouring farmers will get preparation to work together in the technology worked. Although most of the farmers in Bangladesh are illiterate but they can still learn the benefits of using the lures around the traps through demonstrations. They already known about the natural enemy of harmful insects are saved, that the danger of insecticide use is avoided, their environment is safe and that they can have a good crop. Recently, Islam (2012) reported on the knowledge and practices on pheromone used by Bangladeshi farmers, where found the present knowledge on pheromone technology and some important suggestions. Farmers now understood the money that they save in reducing their use of pesticides can be spend instead on herbicides which are less toxic and give a more effective return.

Besides, the above discussion on present status of pheromone technology in Bangladesh agriculture few broad problems are pointed out, such as:

1. Insufficient investment in pheromone research in Bangladesh,
2. Use of IPM technology is limited to rice and few vegetables.
3. Lack of proper knowledge on identification and synthesis of pheromones.
4. Lack of scopes for promotion and training of potential scientists on pheromone research.
5. Limited commercial manufacture of pheromone technology for control of insects of rice and brinjal developed.
6. Training programmes for pesticide dealers, farmers on pheromone and ICM technologies are still limited.
7. Insufficiency of materials required for pheromone technology.

Recommendations

In view of the several disadvantages /limitations associated with the unscientific use of pesticides in agriculture, there is an urgent need for minimizing the use of chemical pesticides in the management of insect pests. Growing public concern ever potential health hazards of synthetic pesticides and steep increase in cost of cultivation/low profit making by farmers has led to the exploration of eco-friendly pest management tactics such as Integrated Crop Management (ICM). Admitting the scarcity of sex pheromones and beneficial insects, researchers commented by using huge dozes of pesticides destroyed our biodiversity. The government is yet to consent to bulk import and mass use of insect pheromones. We expect the government and the private sector entrepreneurs to set up more laboratories to identify and synthesize pheromone components of the harmful insects for developing a sustainable pest management system.

To overcome the limitations related to pheromone technology for crop protection; the government, NGOs and personnel engaged in agro-research/works should be concern about the following suggested recommendations:

1. Establishment of a new institution/ foundation is recommended to meet the need for pheromone techniques and adaptation in Bangladesh agriculture.
2. Investment should be raised in pheromone research works collaborated with different agro-based research institutes and agricultural universities.
3. The fund should be raised to help generate pheromone-based technologies with climate change hazards and disseminate such technologies at farmers' level.
4. Government might encourage establishment of farmer's cooperatives to ensure the availability of the pheromones on time.
5. Researcher should be conduct to test the adoptability of identified pheromone for pest insects available in Bangladesh.
6. Researcher should be take similar attention on harmful pests of other major crops like rice and vegetables.
7. More specific pheromone component(s) for the harmful insect(s) should be developed to control the pest(s).
8. Contribution of private sector and NGOs has to be encouraged to quality pheromone production.
9. It is also necessary to expand IPM practice to other economic crops (e.g. Fruits, Pulses etc.).
10. Attempts on pheromone technologies should now be made to transfer at the field level.
11. Farmers should as well be motivated to reduce their dependence on the use of chemical pesticides to control harmful pest insects.
12. Conduction of appropriate training programs on pheromone technologies with farmers, field level agricultural officers, researchers/scientists are necessary.

It is essential to review the present activities on pheromone tactics to pest control with the relevant experts, professionals, and farmer's representatives and update it based on their comments and suggestions. Author would like to illustrate the high attention on status of pheromone use in Bangladesh agriculture and future activities is addressed by the government to ensure sustainable agriculture and food

security. This issue needs to be spelled out in the present National Agricultural Policy (NAP).

Conclusion

Bangladesh has been enormously successful in increasing its agricultural production in an effort to attain self-sufficiency. This has been largely achieved through new technologies in agriculture. The farmers were immensely benefited by using a combination of pheromones and beneficial insects when they could no longer control harmful pests even after spraying insecticides. Damage to production was 40 to 50 per cent even after spraying cocktail insecticides but after using pheromones and beneficial insects, the rate of damage diminished to 10 per cent. The identification and uses of insect pheromones is an active area of research and new developments continue to be made. Potentially, pheromones may be used to trap out certain Bangladeshi harmful insects and to attract insects to insecticide or chemosterilant baits, reduce the number of insecticide applications, or to confuse insects and disrupt mating. Use of traps as a sampling tool to determine need for and timing of control measures can provide the basis of an ICM/IPM strategy for these pests.

Pheromone programs have been used for several decades around the globe and to date (2012) there is no documented public health evidence to suggest that agricultural use of synthetic pheromones is harmful to humans or to any other non-target species. However, continuing research is being conducted. In order to reduce the pesticidal load in the environment and with sustainability, certain behavioral chemicals could be harnessed. Such an endeavor is the use of sex pheromones. This dynamic and paradigm shift in management strategies satisfies all the bio-safety concern as well as playing a pivotal role in combating insect pests of high-value and damage sensitive crops. The research from socio-economists will help to guide the strategy for promotion of the pheromone technology in Bangladesh successfully. This review facilitates technical ways for identification/confirmation, understanding of the nature of pheromones, their potential for pest control and overview in respect of Bangladesh.

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Pseudomonas fluorescens: A Promising Biocontrol Agent and PGPR for Sustainable Agriculture

Chapter · February 2016

DOI: 10.1007/978-81-322-2647-5_15

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15 *Pseudomonas fluorescens*: A Promising Biocontrol Agent and PGPR for Sustainable Agriculture

Deepak G. Panpatte, Yogeshvari K. Jhala, Harsha N. Shelat, and Rajababu V. Vyas

Abstract

Indiscriminate use of chemicals as fertilizers and pesticide caused incredible harm to the environment and ecosystem including animals and humans. To replace such type of hazardous agrochemicals, biological solution is provided by nature in the form of microorganisms having capacity to promote the plant growth without substantially harming the environment. One of the biological approaches for the control of different phytopathogenic agents is the use of biocontrol plant growth-promoting rhizobacteria (PGPR), which is capable of suppressing or preventing the phytopathogen damage. The best characterized biocontrol PGPR belong to the bacteria genus *Pseudomonas*. Fluorescent pseudomonads are suitable for application as biological control agents due to their abundant population in natural soils and plant root system and their capability to utilize many plant exudates as nutrient. Fluorescent pseudomonads are known to have important traits in bacterial fitness such as the ability to adhere to soil particles and to the rhizoplane, motility and prototrophy, synthesis of antibiotics, and production of hydrolytic enzymes. Moreover, *Pseudomonas* also possesses plant growth-promoting traits such as nitrogen fixation, phosphate solubilization, iron chelation, and phytohormone production. Such multidimensional utility of fluorescent *Pseudomonas* makes them a bioagent of choice to be exploited in the field of agriculture.

Keywords

Biocontrol • Rhizobacteria • Phytopathogens • Fluorescent • Pseudomonas

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D.P. Singh et al. (eds.), *Microbial Inoculants in Sustainable Agricultural Productivity*, DOI 10.1007/978-81-322-2647-5_15

15.1 Need of Biocontrol Agents

Across the world, plant diseases are major cause of yield loss. The global market for phytosanitary products is dominated by synthetic pesticides (Thakore 2006). There are many disadvantages of using such chemical pesticides which include accumulation of toxic residues in environment and adaptation of pathogens to such chemicals which in turn reduce its efficiency and led to undesirable effect on nontarget organisms prevailing in the same niche. Moreover, nowadays, consumers are becoming more and more concerned about pesticide-free safer foods which results in emergence of eco-friendly strategies for plant disease management, i.e., biocontrol agents.

15.2 What Are Biocontrol Agents ?

Biocontrol agents can be defined as living organisms or natural products derived from living organisms (genetically modified crops, insects, nematodes, and microorganisms; Fig. 15.1) that are used to suppress plant pathogen pest populations.

Among these biocontrol agents, microorganism-based products (bacteria, fungi, virus, and yeasts) represent 30 % of total sales (Thakore 2006). Microbial biocontrol agents are having different modes of action for dealing with pathogens. The application of biocontrol agents and disease suppressing chemicals can reduce the possibility of resistance development among pathogen representing an integrated pest management strategy with the goal of minimizing the use of chemicals. Most of the bacterial strains exploited as biocontrol agents belong to the genera *Agrobacterium, Bacillus*, and *Pseudomonas* (Fravel 2005).

15.3 *Pseudomonas* as Biocontrol Agent

Research carried out at the University of California, Berkeley, during the late 1970s (Weller 1988) has awakened the global interest in the *Pseudomonas* sp. as biocontrol agents. Species of fluorescent *Pseudomonas* are capable of utilizing wide range of organic and inorganic compounds which imparts them capacity to live in varied environmental conditions. Members of this genus are found in large numbers in all the major natural environments, viz., terrestrial, freshwater, and marine, and they also form intimate associations with plants and animals. This widespread dispersal suggests a significant amount of physiological and genetic flexibility (Nowak-Thompson et al. 1997). The bacteria belonging to genus *Pseudomonas* are functionally diverse and ecologically noteworthy microorganisms because of their multiple utility as plant growth-promoting agents and bioremediators. Pseudomonads are gram-negative, chemoheterotrophic, and motile rods with polar flagella as defined by Palleroni (1984). *Pseudomonas* has been recognized as a complex collection of a large number of described species (Gardener et al. 2005). The functional and metabolic heterogeneity of *Pseudomonas* has been well documented from comprehensive studies dating to more than 45 years ago. Species of the genus *Pseudomonas* embodies an attractive biocontrol agent because of their catabolic adaptability, their outstanding root-colonizing abilities, and their capacity to produce a wide range of antifungal metabolites. Among various *Pseudomonas* spp., fluorescent pseudomonads have received particular attention as biocontrol agent of choice. *Pseudomonas* exerts its biocontrol activity through direct antagonism of phytopathogens and induction of disease resistance in the host plant (Cartieaux et al. 2003). Fluorescent *Pseudomonas* is a widely studied group among common inhabitants of the rhizosphere. They can be visually distinguished from the other *Pseudomonas* species of soil by their ability to produce water-soluble yellow-green pigments. They comprise of *P. aeruginosa*, the type species of the genus, *P. aureofaciens*, *P. chlororaphis*, *P. fluorescens*, *P. putida*, and the plant pathogenic species *P. cichorii* and *P. syringae* (Landa et al. 2003; De La-Funte et al. 2006). *Pseudomonas* spp. are well adapted for inhabiting in the rhizosphere. Pseudomonads possess many traits that make them well suited as biocontrol and growth-promoting agents (Weller 1988). These include their ability to (1) grow faster which makes them

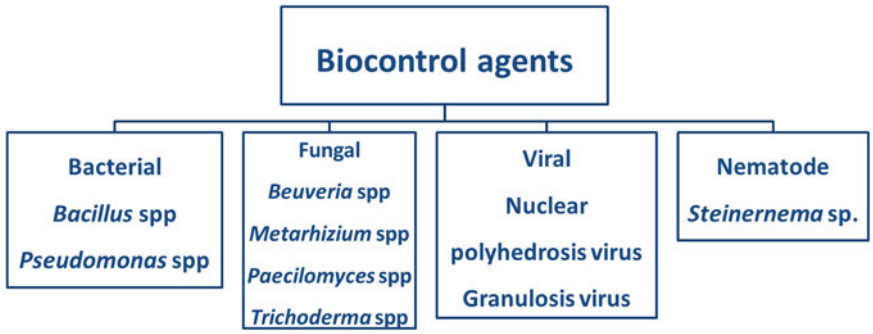

Fig. 15.1 Classification of biocontrol agents

easy to be mass produced in the laboratory, (2) readily consume seed and root exudates, (3) colonize and multiply in the rhizosphere and spermosphere environments and in the interior of the plant, (4) produce a wide spectrum of bioactive metabolites (i.e., antibiotics, siderophores, volatiles, and growth-promoting substances), (5) compete aggressively with other microorganisms, (6) adapt to environmental stresses, and (7) easily colonize plants upon subsequent reinoculation in soil by seed bacterization. The presence of pseudomonads in soil provides natural suppressiveness to the soil against some soil-borne pathogens (Weller et al. 2002).

Several strains live in commensal relationship with plants, protecting them from infection by pathogens that would otherwise cause disease. Control of root diseases by beneficial bacteria involves a blend of possible mechanisms that may complement each other. The primary mechanism of biocontrol includes production of antibiotics or inactivation of virulence trait of pathogens (Diby et al. 2005). Another important mechanism is the indirect inhibition of the pathogen by bacterial stimulation of defense responses in the plant host. Many of the plant-associated strains belong to fluorescent *Pseudomonas* group, which currently includes more than 50 named species (Yamamoto et al. 2000; Mulet et al. 2010).

Pseudomonas plays key role in better growth and development of plant through its capacity to protect plants against pathogens during various developmental stages. The above said benefit of pseudomonads depends on their ability to efficiently consume root exudates and resist predation by soil predators such as nematodes and protozoa (De Mesel et al. 2004; Abuzar and Haseeb 2010). Bacteria have evolved an array of antipredatory mechanisms, such as toxicity. Extracellular metabolites of *Pseudomonas* sp. drive complex interactions with predators, affecting their physiology and behavior. Secondary metabolite works specifically on predators, acting as repellents, stressors, or toxics. Production of such secondary metabolites by biocontrol bacteria serves multiple functions, and metabolites protecting plants against pathogens improve bacterial resistance (Gadoury et al. 1989).

Pseudomonas sp. can utilize variety of organic compounds as energy sources and produce an array of secondary metabolites foremost as 2, 4-diacetylphloroglucinol (DAPG, Phl), lipopeptides, phenazines, pyrrolnitrine, pyochelin, and hydrogen cyanide (Keel et al. 1992; Haas and Defago 2005). Biocontrol strains of *Pseudomonas* sp. with a proven effect in plant bioassays produce one or several antibiotic compounds. In vitro, these antibiotics have been proven as inhibitory compounds, and they are also showing active response for the plant health management in field conditions. Strains that produce the antifungal compound DAPG play an important role in the suppression of some root diseases when introduced into the rhizosphere via seed or soil treatments (Reddy et al. 2009). *Pseudomonas* sp. plays a key role in suppression of plant diseases and commercially exploited for plant disease management in agriculture sector. Biological control of plant diseases through antagonistic bacteria is less popular among the farming com-

munity in comparison to other disease control measures, but it has potential to transform plant disease management strategies.

15.4 Concept of Disease Suppressive Soil

Suppressive soils are soils in which phytopathogens are unable to persist or are present but fail to induce severe disease symptoms on susceptible crops. Plants are protected from diseases generally caused by soil-borne phytopathogens such as bacteria, fungi, and even nematodes in suppressive soils. Suppressiveness in soil is mainly attributed to the presence of high number of antagonistic bacteria having disease suppressive properties. Here the plant roots harbor plant-beneficial microbial communities which are having general beneficial effect on plant health and thereby also known as plant probiotics. Pasteurization of soil results into loss of disease suppressiveness which proves that microorganisms play an important role in disease suppressiveness of soil. Most of the soil pathogens such as fungi, bacteria, and plant-deleterious nematodes get suppressed in such soils. Dominant microfloras of suppressiveness in soil are *Trichoderma*, *Pseudomonas*, and *Bacillus* species. How these bacteria achieve this and what they have, to protect plant from pathogenic fungi, have been analyzed in biocontrol strains of fluorescent *Pseudomonas. Pseudomonas* competitively colonizes plant roots and stimulates plant growth and/or reduces the incidence of plant disease. *Pseudomonas* acts by production of antibiotics or by induction of systemic resistance within the plants during its colonization. It also has reported that growth regulatory compounds and beneficial enzymes are present in them (Haas and Defago 2005). *Pseudomonas* owes their fluorescence due to extracellular diffusible pigments such as pyoverdin (Pvd), pyochelin, and ferripyoverdin (Pvd Fe^{3+} complex) (Paez et al. 2005). The phenomenon of natural suppressive soils has been described for *Gaeumannomyces graminis* var. *tritici* (take-all of wheat), *Fusarium oxysporum* (wilt), *Phytophthora cinnamon* (root rot), *Pythium* spp. and *Rhizoctonia solani* (damping-off of seedling), *Thielaviopsis basicola* (black root rot), *Streptomyces scabies* (bacterial scab), *Ralstonia solanacearum* (bacterial wilt), and *Meloidogyne incognita* (root swelling and root-knot galls) (Haas and Defago 2005).

15.5 Mechanism of Biocontrol by *Pseudomonas*

Over the last few years, a great diversity of rhizosphere microorganisms has been described, characterized, and, in many cases, tested for activity as biocontrol agents against soil-borne plant pathogens. Such microorganisms can produce substances that may limit the damage caused by phytopathogens, e.g., by producing antibiotics, siderophores, and a variety of enzymes or by induction of systemic resistance in host plants. These microorganisms can also function as competitors of pathogens for colonization sites and nutrients. The major mechanisms by which *Pseudomonas* exerts its biocontrol effect are:

1. Competition for niche and nutrient acquisition
2. Antibiotic production
3. Induced systemic resistance

15.5.1 Competition for Niche and Nutrient Acquisition

The high microbial diversity, density, metabolic activity, and competition occurring in the rhizosphere environment represent a challenging "biological buffering" (Keel et al. 1996) that generally limits the establishment of exogenous, foreign microorganisms into the rhizosphere. Thereby, it is essential to evaluate the ability of introduced pseudomonads to colonize roots and provide protection against major and minor soil-borne pathogens. Several definitions of root colonization by rhizobacteria were proposed (Lemanceau et al. 1995; Van Loon et al. 1998), and that defines microbial colonization of plant as movement of the rhizobacteria from an inoculum source to the roots, multiplication, and persistence in the presence of native soil microflora. Weller et al. (2002) defined root colonization as the process

whereby rhizobacteria introduced into the seeds, vegetative propagated plant parts, or soil become distributed along roots growing in raw soil, multiply, and then survive for several weeks in the presence of indigenous soil microflora. Root colonization included colonization of the rhizosphere, rhizoplane, and/or inside the root. Rhizosphere competence describes the relative root-colonizing ability of a rhizobacterium. Bacterial inoculants become more powerful when they multiply on the root and colonize it. So the establishment of inoculant is an important factor for the disease suppression by bio-inoculant. Root colonization not only results in high population densities on the root system, it also functions as the delivery system of antifungal metabolites along the whole root. The extent of colonization ability of applied strain may also be dependent on the mechanism by which a biocontrol agent performs its action. The biocontrol of plant disease can be achieved by antibiosis wherein optimum colonization is needed for delivery of antifungal compounds to entire root system, whereas for ISR colonization of plants by limited number of bacteria is sufficient to induce ISR response in plant. The speed and degree of colonization by biocontrol is supposed to be an important trait. Most of the *Pseudomonas* strains are having short generation time. Microcolonies of *P. fluorescens* WCS365 appeared on the tomato root (Chin-A-Woeng et al. 1997; Bloemberg et al. 2000) 1 day after seed inoculation. Bacterial antagonist generally colonizes intracellular junction between root epidermal cells as they are nutritionally rich which represent small surface area of total root surface area (Chin-A-Woeng et al. 1997). Dhingani et al. (2013) studied colonization of fluorescent *Pseudomonas* isolates as a plant growth-promoting attribute. They isolated 30 isolates of fluorescent *Pseudomonas* from six different locations of Junagadh district, Gujarat, India, and confirmed various PGPR traits present in the fluorescent *Pseudomonas* which may help in the improved plant growth promotion during colonization with suppressive rhizospheric soils. Many of the biocontrol systems are dependent on positive relationship between colonization and pathogen suppression. During the last 40 years, the process of root colonization, the biotic and abiotic factors affecting colonization, and the bacterial genes and traits that contribute to rhizosphere competence has been clearly elucidated from the experimental systems using *Pseudomonas* sp.

Soil area around the root and influenced by root is known as rhizosphere (Hiltner 1904) which is richer in microbes than bulk soil. The rhizospheric microflora is mainly affected by root exudates that contain organic acids, sugars, and amino acids. Biocontrol agents applied to the soil have to race with injurious microorganisms and pathogens for limited available nutrients in root exudates and suitable colonization niches and finally outnumber them. After inoculation, the biocontrol agent can cause inhibition of soil pathogen only for a short period of time. Soil microorganisms have to become highly dependent upon nutrients present in the rhizosphere or root exudates. So, we can assume that there must be strong competition for nutrients between the biocontrol agent and the indigenous microflora in the rhizosphere of the host plant. Native microbial strains or aggressively colonizing biocontrol bacteria can therefore prevent the establishment and consequent deleterious effects of a pathogen. The ability of pseudomonads to establish in niche and rapidly compete for nutrient acquisition is thought to be a general mechanism for antagonistic activity dispersed by biocontrol strains of pseudomonads and thereby acting as plant probiotic. Fungal pathogens can be eliminated from the soil by increasing competition for nutrients such as carbon, nitrogen, or iron which in turn reduce the ability of fungal pathogens to proliferate in the soil (Leong 1986; Loper and Buyer 1991). The generation time of pseudomonads is 3–6 h in rhizosphere which is slower than that in nutrient-rich laboratory media as microorganism in the rhizosphere live under nutrient limiting (Lugtenberg and Kamilova 2009; Haas and Defago 2005). Populations of *Pseudomonas* established on the plant roots could act as a sink for the accessible nutrients and limit the nutrient availability for pathogen and its successive root colonization. This mechanism is generally used by fluorescent pseudomonads because of their nutritional versatility and high growth rates in the rhizosphere

(Walsh et al. 2001). Moreover, the pseudomonads compete with indigenous microbial populations for nutrition in the rhizosphere for successful removal of the pathogens. Siderophores are organic compounds produced by pseudomonads which sequester most of the available Fe^{3+} in the rhizosphere and starve the pathogens for their iron requirement and thereby play a main role in defeating pathogens in the same ecological niche (O'Sullivan and O'Gara 1992). Fluorescent siderophores have high affinity for ferric iron, which forms ferric-siderophore complex that becomes unavailable to other organisms, but the producing strain can utilize this complex via a very specific receptor in its outer cell membrane (Koster et al. 1993, 1995; Buyer and Leong 1986). In this way, fluorescent *Pseudomonas* strains may restrict the growth of deleterious bacteria and fungi on the plant root (Loper and Buyer 1991).

Failure of a pathogen to compete effectively with the biocontrol strain and use the available nutrient sources in same ecological niche will restrict the pathogen's spread. A classical example of niche exclusion is the control of leaf frost injury caused by *P. syringae*, which has an ice nucleation protein on its cell surface (Lindow 1983a, b; Lindow et al. 1983). Well-known example of competition for nutrients is limitation of iron as iron – an essential cofactor for growth in all organisms. The availability of Fe^{3+} in soils is lower at neutral and alkaline pH, which in turn leads to Fe^{3+} limitation. Fluorescent *Pseudomonas* species utilize Fe^{3+} by production of siderophores which are high-affinity iron chelating compounds. The capacity of iron scavenging under iron limitation gives the biocontrol organism a selective advantage over phytopathogens that possess less efficient iron binding and uptake systems. As compared to wild-type parental strains, siderophore-deficient mutants were found to be less effective against pathogens (Bakker et al. 1986).

15.5.2 Antibiotic Production

Antibiotic-producing bacterial biocontrol agents occur frequently and are efficient agents for plant disease management as they can be easily isolated from soil. Many factors affect the production of antibiotics such as temperature, pH, and the levels of various metal ions, particularly of Zn^{2+} (Duffy and Defago 1997). Among the variety of *Pseudomonas* species inhabiting the rhizosphere, certain strains of fluorescent pseudomonads have received particular attention because of their potential to control seed- and soil-borne pathogenic fungi and oomycetes (Keel et al. 1992, 1996). Plant-beneficial microorganisms help in exclusion of plant pathogens from rhizosphere through secretion of antimicrobial metabolites which in turn improves plant health (Haas and Keel 2003; Handelsman and Stabb 1996; Raaijmakers et al. 2002; Thomashow and Weller 1996). A triangular interaction occurs among plants, pathogens, and bacteria for regulation of antifungal traits of *Pseudomonas* (Jain et al. 2011). Due to this reason, efficient colonization is required for antibiosis (Chin-A-Woeng et al. 2003), and that's why it is not unexpected that some strains, which show antifungal activity under laboratory conditions, do not act as biocontrol agents in vivo. The identification and quantification of the antibiotics which are produced during biocontrol in situ are a challenge and have been shown only for a few cases (Thomashow and Weller 1996). The slow growth rate of bacteria in the rhizosphere favors the production of secondary metabolites (Haas and Defago 2005). Most of the identified *Pseudomonas* biocontrol strains produce antifungal metabolites, of which DAPG, phenazines, pyrrolnitrin, pyoluteorin, and volatile hydrogen cyanide are the most frequently detected classes. However, novel antifungal metabolites viscosinamide (Nielsen et al. 1999) and tensin (Nielsen et al. 2001) have been discovered and play a role in protection of plants against phytopathogens. Fluorescent pseudomonads producing antibiotic DAPG are an important group of biocontrol agents for suppressing diseases of roots and young seedlings of various crops, e.g., suppression of black root rot of tobacco by *P. fluorescens* CHA0 (Stutz et al. 1986), take-all of wheat (Keel et al. 1992), and *Fusarium* wilt, crown, and root rot of tomato (Duffy and Defago 1997; Tamietti et al. 1993). Moreover, *Pseudomonas* sp. F113 is found to suppress damping-off of sugar beet (Fenton et al. 1992; Shanahan et al. 1992), and *P. fluorescens* Q2-87 (Harrison et al. 1993;

Pierson and Weller 1994) and Q8r1-96 (Raaijmakers and Weller 1998) suppress take-all of wheat. DAPG-producing strains of *P. fluorescens* are also having a key role in the natural biocontrol of take-all disease (Raaijmakers and Weller 1998; Raaijmakers et al. 1997). The exact mechanism of action of DAPG on pathogens is yet to be discovered. The importance of DAPG as biocontrol molecule has been demonstrated by genetic approaches (Thomashow 1996) as well as direct isolation of disease suppressive strains producing DAPG from rhizosphere of crop plants (Bonsall et al. 1997; Duffy and Defago 1997; Raaijmakers and Weller 1998).

Development of resistance among the human and animal pathogens against the antibiotics used for treatment is believed to be the main risk of using an antibiotic-producing biocontrol agent. Moreover, there is also possibility of transfer of genes encoding the antibiotic production to related strains (Zhang et al. 2003), which seems to be realistic as some conjugative transfers require quorum sensing that are dependent on a high density of microbes. This type of cross transfer of genes is possible in root where pseudomonads form microcolonies under a mucoid layer (Chin-A-Woeng et al. 1997). The genetic material is exchanged at a high frequency in the rhizosphere. These are the reasons for slow process of registration of biocontrol products based on antibiotic-producing microbes.

15.5.3 Induced Systemic Resistance (ISR)

In simple words, ISR can be defined as a broad spectrum plant immune response activated by plant-beneficial bacteria that live in association with plant roots. Few strains of pseudomonads such as *P. fluorescens* (van Loon and Bakker 2006; van Wees et al. 1997; Kamilova et al. 2005) trigger ISR response to combat against a broad spectrum of plant pathogens. Such immunized plants express defense responses faster and stronger after pathogen attack, which results in enhanced level of protection (Van Peer et al. 1991). Such beneficial microbes induce resistance in distant parts of the plants such as leaves, and that's why it is known as ISR response. ISR response induced by beneficial microbes is effective against broad range of pathogens, viz., bacteria, fungi, and viruses (van Loon et al. 1998; van Loon 2007), but the response is believed to be random (Verhagen et al. 2003). There exists the host specificity among the ISR-inducing microbial strains as the ISR induction was found to be dependent on the plant species and cultivar (van Loon and Bakker 2006; van Wees et al. 1997). Generally the plant hormones, viz., jasmonate and ethylene, are believed to be key regulators of ISR response (van Wees et al. 2000). ISR response was observed in many plant-pathogen systems wherein the bacterium and the challenging pathogen remained spatially separated. Many effective biocontrol pseudomonads provoke ISR (Ongena et al. 2004; Ton et al. 2002; Zehnder et al. 2001). ISR does not require complete root colonization. In addition to live microbes, such as *Bacillus*, *Pseudomonas*, and *Trichoderma*, dead microbial cells and some of the products of bacterial metabolites, viz., siderophores, lipopolysachharides, salicylic acid, pyocyanin, and pyochelin as well as organelles such as flagella, are the main inducers of ISR response in plants (Audenaert et al. 2002). Moreover, the volatile 2,3-butanediol (Ryu et al. 2003), the signal molecule AHL (Schuhegger et al. 2006), the antibiotic phloroglucinol (Iavicoli et al. 2003), and some c-LPs (Ongena et al. 2002; Pérez-García et al. 2011) are also believed to be important triggering molecules of ISR response.

15.6 Role of *Pseudomonas* for Plant Growth Promotion

Pseudomonads possess many traits that make them well suited as biocontrol and growth-promoting agents (Weller 2007). There are several ways in which different plant growth-promoting *Pseudomonas* have been reported to directly facilitate the proliferation of their plant hosts. The direct promotion of plant growth by PGPR generally entails providing the plant with a compound that is synthesized by the bacterium or facilitating the uptake of nutrients from the environment. Direct mechanisms of plant growth

promotion are (1) phytohormone production, (2) nitrogen fixation, (3) siderophore production, and (4) phosphate solubilization.

15.6.1 Phytohormone Production

15.6.1.1 Indole 3 Acetic Acid

Many rhizospheric strains of *Pseudomonas* produce indole acetic acid (IAA) which helps in stimulating plant growth (Loper and Schroth 1986). The phytohormone indole-3-acetic acid (IAA) is known to be involved in root initiation, cell division, and cell enlargement. IAA production by microorganisms increases root length and surface area which in turn enables plants to increase absorption of water and nutrients from their ecosystem (Salisbury 1994). Increase in root length as well as the number of secondary roots in young seedlings through IAA production by microorganisms increases the chances of survival of seedlings due to enhanced capacity to anchor to the soil and absorb water and nutrients from the surroundings (Patten and Glick 2002). In IAA-producing bacteria, L-tryptophan-dependent auxin production was observed and reported to increase the grain yield and the number of branches (Asghar et al. 2002, 2004). Patten and Glick (2002) reported the role of IAA-producing *P. putida* in the development of the host plant root system.

15.6.1.2 Cytokinins

Cytokinins promote cell divisions, cell enlargement, and tissue expansion and are believed to be the signals for mediation of environmental stress from roots to shoots. *P. fluorescens* can produce cytokinins as reported by Garcia et al. (2001).

15.6.1.3 1-Aminocyclopropane-1-Carboxylate (ACC) Deaminase

The stress hormone ethylene is the only gaseous phytohormone and produced upon physical or chemical to the plants which causes inhibition of plant root growth. Glick et al. (1998) reported that some of the PGRP strains can produce a stress-relieving enzyme named as ACC deaminase that breaks down ACC, which is the precursor for biosynthesis of ethylene in plants. Production of ACC deaminase enzyme by microorganisms can decrease the concentration of ethylene in the plant roots and thereby elongates plant roots (Glick et al. 1994). Shah et al. (1998) reported that insertion of ACC deaminase gene within *Pseudomonas* spp. aided bacteria with capacity to produce ACC deaminase enzyme and thereby release stress which in turn elongates seedling roots. Pseudomonas strains having capacity to produce ACC deaminase enzyme were reported to promote plant growth under stressful condition such as flood (Grichko and Glick 2001) or heavy metal contamination (Burd et al. 1998).

15.6.2 Nitrogen Fixation

The first evidence for nitrogen fixation by *Pseudomonas* like microorganisms has been reported by Anderson in 1955. Nitrogen-fixing ability of members of the genus *Pseudomonas* is poorly understood. The mechanism of nitrogen fixation and the protection of nitrogenase against oxygen deactivation were also not revealed (Young 1992). However, recently several workers demonstrated among the strains of pseudomonads (Desnoues et al. 2003; Krotzky and Werner 1987). The optimum conditions for the nitrogen fixation and structure of genes encoding nitrogenase enzyme in *Pseudomonas* sp. were studied in detail using *P. stutzeri* A15 (A1501), isolated from rice paddies in China (Desnoues et al. 2003). So, one can classify the *Pseudomonas* spp. as nitrogen fixers based on their physiological properties, nitrogenase assays, phylogenetic studies, and detection of *nifH* DNA by hybridization or PCR amplification (Chan et al. 1994; Vermeiren et al. 1999). After detection presence of nitrogen-fixing traits among the species of *Pseudomonas* genus, nitrogen-fixing strains of *Pseudomonas* spp. were reassigned genera in α- and β-proteobacteria (Chan et al. 1994). Krotzky and Werner in 1987 isolated two nitrogen-fixing *Pseudomonas* strains, viz., *P. stutzeri*. and *P. stutzeri* CMT.9.A, from the roots of sorghum, and You et al. (1991) isolated *P. stutzeri* strain A15 from rice paddies from China (You et al. 1991).

15.6.3 Solubilization of Phosphorus

The second important macronutrient required for plant growth is phosphorous. Phosphorous is present in insoluble forms such as iron and aluminum phosphates in acidic soils and calcium phosphates in alkaline soils. In phosphorous-rich soil, only a small proportion of phosphate (~0.1 %) is available to plants (Stevenson and Cole 1999). Phosphate-solubilizing bacteria (PSB) secrete organic acids and phosphatase enzymes to convert the insoluble phosphates into soluble forms. This process is known as phosphate solubilization which leads to an increase in the content of available phosphate for plants (Gyaneshwar et al. 2002). Almost all the soil types contain phosphate-solubilizing bacteria (Gyaneshwar et al. 2002), among which *Bacillus*, *Enterobacter, Erwinia*, and *Pseudomonas* spp. are most prevalent. Generally rhizospheric region of plant is colonized by phosphate-solubilizing bacteria where they bring about solubilization of insoluble inorganic phosphatic compounds. Most commonly the phosphate-solubilizing ability of PGPR strains is dependent on the availability of other macronutrients such as carbon and nitrogen as well as metal ions (Kim et al. 1998). Generally, phosphate-solubilizing bacteria produce various types of organic acids, among which the most abundant is β-ketogluconic acid, a secondary oxidation product of glucose metabolism. The oxidation of glucose is catalyzed by an enzyme glucose dehydrogenase (GDH) present in cytoplasmic membrane of bacteria, and as a result of the enzyme activity, gluconic acid and β-ketogluconic acid are produced which bring about phosphate solubilization.

15.6.4 Sequestering Iron by Siderophores

Iron is essential for life for all living organisms and is required as a component of proteins involved in important processes such as respiration, photosynthesis, and nitrogen fixation.

Despite the abundance of this element on the earth's surface, soil organisms such as plants and microbes have difficulty in obtaining enough iron to support their growth because iron in soil is largely present as insoluble, ferric hydroxides, which cannot be readily transported into cells. Microorganisms and some plants can secrete low molecular weight, organic, iron binding molecules known as siderophores which help in iron scavenging from soil. Each functional group presents two atoms of oxygen or less commonly nitrogen that bind to iron. In general, catecholate-type siderophores are typical to bacteria. It is known that many bacteria, including *Pseudomonas* spp., react to limiting Fe^{3+} concentrations by inducing a high-affinity iron uptake system (Braun 1985; Neilands 1982) consisting of siderophores, Fe^{3+} chelating molecules, and outer membrane receptor proteins with a high affinity for the matching Fe^{3+} siderophore complex (De Weger et al. 1986). Production of siderophores by plant growth-promoting *Pseudomonas* spp. during iron starvation is considered as the one of the mechanism in inhibition of phytopathogens. But whenever the concentration of iron in the medium is sufficient, such antagonism will not be observed (Geels and Schippers 1983). The following scenario was proposed to account for the enhancement of plant growth by the *Pseudomonas* spp. (Kloepper et al. 1980). After the inoculation of seeds, the *Pseudomonas* bacteria rapidly colonize the roots of the developing plant. The limiting Fe^{3+} concentration in the soil induces the high-affinity iron uptake system. The siderophores bind Fe^{3+}, and as an uptake of this Fe^{3+}, siderophore complex requires a very specific uptake mechanism; this binding makes this essential element unavailable for many other rhizomicroorganisms. These microorganisms, including deleterious species, then are unable to obtain sufficient iron for optimal growth since they produce either no siderophores at all or less efficient ones (Raaijmakers et al. 1995). Thus, the population of deleterious microorganisms is reduced, creating a favorable environment for the development of the plants (De Weger et al. 1986).

Several species of fluorescent pseudomonads produce siderophores, and there is evidence that a number of plant species can absorb bacterial siderophore complexes (Bitter et al. 1991).

Pyoverdines (PVDs) or pseudobactins are fluorescent yellow-green siderophores (Budzikiewicz 1997). *P. aeruginosa* produces siderophore pyochelin having lower affinity for iron. Fluorescent pseudomonad species, viz., *P. fluorescens*, *P. stutzeri*, and *P. putida*, produce siderophore named as pseudonlonine (Lewis et al. 2000; Mossialos et al. 2000; Mercado-Blanco et al. 2001).

15.7 Scope of *Pseudomonas* as Biocontrol Agent

The prospect of manipulating crop rhizosphere microbial populations by inoculation of beneficial bacteria, i.e., *P. fluorescens*, to increase plant growth has shown considerable promise in laboratory and greenhouse studies. The potential environmental benefits of this approach, leading to a reduction in the use of agricultural chemicals, fit with sustainable management practices. We can expect to see new *P. fluorescens* products becoming available to farmers as a biofungicides. The success of these products will depend on our ability to manage the rhizosphere to enhance survival and competitiveness of these beneficial microorganisms. Sequencing the genome provided further information of its environmental interactions and its metabolic capabilities, which can be used to control plant diseases. Though *P. fluorescens* is the most widely used biocontrol agent, the major limitation is not only its shelf life but also inconsistent field performance.

15.8 Conclusion

Unlike chemical pesticides, biocontrol agents need support even after their application to get established in targeted niche. Therefore, for the success of biological control, one has to ensure not only the quality of biocontrol agent applied but also its establishment in natural ecosystem to thrive and compete well with the pathogens. Development of better formulations to ensure survival of activity in the field and compatibility with chemical and biological seed treatments is another area of focus. *P. fluorescens* as bioagent has good prospectus in the future as it gives very high cost-benefit ratio. In view of this, the first assumption is to isolate the *P. fluorescens* bacteria from the rhizosphere of various field crops with enhanced antagonistic activity against soil-borne fungal pathogens under native environmental conditions and determine the ability of selected bacterial isolates to suppress the soil-borne fungal pathogens under in vitro conditions.

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Trichoderma: A bio-control agent for management soil born diseases

Submitted by Rakesh Kumar Singh on Tue, 06/07/2010 - 15:59
Posted in English biocontrol Trichoderma

Trichoderma: A bio-control agent for management soil born diseases

Trichoderma is a very effective biological mean for plant disease management especially the soil born. It is a free-living fungus which is common in soil and root ecosystems. It is highly interactive in root, soil and foliar environments. It reduces growth, survival or infections caused by pathogens by different mechanisms like competition, antibiosis, mycoparasitism, hyphal interactions, and enzyme secretion.

General Characteristics

Colonies, at first transparent on media such as cornmeal dextrose agar (CMD) or white on richer media such as potato dextrose agar (PDA). Mycelium typically not obvious on CMD, conidia typically forming within one week in compact or loose tufts in shades of green or yellow or less frequently white. Yellow pigment may be secreted into the agar, especially on PDA. A characteristic sweet or 'coconut' odor is produced by some species.

Conidiophores are highly branched and thus difficult to define or measure, loosely or compactly tufted, often formed in distinct concentric rings or borne along the scant aerial hyphae. Main branches of the conidiophores produce lateral side branches that may be paired or not, the longest branches distant from the tip and often phialides arising directly from the main axis near the tip. The branches may rebranch, with the secondary branches often paired and longest secondary branches being closest to the main axis. All primary and secondary branches arise at or near 90° with respect to the main axis. The typical *Trichoderma* conidiophores with paired branches assumes a pyramidal aspect.

Phialides are typically enlarged in the middle but may be cylindrical or nearly subglobose. Phialides may be held in whorls, at an angle of 90° with respect to other members of the whorl, or they may be variously penicillate (gliocladium-like). Phialides may be densely clustered on wide main axis (e.g. *T. polysporum*, *T. hamatum*) or they may be solitary (e.g. *T. longibrachiatum*).

Conidia typically appear dry but in some species they may be held in drops of clear green or yellow liquid (e.g. *T. virens*, *T. flavofuscum*). Conidia of most species are ellipsoidal, 3-5 x 2-4 µm. Conidia are typically smooth but tuberculate to finely warted conidia are known in a few species.

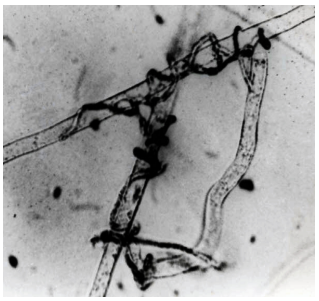
Synanamorphs are formed by some species that also have typical *Trichoderma* pustules. Synanamorphs are recognized by their solitary conidiophores that are verticillately branched and that bear conidia in a drop of clear green liquid at the tip of each phialide.

Chlamydospores may be produced by all species, but not all species produce chlamydospores on CMD at 20° C within 10 days. Chlamydospores are typically unicellular subglobose and terminate short hyphae; they may also be formed within hyphal cells. Chlamydospores of some species are multicellular (e.g. *T. stromaticum*).

Teleomorphs of *Trichoderma* are species of the ascomycete genus *Hypocrea* Fr. These are characterized by the formation of fleshy, stromata in shades of light or dark brown, yellow or orange. Typically the stroma is discoidal to pulvinate and limited in extent but stromata of some species are effused, sometimes covering extensive areas. Stromata of some species (*Podostroma*) are clavate or turbinate. Perithecia are completely immersed. Ascospores are bicellular but disarticulate at the septum early in development into 16 part-ascospores so that the ascus appears to contain 16 ascospores. Ascospores are hyaline or green and typically spinulose.

Trichoderma harzianum

More than 200 species of *Hypocrea* have been described but only few have been grown in pure culture and fewer have been redescribed in modern terms.



Trichoderma's **mycelium parasitising to mycelium of** *Pythium*

Benefits of Trichoderma

1. **Disease Control:** Trichoderma is a potent biocontrol agent and used extensively for soil born diseases. It has been used successfully against pathogenic fungi belonging to various genera, viz. Fusarium, Phytopthara, Scelerotia etc.
2. **Plant Growth Promoter:** Trichoderma strains solubilize phosphates and micronutrients. The application of Trichoderma strains with plants increases the number of deep roots, thereby increasing the plant's ability to resist drought.
3. **Biochemical Elicitors of Disease:** Trichoderma strains are known to induce resistance in plants. Three classes of compounds that are produced by Trichoderma and induce resistance in plants are now known. These compounds induce ethylene production, hypersensitive responses and other defense related reactions in plant cultivars.
4. **Transgenic Plants:** Introduction of endochitinase gene from Trichoderma into plants such as tobacco and potato plants has increased their resistance to fungal growth. Selected transgenic lines are highly tolerant to foliar pathogens such as *Alternaria alternata, A. solani,* and *Botrytis cirerea* as well as to the soil-borne pathogen, *Rhizectonia* spp.
5. **Bioremediation:** Trichoderma strains play an important role in the bioremediation of soil that are contaminated with pesticides and herbicides. They have the ability to degrade a wide range of insecticides: organochlorines, organophosphates and carbonates.

Biocontrol mechanisms of *Trichoderma:*

The Trichoderma may suppress the growth of the pathogen population in the rhizosphere through competition and thus reduce disease development. It produces antibiotics and toxins such as trichothecin and a sesquiterpine, Trichodermin, which have a direct effect on other organisms. The antagonist (*Trichoderma*) hyphae either grow along the host hyphae or coil around it and secrete different lytic enzymes such as chitinase, glucanase and pectinase that are involved in the process of mycoparasitism. Examples of such interactions are *T. harzianum* acting against *Fusarium oxyporum, F. roseum, F. solani, Phytophthara colocaciae* and *Sclerotium rolfsii*. In addition, Trichoderma Enhances yield along with quality of produce. Boost germination rate. Increase in shoot & Root length Solubilizing various insoluble forms of Phosphates Augment Nitrogen fixing. Promote healthy growth in early stages of crop. Increase Dry matter Production substantially. Provide natural long term immunity to crops and soil.

Method of application:

1. **Seed treatment:** Mix 6 - 10 g of Trichoderma powder per Kg of seed before sowing.
2. **Nursery treatment:** Apply 10 - 25 g of Trichoderma powder per 100 m2 of nursery bed. Application of neem cake and FYM before treatment increases the efficacy.
3. **Cutting and seedling root dip:** Mix 10g of Trichoderma powder along with 100g of well rotten FYM per liter of water and dip the cuttings and seedlings for 10 minutes before planting.
4. **Soil treatment:** Apply 5 Kg of Trichoderma powder per hector after turning of sun hemp or dhainch into the soil for green manuring. Or Mix 1kg of Trichoderma formulation in 100 kg of farmyard manure and cover it for 7 days with polythene. Sprinkle the heap with water intermittently. Turn the mixture in every 3-4 days interval and then broadcast in the field.
5. **Plant Treatment:** Drench the soil near stem region with 10g Trichoderma powder mixed in a liter of water

Trichoderma formulations:

Important commercial formulations are available in the name of Sanjibani, Guard, Niprot and Bioderma. These formulations contain 3x106 cfu per 1 g of carrier material. Talc is used as carrier for making powder formulation.

Uses:

Used in Damping off caused by *Pythium* sp. *Phytophthora* sp., Root rot caused by *Pellicularis filamentosa*, Seedling blight caused by *Pythium*, Collar rot caused by *Pellicularia rolfsii*, Dry rot caused by *Macrophomina phaseoli*, Charcoal rot caused by *Macrophomina phaseoli*, Loose smut caused by Ustilago segetum, Karnal bunt diseases, Black scurf caused by *Rhizoctonia solani*, Foot rots of Pepper and betel vine and Capsule rot of several crops. Effective against silver leaf on plum, peach & nectarine, Dutch elm disease on elm's honey fungus (*Armillaria mellea*) on a range of tree species, Botrytis caused by *Botrytis cinerea*, Effective against rots on a wide range of crops, caused by *fusarium, Rhizoctonia*, and *pythium*, and sclerotium forming pathogens such as *Sclerotinia* & *Sclerotium*

Recommended For:

Trichoderma is most useful for all types of Plants and Vegetables such as cauliflower, cotton, tobacco, soybean, sugarcane, sugarbeet, eggplant, red gram, Bengal gram, banana, tomato, chillies, potato, citrus, onion, groundnut, peas, sunflower, brinjal, coffee, tea, ginger, turmeric, pepper, betel vine, cardamom etc.

Precautions:

- Don't use chemical fungicide after application of Trichoderma for 4-5 days.
- Don't use *trichoderma* in dry soil. Moisture is a essential factor for its growth and survivability.
- Don't put the treated seeds in direct sun rays.
- Don't keep the treated FYM for longer duration.

Compatibility:

Trichoderma is compatible with Organic manure Trichoderma is compatible with biofertilizers like *Rhizobium, Azospirillum, Bacillus Subtilis* and *Phosphobacteria*.
Trichoderma can be applied to seeds treated with metalaxyl or thiram but not mercurials. It can be mixed with chemical fungicides as tank mix.

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TRICHODERMA AS POTENTIAL BIOCONTROL AGENT, ITS EXPLOITATION IN AGRICULTURE: A REVIEW

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ARTICLE INFO

Article history
Received: 13th August, 2018
Revised: 18th September, 2018
Accepted: 29th November, 2018

Keywords
Trichoderma,
Biocontrol potential,
Antibiotics,
Activity,
Agriculture

ABSTRACT

The novel technologies in all areas of agriculture have improved agricultural production, but some modern practices affect the environment. The recent challenge faced by advanced farming is to achieve higher yields in an environment-friendly manner. Thus, there is an immediate need to find eco-friendly solutions. Among various types of species being used as biocontrol agents, *Trichoderma* is widely used as biocontrol agent against different kinds of plant pathogens. *Trichoderma* spp. are asexual fungi that are present in all types of agricultural soils and also in decaying wood. The hostile activity of *Trichoderma* species showed that it is parasitic on many soil-borne and foliar plant pathogens. Recent studies showed that this fungus not only acts as biocontrol agent but also stimulates plant resistance, plant growth and development resulting in an increase in crop production. The antagonistic activity involves mycoparasitism, antibiotics, competition for nutrients and also induces systemic resistance in plants. Currently, *Trichoderma* spp. are being used to control plant diseases in sustainable disease management system. This paper reviews the already published information on *Trichoderma* as biocontrol agent, its biocontrol activity and its commercial production and application in plant disease management programs.

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INTRODUCTION

The term 'biocontrol or biological control' was introduced for the first time in 1914 by Tubeuf and Smith in 1919 with special concern to plant pathogens and insects respectively. Biocontrol refers to the reduction in plant pest population by naturally occurring organisms that are part of integrated disease management. Biocontrol agent of plant pathogens known as antagonist inspires development and research work in many fields to meet the needs of rising human population by managing the pest. These antagonistic microorganisms belong to various groups of fungi and bacteria while plant pests include plant pathogens, weeds, and insects.

A diversity of biocontrol agents (BCAs) or bio-fungicides are present in the ecosystem and there is need to isolate for bringing into play because BCAs have a low cost of production, long lasting effect on the growth of pathogen and no effect on human health. Virtually, all plant pathogens of plant diseases are subjected to biological control or natural microorganisms having antagonistic effect that are already present in soil and environment. Only between 1-10% microbes show the ability to inhibit pathogen growth *in vitro* when fungal and bacterial isolates tested for biocontrol activities. Some of these have the ability to suppress plant pathogen under *in vivo* favorable conditions while few have broad-spectrum

activities against miscellaneous pathogens texa. Some important microbes belonging to different genera that are currently being marketed worldwide for promoting trend of organic farming among farmers by reducing the use of chemical pesticides include *Agrobacterium, Ampelomyces candida*, *Bacillus*, *Coniothyrium*, *Pseudomonas* (Haas and Défago, 2005), *Streptomyces* and *Trichoderma*. These BCAs interact with plant and pathogens that suppress the pathogen growth by direct and indirect mechanisms involved in the working of antagonist include hyperparasitism, competition, induction of host resistance, production of antibiotics (Phenazines, 2,4-diacetyle phloroglucinol), lytic enzymes (Chitinases, proteases, glucanases), non regulatory waste products (Hydrogen cyanide, ammonia, carbon dioxide) and physical/chemical interference (blockage of soil pores) that leads to biocontrol of plant parasitic pathogens (Pal and Gardener, 2006).

***Trichoderma* as biological control agent:** In the early 1930s, *Trichoderma* was firstly reported as biocontrol agent (Weindling, 1932) and species of genus *Trichoderma* are free-living and cosmopolitan fungi in soils, decaying organic and vegetable matter (Harman et al., 2004a). *Trichoderma* species are successful antagonists having biocontrol abilities against economically important plant parasitic soil-borne pathogens and present abundantly in almost all type of soils (Kushwaha and Verma, 2014; Olabiyi and Ruocco, 2013; Shahid et al., 2014). Biocontrol antagonists played important role in the management of plant diseases and parasitic microorganisms (Alwathnani and Perveen, 2012; Hajieghrari et al., 2008; Zhang et al., 2013). *Trichoderma* attacked other plant pathogenic fungi and promotes plant and root growth. It uses different mechanisms for the control of plant pathogenic pathogens including antibiosis, mycoparasitism, the induced resistance of host cell and competition for nutrient and space. Species of *Trichoderma* can control and antagonize broad range of economically important postharvest phytopathogenic fungal pathogens and plant-pathogenic fungi as well as also control bacteria and viruses (Harman, 2006; Yedidia et al., 2003). Significant information on nutrition of *Trichoderma* are available in literature but very little is well-known about specific carbon and nitrogen nutrients on mass production of *Trichoderma* antagonists (Rajput et al., 2014). Generally, it is considered aggressive competitor that rapidly colonizes the pathogen especially soil-borne pathogens such as *Fusarium* spp., *Phytophthora* spp. and *Rhizoctonia* etc.

***Trichoderma* biology:** Mycoflora belonging to genus *Trichoderma* usually cosmopolitan shows a high level of genetic diversity and frequently found in varying habitats (Grinyer et al., 2004; Samuels, 2006; Zhang et al., 2007). *Trichoderma* species have been easily isolated from natural soil, decaying plant organic matter and wood and is classified as imperfect fungi belonging to order Hypocreales of Ascomycota (Howell, 2003; Küçük and Kivanç, 2003). *Trichoderma* multiplies and grows very fast in different nutrient sources such as Malt Agar (MA), Czapek Dox Agar (CDA) as well as Potato Dextrose Agar (PDA) and produces conidia/spores of various shades characterized by green color (Chaverri et al., 2003; Rey et al., 2001) and some species produce thick walled chlamydospores (Lu et al., 2004). The salient feature of this genus is the ability to parasitize other pathogenic fungal mycoflora specially associated with root rot and wilt diseases (Santoro et al., 2014; Verma et al., 2007). *Trichoderma* species have been reported as endophytic fungi while generally found in all types of soils such as agricultural soil, orchard soil as well as forest soil as opportunistic plant symbionts (Chaverri et al., 2011) and usually considered successful competitor of plant pathogens (Kim et al., 2012; Woo et al., 2006).

Morphological characteristics: Morphological based identification of *Trichoderma* species is a primary method of identification that is not a precise method to differentiate diversity between species (Zhang et al., 2005). *Trichoderma* species are fast growing under the optimum range between 25-30 ºC (Latifian et al., 2007). *Trichoderma* used a variety of compounds such as carbon and nitrogen sources as a growth medium for its sporulation (Gao et al., 2007; Seyis and Aksoz, 2005) and sporulates of *Trichoderma* abundantly produce powder masses characterized by green conidia (Chaudhari et al., 2011) which is diagnostic tool that is also found in related and unrelated genera such as *Myrothecium*, *Clonostachys* and *Aspergillus* as well as *Penicillium* respectively (Alvindia and Hirooka, 2011). Conidiophore is not well defined but mostly branched contains unicellular conidia and phialides at the tip of branched hyphal system that cannot be seen on one week old media (Lu et al., 2004). Generally, conidia shape is ellipsoidal to oblong and some *Trichoderma* species have globose to subglobose with the length/width ratio 1.4 and 1-3 respectively (Bissett et al., 2003; Jaklitsch et al., 2006) while few species have smooth conidia

(Samuels et al., 2002). Conidia color morphology varies from species to species but typically green or may be gray, white and yellow (Jaklitsch et al., 2006).

***Trichoderma* ecology:** *Trichoderma* is usually widely distributed and ubiquitous in almost all types of soils (Olabiyi and Ruocco, 2013; Röhrich et al., 2013; Singh et al., 2014) and found on decaying bark and plant root surface when damaged by phytopathogens (Brotman et al., 2013; Samolski et al., 2011). Species of this genus are fast growing saprophytes that can be detected by coconut smell due to volatile compound such as 6-pentyl-2pyrone and competitors for nutrient as well as space (Tsai et al., 2008). They comprise up to 3.1% and 15% of total fungal propagules from forest and pasture soil respectively (Hagn et al., 2003). Climatic conditions affect distribution of *Trichoderma* species. For instance, *T. harzianum* favors warm climate while *T. viride* and *T. polysporum* characteristic of cool temperature (Sarhy-Bagnon et al., 2000). Similarly, *T. citrinoviride* has been reported in South East Asia but not found in India (Zhang et al., 2005). In general, *Trichoderma* is more ubiquitous in acidic soil condition (Carreras-Villasenor et al., 2012) while *T. pseudookoningi* and *T. hamatum* show more tolerant behavior with excessive moisture as compared to other species (Kumar, 2007).

Effect of environment on *Trichoderma*: The environmental and nutritional parameters play an important role in enhancing mycelial growth and biomass production of *Trichoderma* species and growth and multiplication of biocontrol agents varies with the substrates (Romero-Arenas et al., 2012). The maximum mass production of *Trichoderma* for commercialization product against soil-borne phytopathogen mainly relies on its physiological and environmental factors such as temperature, pH, light, nitrogen and carbon sources (Jayaswal et al., 2003).

Species of *Trichoderma* spp. are reported to be more sensitive to light and nutrient media (Steyaert et al., 2010) and *Trichoderma* conidial response not restricted to light. Light affects many pathways of *Trichoderma* including oxidative stress, development, vegetative growth, sulfur and carbon mechanism and reproduction that involves in the signaling process. Today, BLR-1 and BLR-2 are known to be photoreceptor-orthologs and light regulatory protein ENVOY that regulates expression of cellulase gene established connection between nutrient signaling and light response in *Trichoderma* (Schmoll et al., 2009). Sun emits radiation of various wavelengths that initiates photochemical reactions and wavelength response of photo-conidiation lies within blue or UV spectrum that describes the fungus belonging to blue-light fungi. *Trichoderma* spp. light regulator proteins BLR-1 and BLR-2 are the key regulators of this response (Casas-Flores et al., 2004). *Trichoderma* can sporulate and grow on a variety of artificial nutrient media but not all of them. Aslam et al. (2010) compared cellulase activity of *Trichoderma* spp. and different fermented media with carbon sources for the production of cellulase. *Trichoderma* exhibited maximum mycelial growth on glucose culture but no production of cellulase enzyme was observed. Macroscopic characteristic like mycelial growth and development rate of *Trichoderma* spp. on yeast complete medium (YCM) and potato dextrose agar (PDA) adjusted with sodium hydroxide (SQ) and industrial chemical products (PQIND) at approximately 7, 9 and 11 pH and resulted that development rate of *Trichoderma viride* strain CP-50 and *Pleurotus ostreatus* strain CP-T4 at pH 11.2 were 0.41 mm/day and 6.10 mm/day respectively. *T. viride* development and growth rate was negative in an alkaline medium (Romero-Arenas et al., 2012). Compared mycelial growth, biomass yield and conidia production of *T. harzianum, T. longibrachiatum* and *T. viride* checked on different nutrient media including PDA, Waksman Agar, Agar Agar, Corn Meal Agar, Czepak's Agar and tested their efficacy by dual culture technique against seed borne pathogens such as *Alternaria alternata, Botryodiplodia theobromae, Fusarium moniliforme, F. oxysporum, F. solani* and *Rhizoctonia solani*. Maximum mycelial growth inhibition of these pathogenic fungi was noted by *T. harzianum* and PDA medium was the best for biomass and spore production of *Trichoderma* species (Mustafa et al., 2009). All microorganisms growth including *Trichoderma* species is influenced by environmental parameters that affect antagonistic potential of biocontrol agents. Among the environmental parameters pH is the most important factor that affects mycelial growth of *Trichoderma*. Onilude et al. (2013) described effect of cultural and environmental parameter such as carbon (Mannitol, wheat and rice bran), nitrogen (peptone, $NaNO_3$ and NH_4SO_4) sources and pH as well as temperature respectively on sporulation and growth of *T. viride* under batch and fed-batch culture. Mycelial growth was good at the temperature range of 30-37ºC while sporulation was favored by 30-45ºC. Moreover, maximum growth and sporulation of *T. viride* was observed at 4.5-6.0 pH. The influence of pH on biomass and mycelial growth

demonstrated that acidic pH is the most important key factor for biomass production of all *Trichoderma* species (Singh et al., 2014).

da Silva et al. (2012) evaluated temperature and pH effects on Trichoderma spp. like *T. harzianum*, *T. polysporum, T. koningii and T. viride for chitosanase production under solid state fermentation. T. viride, T. harzianum and T. polysporum, T. koningii showed optimum chitosanase activity at 5.0 and 5.5 pH respectively with maximum chitosanase with enzymes production about* 1.4 IU/gds from *T. polysporum* followed by *T. viride* (~1.2 IU/gds) and *T. harzianum* (1.06 IU/gds). Temperature between ranges of 40-50 ºC did not affect the activity of the enzyme.

Inam-Ul-Haq et al. (2009) scrutinized *Fusarium oxysporum* survival on chickpea plants under the three parameters such as moisture, temperature and *Trichoderma* spp. when *Trichoderma* spore suspension incubated on chick pea branches at temperature 25 and 35ºC in sandy clay loam soil for the period of 4 months with different water potential -0.03 MPa, -0.3 MPa and less than -50 MPa then resulted that maximum *F. oxysporum* growth reduced in moist soil as compared to others water potentials. The survival of *F. oxysporum* was 100% and completely eradicated in air-dry and moist soil respectively after 6 months but up to 10-12% killed by antagonist at 35 ºC on branches chickpea plant.

Efficacy of *Trichoderma* spp. against pathogenic microflora: Species of *Trichoderma* are reported as ubiquitous and cosmopolitan soil-borne Ascomycetes (Singh et al., 2014) that reproduce asexually which are present in almost all types of soil habitats and other environment such as manure, decaying plant tissues, and wood. It produces as discrete colonies with various shades of green or white conidia and colony color from the reverse side of Petri plate often uncolored, yellow, amber, buff, and yellow green. Some species can be identified from their aroma which include *T. atroviride*, *T. lignorum* and *T. fertile* produces odor of coconut and mold respectively (Gams and Bissett, 2002). Conidia become mature after initial production of conidia within 7 to 14 days thus branching pattern and conidiophore morphology is critical for identification. *Trichoderma* used as biological control agent and have the ability to compete with other parasitic and saprophytic pathogens (Table 1) specially soil-borne fungi due to metabolic activities and competitive nature that make them decomposer of herbaceous and woody material (Elad, 2000) in which sexual perfect stage (*Teleomorph* genus *Hypocrea*) has frequently found (Harman et al., 2004b). *Trichoderma* readily obtained by Multiple Tube Dilution Technique (MTDT) due to its formation of chlamydospores and colonization of organic substrates (Khandelwal et al., 2012).

Importance of Trichoderma: The perspective character of *Trichoderma* species as biological control of fungal plant pathogens was first introduced in the early 1930s and later on research indicates that it can control effectively foliar, seed and specially soil-borne pathogenic fungi belong to various genera (El-Mohamedy and Alla, 2013; Gveroska and Ziberoski, 2012).

Trichoderma species are opportunistic, avirulent, and plant symbionts that can compete as well as survive in the complex ecosystem (Harman et al., 2004b). Although, these are capable of successful root colonizer and their number increases when abundant healthy roots are present in the ecosystem (Brotman et al., 2008) and protect the roots and plants from pathogens as well as diseases (Howell, 2003). They increase plant resistant ability against drought conditions and promote the growth of a plant by phosphate, micro-nutrients, and solubilization (Kumar, 2013). Some species of *Trichoderma* are efficient producer of extracellular enzymes that degrade complex compounds of polysaccharides and also used commercially (Samanta et al., 2012). *Trichoderma* species are environmental friendly (Singh et al., 2008) and an alternative to synthetic chemicals (Gupta and Dikshit, 2010) that developed symbiotic relationship with plants rather than parasitic relationship reduced chances of behavioral changes in human caused by the use of synthetic chemicals (Brimner and Boland, 2003).

Plant growth enhancement by *Trichoderma* specie: *Trichoderma* spp. not only controlled pathogens, they also enhance plant growth and root development (biofertilizer) and stimulate plant defense mechanisms (Harman et al., 2004b). Some *Trichoderma* strains have been shown to penetrate the epidermis and establish robust and long-lasting colonization of root surfaces. *Trichoderma* spp. has been shown to improve growth of lettuce, tomato, and pepper plants (Vinale et al., 2008). In a study of maize plants, several months after treatment with *T. harzianum* strain T-22, the plant roots were about twice as long when compared to untreated plants. *Trichoderma* spp. also produced gluconic and citric acids, decreased the soil pH, and enhanced the solubilization of phosphates, micronutrients, and mineral components such as iron, magnesium, and manganese (Vinale et al., 2008).

Table 1: Efficacy of *Trichoderma* species against soil-borne fungal pathogens.

Trichoderma strains	Pathogen(s)	Plant/ Crop	Disease	Efficacy (Inhibition)	Experiment	Reference
T. harzianum TH-H-3 *T. virens* TV-K-3	*Rhizoctonia solani*	Tomato	Wilt	5 %	Pot Exp.	Kumar (2013)
T. harzianum *T. viride*	*Fusarium solani*	Tomato	Root rot	70-72%	*In vitro*	Haggag and El-Gamal, 2012
T. harzianum *T. viride*	*R. solani*	Tomato	Damping off	51% 39%	*In vitro*	Haggag and El-Gamal (2012)
T. harzianum Mutants	*R. solani*	Tomato	Damping off	40% in greenhouse 100% in field	Greenhouse and field	Montealegre et al. (2010)
T. viride (Tv-R)	*M. phaseolina*	Chickpea	Dry root rot	62%	laboratory conditions	Manjunatha et al. (2013)
T. harzianum *T. viride* *T. virens*	*R. bataticola*	Mungbean	Dry root rot	87%	Pot and field conditions	Dubey et al. (2009)
T. harzianum T22	*Pythium ultimum*	Tomato	Wilt	74%	*In vitro*	Mastouri et al. (2010)
T. harzianum T-22	*F. verticillioides*	Maize	Ear and kernel rot	65% reduce size of necrotic area		Ferrigo et al. (2014)
T. harzianum *T. viride*	*F. oxysporum* f. sp. *Ciceris*	Chickpea	Chickpea wilt	44-60%	Field exp.	Dubey et al. (2007)
T. harzianum	*F. oxysporum* f. sp. *Radicis cucumerinum* *Botrytis cinerea*	Cucumber *Arabidopsis thaliana*	Stem and root rot	12-79%	Pots experiments	Alizadeh et al. (2013)
T. viride	*F. oxysporum* f. sp. *adzuki*	Soybean	Root rot	-	*In vitro*	John et al. (2010)

	Pythium arrhenomanes		Damping off			
Trichoderma spp.	*Phytophthora cactorum*	Strawberry	Leather rot	88% in 2001 97.6% in 2002 99.0% in 2003	Field Exp.	Porras et al. (2007)
T. harzianum *T. viride* *T. virens* T. *koningii* *T. pseudokoningii*	*Alternaria tenuissima*	Sorrel	Leaf spot	67-76% 78-80% 72-77% 77-80% 72-80%	*In vitro*	Ambuse et al. (2012)
	M. phaseolina		Charcoal rot	72%		
T. harzianum 1	*F. solani*	Cotton	Wilt and boll rot	71%	*In vitro*	Asran-Amal et al. (2010)
	R. solani		Boll rot and leaf spot	58%		
T. atrovirde *T. Longibrachiaum* *T. virens* *T. hazianum*.	*F. sambucinum*	Potato	Potato dry rot	*T. longibrachiatum* showed the strongest inhibition	*In vitro*	Ru and Di (2012)
T. harzianum	*A. alternate*	Tobacco	Brown spot	Diffusible metabolites more effective than volatile diffusible	*In vitro*	Gveroska and Ziberoski (2012)
	B. cinerea,		Grey mould	35-44% on fruit and 43-64% on stem		
T. harzianum T39	*Pseuperonospora cubensis*, *Sclerotinia sclerotiorum*	Cucumber	Downy mildew white mould	48-78% 64 on fruit and 30-35% on stem,	Greenhouse	Elad, 2000
	Sphaerotheca fusca		Powdery mildew	45-71 %		

T. hazianum.	*R. solani*	Bean	Root rot	Reduced disease severity and protect seedlings from pre-emergence damping-off	Greenhouse conditions	Júnior et al. (2007)
T. hazianum.	*F. oxysporum* f. sp. *lycopersici*	Tomato	Tomato wilt	44.4%	*In vitro* and pot conditions	Alwathnani and Perveen (2012)
T. hazianum	*Botryodiplodia theobromae* *F. solani*	Yam	Root rot	53-84% 59-87%	*In vitro*	Okigbo and Emeka (2010)
T. hazianum BHU-51 *T. hazianum* BHU-105	*S. Sclerotiorum*	Brinjal	Damping-off	38-44%	Field Exp.	Singh and Singh (2012)
T. hazianum	*F. solani* *F. oxysporum* *R. solani* *Pythium spp.* *Sclerotium rolfsii*	Green bean	root rot	58-100% 56-100% 46--100% 42--100% 40-100%	*In vitro* and greenhouse	El-Mohamedy and Alla (2013)
T. harzianum (Th azad) *T. viride* (01 PP)	*F. oxysporum udum*	Pigeon Pea	Wilt	58% 60%	*In vitro*	Shahid et al. (2014)
T. hazianum *T. viride*	*F. oxysporum*	Banana	Wilt	1.4cm inhibition zone 1.0cm inhibition zone	*In vitro*	Thangavelu et al. (2004)
T. hamatum	*F. oxysporum*	Lentil	Vascular wilt	33%	Glasshouse	El-Hassan et al. (2013)
T. hazianum	*F. oxysporum* *R. solani*	Chickpea	Root rot and wilt	Significantly inhibit growth of phytopathogens	*In vitro*	Verma et al. (2014)

T. virens GL3 and GL21	*Pythium ultimum* and *R. solani*	Cucumber	Damping-off	Most effective in greenhouse Most consistent & effective in growth chamber	Field and greenhouse	Roberts et al. (2005)
T. hazianum ITEM 3636 *T. longibrachiatum* ITEM 3635	*F. solani*	Peanut	Brown root	*T. harzianum* effective than *T. longibrachiatum* in decreasing mean disease severity index and boosting yield	Field exp.	Rojo et al. (2007)
T. harzianum Th908	*F. oxysporum* Fo2797	Tomato	Wilt	15-35%	*In vitro*	Marzano et al. (2013)
T. harzianum	*F. udum*	Pigeon pea	Wilt	Soil application of *T. harzianum* more effective than seed treatment	Field exp.	Prasad et al. (2002)
T. citrinoviride	*S. sclerotiorum*	Soybean	Stem Rot (White Mold)	96%	*In vitro*	Thakkar and Saraf (2014)
	M. pheseolina		Charcoal rot			
T. atroviride *T. koningiopsis* *T. asperellum* *T. spirale* *T. brevicompactum* *T. longibrachiatum*	*S. sclerotiorum*	-	-	70% germination promoted by *T. asperellum* Th034, *T. atroviride* Th002 and *T. harzianum* Th203	*In vitro*	Smith et al. (2013)
T. harzianum	*F. solani*			33% by volatile metabolites and showed highly efficient antagonism		
	R. solani			41% by volatile metabolites and showed highly efficient antagonism		

	S. sclerotiorum			66% by volatile metabolites and showed highly efficient antagonism		
	F. solani	-	-	32% by volatile metabolites and showed highly efficient antagonism		
	R. solani			33% by volatile metabolites and showed highly antagonism		
T. ghanense						
	S. sclerotiorum			33% by volatile metabolites and showed efficient antagonism		
	F. solani			71% by volatile metabolites and showed highly efficient antagonism		
T. asperellum	*R. solani*			66% by volatile metabolites and showed highly efficient antagonism	*In vitro*	Qualhato et al. (2013)
	S. sclerotiorum			17% by volatile metabolites and showed highly efficient antagonism		
	F. solani			28% by volatile metabolites and showed efficient antagonism		
T. tomentosum	*R. solani*			52% by volatile metabolites and showed highly efficient antagonism		
	S. sclerotiorum			0% by volatile metabolites and showed efficient antagonism		
T. harzianum	*S. delphinii*	Cotton				

T. atroviride			Seed rot and seedling rot	*T. harzianum* exhibited highest disease suppression	*In vitro* and greenhouse	Mukherjee et al. (2013)
T. hazianum *T. asperellum*	*R. solani*	Sugar beet	Damping-off	1.88-3.04% increase in healthy seedlings as compared to control	*In vitro* and Greenhouse	Kakvan et al. (2013)
T. viride *T. harzianum* *T. aurepviride*	*F. moniliforme*	Maize	Stalk rot	*T. harzianum* showed maximum antagonism and seed germination	*In vitro* and *In vivo*	Harleen and Chander (2011)
T. viride	*Colletotrichum capsici*	Chilli	Fruit rot	58%	*In vitro*	Sangeetha et al. (2011)
T. harzianum	*F. oxysporum* f. sp. *lycopersici*	Tomato	Wilt	5-7.5%	*In vitro*	Sriram et al. (2010)
	Phytophthora capsici	Chilli	Damping-off	50%		
T. harzianum	*A. porri*	Onion	Purple blotch	73%	*In vitro* under greenhouse	Abo-Elyousr et al. (2014)
T. longibrachiatum				70%		
T. viride	*R. solani*	Tomato	Root rot and wilt	80%	*In vitro*	Hafez et al. (2013)
	F. oxysporum			77%		
	F. verticilloid			67%		
	A. alternata			80%		
	Mucor racemosus			40%		
T. harzianum *T. pseudokoningii*	*A. porri*	Onion	Onion blotch	73-75% inhibition of mycelial growth 71-73% inhibition of mycelial growth	*In vitro*	Imtiaj and Lee (2008)
T. virens				75% inhibition of mycelial growth		
T. viride	*Fusarium sp.*	-	-	40-45%	*In vitro*	

	Curvularia sp.			38-50%		
	Aspergillus niger			41%		Reena et al. (2012)
	Rhizopus			41%		
	Aspergillus flavus			45%		
	Aspergillus fumigates			39-50%		
T. viride				73%		
T. harzianum				71%		
T. longibrachiatum	*S. rolfsii*	Sugarbeet	Damping-off	53%	*In vitro*	Paramasivan et al. (2013)
T. reesei				52%		
T. koningii				55%		
T. harzianum						
T. harzianum T100	*F. oxysporum* and *F. proliferatum*	-	-	Antagonistic activity of *Trichoderma* spp. was more on *F. proliferatum* than on *F. oxysporum*.	*In vitro*	Ghanbarzadeh et al. (2014)
T. viride						
T. hamatum						
T. harzianum	*F. solani* f. sp. Melongena and *F. oxysporum* f. sp. *lycopersici*	Brinjal and Tomato	Wilt	*T. Longibrachiaum* showed 100% inhibition than others	*In vitro*	Enespa and Dwivedi (2014)
T. atroviride						
T. Longibrachiaum						
T. harzianum T-39	*Plasmopara viticola*	Grapevine cultivars	Downy mildew (D.M)	Reduced D.M symptoms, but degree of efficacy differed among cultivars	Field experiment	Banani et al. (2013)

Plant root colonization by *Trichoderma* spp.: Studies of the early invading fungi *Trichoderma* spp. showed that root colonization stimulated plant defense responses such as induction of peroxidases, chitinases, β-1, 3 glucanase, phenylalanine, and hydroperoxidase lyase; activated signaling of biosynthetic pathways; and caused accumulation of low-molecular weight phytoalexins (Yedidia et al., 2003).

Therefore, the interaction appears to be a symbiotic relationship in which *Trichoderma* lives in the nutritional niche provided by the plant, and the plant was protected from disease.

Uses of *Trichoderma* spp.: The discovery of cellulase production by *Trichoderma reesei*, which was isolated by Reese (1976), led to it becoming a very important cellulase or enzyme producer.

The cellulase produced by *Trichoderma* spp. is used mainly for malting, baking, and grain alcohol production. The filamentous cellulolytic *Trichoderma* spp., produce a broad range of cellulases and hemicellulases. The main application of lignocellulosic biomass is the production of biofuels such as ethanol although it is also used in the pulp, paper and textile industries. *Trichoderma* is also used for safe industrial enzyme production. Macerating enzymes are used to improve the brewing process for fruit juice production and as a feed additive for livestock and pet food. *Trichoderma* also used for seed germination, for example, a study showed that sunflower seeds germination significantly increased in *T. viride* or *T. resei* treated plants compared to control plants. The commercial use of several *Trichoderma* species for the protection and growth enhancement of a number of crops is ongoing (Samuels, 2006). Currently, the commercially available formulations are RootShield TM, BioTrek 22 TM, T- 22G TM, and T-22 HBTM (Bio-works, USA); Suprevisit TM (Borregaard BioPlant, Denmark); Binab TM (Bio-Innovation Sweden); Trichopel TM, Trichojet TM, Trichodowels TM, and Trichoseal TM (Agimm, New Zealand); Trieco TM (Ecosense Labs, India), and Tricho-green (Mycology Lab, Malaysia). Not all of these products are registered as a biocontrol agent, but they are marketed as plant growth promoters, plant strengtheners, or soil conditioners.

Interaction of *Trichoderma* with other microorganisms and plants: Fungi from the genus *Trichoderma*, due to their colonization of different environments, are forced to compete for nutrients and space with many other organisms. The mechanisms facilitating colonization of different ecological niches are well-developed and highly diverse in *Trichoderma* spp. (Vinale et al., 2008).

Hyperparasitism is connected with the direct contact of an antagonist with a pathogen and is composed of such stages as pathogen recognition, attack, gradual penetration of the pathogen cells and death (Vinale et al., 2008). In this process, a considerable role is played by CWDE (Cell Wall Degrading Enzymes) lytic enzymes, synthesized by *Trichoderma* species that facilitate hydrolytic degradation of pathogen cell walls, composed of chitin and glucan polysaccharides. *Trichoderma* species are also capable of producing cell wall degrading enzymes such as cellulase, xylanase, pectinase, glucanase, lipase, amylase, arabinase, and protease as well as many volatile metabolites, such as 6-n-pentyl-2H--pyran-2-one (6-PAP). Chitinases are the most important lytic enzymes playing a key role in the degradation of cell walls of other plant pathogenic fungi. Other enzymes determining the capacity of *Trichoderma* fungi for hyperparasitism, mainly in relation to fungus-like organisms, i.e. *Phytophthora* sp. and *Pythium* sp. are *β*-1, 3- and *β*-1, 6-glucanases. Cellulases form yet another group of enzymes produced by the *Trichoderma* species. These enzymes are capable of hydrolyzing lignocellulose biomass and comprise three types of enzymes which act synergistically.

When considering the interactions of *Trichoderma* fungi with plants, it was found that these fungi have an advantageous effect on plants. Stimulation of plant growth and yield takes place thanks to this interaction and the advantageous effects are seen in the production of vitamins, the increased availability of biogenic elements (nitrogen, phosphorus), the mobilization of nutrients from the soil and organic matter, and the enhanced intensity of mineral uptake and transport. Furthermore, *Trichoderma* fungi are capable of producing zeaxanthin and gibberellin, i.e. compounds accelerating seed germination. Many *Trichoderma* strains produce acids, e.g. gluconic, citric, and coumaric acids, causing the release of phosphorus ions and microelements, which subsequently become available to plants (Harman et al., 2004a).

Application of *Trichoderma* in biological plant protection: *Trichoderma* fungi are microorganisms that have been most frequently tested and applied in biological plant protection. The use of *Trichoderma* fungi may cause a considerable limitation in the use of chemical fungicides in agriculture (Akhtar et al., 2012). It is estimated that 90% of all the antagonistic fungi used in plant protection belong to the genus *Trichoderma*. Recent studies showed the potential of *Trichoderma* spp. to control stem canker of brassicas, severity greatly depending on avirulence genes in fungi *vs.* resistance genes in plants. The species of *Trichoderma* significantly differed in their hyperparasitic effects towards pathogen.

Prospects for the application of *Trichoderma*: Increased ecological awareness of whole societies and growing interest in alternative sources of energy make it possible to use fungi from the genus *Trichoderma* in the production of the so-called second generation biofuels (Schuster and Schmoll, 2010). The development of an adequately high efficiency of this process to ensure its economic viability poses a serious challenge for

researchers. Fungi from the genus *Trichoderma* may also be applied in modern plant cultivation technologies, in which considerable emphasis is placed on the environmental impact.

***Trichoderma* as a pathogen of humans:** Apart from the beneficial species used for human needs, the genus *Trichoderma* also comprises species which are highly dangerous to human health (Druzhinina et al., 2008). The pathogenic species include *Hypocrea orientalis*, genetically close but clonal *Trichoderma citrinoviride* Bis- set as well as *T. harzianum*, and *T. longibrachiatum,* with the prevalence of the first two closely related species. They constitute a lethal hazard for individuals with reduced resistance, including patients with leukemia, HIV- positive or having transplants (Kredics et al., 2003). Infections caused by *Trichoderma* are typically diagnosed late and are difficult to treat, as these fungi exhibit a low sensitivity to commonly applied antifungal drugs (Kratzer et al., 2006) and combined treatment is frequently necessary (Alanio et al., 2008).

Genomes of *Trichoderma*: Contemporary techniques allow one to sequence and compare whole genomes of different organisms, including fungi. In recent years three species of *Trichoderma* (*Hypocrea*); *T. reesei* (*H. jecorina*), *T. atroviride* (*H. atroviri- dis*), and *T. virens* (*H. virens*) have been sequenced, and the results are publically available. The smallest genome size (34 Mb) was found in the weakly mycoparasitic *T. reesei*. The largest genome (38.8 Mb) was one of the highly parasitic *T. virens* (Mukherjee and Kenerley, 2010). The genome of *Trichoderma* was of intermediate size (36.1 Mb). Similarly to some fungi, such as *Neurospora crassa* (Irelan et al., 1994) or *L. maculans* (Fudal et al., 2009), the genomes of *Trichoderma* contain fragmented transposable elements, called Repeat Induced Point (RIP) mutations. However, the comparative genomics have also revealed great differences between the genomes of *Trichoderma* and even closely related fungi, such as *Giberella zeae*. These are differences in respect to the decreased number of repetitive DNA sequences and numerous unique genes or gene families.

Studies on the expression of some genes produced by *Trichoderma* have proved difficult, as their activity may be connected solely with defense against other microbes or multicellular organisms (Osbourn, 2010). Brakhage and Schroeckh (2011) suggested some strategies to activate silent gene clusters by cultivating fungi in conditions that simulate competition and allow the usual biosynthetic pathways to be initiated. A detailed metabolomic-genomic study is suggested for elucidating the roles of the numerous gene products of *Trichoderma* (Mukherjee et al., 2012).

Tools for genetic manipulation of *Trichoderma*: Due to industrial application of *Trichoderma*, the genetic tool kit for this fungus is the most extensive of the genus, although also research with other species is not limited by technical obstacles and most tools can also be used for all species with slight modifications. Transformation of many species is possible, and different approaches such as Agrobacterium-mediated transformation (Zeilinger, 2004), or biolistic transformation were developed. The range of selectable marker cassettes, which includes hygromycin and benomyl resistance (Schuster et al., 2007), the *Aspergillus nidulans* and S gene, which enables growth on acetamide as sole nitrogen source as well as the auxotrophic markers, pyr4, arg2, and hxk1 allows for construction of multiple mutants, which is now facilitated by the availability of a *Trichoderma* spp. strain with perturbed non homologous end-joining pathway (Guangtao et al., 2009; Guangtao et al., 2010). Sequential deletions despite a limited number of selection markers became possible by the use of a blaster cassette comprising direct repeats for homologous recombination and excision of the marker gene (Hartl and Seiboth, 2005). Besides knockout strategies for functional analysis of genes, also expression of antisense constructs for knockdown (Rocha-Ramírez et al., 2002; Schmoll et al., 2009) was reported for *Trichoderma*, and RNAi has been shown to function in *Trichoderma* (Brody and Maiyuran, 2009). Last but not least, the recent discovery of a sexual cycle in *Trichoderma* spp. (Seidl et al., 2009) further boosts the versatility of this fungus for research and industry.

Mechanisms of *Trichoderma*: The most important and fascinating feature of *Trichoderma* is the study of mechanisms varying for management of phytopathogens and plant diseases in which pathogen antagonized by biocontrol agent results from different types of interaction between organisms (Pal and Gardener, 2006). The followings are direct and indirect biocontrol mechanisms to control plant pathogens (Figure 1).

Direct Antagonism

Direct antagonism results in the physical contact of biocontrol agent with the pathogen. It includes;

Mycoparasitism: Mycoparasitisim or hyperparasitism is

a complex process (Harman et al., 2004b) which involves the parasitic interaction of two or more fungi in which one parasitize mycelia of other (Druzhinina et al., 2011) and species of *Trichoderma* parasitize a wide range of mycoparaites especially soil-borne pathogens (Hajieghrari et al., 2008). In this process, firstly *Trichoderma* species sense the pathogen and come into contact with host involves morphological changes such as coiling and appressorium formation which developed hole on the surface of host or target pathogen (Omann and Zeilinger, 2010). Secondly, *Trichoderma* species recognize signals from host fungus that activate penetration of *Trichoderma* hyphae into the lumen of target parasitized fungus (Kubicek and Druzhinina, 2013). Thirdly, active multiplication takes place inside the hyphae of target fungi. *Trichoderma* and pathogen attachment mediated by binding of carbohydrates and lectin that are present in *Trichoderma* cell wall and target pathogen respectively.

Interestingly, *Trichoderma* extracellular enzymes are produced during the penetration process that inhibited the hyphael growth of the target pathogen. It is believed that a variety of chitinolytic enzymes and *ß*-1,3-glucanases are key enzymes in mycoparasitism (Kamala and Devi, 2012) of *Trichoderma* species that has shown great potential against phytopathogens. In the recent studies, more than 1100 strains of *Trichoderma* have been found to be mycoparasite from molecularly defined 75 species (Druzhinina et al., 2011).

Mixed- path Antagonism

Antibiosis and secondary metabolites: *Trichoderma* species cause decay of phytopathogenic fungi without any physical contact between microorganisms by producing the antimicrobial compounds. This process generally called as "antibiosis" and term secondary metabolites is a group of heterogeneous chemically divergent natural compounds might be associated with survival functions such as symbiosis, differentiation, and competition against organisms etc. for the producing organism (Wu et al., 2014). Antibiotics are natural products having the ability to inhibit target pathogen growth correlated with biocontrol activity. Antibiosis occurs during the interaction process of *Trichoderma* strains with pathogenic microbes involving antibiotics or low molecular diffusible compounds that retard the growth of pathogen (Reino et al., 2008). Most of the *Trichoderma* species produced volatile and non-volatile metabolic compounds including tricholin, massoilactone, heptelidic acid, gliovirin, 6-penthyl-α-pyrone, harzianic acid, glisoprenins, peptaibols, alamethicins, and others have been studied (Qualhato et al., 2013) which are toxic to target pathogen. The synergetic effect of antibiotics and hydrolytic enzymes achieve maximum level of antagonism rather than alone mechanism (Monte, 2001). *T. harzianum* and *T. virens* are the most effective biocontrol agents with respect to antibiotics that produce gliovirin and pyrone respectively.

Indirect Antagonism

Competition: Malnourishment is the foundation of death for every living organism. Competition is a phenomenon in which *Trichoderma* species and pathogen compete for limited nutrient and space availability. *Trichoderma* is generally considered as an aggressive competitor against soil-borne fungal pathogens that grow very fast towards pathogen and rapidly colonize it (Cuervo-Parra et al., 2014). Nutrients competition linked to soil rhizosphere and competition for infection sites appear inside or on roots of the plant. In most of the filamentous fungi, iron uptake is necessary for viability and most fungi produced low molecular ferric iron specific chelators called as siderophores under iron starvation that mobilize environmental iron (Eisendle et al., 2004). Species of *Trichoderma* have more ability to mobilize soil nutrients and their take up as compared to other microbes. Among all the mechanisms, nutrient competition is the most important (Verma et al., 2007) that prevent infection from the pathogen.

Induced systemic host resistance: Induction of host resistance in the plant is a complex mechanism. Generally, there are three pathways to induce resistance in the host plant. Two of these involve direct assembly of pathogenesis-related (PR) proteins that can be induced by the mechanism of other organisms. In one pathway PR proteins production results by an attack of pathogenic microbe while in other pathway PR proteins production as a result of necrosis or wound i.e. herbivory by insects. In the third type pathway of resistant induced by root associated with non-pathogenic bacteria such as rhizobacteria referred to as Rhizobacteria-Induced Systemic Resistance (RISR).

Pathogen-related induced pathway and herbivory pathway depends upon salicylic acid and jasmonic acid that are signaling molecules produced by plant respectively and their exogenous application induces a similar response as are produced in naturally produced molecules (Bostock et al., 2001). The pathway linked with

jasmonate referred to as induced systemic resistance that is relatively different in the process initiated by rhizobacteria. Both pathways (Jasmonate- and salicylate-induced pathways) characterized by cascade of pathogenesis-related (PR) proteins production that include antifungal enzymes including chitinases, thaumatins, glucanases and oxidative enzymes. The triggering molecules produced in *Trichoderma* response are still unknown (Druzhinina et al., 2011) that results in direct accumulation of phytoalexins or PR proteins referred to as Systemic Acquired Resistance (SAR).

The third kind of resistant induced by nonpathogenic rhizobacteria associated with roots of the plant described as rhizobacteria-induced systemic resistance (RISR) that is phenotypically similar to salicylic and jasmonate-induced resistant and functionally different results reduction of plant disease by systemic resistant. However, the Induced systemic resistance elicited by some important *Trichoderma* spp. mentioned in Table 2.

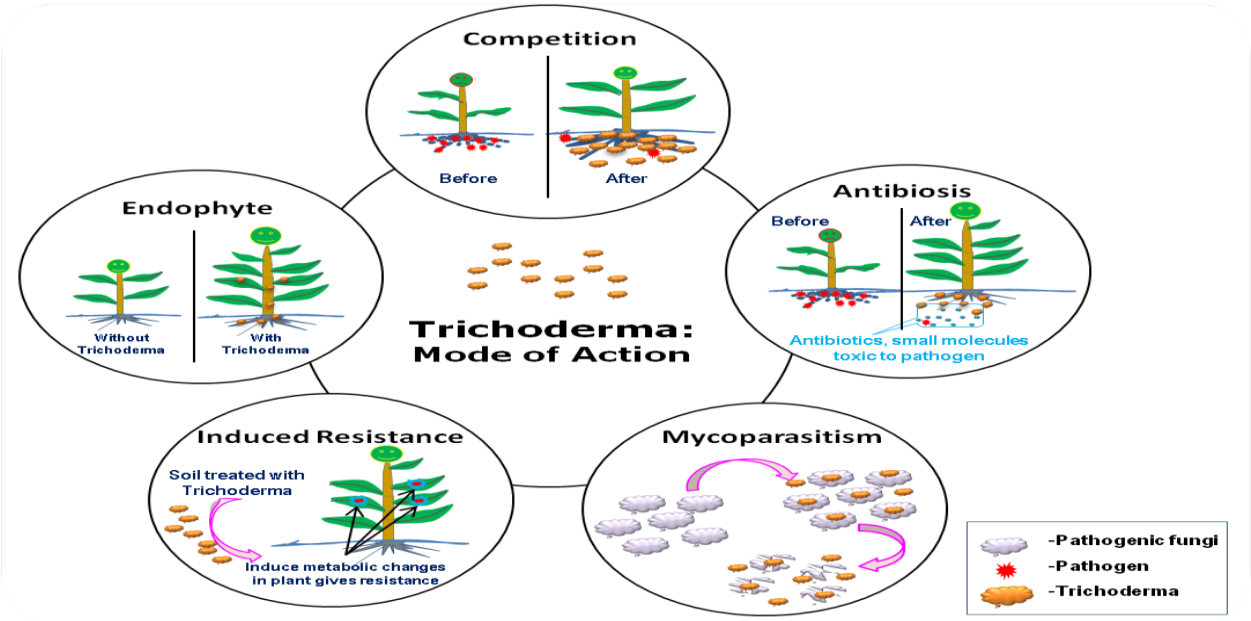

Figure 1. Model depicting mode of action of Trichoderma spp. against pathogen and plant growth improvement.

Table 2. Induced systemic resistance elicited by some important *Trichoderma* spp.

Species	Plant species	Pathogens	Outcome	References
T. virens	Cotton	*Rhizoctonia solani*	Protected plant by inducing terpenoid phytoalexins toxic to fungi	Kumar et al. (2009)
T. harzianum	Pepper	Phytophthora capsici	Improved production of the phytoalexins capsidiol toxic to pathogen	Ahamed and Vermette (2009)
T. virens	Tomato	*Pseudomonas syringe*	Secreted proteins-Sm1 and Ep11 both induced systemic acquired resistance	Salas-Marina et al. (2015)
T. asperellum	Cucumber	*Pseudomonas syringe*	Modulated the expression of proteins related to jasmonic acid/ethylene signaling	Shoresh et al. (2005)

Endophytic activity: Endophytic activity of many microorganisms (growth inside plant tissue without any harm) may useful to host plant by stimulating of plant growth, a postponement to the beginning of drought stress and the obstruction to pathogens. Endosymbiotic species are capable of establishing colonies in plant roots and triggers the expression of many plant genes affecting stress responses. Recently, there are reports showing *Trichoderma* isolates acting as endophytic plant symbionts in some woody plants (Chaverri et al., 2011).

Interestingly, strains forming an association with roots are altering the gene expression pattern in shoots. These changes are the key points in altering plant physiology and this can be exploited in the improvement of many important traits like uptake of nitrogen fertilizer, abiotic/ biotic stress resistance, and photosynthetic efficiency leading to higher yields (Harman et al., 2012). Phylogenetic analysis classifies all known endophytic species as a separate taxon with the exception of *T. koningiopsis, T. stilbohypoxyli* and *T. stromaticum* within their clades at terminal position suggesting endophytism is not an old trait but recently evolved in *Trichoderma* species (Druzhinina et al., 2011).

Efficacy of *Trichoderma* spp. against soil-borne mycoflora: Biological control of phytopathogens is a prospective non-chemical way for disease control. *Trichoderma* species found to be most effective biocontrol under greenhouse and field conditions against many plant diseases caused by soil-borne pathogens (Srinivasa and Devi, 2014) and their efficacy highly depend upon physiological and environmental parameters such as temperature (Colussi et al., 2012), pH (Romero-Arenas et al., 2012), growth medium (Blaya et al., 2013), light (Schmoll et al., 2009), carbon and nitrogen sources (Onilude et al., 2013). However, hyphal establishment, growth and biocontrol potential of *Trichoderma* greatly depend upon the biotic component interaction of various agricultural soil and all climatic zones (Bae and Knudsen, 2005) and *Trichoderma* isolates reported as successful biocontrol that retard growth of soil fungi (Keswani et al., 2014).

***Trichoderma* as a protector of plant health:** The beneficial action of *Trichoderma* spp. is not limited to fighting pathogens; they have also been shown to be opportunistic plant symbionts, enhancing systemic resistance of plants (Shoresh et al., 2010), a response which is improved by ceratoplatanin family proteins (Djonović et al., 2006). Perception of the signals transmitted by *Trichoderma* in the plant requires the function of a MAPK and also in the fungus itself, a MAPK signaling is crucial for full induction of systemic response in the plant (Viterbo et al., 2005). By colonizing plant roots, which is significantly enhanced by swollenin (Brotman et al., 2008) or invading them, they are also carried through soil and occupy new niches. This interaction with plants as well as their rhizosphere competence leads to enhanced root proliferation, better growth, and protection of the plants against toxic chemicals, against which *Trichoderma* spp. themselves show a remarkable resistance. Hence, these fungi are promising agents that can be applied for remediation of polluted soil and water by treatment of appropriate plants with spores (Harman et al., 2004a).

Food Industry: With their long history of safe industrial scale enzyme production, *Trichoderma* spp. has also been extensively applied for production of food additives and related products (Blumenthal, 2004). Currently, various *Trichoderma* enzymes are applied to improve the brewing process (β-glucanases), as macerating enzymes in fruit juice production (pectinases, cellulases, hemicellulases), as a feed additive in livestock farming (xylanases) and for pet food. Cellulases are mainly applied in baking, malting, and grain alcohol production. However, not only enzymes but also metabolites of *Trichoderma* spp. are used as additives. One of the first products isolated from *T. viride* was a chemical with characteristic coconut-like aroma, a 6-pentyl-α-pyrone with antibiotic properties, the production of which was constantly improved to reach concentrations of more than 7 g/L in extractive fermentation cultures in *T. atroviride* nowadays (Oda et al., 2009). An interesting idea is the application of cell wall-degrading enzymes, for example of *T. harzianum*, as food preservatives because of their antifungal effect, but so far this suggestion has not found broad application. With a similar aim, *T. harzianum* mutanase can be used in toothpaste to prevent accumulation of mutan in dental plaque (Wiater et al., 2005).

Commercialization of *Trichoderma* products: Commercialization of *Trichoderma* or biocontrol agents depends upon the screening process of biocontrol microorganism and its efficacy against pathogenic mycoflora. The first species of *Trichoderma* (*Trichoderma harzianum*) registered with EPA in 1989 for control of plant pathogens and diseases (Fravel, 2005).

Commercialization of biocontrol products is a multi step process and includes (Table 3):

a) Isolation of microorganisms
b) Evaluation of antagonists in lab and field conditions
c) Selecting best isolate in field conditions
d) Mass production
e) Formulation
f) Delivery
g) Compatibility
h) Registration and release

Methods of application *Trichoderma* species

Seed treatment: Seed treatment is also known as seed priming used for multiple purposes on many crops to provide inexpensive cover against wilting and rotting of planted seeds by soil-borne fungi such as *Rhizoctonia*, *Sclerotinia,* and *Macrophomina* species. For this purpose, mix 10 grams *Trichoderma* formulation for 1 kg of seed to per liter of cow dung slurry before sowing especially for pulses and cereal crops. *Trichoderma* multiply, reproduce and move towards the root of germinating seed where it fixes nitrogen and increases nutrients, various toxic metals and metabolites uptake and (Harman, 2006) root colonization by *Trichoderma* species promote root growth and increases resistant to abiotic stresses. *Trichoderma* seed treatment enhance chances of germination, vigor index and defense mechanism of the plant (Harman et al., 2004b).

Table 3. *Trichoderma* based commercial products against various diseases.

Commercial Product/ Trade name	*Trichoderma* species	Target disease	Company/ Manufacturer or distributor
Anti-Fungus	*Trichoderma* spp.	Root rot	Grondoontsmettingen De Ceuster, Belgium
Binab	*Trichoderma* spp.	Root rot and wilt	Binab, Sweden
Biofungus, Superesivit	*Trichoderma* spp.	Root rot and wilt	Bioplant, Denmark
Root Pro	*T. harzianum*	Root rot	Efal Agri, Israel
Root Shield, Plant Shield, T-22 Planter box	*T. harzianum* T-22	Root rot	Bioworks, Geneva, USA
T-22B, T-22G	*T. harzianum T-22*	Root rot	TGT Inc. New York, USA
T35	*T. harzianum*	Wilt	Makhteshim-Agan Chemical Israel
ECO T/T22	*T. harzianum*	Root rot	PHP Ltd., South-Africa
GlioGard and SoilGard	*T. virens*	Root rot	Grace-Sierra Co. Maryland
Harzian 20, Harzian 10	*T. harzianum*	Wilt	Natural Plant Protection, Noguerres, France
Biospark Trichoderma	*T. parceramosum T. pseudokoningii*	Wilt	Biospark Corporation, Phillipines
F-stop	*T. harzianum*	Root rot	Eastman Kodal Co. TGT Inc., New York, USA
Soil Gard	*T. virens* GL-21	Root rot	Certis, USA
Tricho-X	*T. viride*	Root rot	Excel Industries Ltd., ,India
Trichodex	*T. harzianum*	Grey mold	Makhteshim Chemical Works, Israel
Trichopel	*Trichoderma* spp.	Root rot	Agrimm Technologies, New Zealand
Bip T	*T. viride*	Wilt	Poland
Trieco	*T. viride*	Root rot, wilt	Ecosense Laboratories, India
Pant biocontrol agent-1	*T. harzianum*	Root rot, wilt	Deptt. of Plant Pathology, GB plant University of Agriculture & Technology, Panatnagar, Uttarakhand
Plant helper	*T. atroviride*	Root rot	Ampac, California

Biobus 1.00WP	*T. viride*	Root rot	Nam Bac, Vietnam
Biocon	*T. viride*	Root rot, wilt	Tocklai Experimental Station Tea Research Association, India
TRICO-DHCT	*Trichoderma* spp.	Sheath blight	Can Tho University, Vietnam
Defense SF	*T. viride*	Wilt	Wockhardt Life Science Ltd., India
NLU-Tri	*T. virens*	Wilt	Ho Chi Minh University of Agriculture and Forestry, Vietnam
Ecoderma	*T. viride+ T. harzianum*	Root rot and wilt	Morgo Biocontrol Pvt. Ltd., India
Bio – Humaxin Sen Vàng 6SC	*Trichoderma* spp.	Cottony rot	An Hung Tuong, Vietnam
Trichogourd	*T. viride*	Damping off	Anu Biotech international Ltd. India
Promot PlusWP and Promot PlusDD	*Trichoderma* spp. *T. koningii* *T. harzianum*	Damping off, Root rot and wilt	Tan Quy
Vi-DK	*Trichoderma* spp.	Root rot and wilt	Pesticide Corp.
Fulhumaxin 5.15SC	*Trichoderma* spp.	Root rot	An Hung Tuong, Vietnam
Trichotech	*Trichoderma* spp.	Wilt	Finlay International Kenya Ltd. Dudutech, Laboratory
Ecofit	*T. viride*	Root rot	Hoechest and Schering Agro. Evo. Ltd., India
Funginil	*T. viride*	Root rot	Crop Helath Bioproduct Research Centre, India
Trichopel Trichodowe Trichoject	*T. harzianum +T. viride*	Wilt	Agrimm, Technologies Ltd., New Zealand
Binap- T&W	*T. harzianum+ T. polysporum*	Wilt	Bio Innovation AB, Toreboda, Sweden
Antagon-TV	*T. viride*	Seed and soil-borne diseases	-
Dfence-SF	*T. viride*	Seed and soil-borne diseases	-

Nursery treatment: Young seedlings are more susceptible to disease that leads to the development of the diseased plant. Moist soil during the germination period increases the chances of infection by root rot and wilt fungi. Nursery beds amendment with the application of *Trichoderma* spore suspension or product facilitate management of soil-borne pathogens and protect the crop results significant increase in the yield under field condition (Reglinski and Dick, 2005). Before sowing of the crop, drench nursery bed treated with @ 5kg *Trichoderma* formulation per liter of water (Ranasinghe et al., 2005). Effect of *T. harzianum* on artificially inoculated *Phaeomoniella chlamydospora* on grapevine under greenhouse and nursery trial has been reported that *T. harzianum* level of the necrotic area which was higher in old inoculating nurseries and also increase tolerance in plants against stress condition.

Cutting and seedling root dip: Dipping of plant cuttings and seedling roots in *Trichoderma* suspension is another way of *Trichoderma* application aims to protect seedling and cuttings from pathogen infection. It is done by dipping of cutting and seedling roots before planting for 10 minutes in a mixture of 10 g *Trichoderma* formulation per liter of water.

Soil application: *Trichoderma* is an actively growing fungus and can be applied in the soil and nursery as drench as well as granular form. Soil can also be treated with 1 kg *Trichoderma* formulation mix with 100 kg FYM

and cover it with polythene for 7 days. Turn the position of the mixture after 4-5 days interval and apply in the field.

Application recommendation and precautions

Recommendation: All type of plants and vegetables can be treated with *Trichoderma* for better production such as tomato, potato, pepper, tobacco, sugar beet, sugarcane, brinjal, turmeric, ginger, betel vine, banana, eggplant, cotton, chilies, cardamom, onion, maize, cucumber, peanut, red gram, white gram, Lentil, chickpea, cassava, citrus etc.

Precautions: Some precautionary measures should be kept in mind regarding the application of *Trichoderma* inoculums in the field condition. These are given below:

1. Don't settle treated Farm Yard Manure (FYM) for a longer time.
2. Don't place *Trichoderma* treated seed in direct sunlight.
3. Don't apply chemical pesticides or fungicides after application of *Trichoderma* for 5-6 days.
4. Moisture is an important factor for *Trichoderma* growth and reproduction so don't try to use it in dry soil.

Sensitivity against agrochemicals: The efficiency of the bioagents is hampered due to poisonous nature of fungicides which are used simultaneously in crop production technology. Therefore, the sensitivity and tolerance of *Trichoderma* have been tested by our group and many others (Madhusudhan et al., 2010). The effect of different fungicides together with *Trichoderma* spp. has been studied for integrated disease management. *Trichoderma* spp. have shown greater tolerance for broad spectrum fungicides than many other soil microbes as it has the capacity to colonize the pesticides treated soil more rapidly (Oros et al., 2011). *Trichoderma* alone or their combinations with bacteria or their immobilized formulations can have great potential, as more than a few unusual contaminants can be treated at the same time and will have wider applicability, hence improving the overall cost effectiveness of the technology.

Future prospects: Sustainability is also the major driving force for the investigation of biocontrol with *Trichoderma*. As opportunistic plant symbionts and effective mycoparasites, numerous species of this genus have the potential to become commercial biofungicides. The challenge in this field of research will be the development of reliable screening techniques, which allow for prediction of the biocontrol efficiency of a given isolate by determination of the key factors for this process. Nevertheless, also the ecological effects of the widespread application of a single (or few) fungal species in agriculture remain to be investigated in order to ensure a truly beneficial effect for the environment. *Trichoderma* as biocontrol agent is utmost important part of integrated plant disease management that can be used against soil-borne phytopathogens but its biocontrol potential is yet to be limited to laboratory experiments and very diminutive attention has been paid to its commercial formulation. Moreover, farmers also have lack of information concerning its utilization. So, the concept of *Trichoderma* commercialization needs to be improved and cost-effective production formulation should be popularized. Some biocontrol agents or *Trichoderma* species unsuccessful to compete with phytopathogens that attributed to physiological and environmental parameters influences the effectiveness of BCAs. Thus, molecular tools and genetic engineering need to be performed for improvement of BCAs that can be able to proliferate and compete against a wide range of phytopathogens. So, this is necessary to give the support to agencies that are engaged in this field.

CONCLUSION

Biological control gives the impression of an alternative to chemical-based pesticides for disease suppression and control. Scientists and their research have proved that *Trichoderma* is non-pathogenic to plants and need to be formulated in a way that favors the activity and survival of microbes. Moreover, the novel concept of biocontrol needs a space outside the laboratory to see its fruits in present production systems.

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Journal of Plant Interactions

ISSN: 1742-9145 (Print) 1742-9153 (Online) Journal homepage: https://www.tandfonline.com/loi/tjpi20

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To cite this article: Girija Ganeshan & A. Manoj Kumar (2005) *Pseudomonas fluorescens*, a potential bacterial antagonist to control plant diseases, Journal of Plant Interactions, 1:3, 123-134, DOI: 10.1080/17429140600907043

To link to this article: https://doi.org/10.1080/17429140600907043

Journal of Plant Interactions, September 2005; 1(3): 123–134

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REVIEW

Pseudomonas fluorescens, a potential bacterial antagonist to control plant diseases

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(Received 24 April 2006; accepted 11 July 2006)

Abstract
Fluorescent Pseudomonads belong to plant Growth Promoting Rhizobacteria (PGPR), the important group of bacteria that play a major role in the plant growth promotion, induced systemic resistance, biological control of pathogens etc. Many strains of *Pseudomonas fluorescens* are known to enhance plant growth promotion and reduce severity of various diseases. The efficacy of bacterial antagonists in controlling fungal diseases was often better as alone, and sometimes in combination with fungicides. The present review refers to occurrence, distribution, mechanism, growth requirements of *P. fluorescens* and diseases controlled by the bacterial antagonist in different agricultural and horticultural crops were discussed. The literature in this review helps in future research programmes that aim to promote *P. fluorescens* as a potential bio-pesticide for augmentative biological control of many diseases of agriculture and horticultural importance.

Keywords: Pseudomonas fluorescens, *strains, bacterial antagonists, plant diseases, biological control, plant pathogenic fungi*

Introduction

Pseudomonas fluorescens encompasses a group of common, nonpathogenic saprophytes that colonize soil, water and plant surface environments. It is a common gram negative, rod-shaped bacterium. As its name implies, it secretes a soluble greenish fluorescent pigment called fluorescein, particularly under conditions of low iron availability. It is an obligate aerobe, except for some strains that can utilize NO_3 as an electron acceptor in place of O_2. It is motile by means of multiple polar flagella. *Pseudomonas fluorescens* has simple nutritional requirements and grows well in mineral salts media supplemented with any of a large number of carbon sources (Palleroni 1984). Because they are well adapted in soil, *P. fluorescens* strains are being investigated extensively for use in applications that require the release and survival of bacteria in the soil. Chief among these are biocontrol of pathogens in agriculture and bioremediation of various organic compounds.

Certain members of the *P. fluorescens* have been shown to be potential agents for the biocontrol which suppress plant diseases by protecting the seeds and roots from fungal infection. They are known to enhance plant growth promotion and reduce severity of many fungal diseases (Hoffland et al. 1996, Wei et al. 1996). This effect is the result of the production of a number of secondary metabolites including antibiotics, siderophores and hydrogen cyanide (O'Sullivan & O'Gara 1992). Hass and Defago (2005) reviewed the mechanisms by which *P. fluorescens* control pathogenic microorganisms in detail. Competitive exclusion of pathogens as the result of rapid colonization of the rhizosphere by *P. fluorescens* may also be an important factor in disease control. The present review discusses the occurrence, distribution, growth requirements of *P. fluorescens* and diseases controlled by the bacterial antagonist in different agricultural and horticultural crops.

Occurrence and distribution

A study during storage of broccoli at 5, 15 and 20°C in Australia (Victoria and New South Wales) indicated *P. fluorescens* to be predominant. Of the 31 bacterial isolates from phylloplane of *Solanum melongena* and 20 from *Ipomoea batata* were characterized, out of that one isolate belonging to *P. fluorescens* was

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ISSN 1742-9145 print/ISSN 1742-9153 online
DOI: 10.1080/17429140600907043

found to be antagonistic to *Macrophomiea phaseolina*, *Helminthosporium tetramere*, *Alternaria tenuis* and soil-borne *Fusarium solani* and *Sclerotinea rolscii* (Atef 2000). Rangeshwaran and Prasad (2000b) collected 300 isolates from different regions of Karnataka. Four isolates belonging to *P. fluorescens* viz., PDBCAB 2, PDBCAB 19, PDBCAB 29, PDBCAB 30, were most prominent. Total rhizosphere population was higher (log cfu 6.4) for *P. fluorescens* isolate PDBCAB 29 after 14 days of germination of chickpea. Strains PDBCAB 19 and 30 were able to fully control *F. oxysporum* f. sp. *ciceris*. All above four antagonists promoted growth of chickpea. Out of 11 pea cultivars screened at flowering stage (Netherlands), five showed colonization. Pea cv. Twiggy at pod stage showed the highest and most consistent colonization (Elvira-Recuenco & van Vuurde 2000).

In Poland, 94 antagonistic agents from various vegetables and potato were isolated and the most frequent strain was *P. fluorescens* biovar I. It formed 31.9% of the tomato isolates (Zolobowska & Pospieszny 1999). Mabagala (1999) isolated naturally occurring epiphytic non-pathogenic bacteria from reproductive tissue of various bean genotypes grown in field. Among that one isolate was identified as *P. fluorescens* which was antagonistic to *Xanthomonas axonopodis* pv *phaceoli in vitro*. In Italy, Cirvilleri et al. (1999) collected 182 bacterial isolates from rhizosphere of tomato, lettuce, cauliflower and artichoke grown in 10 commercial green houses. They found that most isolates belonged to *P. fluorescens* biover I, II, III, V and to *P. putida*. Zalewska (1999) isolated and tested 138 epiphytic bacteria, out of that 25 isolates belonging to *P. fluorescens* had limited the growth of *Monilia coryli* on hazel (*Corylus avellana*) during 1994–1997 in Poland.

Bonaterra et al. (1998) isolated in Spain several strains of *P. fluorescens* from a wide range of environments which were antagonistic to brown shoot of pear (*Stemphyllium vesicarium*). Johnson et al. (1999) observed *P. fluorescens* up to 10 m from inoculated pear and apple trees as bloom progressed, which suggested that insect vectors were involved in the movement of bacteria. Bacterial population associated with outer florets and the cut surfaces of the stem were ten-fold higher than those associated with inner florets and non cut stems (Padaga et al. 2000).

Klyuchnilov and Kozhevin (1990) in former USSR, found that the most numerous and active population of *P. fluorescens* were found in the exorhizosphere of potato. Hebbar et al. (1991a) studied bacteria associated with sunflower leaves and roots inhibited *in vitro* growth of *Alternaria helianthi, Sclerotium rolfsii, Rhizoctonia solani* and *Macrophomina phaseolina* in France. The root-associated bacteria were identified as *P. fluorescens* and could be used as seed inoculum to improve plant growth through disease control. Hoflich (1992) isolated 105 bacteria from the rhizosphere of winter wheat; only one *P. fluorescens* strain (PsIA12) stimulated reproductive growth of winter wheat, winter rape, oil radish, mustard and peas in pot and field experiments. It also inhibited soil borne root pathogens (*Gaeumannomyces graminis, Fusarium oxysporum* f. sp. *pisi and F. solani*) in plate tests. Thara and Gnanamanickam (1994) isolated 1757 isolates of bacterial antagonists and identified both fluorescent and non fluorescent groups, 12% inhibited *R. solani* and 13% (of the remaining total 1366) tested positive for chitinase activity. Tzeng et al. (1994) isolated a total of 151 strains of fluorescent pseudomonads from various crop at different localities in Taiwan. They also found most of the foliar fluorescent pseudomonad strains to be saprophytic.

Mechanism of action

Particular bacterial strains in certain natural environments prevent infections diseases of plant roots. How these bacterial strains achieve this protection from pathogenic fungi has been analysed in detail in biocontrol strains of fluorescent pseudomonads (Hass & Defago 2005). The anti-fungal metabolite 2,4-diacetyl phloroglucinol play a major role in the biocontrol capabilities of *P. fluorescens* (Delany et al. 2000). Karunanithi et al. (2000) observed a native isolate of *P. fluorescens* producing an antibiotic compound, pyrollnitrin, which inhibited growth of *M. phaseolina* by producing an inhibition zone of 12 mm. Meena et al. (1999) got a substantial increase in phenyl alanine ammonia lyase activity in rice leaves sprayed with *P. fluorescens* Pf 1 strain one day after treatment, whereas maximum activity was observed 3 days after treatment. Steijl et al. (1999) working with radish and carnation for control of *F. oxysporum* f. sp. *raphani* and *F. oxysporum* f. sp. *dianthi* found that fungal infection led to degradation of cell walls in host cells. The lignin component in infected wall was demethoxylated, oxidized and depolymerized. It was also found that infecting radish and carnation with *P. fluorescens* WCS 417r before pathogen infection led to reduced cell wall degradation. *Pseudomonas fluorescens* strain CHA0 requires sufficient iron, and iron competition is not a suppressive mechanism in this system. It is thought that HCN production has a role in disease suppression (Kell et al. 1989).

Recently, Imran et al. (2006) reviewed the role of cyanide in controlling root knot disease of tomato. *Pseudomonas fluorescens* 2-79 produce the antibiotic phenazine-1-carboxylic acid and suppress take-all of wheat caused by *Gaeumannomyces graminis* var *tritici*. The antibiotic was isolated only from roots of wheat colonized by strain 2-79 in both growth chamber and field studies in USA (Thomashow

et al. 1990). Kell et al. (1992) indicated that the importance of 2,4-diacetyl phloroglucinol production by strain CHA0 (*P. fluorescens*) is the suppression of soil-borne plant pathogens in the rhizosphere. Borowicz et al. (1992) in Poland observed that the ability of plant growth-promoting fluorescent *Pseudomonas* inactivate cell wall degrading enzymes of plant pathogenic fungi should be taken into account as an additional mechanism explaining the biocontrol properties of this group. Bacteria isolated in the rhizosphere and roots of maize exhibited varying degrees of antagonism towards *F. moniliformae*, which was dependent upon the soils from which the bacteria were isolated. It is suggested that antibiotic production rather than siderophores is responsible for anti-fungal activity of antagonistic bacteria (Hebbar et al. 1992). The antagonism was connected to siderophore production on fluorescent *Pseudomonas* strains and to production of antibiotic like substance in non-fluorescent strains (Cassinelli et al. 1993). Sacherer et al. (1994) at Switzerland found antibiotic metabolites synthesized by *P. fluorescens* strain CHA0 play an important role in the suppression of root diseases of plants. The production of there metabolites is activated by the global activator gene *gacA*. Nowak Thompson and Gould (1994) standardized detection methods for several iron binding metabolites (siderophores) of *P. fluorescens* B10 (JL 3133) using C18 reverse phase HPLC coupled with photodiode array.

Growth studies

Turnbull et al. (2001) studied the bacterial motility in the survival and spread of *P. fluorescens* SBW25 and attachment and colonization of wheat roots. They constructed motile and non-motile strains and allowed their detection in both soil and water. Although there was no difference between strains in water, the motile strain survived in significantly greater numbers than the non-motile strain after 21 days in soil. The motile strain had a significant advantage in attachment to sterile wheat roots in both non-competitive and competitive studies. They concluded that bacterial motility could contribute to survival in soil and the initial phase of colonization where attachment and movement onto the root surface are important. Pujol et al. (2005) screened *P. fluorescens* EPS62e for its high efficacy controlling *Erwinia amylovora* infections in flowers, immature fruits and young pear plants. The population level of EPS62e after treatment was 7 log CFU (g f.w.)$^{-1}$, which in turn decreased progressively to 4–5 log CFU (g f.w.)$^{-1}$ after 17 days and then remained stable until the end of the assay 11 days later. Knee et al. (2001) in the USA purified pea root mucilage which appeared to contain an unusually high amount of material that was similar to arabinogalactin protein. Purified pea mucilage was used as the sole carbon source for growth of *P. fluorescens* PRA 25.

Ripp et al. (2000) showed that utilizing bioluminescence of a population monitoring tool for lux-based microorganisms, was more effective and precise than standard selective plating techniques, and provided an accurate ecological analysis of *P. fluorescens* HK44 population dynamics. Srivastava et al. (1999) studied root colonization of wheat in non-sterile soils employing two promising growth promoter strains of *P. fluorescens* (GRP3 and PRS9). Both strains showed parallel colonization patterns in wheat rhizosphere and rhizoplane. Both strains GRP3 and PRS9 promoted wheat growth in terms of root-shoot length and weight, however, bacterial population decreased towards the root tip. Hase et al. (2000) studied the effect of cucumber roots on the survival pattern of *P. fluorescens* CHA0-Rif for 22 days in two non sterile soils. They found that soil types had a significant influence on the occurrence of VBNC (viable but non-culturable) cells of CHA0-Rif, although these cells were found in root-associated habitats (i.e., rhizosphere and root tissues) and not in bulk soil.

Gandhi Kumar et al. (2001) found peat, coir pith and talc to be the best substrates for growing *P. fluorescens*. McGuire (2000) found that surface populations of *P. flurorescens* were stable between 103 and 104 cfu/cm^2 on shellacked fruit over 4 months at 13°C. Keinath et al. (2000) showed that zinc and medium dilution were effective for improving genetic stability of other *P. fluorescens* biocontrol strains obtained from Guana island and Italy. McEldowney (2000) obtained results which suggested that the characteristics of heavy metal (cadmium) accumulation by *P. fluorescens* H2 was substantially affected by attachment to solid surfaces (glass surface). Nielsen and Sorensen (1999) found 12 isolates of *P. fluorescens* from barley and sugar beet rhizosphere showing chitinolytic activity in batch cultures when grown in media without exogenous chitin. Nakata et al. (2000) obtained results (radish) which suggested that polysaccharide (Flocculent 1–100 ppm) enhanced adhesion of *P. fluorescens* S272 cells that might be useful for promoting plant growth through the increased antibiotic activity. Mercier and Lindow (2000) illustrated that plants capable of supporting high bacterial population sizes were proportionally more depleted of leaf surface nutrients than plants with low epiphytic populations. Naseby and Lynch (1999) working with pea (*P. sativum* var. *Montana*) in a sandy loam soil of pH 5.4, observed that increasing pH increased the indigenous population of *P. fluorescens* strain F11$_3$ compared to reduced pH treatment.

Pseudomonas fluorescens strain CHA0 stored in MS1 clay or in pure vermiculite clay could be re-isolated up to 6 months and survived exposure to

60°C for 24 h in that clay. In clay from MC1, on king's B medium, CHA0 survived less than one month and was killed by exposure to heat (Stutz et al. 1989). The suppressive ability of *P. fluorescens* strain CHA0 to decrease take-all of wheat (caused by *G. graminis* var. *tritici*) and black root of tobacco (caused by *Thielaviopsis basicola*) was dependent on soil quality and the host-pathogen systems (Wuthrich & Defago 1991). The colonization of potato and radish root system by strain of *P. fluorescens* was studied in pot culture experiment at UK. Root colonization was extensive but populations were highest on the upper root system and their distribution throughout the root system was greatly affected by environmental factors. Percolation of water through the soil and partial soil sterilization enhanced colonization (Davies & Whitbread 1989). Preliminary analysis of soil samples from Victoria, Australia, indicated *P. fluorescens* to be a dominant species (63%) (Pascoe & Premier 2000).

Elsherif and Grossmann (1991) found that *P. fluorescens* applied to seed or root treatment or by placing in the planting hole in pot trials against *Plasmodiophora brassicae* in Chinese cabbages, it significantly controlled the pathogen. Soil bulk, density and temperature had significant effect on colonization of the rhizosphere by *P. fluorescens*. Greatest rates of colonization occurred at lower bulk density (0.82g/cm^3) and highest temperature (22°C) (Rattray et al. 1993). Thomashow et al. (1990) studied roots of wheat plants grown in steamed soil yielded larger bacterial populations (*P. fluorescens* 2-79) compared to roots from natural soils. Weidenborner and Kunz (1993) showed that fermentation of *P. fluorescens* for 24 h at 20°C a Plate Count Broth (PCB; 0.5% casein-peptone, 0.25% yeast extracts, 0.1% dextrose) concentration of 100% enhanced effectively to 93.6% while number of nematodes was reduced to 88.1% in a PCB medium of 50%. Best results were obtained if fermentation was done in a medium containing 0.5% casein-peptone and 0.25% yeast extract. Out of 253 bacterial strains (mostly *Pseudomonas* spp.) isolated from rhizosphere of plants growing in suppressive soils, 18 strains (50% being *P. fluorescens* and *P. putida*) gave more than 35% inhibition of the pathogen, *P. ultimum* (Cassinelli et al. 1993). Survival of indigenous heterotrophic bacteria (*P. fluorescens* R_2f, RP_4) depends on the concentration of Cu (IL) and time of incubation (Kozdroj 1994). Casale et al. (1995) found that mulch characteristics which favour healthy growth of citrus and avocado also favour the growth of *P. fluorescens*.

Control of plant diseases in crops

Cereals

When rice seeds were treated with the formulation of *P. fluorescens* Pf1 before sowing, at sown and at 30 days, seedlings showed resistance to *X. oryzae* pv. *pryzae*, where the disease incidence decreased from 6.8–1.2 (Vidhyasekaran et al. 2001). In a field trial, Karpagavalli et al. (2002) investigated the complementary effect of silica (6 t lignite fly ash (LFA)/ha) along with a foliar spray of the biological control agent, *P. fluorescens*, on the blast [*Magnaporthe grisea*] incidence of rice cultivars IR50 and White Ponni. Foliar spray of *P. fluorescens*, in addition to LFA and 45 kg potash/ha significantly reduced the rice blast incidence and increased crop yield. When seeds were treated with *P. fluorescens* strain PfALR2, Mishra and Sinha (2000) got increased seed germination of rice from 26.3–52.6%. Two *P. fluorescens* strains, viz. PF1 and FP7, inhibited the mycelial growth of sheath blight fungus *R. solani* and increased the seedling vigour of rice plants and yield under green house and field conditions. Pseudomonas treatment of rice cv. IR50 led to induction of systematic resistance against *R. solani* as a result of increase in chitinase and peroxidase activity (Nandakumar et al. 2001).

Field trials with rice cultivars Co 43, ADT 36 and ADT 38 were conducted during Kuruvai (June–September), Samba (August–January) and Navarai (January–April) seasons, to evaluate the efficacy of *P. fluorescens* strain Pf-1, in controlling *Hirschmanniella gracilis*. This was done by giving seed treatment (10 g/kg) and nursery soil application either with or without soil application of carbofuron 3g @ 1.3g ai./m^2. Among the treatments, 10 g/kg of the biocontrol agent was found to be superior to all other treatments. Maximum bacterial colonization and nematode suppression along with rice yield/increase (13%) was observed in this treatment (Ramakrishnan et al. 1998). Rajbir Singh and Sinha (2005) studied the effect of *P. fluorescens* strains 1 and 5 against sheath blight, *R. solani* on rice under glasshouse conditions. They found that *P. fluorescens* of higher rate, i.e., 8 g/l was highly effective in reducing disease severity (60.0%) and incidence (35.6%) and increasing grain yield (33.8%) and 1000-grain weight (12.9%).

In wheat (*Triticum aestivum* L.), two strains of *Pseudomonas* GRP3 and PRS9 promoted wheat growth in terms of root-shoot length and weight. Kita et al. (2004) found *P. fluorescens* strain PSR21 to be a good measure for a wheat seed treatment and later, during the spring, spraying resulted with a noticeable decrease of the average degree of culms damage. Wang-Ping et al. (1999) observed that *P. fluorescens* biovar I and III successfully reduced the disease incidence (*Helminthosporium sativum*) and increased plant height and dry weight. Generally, bacterial (*P. fluorescens*) population decreased towards the root tip (Srivastava et al. 1999). In a glasshouse experiment, the effects of different fly ash concentrations (0, 20, 40%) and soil microorganisms (*P. fluorescens*, *Azotobacter chroococcum*, *Glomus*

mosseae and *Aspergillus awamori*) on the plant growth, photosynthetic pigments and leaf blight of wheat caused by *A. triticina* were tested. *Glomus mosseae* caused the greatest increase in plant growth and photosynthetic pigments and greater reduction in the percentage of infected leaf area followed by *P. fluorescens* (Siddiqui Singh 2005). The bacteriarization of wheat weeds with *P. fluorescens* strain WCS 417 in the preceding year had limited the natural build up of *G. graminis*. Only 6% white heads had developed in these fields and yield was increased significantly (Lamers et al. 1988). McManus et al. (1993) found that common bunt disease (*Tilletia laevis*) was reduced by 65 and 50% during consecutive seasons when wheat seeds and 2-week-old seedling were treated with Pf2-79r. Whereas, the strain D7 suppressed germination of seeds and reduced root and shoot growth of downy brome (*Bromus tectorum*) in agar diffusion assay. The inhibition was complete at concentrations as low as 1 mg total dry matter/liter agar. Also, the active fraction inhibited the plant pathogenic fungus *Gaemannomyces graminis* var. *tritici* (Gurusiddaiah et al. 1994) on wheat.

Seeds of ragi (*Elengine coracana* var. *paiyur* 1) treated with *P. fluorescens* against blast disease caused by *Pyricularia grisca* indicated that it was not significantly better than fungicidal treatments (Vanitha 1998). Umesha et al. (1998) carried out an experiment under greenhouse and field conditions in Karnataka, India. Where they treated seeds of pearl millet (*Pennisetum glaucum*) with *P. fluorescens* formulated in talc powder which increased seedling vigour and inhibited sporulation of the downy mildew pathogen. *P. fluorescens* controlled downy mildew by both seed treatment and foliar application, but efficacy was significantly higher when seed treatment was followed by a foliar application.

Pulses

P. fluorescens strain RPB14 showed potential biological control activity against major diseases of *Phaseolus vulgaris* cv. Baspa both *in vitro* and under field conditions (Himachal Pradesh, India). Two foliar applications of RPB14 and a single spray of carbendazim (0.05%) were best in protecting from both diseases by 59.6% (floury leaf spot) and 51% (fuscous blight) and in increasing the seed yield by 81.3% over the control (Mondal 2004). *Pseudomonas fluorescens* strain Pf1, effectively inhibited the mycelial growth of *Macrophomira phaseolina*, the pathogen causing dry root rot in Black gram cv.Co5 application of strain Pf1 (10 g/kg seed) followed by soil application (2.5 kg/ha) against root rot effectively supported higher plant growth, better *Rhizobium* nodulation and grain yield (Jayashree et al. 2000). Field trials in *Vigna mungo* to control root rot disease complex caused by *Macrophomina phaseolina* and cyst nematode, *Heterodera cajani* was carried out at Coimbatore. *Pseudomonas fluorescens* was applied as seed treatment (2 g/kg seed) and the results showed less root rot incidence and nematode population along with increased pod yield (Latha et al. 2000). Siddiqui et al. (1998) used *P. fluorescens* alone or in combination with pesticides to control wilt disease complex of Pigeon pea, *H. cajani*. *Pseudomonas fluorescens* alone increased plant growth, nodulation, phosphorus content and decreased nematode multiplication and wilting in infected plants. Shaid Ahamad et al. (2000) conducted field trials at Kanpur, India for controlling root rot disease (*R. bataticola*) in Chick pea cv. C235 using bacterial antagonist *P. fluorescens* at 500 g/ha. It gave some control compared to untreated control when given as soil inoculation plus seed treatment.

In chickpea *P. fluorescens* (PDBCAB 2) treated plots exhibited low root-rot (4.4%). However, at day 60 lowest root-rot incidence (5%) was recorded in strain PDBCAB 2 treated plots and highest root-rot incidence (13.9%) was observed in control (Rangeshwaran et al. 2001). Plant stand and fresh weight of navy beans (*Phaseolus vulgaris*) were survived well on the *P. fluorescens* treated seeds for more than 10 weeks in storage (Tu & Zheng 1997).

Fruits

In grape vine cv. Muscut Hamburg, a talc formulation of *P. fluorescens* (15×10^8 cfu) was applied around 15 cm soil to root knot infested vines. Depth is the basic one at the time of pruning in July, 1996 at Coimatore, India. They found all three levels significantly reduced the severity of root knot infection in roots. Yield of grape increased under *P. fluorescens* treatment which ranged from 45% at dosage 1g/vine to 166% at 4g/vine (Shanthi et al. 1998). In sweet orange (*Citrus sinensis*) and lemon (*C. limon*), application of talc-based formulation of *P. fluorescens* strain Pf1 40 g/tree, retarded multiplication of *T. semipenetrans* significantly in field trials at Bhavanisagar, Tamilnadu. Combining this treatment with carbofuron 2g ai./tree further enhanced the suppression of the nematode. The recovery in the declining trees was also accompanied by a significant decrease in the nematode parasites in roots (Shanthi et al. 1999). Kucheryava et al. (1999), proposed five strains of *P. fluorescens* as biocontrol agents for the control of *Venturian equalis*, the causal agent of apple scab disease in apple at North Germany. They isolated and characterized epiphytic bacteria from phyllosphere of apple. In blueberry (Silva et al. 2000) (*Vaccinuim corymbosum* cv. *Blue crop*), *P. fluorescens* strain Pf 5 increased leaf area and number and shoot and dry weight in pasteurized root. PRA 25 strain increased copper and phosphoras uptake.

Pseudomonas fluorescens A506 suppressed the epiphytic growth of *A. avenae* subsp. citrulli when

applied to attached watermelon blossoms 5 h prior to inoculation (Fessehaie & Walcott 2005). Application of *P. fluorescens* strains 558 significantly reduced anthracnose in mango caused by *Colletotrichum gloeosperoides* when the fruits were inoculated by the antagonist (Koomen & Jeffries 1993) in the UK. Strain Pfcp protected banana plants from wilt disease caused by *P. solanacearum* up to 50% in the greenhouse and in the field (Anuratha & Gnanamanickam 1990) at Madras, India. Wilson and Lindow (1993) concluded that strain A506 probably prevents fire blight infection of pear in the field by preventing epiphytic build-up of pathogen inoculum on pistils and by inhibiting the growth of inoculum deposited on nectarines. Whereas, strains 1-1-4 provided more than 95% crown gall disease control in peaches at China (Zhang et al. 1991). *Pseudomonas fluorescens* provided 0–60% disease control of apricot and peach cutting inoculated with *Leucostoma cinctum*, and treatment of cutting with the bacteria before inoculation provided better control (Rozsnyay et al. 1992).

Vegetables

Keinath et al. (2000) isolated 13 bacterial strains from field in the southern USA having damping off history. The seeds of snap bean were treated in bulk with *P. fluorescns* C 200 and BD4-13. Analysis of variance of percent plant stand at 28 day after sowing revealed highly significant ($p < 0.01$) effects of location and treatment in 1996, 1997 and 1998. Data collected for 2 years indicated that no biological seed treatment significantly affected plant stand. In *Phaseolus vulgaris*, seed-borne *Colletotrichum lindmuthianum* was controlled by *P. fluorescens* but it was only next to *T. viride* in efficacy (Ravi et al. 1999). Naseby et al. (2001) assessed four strains of *P. fluorescens* against *P. ultimum* in pea and found the disease incidence to reduce, seed bactrerialization of pea by *P. fluorescens* in combination with a aerial spray of their cell suspensions or neemazol (product of neem) at different concentration were tried under field conditions at UP, India. The spray combination increased dry weight of aerial parts, number of nodes and pods and seed weight of pea. Colonization of rhizosphere was fairly good in strain Pf 5 (Singh et al. 2000).

Khan and Akram (2000) achieved enhancement of plant growth and yield in tomato from nematode- (*M. incognita*) and fungus- (*F. oxysporum*) infected plants by treating them with *P. fluorescens* in field trials at Aligarh, India. Thiribhuvanamala et al. (1999) got significantly reduced mycelial growth and sclerotial production of *Sclerotium rolfsii* (*in vitro*), the causal agent of stem rot in tomato. Park Chang Seuk et al. (2001) confirmed that the root dipping of tomato seedlings in strain B16 cell suspension (109 cfu/ml) when transplanting was sufficient to suppress bacterial wilt till fruit bearing. *P. fluorescens* was found effective in reducing (54.30%) the fruit rot disease of tomato (Hegde & Anahosur 2001). Dekkers et al. (2000) found *P. fluorescens* WCS 365 to be an efficient root colonizer when tomato seeds were inoculate with this strain to control root rot, *F. oxysporum* f. sp. *rakius-lycopersici*. Hultberg et al. (2000) found *P. fluorescen* Pf 5-014 and its mutant strain 5-214 to significantly reduce fungal population (damping off by *P. ultimum*) in *in vitro* cultures containing both high and low inoculums. Spraying tomato cv. PKM-1 with *P. fluorescens,* 48 h after inoculation with *A. solani* reduced leaf blight in tomato by 15–38% (Whistler et al. 2000). Hanna et al. (1999) carried out bioassay and greenhouse tests to control *M. incognita* in tomato. When broth culture (150 ml) was adjusted to 10^8 c.f.u/ml *P. fluorescens* showed the most nematicidal activity against hatched juveniles and adults of *M. incognita*. They found that increased concentrations (10^5 log and 5×10^8 cfu/ml.) increased mortality. Tomato cv. Solan Gola is susceptible to wilt (*R. solanacearum*); least wilt incidence (27.77 and 22.22%) was observed when $NaNO_3$ was combined with *P. fluorescens* during 1998 and 1999, respectively, and the wilt population was reduced, when compared to the control (Pradeep Kumar et al. 2003). In Algeria (Botero Ospina & Aranzazu Hernandez 2000) got significant antagonism against *F. oxysporum* f. sp. *lycopersici* in tomato by *P. fluorescens*, in pot culture trials (Manoranjitham et al. 2000a), and it also decreased disease population of *P. appomidermatum*, the causal agent for damping off on tomato. It also increased shoot, root length and dry weight of seedling (Manoranjitham & Prakasam 1999).

Two strains of *P. fluorescens*, B1 and B2, were evaluated in a growth chamber and in the field against *R. solani* in potato and in lettuce. The greatest disease suppression effect on potato was achieved by strain B1 (37%), followed by B2 (33%), whereas the marketable tuber yield increased up to 12% (B1) and 6% (B2) (Grosch et al. 2005).In potato cv. Russet Burbank inoculated with *P. fluorescens* bv V and bv I, in the first year, *P. fluorescens* S22: T: 04 at 1×10^8 cfu/ml decreased dry root rot caused by *G. pulicaris* by 19%. In the second year, *P. fluorescens* P.22:Y:05 at 4×10^8 cfu/ml reduced severity of disease by 25% (Schisler et al. 2000). Manoranjitham et al. (2000b) treated chili seeds with *T. viridae* (4g kg^{-1}) + *P. fluorescens* (5 g kg^{-1}) and found 7.0 and 12.5% pre- and post-damping off respectively as against 27.5% and 54.75% in control. There increased root, shoot length and dry weight of chili seedling and decreased the population of *P. apnanidermatum* from 16.75×10^2 cfu g^{-1} at the time of sowing to 13.41×10^2 cfu g^{-1} at 20 days after sowing compared to 17.5×10^2 cfu^{-1} and 17.08×10^2 cfu^{-1} in control. Manoranjitham and Prakasam (2000) continuing their studies, found

that seed treatment of chili with talc-based formulation of *P. fluorescens* effectively reduced damping off and the combination with *T. viridaae* at 5 g/kg recorded 31.65, 66.6 and 37.58% increase in root, shoot length and dry matter production over control respectively. Sharifi-Tehrani and Omati (1999) used two strains of *P. fluorescens in vitro* and *in vivo* to control damping off in bell pepper. They inoculated 12.5 mg of fungal inoculum and about 10^9 cfu of bacteria (*P. fluorescens*)/g of soil prior to filling pots and transplanting of seedlings. When results were analysed 30 days after transplanting it was found that *P. fluorescens* strains provided some protection but the level of protection was less than those provided by *B. subtilis*.

Rajappan and Ramaraj (1999), working on cauliflower to control wilt disease caused by *F. moniliformae*, found that soil application of talc-based formulation of *P. fluorescens* effectively controlled the disease under field conditions. Control of Pythium root rot in cucumber was done in hydrponic systems by Zheng et al. (2000) where, *P. fluorescens* reduced mucilage and root discoloration more effectively than *P. chlororaphis* but did not significantly promote marketable yield (on a fresh mass basis). Brovko and Brovko (2000) in Russia, estimated cucumber yield loss ranging from 10–15% to 91–95% due to *Fusarium* root rot caused by *Fusarium* species. Treatment with Rhizoplan (*P. fluorescens*) diluted to 1:50 with water, improved disease resistance and increased yield by 8.5–16.2%.

When sugar beet seeds were inoculated with *P. fluorescens* DR54 in soil microorganisms, an improvement in plant emergence was found after 7 days. Strain DR54 reduced mycelial density, oospore formation and intracellular activity of *P. ultimum* (Thrane et al. 2000). In lentil (*Lens culinaris*) var. PDL 2 and Sehore 74-3, field experiments were conducted to control lentil wilt (*F. oxysporum* f.sp. *lintis*) during 1995–1996 and 1996–1997 (De & Chaudhary 1999). *Pseudomonas fluorescens*+*T. viridae* and carboxin reduced wilt disease by 65% and increased yield by 229%. In commercial greenhouse trials, *P. fluorescens* strain WCS 374 suppressed *Fusarium* wilt and increased radish yield (Leeman et al. 1991). Two isolates of *P. fluorescens* (PF1 and PF2) inhibited *P. solanacearum*, which causes bacterial wilt of potato *in vitro* (Shekhawat et al. 1993). PF2 failed to reduce wilt incidence. Stanlit et al. (1994) found that the post harvest rooting of Dutch white cabbage was reduced by post-harvest treatment with *P. fluorescens* strains CL 42, CL 66, CL 82 in three storage trials carried out in an experimental cold store. *Pseudomonas fluorescens* gave a consistently high level of control of damping off (*Pythium debaryanum* and *P. ultimum*) in beet root at both 2 and 4 weeks (Dodd & Stewart 1992) at New Zealand. And it is found effective against the five pathogenic seed-borne fungi of okra and increased the percentage of germination, seedling vigour and reduced seedling mortality (Gurjar et al. 2004). In peas (*Pisum sativum*), *P. fluorescens* used without captan was very effective, increasing both emergence and yield of 33% when compared to captan alone (Parke et al. 1991).

Flowers

Carnation plants grown in hydroponics solution inoculated with *P. fluorescens* showed a significant reduction in the number of plants infected with *F. oxysporum*, whereas strain M24 (Collected from 15-year-old avocado roots at Queensland) gave significant protection of roots of *Jacaranda acutifolia* from infection by *Phytophthora cinnamomi* when the fungal inoculum level was 0.0016 g colonized bran/sand per gram (Sorokina et al. 1999).

Oil crops

As biological control agent, *P. fluorescens* have been shown to have beneficial effect on plant growth and health. Shanmugam et al. (2002) conducted a study to test the effect of *P. fluorescens* Pf 1 on co-inoculation in peanut [groundnut] to control root rot, a severe soil-borne disease caused by *Macrophomina phaseolina*. Strain Pf 1 resulted in significantly inhibition of *M. phaseolina* mycelial growth under *in vitro* conditions. Leaf shoot disease caused in groundnut by *Cercosporidium personatum* was reduced when the groundnut seeds were treated with *P. fluorescens* Pf 1, along with a foliar spray at seedling stage. The talc-based powder formulation effectively controlled the disease under field condition in Madurai in 1999. The pod yield increased and maximum disease protection was observed 30 days after sowing by seed treatment with *P. fluorescens* (Meena et al. 2000a). Meena et al. (2000b) continuing their studies on groundnut found that foliar application of *P. fluorescens* strain Pf1 significantly controlled late leaf spot and rust (*Puccinia arachis*) in greenhouse conditions. An increase in activity of phenyl alanine ammonia lyase one day after application of bacterial antagonist was seen, and maximum activity was observed 3 days after treatment. Vanitha (1998) used 6 carriers (farm yard manure, gobar gas effluent, peat soil, groundnut shell, municipal compost and coconut coir pith compost) for soil application of *P. fluorescens* against groundnut collar rot incidence. The lowest disease incidence (23.33%) was found in peat soil inoculums followed by farm yard manure and goober gas effluent inocula both at 30% as against 85% in control. In Dharwad (Patil et al. 1998), *P. fluorescens* strain FDP-15, isolated from groundnut roots was most efficient and ecologically fit strain. This strain improved seed germination, nodulation, dry weight and pod yield as well as protected plants from sclerotial infection compared with captan. FDP15

increased seedling emergence by 16%, nodulation 18%, dry weight 40%, total pod yield 65% and resulted in 18% greater survival of plants up to harvest.

In safflower (Prashanthi et al. 2000), *P. fluorescens* strain Pf1 produced the highest inhibition zone of *Macrophomina phaseolina*, the causal agent for root rot of safflower, in sesame cv. TMV6, and strain Pf1 effectively inhibited the mycelial growth of *M. phaseolina*, the pathogen causing dry root rot in sesame (Jayashree et al. 2000). In sunflower, the highest sclerotium root/collar rot disease suppression was exhibited by *P. fluorescens* strain PDCAB 2. They also found that relying on the greenhouse test was better than the laboratory test (Rangeshwaran & Prasad 2000a). A positive correlation between *in situ* tests and field trial demonstrated that significant protection of sunflower from *Sclerotini sclerotirum* could be obtained by seed bacteriarization with *P. fluorescens* which gave a satisfactory level of root colonization and biocontrol was obtained in three different soil types (Expert & Digat 1995). Hebbar et al. (1991b) found that the antagonistic bacteria (*P. fluorescens*) associated with sunflower roots and leaves when used as a seed inoculum in the presence of *C. rolfsii* increased seedling emergence by 28%. It also improved plant yield through disease suppression.

Cotton

In cotton (Mondal et al. 2000), *P. fluorescens* strains CRb 26, CRb 39, protected plants from bacterial blight caused by *X. axonotrodis* pv. *Malvacearum* (Xam R-32). CRb 26 produced four major phenolic compounds I, II, III, IV. Compounds I and IV were fluorescent. Compounds II and IV completely inhibited growth of Xam R-32 at 200 and 100 µg/ml *in vitro*. Demir et al. (1999) isolated 128 isolates of fluorescent Pseudomonads from healthy cotton seedlings and rhizosphere soils and tested against *Rhizoctonia solani*. *P. fluorescens* (Gh/R 1810) was the most effective strain resulting in 16.36% greater emergence and 57.94% greater survival of cotton seedlings. Hagedorn et al. (1990) found that application of *P. fluorescens* strain EG1053 provided larger plant stands and reduced seedling disease symptoms (caused by *P. ultimum* and *R. solani*) on surviving plants of cotton in both potting mix with amended pathogens and naturally infected cotton soils.

Other crops

P. fluorescens was effective in reducing *R. solani* disease incidence of *Phyllanthus niruri* in greenhouse conditions (Ayyanar Kamalakannan et al. 2004). In forest nurseries at Haruana, India, *P. fluorescens* was more effective against damping off than *B. subtilis* (Kaushik et al. 2000). Bora et al. (2000) found *P. fluorescens* M15 to control brown plaster disease in mushroom at the rate of 86.6%. Anith et al. (2000) found seed treatment of ginger with *P. fluorescens* strain EM85 along with solarization decreased wilt (*R. solanacearum*) incidence to 7.42% and increased yield to 29.43 t/ha compared to 19.51 t/ha in control.

Pseudomonas tolassi is a major cause of mushroom spoilage in Australia. Growth of *P. tolassi* was greatly reduced by prior colonization of the mushroom beds by *P. fluorescens* (Healey & Harvey 1989). In cocoa, *P. fluorescens* isolates from the surface of healthy cocoa were antagonistic to *P. palmivera* (*in vitro* and in the field) and were more effective than copper oxide or chlorothalonil in controlling black pod (Galindo et al. 1992). *Nicotiana flutinosa* and 2 cultivars of *N. tobacum* were grown in autoclaved natural soil previously inoculated with *P. fluorescens* strain CHA0. After six weeks, all tested plants showed resistance in leaves to infection with Tobacco Necrosis Virus (TNV) to the some extent as plants previously immunized with TNV. Root colonization of tobacco plants with strain CHA0, as well as leaf infection with TNV, caused an increase in salicylic acid in leaves (Maurhofer et al. 1994).

Berg et al. (2000) found that *P. fluorescens* isolate P6 and P10 were found to be antagonistic to *Verticillium* wilt pathogen and increased the yield from 117–344% (P10) in greenhouse trials and 113–247% (P6) in field trials. *Pseudomonas fluorescens*, isolates from rhizosphere of winter rape, was antagonistic to pathogenic and saprophytic fungi on rape and flax and protected germinating plants against infections by *Phoma lingam* (*Leptosphaeria maculans*), *F. acenaceam* (*Gibberella avanacea*), respectively. The antagonistic effect of *P. fluorescens*+ nutrient medium was increased by 20% with treatment with the bacterium alone. *Leptosphaeria maculans* was suppressed more than *G. avenacea*. The bacterium was non-pathogenic to rape and flax and tolerant of all herbicides tested (Novotna 1990).

Conclusions

Environmental and consumer concerns have focused interest on the development of biological control agents as an alternative, environmentally-friendly strategy for the protection of agricultural and horticultural crops against phyllopathogens (Dunne et al. 1998). *Pseudomonas fluorescens* is one such proven biological control agent. Many success reports by several scientists around the world have described different *Pseudomonas* strains able to significantly control a number of fungal, bacterial and nematode diseases in cereals, horticultural crops, oil seeds and others. The efficacy of bacterial antagonism in controlling diseases was often better than with fungicides. However, the bacterial antagonism in combination with fungicides sometimes improved efficacy in controlling diseases. Besides disease control, treatments also improved seedling health and yields of crops. Peat soil was found to be the best

substrate followed by farmyard manure and gobar gas for colonization of *P. fluorescens*. Polysaccharides enhanced the adhesion of *P. fluorescens* S272 which promoted plant growth through increased antibiotic activity. The present review contributes to future research programmes that aim to promote *P. fluorescens* as a potential bio-pesticide for augmentative biological control of many diseases of agriculture and horticultural importance. However, a better understanding of the factors involved, the signalling interaction among antagonist, pathogen, soil and plants, are yet to be revealed to promote the biocontrol agents as wide applicable bio-pesticides in future.

Acknowledgements

The authors are grateful to Dr S. D. Shikamani, Director, Indian Institute of Horticultural Research, Bangalore and also thankful to Dr Poonam Sinha, (IIHR) Senior Scientist, for her help in literature selection.

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Symposium

The Nature and Application of Biocontrol Microbes III: *Pseudomonas* spp.

Pseudomonas Biocontrol Agents of Soilborne Pathogens: Looking Back Over 30 Years

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ABSTRACT

Weller, D. M. 2007. *Pseudomonas* biocontrol agents of soilborne pathogens: Looking back over 30 years. Phytopathology 97:250-256.

Pseudomonas spp. are ubiquitous bacteria in agricultural soils and have many traits that make them well suited as biocontrol agents of soilborne pathogens. Tremendous progress has been made in characterizing the process of root colonization by pseudomonads, the biotic and abiotic factors affecting colonization, bacterial traits and genes contributing to rhizosphere competence, and the mechanisms of pathogen suppression. This review looks back over the last 30 years of *Pseudomonas* biocontrol research and highlights key studies, strains, and findings that have had significant impact on shaping our current understanding of biological control by bacteria and the direction of future research.

Pseudomonas spp. are aerobic, gram-negative bacteria, ubiquitous in agricultural soils, and are well adapted to growing in the rhizosphere. Pseudomonads possess many traits that make them well suited as biocontrol and growth-promoting agents (135). These include the ability to (i) grow rapidly in vitro and to be mass produced; (ii) rapidly utilize seed and root exudates; (iii) colonize and multiply in the rhizosphere and spermosphere environments and in the interior of the plant; (iv) produce a wide spectrum of bioactive metabolites (i.e., antibiotics, siderophores, volatiles, and growth-promoting substances); (v) compete aggressively with other microorganisms; and (vi) adapt to environmental stresses. In addition, pseudomonads are responsible for the natural suppressiveness of some soils to soilborne pathogens (137). The major weakness of pseudomonads as biocontrol agents is their inability to produce resting spores (as do many *Bacillus* spp.), which complicates formulation of the bacteria for commercial use. The purpose of this review is to look back over the last 30 years of *Pseudomonas* biocontrol research and identify some key studies and findings that have helped to shape our current understanding of the biocontrol activity of these bacteria and the direction of future research.

CLASSIC STRAINS AND NOVEL CONCEPTS

Berkeley strains. One lineage of contemporary *Pseudomonas* biocontrol research can be traced to bacterization studies with fluorescent pseudomonads beginning in the 1970s at the University of California, Berkeley. Bacterization is the process of inoculating plant seeds, seed pieces, or roots with bacteria to enhance plant growth (54). These studies demonstrated that certain fluorescent *Pseudomonas* spp. improved the growth of potato sugar beet and radish when applied to seeds or seed pieces (108,109). Examples of strains that have provided enhanced growth include TL-3 (13), B10, A1, and E6 (58), isolated from potato tubers and roots, and SH-5, isolated from sugar beet roots (116). All of these strains were classified as *P. fluorescens-putida* types. For example, in 11 trials conducted at multiple field sites over 3 years, bacterization of potato seed pieces with TL-3 resulted in an average yield increase of 10% compared with the noninoculated control. These results were statistically significant at 6 of 11 sites (13,58). In five of nine field trials, SH-5 significantly increased the yield of sugar beet an average of 12%. Growth promotion following bacterization also was demonstrated for radish (56) and ornamental plants (145). Growth promotion in these studies apparently resulted from suppression of "minor pathogens." These studies, and many others, resulted in the following new terms, findings, and concepts.

- Rhizobacteria: plant-associated bacteria that are able to colonize and persist on roots (54).
- Plant growth-promoting rhizobacteria (PGPR): rhizobacteria that have the ability to promote the growth of plants following inoculation onto seeds or subterranean plant parts (54). Initial studies of PGPR focused primarily on fluorescent pseudomonads, but it is now known that PGPR include a diverse assemblage of bacteria representing a broad spectrum of genera.
- PGPR strains are aggressive colonists of the rhizosphere environment and they can persist for the duration of the growing season (5,58).
- PGPR can preempt the establishment of other rhizosphere microorganisms through competition for favored sites on the root and in the rhizosphere (57,116).
- Production of siderophores (e.g., pyoverdine and pseudobactin) by PGPR, which can limit the amount of iron available to pathogens for growth, was identified as a new mechanism of biological control (53). Strain B10 was used as a model organism in studies of siderophore production and the role of siderophores in biological control (58).

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DOI: 10.1094/PHYTO-97-2-0250

- Pseudomonads improve plant growth by suppressing either "major" (produce well-known root or vascular diseases with obvious symptoms) or "minor" (parasites or saprophytes that damage mainly juvenile tissue such as root hairs and tips and cortical cells) pathogens (103).

Dutch strains. A second lineage of contemporary *Pseudomonas* biocontrol research can be traced to bacterizaton studies with fluorescent pseudomonads initiated at the Phytopathologisch Laboratorium, "Willie Commelin Scholten" (WCS), Baarn, The Netherlands. Dutch researchers observed that as the frequency of potato production in a field increased, the yields decreased. Potatoes grown every third year (short potato rotation) or continuously in a field yielded 10 to 15% and 30% less, respectively, than potatoes grown in a field every sixth year (long potato rotation) (41,103,104). They showed that bacterization of seed tubers with pseudomonads such as *P. fluorescens* strains WCS374 and WCS365 and *P. putida* strain WCS358 resulted in an increase in yield in short- but not long-rotation soils (7,33–35). Deleterious rhizosphere microorganisms (DRMO), particularly hydrogen cyanide (HCN)-producing pseudomonads, were thought to be the targets of the PGPR. DRMO increased to population densities sufficient to cause damage in the short but not the long rotations, thus accounting for the influence of crop rotation on PGPR activity (103). Other major pathogens probably also contributed to the poor growth of potatoes in short rotations. Siderophore production and induced resistance were identified as the primary mechanisms of pathogen suppression by the Dutch pseudomonads (7,8,97). WCS strains (i.e., WCS374, WCS365, WCS417, and WCS358) are especially notable because they have been used extensively during the past 25 years as model organisms in studies of siderophore production and uptake, bacterial traits and genes involved in root colonization, and induced systemic resistance (ISR) (25–27,81,124,125).

Antibiotic producers. A third lineage of contemporary *Pseudomonas* biocontrol research can be traced to bacterization studies conducted at several laboratories with fluorescent pseudomonads that produce antibiotics such as phenazine-1-carboxylic acid (PCA) and other derivatives, 2,4-diacetylphloroglucinol (DAPG), pyrrolnitrin (Prn), and/or pyoluteorin (Plt). Biocontrol agents produce a wide variety of antibiotics; however, the lack of definitive experimental evidence for the role of antibiotics in the biocontrol process led to an ongoing debate over most of the last century (30,141). However, this changed, beginning in 1988, with definitive studies showing an important role for antibiotics in biocontrol mediated by pseudomonads (119).

P. fluorescens strain 2-79 and *P. chlororaphis* 30-84 (formerly *P. aureofaciens*) were isolated from wheat grown in take-all suppressive soils from fields near Lind, Washington (136) and Glen Elder, Kansas (96), respectively. Bacterization of spring or winter wheat seeds with either of these two strains resulted in significant suppression of take-all in about 60% of field trails. For example, strain 2-79 increased yields an average of 17% in experimental plots and 11% in commercial scale tests (135). Both strains produce PCA and a pyoverdine siderophore (144). In addition, 2-79 produces anthranilic acid; and 30-84 produces two other phenazines, 2-hydroxyphenazine-1-carboxylic acid (2-OH-PCA) and 2-hydroxyphenazine (2-OH-PZ) as well as HCN (96).

P. fluorescens strains CHA0, Pf-5, Q2-87, and F113 have been used as model strains in studies of the biosynthesis of DAPG, Prn, and Plt, and in studies of the role of these antibiotics in pathogen suppression. *P. fluorescens* strain CHA0 was isolated from roots of tobacco grown near Payern, Switzerland, in a soil naturally suppressive to black root rot of tobacco caused by *Thielaviopsis basicola* (115). CHA0 produces DAPG, Plt, Prn, HCN, indoleacetic acid, salicylic acid, pyochelin, a pyoverdine siderophore (pseudobactin), and other bioactive metabolites (129). Thus, this strain has one of the broadest repertoire of potential biocontrol and growth-promoting mechanisms of any PGPR described so far. CHA0 suppresses root rots of tobacco and tomato, Pythium damping-off of cucumber, and take-all of wheat (28,52,106). The contribution of each metabolite to disease suppression is dependent upon the host crop and target pathogen. For example, production of DAPG was the primary mechanism of suppression of take-all of wheat by CHA0, whereas both DAPG and HCN contributed to suppression of black root rot of tobacco (37,52,130). Plt was involved in suppression of damping-off of cress and cucumber by this bacterium (77). Here it is interesting to note that HCN production by pseudomonads provides a beneficial effect in terms of biocontrol activity. Thus, HCN is an example of a metabolite that can differentially affect plant growth depending on the producer strain, the amount of HCN accumulating in microsites in the rhizosphere, and the crop species grown.

P. fluorescens Pf-5 was isolated from the rhizosphere of cotton and is quite similar to CHA0 in that it produces DAPG, Plt, and Prn (46,47,86). Strain Pf-5 suppressed damping-off of cotton caused by *Pythium ultimum* or *Rhizoctonia solani*. Purified Prn and Plt obtained from Pf-5 provided the same protection against Rhizoctonia and Pythium damping-off, respectively, as did the bacterium. *P. fluorescens* Q2-87 was isolated from wheat roots grown in a suppressive soil from a field near Quincy, Washington. Q2-87 produces DAPG and HCN, but only DAPG contributed to its biocontrol activity against take-all (39,128). In field studies, take-all suppression by Q2-87 was greatest when it was used in combination with three other strains also isolated from the Quincy suppressive soil (92). *P. fluorescens* F113 was isolated from sugar beet in Ireland and suppressed damping-off of sugar beet caused by *Pythium ultimum* and cyst nematode and soft rot of potato (18, 19,29,111). These studies, and many others, resulted in the following novel findings and concepts.

- The research with Pf-5 by Howell and Stipanovic (46,47) sparked interest in the role of antibiotic production in *Pseudomonas* biocontrol activity.
- Studies of the suppression of take-all by *P. fluorescens* 2-79 provided the first unequivocal evidence that production of an antibiotic in situ contributed to biocontrol activity (119). This work outlined a genetic strategy known as "Molecular Koch's Postulates" that is still commonly used to determine the role of a specific metabolite in the biocontrol process: (i) mutagenesis of a biocontrol agent (e.g., transposon mutagenesis), (ii) screening for loss of the trait, (iii) genetic complementation of mutants to restore the target trait, and (iv) comparison of the biocontrol abilities of the parental strain, mutant, and complemented mutant (138).
- Studies with the phenazine producers 2-79 and 30-84 demonstrated that antibiotics can be readily isolated from the rhizosphere environment (120), providing further evidence of the importance of antibiosis in biological control. It is now common to isolate and quantify antibiotic production in the rhizosphere and spermosphere (118).
- Phenazines, DAPG, Prn, and Plt are four of the most common antibiotics produced by *Pseudomonas* biocontrol agents. *Pseudomonas* spp. that produce these antibiotics became a major focus of biocontrol research, and many genes involved in the regulation and synthesis of these compounds are now known (1,4,9,17,23,29,32,38,40,51,60,62,76,78, 85,87,91,94,95,101,106). Strains CHA0, Pf-5, 30-84, and F113 have been especially valuable in the identification and characterization of regulatory genes of metabolite production: (i) *gacS*/*gacA*—a two-component sensor-regulator pair controlling extracellular metabolites and exoenzymes (62, 140); (ii) *rsmZ*, *rsmY*, and *rsmX*—small untranslated regulatory RNAs (51) that modulate activity of translational repressors RsmA and RsmE (99,122,123); (iii) *rpoS* and *rpoN*—alternative sigma factors (101); (iv) *phzI* and *phzR*—pathway-specific regulators of phenazine biosynthesis and quorum sensing (95); (v) *phlF*—repressor of DAPG syn-

thesis (9); and (vi) *pltR*—repressor of pyoluteorin production (85).
- The complete genome of the biocontrol agent *P. fluorescens* Pf-5 has been determined (90).

ISR pseudomonads. A fourth lineage of contemporary *Pseudomonas* biocontrol research can be traced to independent demonstrations in 1991 by research groups in The Netherlands, the United States, and Sweden that some pseudomonads colonizing the roots protected plants from various pathogens by inducing systemic resistance. For example, van Peer et al. (126) reported that *Pseudomonas* strain WCS417 induced resistance in carnation against Fusarium wilt caused by *Fusarium oxysporum* f. sp. *dianthi* when the roots were inoculated with bacteria 1 week prior to stem inoculation with the pathogen. This strain was isolated from the wheat rhizosphere and also promoted the growth of several crops. Subsequently, strains WCS417 and WCS374 were shown to induce resistance in radish against *F. oxysporum* f. sp. *raphani* and other pathogens (42). The O-antigenic side chain of the lipopolysaccharide, present on the outer membrane of strains WCS374 and WCS417, appeared to be the determinant responsible for the induction of resistance in radish (63). Strain WCS374 applied as a seed treatment to radish seeds provided an average reduction in Fusarium wilt of 42% and an average yield increase of 45%. Radish seeds coated with this strain, trade name BioCoat, were sold for a short time (63). Wei et al. (131) demonstrated that *P. putida* 89B-27 and other nonpseudomonads induced resistance in cucumber leaves to anthracnose, caused by *Colletotrichum orbiculare*. Strain 89B-27 also induced resistance in cucumber against angular leaf spot, caused by *P. syringae* pv. *lachrymans* (65), and Fusarium wilt, caused by *F. oxysporum* f. sp. *cucumerinum*. This strain also induced resistance against cucumber pathogens in the field (132). Alström (2) reported ISR in bean against halo blight caused by *P. syringae* pv. *phaseolicola* by seed bacterization with *P. fluorescens* strain S97. Here, there was a correlation between reduction in symptom expression and lower population density of *P. syringae* pv. *phaseolicola* in the leaves (3). These studies are highly significant because they identified an entirely new mechanism of biological control by pseudomonads and other PGPR; ISR is now intensively studied worldwide.

ROOT COLONIZATION AND NOVEL CONCEPTS

The dynamics of colonization. The high microbial diversity, density, metabolic activity, and competition occurring in the rhizosphere environment represents a formidable "biological buffering" (137) that generally limits the establishment of introduced, foreign microorganisms into the rhizosphere. Thus, one must marvel at the ability of introduced pseudomonads and other PGPR to colonize roots and provide protection against major and minor soilborne pathogens. Several definitions of root colonization by rhizobacteria were proposed (54,55,88,109) and most included components of movement of the rhizobacteria from an inoculum source to the roots, multiplication, and persistence, all in the presence of native soil microflora. Weller and Thomashow (139) defined root colonization as the process whereby rhizobacteria introduced on seeds, vegetatively propagated plant parts, or into the soil become distributed along roots growing in raw soil, multiply, and then survive for several weeks in the presence of indigenous soil microflora. Root colonization included colonization of the rhizosphere, rhizoplane, and/or inside the root. Rhizosphere competence describes the relative root-colonizing ability of a rhizobacterium (135).

During the last 30 years, experimental systems using pseudomonads have made significant contributions to our understanding of the process of root colonization, the biotic and abiotic factors affecting colonization, and the bacterial genes and traits that contribute to rhizosphere competence. Root colonization has remained a focus of much research because of the positive relationship between colonization and pathogen suppression in many biocontrol systems. Arguably, the work of Bahme and Schroth (5) was the "gold standard" of root colonization studies. In a pair of elegant experiments conducted at Tulelake, CA (Osborn silty clay-loam) and at Bakersfield, CA (Hesperia sandy loam), they determined the spatial-temporal colonization pattern of seed piece-applied *P. fluorescens* strain A1-B at all stages of potato development and on all below-ground plant parts. The comprehensiveness and attention to details of this study were especially notable. For example, early in the growing season the authors could remove an entire root system with a spade but in order to insure that an entire root was removed later in the season, they dug trenches alongside the plants. Other notable colonization studies included the use of (i) *Pseudomonas* strains A1 and SH5 to describe the distribution of introduced pseudomonads on and among root systems (70); (ii) *P. fluorescens* PRA25 to describe the movement of rhizobacteria through soil and the effect of temperature on colonization (11,64); (iii) *P. fluorescens* 2-79 to describe the relationship between inoculum dose, colonization, and biocontrol activity, and the effect of matric potential on colonization (12,48,133); and (iv) *P. fluorescens* WCS365 to identify rhizosphere competence traits and genes (71). These studies, and many others, resulted in the following novel findings and concepts.

- Passive carriage on the root apex (48,110) and with percolating water (64,72,84,89,117,121) function in concert to move rhizobacteria from inoculum sources on seeds and planting material throughout the root system and into the bulk soil (long-distance transport). Active bacterial movement (10,24,102) plays a role in colonization on a much smaller scale.
- Rhizobacteria, when applied to seeds or planting material, can become widely distributed throughout a root system (5,133,134).
- Population sizes of introduced rhizobacteria are greatest on roots and in soil nearest the inoculum source and decline with increasing distance from the source of inoculum (5,134).
- Populations of introduced rhizobacteria on roots and other underground plant parts are not normally distributed (5,70).
- Root colonization by rhizobacteria varies among fields, soil types, and crops (5).
- The method of inoculum delivery affects spatial-temporal colonization patterns of rhizobacteria on roots and underground plant parts (6).
- Population densities of introduced rhizobacteria in the rhizosphere usually are greatest soon after planting and gradually decline throughout the growing season, often dropping below the detection limit (8,48,59,70,73,79,97).

Bacterial traits and genes contributing to rhizosphere competence. During the last 25 years, studies of rhizosphere competence traits and genes have focused extensively on pseudomonads and have resulted in three major conclusions. First, rhizosphere competence is governed by many genes and traits, and in a single strain, multiple traits may be involved in the process. This should not be surprising because root colonization is a multistage process. Secondly, the contribution of a given trait or gene to rhizosphere competence may be strain-specific. Finally, the relative importance of a trait or gene is affected by the plant species, soil type, environmental conditions, and the type of assay used to study the trait. The following are traits or genes that have been shown to contribute to rhizosphere competence in at least one rhizobacterium.

- Ability to compete for or produce limiting resources including the following: vitamins (biotin, thiamine) (114), amino acids (16,71,112,127), organic acids (71), sugar phosphates (67), and iron (43,45,69,82,83,98).
- Rapid growth rate (22,31,61,113).

- Cell surface structures and traits: lipopolysaccharide (22, 27,113), flagella/motility (16,22,26,71,113), chemotaxis (24), and NADH dehydrogenase (14,22).
- Ability to survive exposure to physical and chemical stresses: heat, desiccation, presence of reactive oxygen species, high osmolarity, low matric potential, and bacteriostatic levels of putrescine (36,49,50,61,68,74,101,107).
- Global regulators facilitating responses to environmental change: GacS and GacA (40,75,84,105), sigma factor (75,107,140), and quorum sensing (interpopulation and intrapopulation signaling) (15,66,93,142,143,146).
- Ability to create a phenotypically diverse population: phase variation/site-specific recombinase (16,20,21,44,100).
- Production of phenazine antibiotics (80).

FUTURE PROSPECTS AND DIRECTIONS

Tremendous progress has been made over the past 30 years in understanding the process of root colonization by pseudomonads and in characterizing the biotic and abiotic factors affecting colonization, the genes contributing to rhizosphere competence, and the diverse mechanisms by which pseudomonads suppress soilborne pathogens. This wealth of knowledge has provided a firm foundation for *Pseudomonas* research in the 21st century that must now be applied to advance broader incorporation of these bacteria into sustainable strategies for the management of soilborne pathogens. In the short term, the technology already exists to directly identify biocontrol agents active against target pathogens, to select strains with an affinity for particular crops or cultivars, to engineer strains for greater efficacy and reliability, and to develop and exploit soils naturally suppressive to particular pathogens. New insights are certain to be gained from the recently published genomic sequence of *P. fluorescens* Pf-5, which already has revealed biosynthetic potential for many previously undetected compounds likely to contribute to the broad antifungal activity of this strain (90). Perhaps the greatest remaining challenge facing *Pseudomonas* biocontrol research is the development of new formulations. Even here, progress has resulted from recognition of the impact of the production process on the quality of biocontrol products, and high-throughput methods have been developed to identify factors that affect efficacy and shelf life. In total, tremendous progress has made over the last 30 years, which bodes well for the future of biocontrol with *Pseudomonas* spp.

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USE OF NEEM AS A BIOLOGICAL PEST CONTROL AGENT

FACT 98-01, January 1998
A quick guide to multipurpose trees from around the world

The neem tree (*Azadirachta indica*) has been introduced and established throughout the tropics and sub-tropics for its highly valued hardiness, its almost year-round shade, and its multiple wood and non-wood products. Agroforesters have promoted it for use in windbreaks, fuelwood plantations, and silvo-pastoral systems, especially for dry zones and infertile, rocky, sandy or shallow soils. People have long recognized that the leaves, bark, wood and fruit of the neem tree either repel or otherwise discourage insect pests, and they incorporated these plant parts into traditional soil preparation, grain storage, and animal husbandry practices. Through more recent chemical analysis the active compounds in neem tissues have been identified. Several neem-based biological pest control (BPC) products have been developed and approved for commercial distribution in some countries. The neem tree can provide an inexpensive integrated pest management (IPM) resource for farmers, the raw material for small rural enterprises, or the development of neem-based industries.

Neem's active ingredients and their impact on pests
Azadirachtin has been identified as neem's principal active compound. It acts on insects by repelling them, by inhibiting feeding, and by disrupting their growth, metamorphosis and reproduction. Neem-based formulations do not usually kill insects directly, but they can alter their behavior in significant ways to reduce pest damage to crops, and reduce their reproductive potential. Azadirachtin affects insect physiology by mimicking a natural hormone. It has been shown to affect egg production and hatching rates. In larvae, azadirachtin can inhibit molting, preventing them from developing into pupae.

Many foliage feeding species will avoid plants treated with neem compounds or will cease eating after ingesting them (NRC, 1992). Its has proven effective as an antifeedant on about 100 insect species (Read & French, 1993). Thus, the extracts work especially well to protect plants from defoliation without affecting beneficial pollinating insects like honeybees.

Overall tests of neem extracts have shown results on about 300 insect species, mostly in the Orthoptera (grasshoppers, katydids, etc.); Homoptera (aphids, leafhoppers, etc.); Dictyoptera (cockroaches and mantids); Lepidoptera (moths and butterflies); Heteroptera (true bugs); Diptera (flies); Coleoptera (beetles and weevils); Hymenoptera (bees, wasps and ants); Isoptera (termites); Thysanoptera (thrips), and Siphonaptera (flea) orders (NRC, 1992; Randhawa and Parmar, 1993).

Even crudely produced neem extracts can provide excellent control of caterpillars and beetle larvae, and are effective on grasshoppers, leaf miners, and leaf and plant-hoppers. Commercially produced neem preparations can suppress a broad range of pests including insects, centipedes, millipedes, mites, and nematodes.

Traditional uses of neem
Farmers have traditionally used various components of the neem tree such as oil extracted from the seed, neem cake, (the residue left after pressing the oil) and the leaves as well as the wood. Farmers in India use neem cake as an organic manure and soil amendment. It is believed to enhance the efficiency of nitrogen fertilizers by reducing the rate of nitrification and to inhibit soil pests including nematodes, fungi, and insects (Gupta, 1993). Neem leaves and small twigs are also used as mulch and green manure.

Neem leaves and neem oil have also been used traditionally to protect stored grains and legumes. Neem leaves are mixed with the grain in storage or the grain is stored in jute bags treated with neem oil or other neem extracts. These methods can protect food and seed stores from insect pests for several months.

Another traditional agricultural practice involves the production of "neem tea." The seeds are dried, crushed and soaked in water overnight to produce a liquid

the genetic source of the seeds. It can also be affected by the process of handling and drying the seeds, contaminants in the water, and exposure to high temperatures or sunlight. The active compounds break down quickly, so an application of neem tea can generally provide protection for only about one week.

Neem is a species of the Mahogany family, and although it has some of the characteristics of a cabinetry wood, its grain is rough and does not polish well. Neem wood is, nevertheless, used to make wardrobes, book cases and closets, as well as packing cases because the wood helps to protect the contents from insect damage (Read & French, 1993). The main stem of the tree is also widely used to make posts for construction or fencing, because the wood is termite resistant.

Farm-level production and use of neem extracts
Farmers with ready access to seed producing neem trees can prepare their own "neem tea" using simple procedures to extract the active compounds. Ripe seeds should be collected from the trees, and the seeds should be depulped, washed clean and dried as soon as possible after harvesting. Seeds should be dried in the shade for 3-7 days. Seeds should be checked, and any that have been contaminated by mold or fungus should be rejected. The dried seeds are then finely crushed in a mortar or mill. About 500g crushed seeds should be mixed with 10 liters water and the mixture should be left to sit overnight. The next day the mixture should be filtered through fine cloth or gauze. It is then ready to be applied directly to crops using a spraying, brush or swab technique. The mixture should not be applied more than once a week, and treatments every 10-15 days is usually adequate for control of normal pest problems. Unused extract should be carefully stored in a closed container in a cool dark protected area (GTZ, n.d.).

Neem extracts can be made from leaves and other tissues, but the seeds contain the highest concentrations of azadirachtin. Industrial scale extraction processes use solvents such as alcohol, ether, and hydrocarbons instead of water. Some sources claim that the waterbased extracts work nearly as well, although using the method described above it is difficult to determine the concentration and therefore the appropriate amount to be applied. In Pakistan a process of freeze drying the water-based neem extract produces a crystalline powder called "neem bitters" that is water soluble (Read & French, 1993).

Small-scale processing for use in rural enterprises
Although efforts have been undertaken by NGOs to promote neem-based micro-enterprises in rural areas to increase employment opportunities, few have succeeded. Challenges they have faced include difficulty in producing uniform concentrations; problems with packaging, storage, and transportation; and lack of information about potential markets. These are common constraints to the development of small agro-enterprises and probably can be overcome. Neem-based enterprises have special potential where it is possible to reach producers who have a market for organic produce and in areas where commercially distributed pesticides are unavailable or too expensive for the average farmer to afford.

Commercial uses of neem
Neem oil has long been produced in Asia on an industrial scale in soaps, cosmetics, and pharmaceuticals. During the 1980s companies began commercial production and distribution of pest control formulations that use azadirachtin as the principal active ingredient. Interest in BPC agents has developed along with the environmental and consumer rights movements, and the recognition that IPM strategies are needed to sustain agricultural production. New markets for organically grown produce and "natural" products also spurred the development of the BPC industry, and azadirachtin was among the first to be commercialized.

In the United States neem-based BPCs were first approved for use on non-food crops in 1985. After subsequent testing, the Environmental Protection Agency (EPA) regulated the use of Dihydroazadirachtin (DAZA), a reduced derivative of azadirachtin, for use on food crops. In 1996 the EPA exempted raw agricultural commodities from meeting DAZA residue requirements, as long as the chemical is applied as an insect growth regulator or antifeedant at no more than 20gms/acre with a maximum of seven applications per growing season (EPA, 1997). The EPA only allows this exemption if approved commercial products are used; food products treated with home-made extracts would not meet these requirements.

Environmental issues
Neem compounds do not persist or accumulate in the environment after being applied as pesticides. They break down quickly when exposed to sunlight, usually within one week. Commercial preparations contain sunscreens which maintain their effectiveness for 2-3 weeks. Neem extracts may have toxic effects on fish, other aquatic wildlife, and some beneficial insects. Therefore, care should be taken in disposing of any unused extracts, by exposing them to heat or sunlight to break down the active compounds.

Neem fruits are an important source of food for some wildlife, especially birds and bats, although they only digest the pulp, not the seed. Neem compounds have been judged to be relatively non-toxic to mammals. Azadirachtin is so effective against insects, because it imitates a naturally occurring hormone that disrupts insect life cycles, however, this hormone does not occur in vertebrates. The United States EPA has concluded that if approved procedures for its application are followed that, "no unreasonable adverse effects to human health are expected from the use of DAZA" and "there is a reasonable certainty of no harm from dietary exposure" (EPA, 1997). Neem oil has been used traditionally as a topical treatment for skin symptoms in both humans and livestock, but it should not be injested orally.

Issues for research
Researchers believe that even modest efforts at genetic improvement could result in higher seed yields, higher levels of azadirachtin, and other useful compounds in the seed. Management of neem plantations for BPC production will require research to determine appropriate silvicultural practices, such as tree spacing, pruning or lopping to promote seed production, as well as possibilities for intercropping within the plantations. More research is also needed on the other bioactive compounds found in neem and on how they interact to repel or deter insect predators. Some of these also have shown anti-fungal and anti-viral properties. Farmers need better methods for preparing neem extracts to ensure uniform concentrations and quality. They also need better information about how to apply the extracts to maximum effect on different insect species at different life cycle stages. Long-term environmental impacts of the use of neem-based BPCs should be monitored and assessed.

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Written by Carol Stoney, Program Officer and Erin Hughes, Program Associate
Winrock International

A publication of the Forest, Farm, and Community Tree Network (FACT Net)

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frontiers in Plant Science

REVIEW
published: 08 May 2017
doi: 10.3389/fpls.2017.00610

Progress on *Azadirachta indica* Based Biopesticides in Replacing Synthetic Toxic Pesticides

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Over the years, extensive use of commercially available synthetic pesticides against phytophagous insects has led to their bioaccumulation in the environment causing increased resistance and reduction in soil biodiversity. Further, 90% of the applied pesticides enter the various environmental resources as a result of run-off, exposing the farmers as well as consumers of the agricultural produce to severe health issues. Therefore, growing attention has been given toward the development of alternate environmentally friendly pesticides/insecticides that would aid an efficient pest management system and also prevent chronic exposures leading to diseases. One such strategy is, the use of neem plant's (Binomial name: *Azadirachta indica*) active ingredients which exhibit agro-medicinal properties conferring insecticidal as well as immunomodulatory and anti-cancer properties. The most prominent constituent of neem is azadirachtin, which has been established as a pivotal insecticidal ingredient. It acts as an antifeedant, repellent, and repugnant agent and induces sterility in insects by preventing oviposition and interrupting sperm production in males. This review discusses, key neem pesticidal components, their active functional ingredients along with recent strategies on employing nanocarriers, to provide controlled release of the active ingredients and to improve their stability and sustainability.

Keywords: azadirachtin, pesticides, biopesticide, *Azadirachta indica*, agro-medicinal components, nanocarriers, sustained delivery

OPEN ACCESS

Edited by:
David W. M. Leung, University of Canterbury, New Zealand

Reviewed by:
Taras P. Pasternak, Albert Ludwig University of Freiburg, Germany
Hossein Alizadeh, Lincoln University, New Zealand

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Specialty section:
This article was submitted to Plant Biotechnology, a section of the journal Frontiers in Plant Science

Received: *25 August 2016*
Accepted: *04 April 2017*
Published: *08 May 2017*

Citation:
Chaudhary S, Kanwar RK, Sehgal A, Cahill DM, Barrow CJ, Sehgal R and Kanwar JR (2017) Progress on Azadirachta indica Based Biopesticides in Replacing Synthetic Toxic Pesticides. Front. Plant Sci. 8:610. doi: 10.3389/fpls.2017.00610

INTRODUCTION

Pesticides are chemical substances used in agricultural practices to aid the production and yield by repelling, preventing, and destroying pests (Kumar et al., 2012). However, over the years, continuous application of synthetic pesticides in agriculture has caused accumulation of pesticidal residues in the environment leading to various chronic illnesses (Bag, 2000). According to a report by the United Nations Environment Programme (UNEP) and the World Health Organization

Abbreviations: UNEP, United Nations Environment Programme; WHO, World Health Organization; NLGP, Neem leaf glycoprotein; EC, Ehrlich carcinoma; CD, Cluster of differentiation; NK, Natural killer; IFNγ, Interferon gamma; TNFα, Tumor necrosis factor alpha; SarAg, Sarcoma antigen; PCL, Poly (ε-caprolactone); DEG, Differentially expressed genes.

(WHO), pesticides are responsible for poisoning around three million people and causing ∼200,000 deaths each year, worldwide. Such cases are reported more in developing countries (95%) than in developed countries (World Health Organisation, 1990; Yadav et al., 2015). On the basis of the types of pest controlled, pesticides are divided into subcategories including insecticides, fungicides, herbicides, rodenticides, pediculicides, and biocides (Gilden et al., 2010). Most of these pesticides are stable compounds with long half-lives ranging from a few weeks to years due to their persistence in soil and water sources (**Table 1**), and they also enter the food chain leading to increased health risks (Pimentel et al., 1992). Pesticide exposure can occur via various means, such as inhalation of aerosols or droplets of pesticides smaller than 5 μm in diameter, which can be absorbed physiologically through the respiratory system. Dermal contact can also lead to exposure and poisoning, through the consumption of directly contaminated food or through food coming in contact with contaminated hands that can lead to pesticide poisoning (Yadav et al., 2015). Further, they can cross the placenta that can cause structural and functional defects to the fetus or in some cases death (Woodruff et al., 2008).

Most of the highly toxic pesticides are readily metabolized and eliminated by the body, however, acute short term exposure can lead to their accumulation. The active ingredients, carriers, solvents, and emulsifiers present in pesticides can cause severe side-effects (World Health Organisation, 1990). The severity of the effects of exposure is dependent on various factors such as the intake dose, route of exposure, pesticide absorption in the body, their accumulation efficacy and persistence. In most cases, metabolism of pesticides in the body makes them water-soluble, so that the body can readily excrete them. However, sometimes metabolism can increase the toxicity, for example the metabolism of carbosulfan and furathiocarb produces carbofuran which is more toxic than the native form of the pesticide. Furthermore, some fat-soluble substances are not metabolized by the body and get stored in the fatty tissues leading to their accumulation. They become even more concentrated while passing through the food chain (Ntow et al., 2008). Such cases cause various toxic effects including, skin sensitization, allergic reaction rashes, neurotoxicity, carcinogenic, reproductive, and endocrine defects, cataract formation and defects in the immune system (Alavanja et al., 2004; Owens et al., 2010). Among these, the carcinogenicity of pesticides have been well documented, and there are many reports that have linked synthetic pesticides to various types of cancers with exposure to various insecticides, herbicides and fungicides (**Table 1**).

Moreover, the use of synthetic pesticides has led to disturbances in the environment, causing pest resistance and toxicity to non-target organisms. In some cases, these synthetic pesticides have caused acute and chronic poisoning to farmworkers, applicators and even consumers, thus making it imperative to adopt alternative means. One of the significant alternative strategies is employing botanical pesticides, which is the most efficient means to replace the wide use of synthetic pesticides. Among these, plant based biopesticides using plant extracts and oils have proved to be the most efficient way of insect control. These herbal pesticides aid the agricultural yield (**Table 2**), as they can be used as insecticides, fumigants, manures, urea coating agent or soil conditioners. They can be used alone, or in combination with other herbs, so as to increase the insecticidal efficacy.

Among these herbs, Neem (*Azadirachta indica*) belonging to the Meliaceae family has emerged as a highly potent bio-pesticide (**Figure 1**). This evergreen, fast-growing plant known as the Indian lilac (Schmutterer, 1990) offers immense antifeedant properties due to its efficacy in suppressing the feeding sensation in insects, at concentrations even less than 1 parts per million (Isman et al., 1991). It is a draft resistant tree that thrives in a sub-humid to sub-arid climate with an annual rainfall of 400–800 mm (Schmutterer, 1990). It comprises of more than 200 allelochemicals prevalent in variable concentrations in the different parts of the plant, providing a variety of pesticidal properties (Koul and Wahab, 2004). Seeds from this tree comprises of 40% of oil with azadirachtin as the major active ingredient, that is mainly responsible for the insecticidal activity of neem (Isman et al., 1991). Further, the seed cake obtained during the processing of neem oil is a vital natural fertilizer used in the common agricultural practices. Additionally, neem leaves have been employed for centuries against the stored grain pests due to its repellent properties (Koul et al., 1990). Collectively, all parts of this plant are known to exhibit by-products that inherently impart an internal chemical defense making neem free from the pest attack, which can also be exploited to develop an efficient pest control strategy. Further, the functional ingredients of neem, exhibit, therapeutic significance as neem oil, bark, leaves and their purified biochemicals are documented to have anticancer (Paul et al., 2011) and antimicrobial (Raut et al., 2014) properties. Neem leaf extract possesses anti-inflammatory properties (Kumar et al., 2015), while neem oil acts as an antifertility agent (Kaushic, 2004). Most importantly an active ingredient of neem known as NLGP has now evolved as a potent immunomodulatory agent (Mallick et al., 2013), thus making it an ideal agro-medicinal plant (**Figure 1**). This unique attribute of neem makes it an ideal bio-pesticidal agent, as it does not cause non-specific toxicity to mammals.

BIO-PESTICIDAL ACTIVITY OF NEEM

Neem Oil

Neem oil extracted by cold-pressing the seed kernels of neem is highly effective against soft-bodied insects and mites. The presence of disulphide in neem oil is a major contributor to its bioactivity. The most significant insecticidal and therapeutic properties of this agro-medicinal neem component are illustrated in **Figure 2**. Neem oil contains more than a dozen azadirachtin analogs, but the major contributor to the insecticidal activity is azadirachtin. The remaining triterpenoids including nimbin, salannin, and their derivatives contribute little to the efficacy (Isman, 2006). Interestingly, neem oil is non-toxic to mammals, birds and fishes and exhibits fewer chances of resistance, due to its multiple mode of action on insects. Many formulations of neem seed oil exhibit antifeedant, ovicidal, larvicidal, insect growth regulatory, and repellent activity against insect pests. The larvicidal property of neem oil against mosquitoes has long been investigated.

TABLE 1 | List of commercially available synthetic pesticides, with their toxicity and carcinogenic profiles.

Name	Side effects	Type of toxicity	Half life	Solubility	Carcinogenic properties	References
Aldicarb (Insecticide)	Acutely toxic pesticide, causes excessive sweating. Salivation, vomiting, diarrhea, muscle twitching and difficulty in breathing.	Suppression of immune system, mutagenic, carcinogenic, effects on reproduction and development.	1.5 and 2 months	Highly soluble in water, also soluble in ethyl benzoate, acetone, xylene, and other organic solvents (17 mg/L of water at 25°C)	Colon cancer	Weichenthal et al., 2012
Chlorpyrifos (Insecticide)	Cholinesterase inhibition, salivation, dyspnoea, vomiting, diarrhea and exothalmia.	Nervous system damage, endocrine disruption	7–120 days	Soluble: (1.4 mg/L at 25°C)	Lung, Leukemia	Slotkin et al., 2006; Weichenthal et al., 2010
Parathion (Insecticide)	Headache, nausea, adverse effects on reproductive system	Severe poisoning can cause psychosis, unconsciousness, convulsions, cardiac arrest and coma	3–6 months	Soluble: (12.4 mg/L at 25°C)	Breast cancer	Garcia et al., 2003; Calaf and Roy, 2008
Monocrotophos	Headache, nausea, weakness, hypersalivation, blurred visions	Hazardous, accidental or intentional exposure can lead to death. Poisoning affects the central nervous system and causes loss of reflexes, involuntary muscle contractions and paralysis	7 days	Soluble: (1 kg/L, 20°C, water)	Lung cancer	Krause et al., 2013
Carbofuran (Insecticide)	Headache, nausea, sweating, chest pains, anxiety, blurred vision due to the rapid inhibition of cholinesterase activity by carbofuran	Poisoning can lead to various neurological, psychological and cognitive effects such as anxiety, depression, short-term memory loss, blurred vision	2–72 days	Slightly soluble in water (0.7 g/L of water at 25°C)	Lung cancer	Bonner et al., 2005
Endosulfan (Insecticide)	Difficulty in breathing, incoordination, vomiting, diarrhea	Chronic toxicity can lead to seizures, changes in kidney structure, blood chemistry	35–67 days	Slightly soluble (0.33 mg/l)	Breast, liver	Kumar et al., 2014
Atrazine (Herbicide)	Abdominal pain, vomiting, diarrhea, eye irritation, slowed breathing, muscle spasms, breathing difficulty	Animals with an oral dose: paralysis of limbs, respiratory distress, structural and chemical changes in lungs, liver, kidney, ovaries and growth retardation	In surface water: >200 days, Atmosphere: 14–109 days	Slightly soluble (0.030 g/liter in water at 20°C)	Colorectal cancer	Lerro et al., 2015
Paraquat (Herbicide)	Acute respiratory distress, thirst, nausea, headache, fever, muscle pain, nail damage, temporary nail loss	Leads to the production of free radicals and oxidative stress, causing cell death. Accelerates the development of Parkinson's disease. Paraquat can cross the placenta causing acute toxicity and death of the fetus	16 months to 13 years	Soluble: (700 g/L at 20°C)	Melanoma, Ovarian and Lung cancer	Park et al., 2009
Glyphosate (Herbicide)	Anorexia, vomiting, hypersalivation and diarrhea, dysphagia, gastrointestinal hemorrhage	Decreases body weight, increases incidence of cataract, lens degeneration, mutagenicity and reduces sperm count	2–197 days	Slightly soluble: (12 g/L at 25°C)	Breast cancer	Cox, 1995; Thongprakaisang et al., 2013
Carbendazim (Fungicide)	Acute toxicity is low, but direct contact can lead to discomfort in eye, skin irritant, irritation of respiratory tract, chronic bronchitis	Minor effects on cellular respiratory function, interference with the mitotic spindle proteins, no teratogenicity concern for dietary exposure	Soil: 8–32 days Water: 2–25 days	(8 mg/L water)	Prostate cancer	Tessier and Matsumura, 2001; Peyre et al., 2014
Mancozeb (Fungicide)	Cholinesterase inhibitor. Causes, headache, nausea, blurred vision, skin rash	Impairs thyroid function, and is mutagenic	Soil: 1–2 days Water: 4–8 weeks	Insoluble in water and most organic solvents	Thyroid cancer	Nordby et al., 2005

Mosquitoes are responsible for causing serious human diseases, that have led to millions of deaths per year, including malaria, dengue, and chikungunya. As a result, botanical origin insecticides are increasingly gaining interest, as they exhibit a multitude of components that minimize the chance of resistance to synthetic insecticides in mosquitoes. One such study investigated the potential of neem oil as an eco-friendly alternative for the control of malaria. Neem oil formulations at

TABLE 2 | Common herbs with active ingredients containing insecticidal properties.

Herb	Active ingredient	Agricultural: Mechanism of action	References
Plant essential oils: (Clove Eucalyptus Lemon grass Mentha species *Thymus vulgaris*)	Eugenol 1,8-cineole Citronellal Menthol Thymol and carvacrol	Fumigant and contact insecticidal property. It interferes with the neuromodulator: octapamine and GABA-gates chloride channels. (Volatile thus, limited persistence in field)	Koul et al., 2008
Tanacetum parthenium (Feverfew)	Pyrethrum	Neurotoxic: causes rapid knockdown effect, along with hyperactivity and convulsions. Pyrethrum blocks voltage-gated sodium channels in nerve axons (Half-life: 2 h)	Isman, 2006
Turmeric (*Curcuma longa*)	ar-turmerone and turmerone	Inhibitory activity on insect growth, antifeedant	Tripathi et al., 2002
Ferula asafoetida (Hing)	Asafoetida (oleo-gum-resin)	It acts as an insect repellent and consists of a characteristic unpleasant smell	Kavianpour et al., 2014
Henna (*Lawsonia inermis*)	Quinones (gives dying properties to henna)	Ethyl acetate and ethanol extracts of Henna exhibits an antifungal effect. Quinones are a source of free radicals, which are stable and complex irreversibly with the protein's nucleophilic amino acids and cause an inactivation of protein, thus exhibiting potential antimicrobial functions	Lee et al., 2001; Jeyaseelan et al., 2012
Allium sativum	Allicin (gives the pungent characteristic odor to crushed garlic)	Antifeedant, repellent, inhibitor of molting and respiration, cuticle disruption and fecundity reduction	Prowse et al., 2006
Momordica Charantia (Bitter Melon)	Crude leaf extract, bitter Momordin	Antifeedant	Devanand and Rani, 2008; Ling et al., 2008

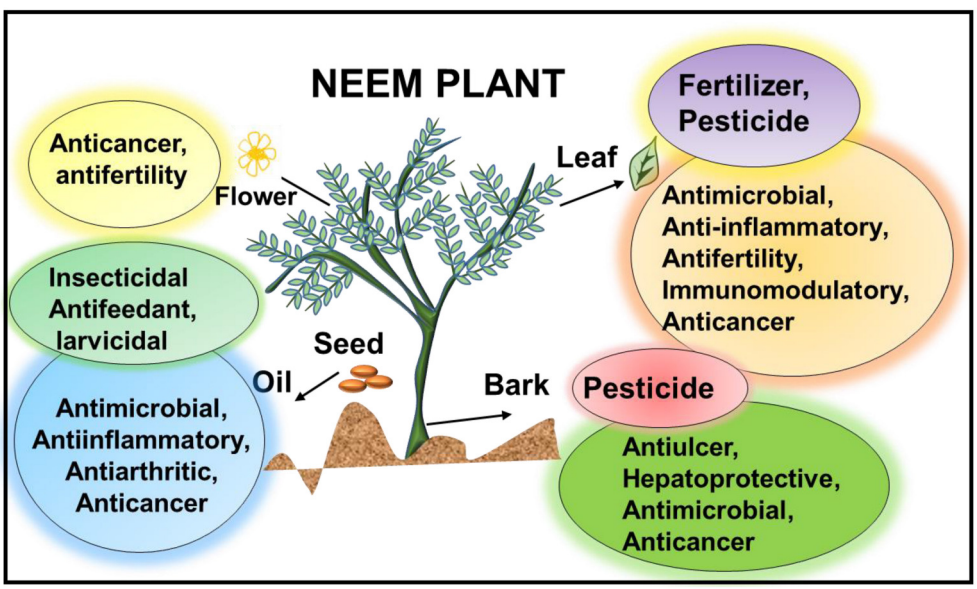

FIGURE 1 | Schematic representation of the agro-medicinal tree, *Azadirachta indica* indicating the potential of this tree as a biopesticidal and therapeutic agent. Active components of neem leaf, bark, seed, and oil have anti-inflammatory, antimicrobial, anticancer, hepatoprotective, anti-arthritic, and immunomodulatory properties. Further, oil extracted from the seeds, neem bark, and neem leaf exhibits insecticidal properties and can be used as a pesticide, herbicide, fungicide and weedicide. Neem leaf can also be used as a biofertilizer as they are capable of increasing the yield of the vermicompost.

different concentrations were evaluated against *Aedes, Anopheles,* and *Culex* mosquitoes (Dua et al., 2009). Results indicated a decrease in the mortality rate which was 98.1% reduction in *Anopheles*, a 95.5% reduction in *Culex* and a 95.1% reduction in *Aedes* on day 1, and thereafter by day 7, 100% larval control was observed. The anti-ecdysteroidal activity observed was due to the presence of azadirachtin in neem oil that kills larvae via a growth inhibition effect. Even though the neem oil formulations employed were costlier than the synthetic larvicides, neem oil was more effective for preventing pest resistance (Dua et al., 2009). Hirose and co-workers evaluated the fungitoxic effect of neem oil, along with three other biofertilizers Supermagro, E.M-4 and Multibion^{TM} against two entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana.* The study indicated a

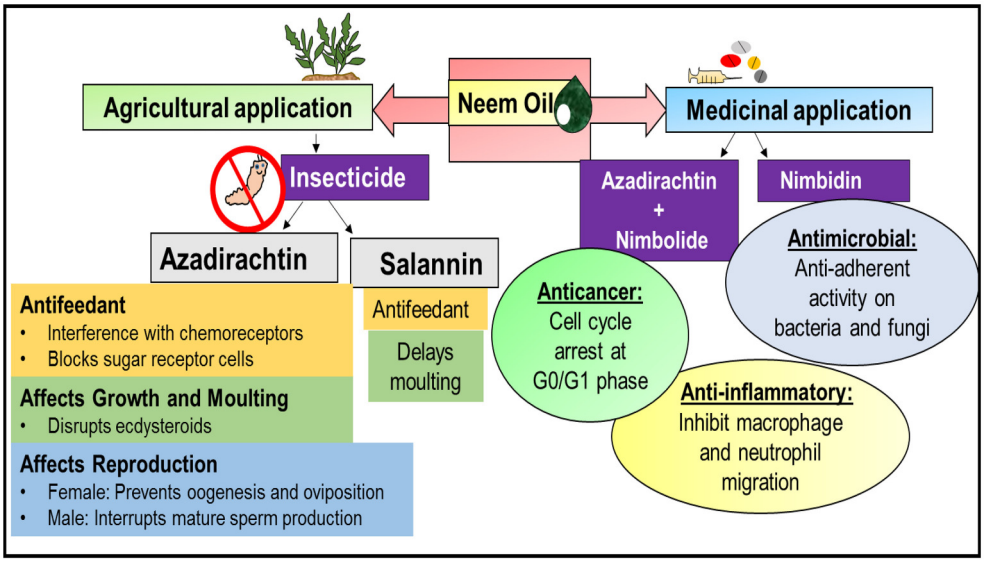

FIGURE 2 | Illustration of agro-medicinal applications of neem oil. Azadirachtin and salannin are the major components of neem oil with insecticidal properties. They both act as antifeedants and delay the process of molting in insects. Azadirachtin and nimbolide also exhibit significant medicinal properties as they act as an anticancer agent by arresting the cell cycle. Another compound, nimbin, can also be extracted from neem oil, and demonstrates anti-inflammatory and antimicrobial properties.

significant negative effect of neem oil on the germination, conidial production, and vegetative growth of the two fungi, which was more significant in Multibion^{TM} (Hirose et al., 2001). The efficacy of neem oil was evaluated in a study against *Sarcoptes scabiei* var. *cuniculi* larvae, which are ectoparasites with high possibility of causing zoonotic infections. The acaricidal activity was observed to be 100% after 4.5 h of exposure to four fractions of neem oil obtained by chloroform extraction (Du et al., 2009). However, the study lacked in analyzing the long term effect of these fractions which requires special emphasis since neem oil has a low shelf life (Immaraju, 1998; Javed et al., 2007). The role of neem oil as an insect growth regulator was further evaluated by Kraiss and Cullen (2008), on a pest of soybean *Aphis glycines* Matsumura. Direct spray of two neem formulations, neem seed oil, and azadirachtin were evaluated under controlled conditions for their efficiency in deterring the fecundity, development time and survivorship of *A. glycines and its predator Harmonia axyridis.* It was observed that both neem formulations were effective in causing nymphal mortality (80% by azadirachtin and 77% by neem oil), with a significant increase in the development time of the surviving adults. However, neither of the formulations caused any significant effect on the fecundity of the insects and the mortality rate was not immediate. Further, a non-target effect of neem treatments on the larval survival and development time of *H. axyridis* was observed, which requires further investigation (Kraiss and Cullen, 2008). A recent study conducted on *Idioscopus clypealis*, a mango pest, compared the efficacy of three synthetic pesticides, endosulfan, cypermethrin, and imidacloprid, along with environmentally friendly neem oil, against the mango hopper. Although among the three insecticides tested, imidacloprid exhibited the highest efficiency against the pest. Biopesticides based on a neem oil formulation also presented significant efficacy. Therefore, the sole dependency synthetic pesticides can be easily modified by inculcating an eco-friendly management program through incorporation of neem oil for controlling the mango hopper (Adnan et al., 2014). Along with growth deterring properties, neem oil also significantly delays reproduction in pests. It causes lethal toxicity during the pupal stage leading to various morphological deformations such as malformed adults, partial ecdysis, and molt blocking, that defers and inhibits adult formation (Boulahbel et al., 2015). However, recently it has been reported that neem oil along with its pest deterrent attributes, also causes malformations in the growth and survival of a non-target predator, *Podisus nigrispinus* which is a zoophytophagous pest commonly used in the biological control of pests. An increasing morphological deformation in the wings, legs, and scutellum along with mortality was observed with increasing concentration of neem oil. Thus, it is imperative to consider, effect of neem based pesticides on, non-target predators (Zanuncio et al., 2016).

Seed cake that is obtained during the processing of neem oil can be used as a bio-fertilizer, as it provides nutrients to the plant. It performs a dual function, as a pesticide and as a fertilizer. The seed cake produces high-quality natural manure, since neem cake compounds increase nitrogen and phosphorous content in soil which, also increases the soil fertility. Powdered seed granules are used as soil conditioners to improve the quality of soil enhances plant growth (Lokanadhan et al., 2012).

Neem Leaf

Neem leaf is a source of vermicompost with fertilizer and pesticidal properties. Adding neem leaves while vermicomposting with earthworms facilitates faster growth and reproduction of earthworms in neem-fed vermireactors.

They are capable of converting 7% of the feed into vermicompost per day thus, increasing the yield (Gajalakshmi and Abbasi, 2004). However, while using neem-fed vermireactors it is important to consider the powerful nematicidal potential of neem, which can have a detrimental effect on annelids (Akhtar, 2000). It increases the shelf life of mungbean grain by providing protective efficacy against *Callosobruchus chinensis,* pulse beetle. A neem leaf dose of 1.5 mg/100 g seed presents a significant decrease in the number of eggs laid as well as it increased the mortality in adults by 62% suggesting its potential as a bioactive anti-repellent during post-harvest grains/seeds storage (Ahmad et al., 2015). Recently, the antifeedant and repellent efficacy of neem leaves was validated in a study where enrichment of organic fertilizers with neem leaf powder and boiler ash was observed to significantly improve resistance of plants against infestation by aphids (Brotodjojo and Arbiwati, 2016).

Neem Bark

The use of neem bark as a bio-insecticide is limited, as its pesticidal efficacy is lower than the other components of the neem tree including neem seed and leaves in controlling insect pests (Sirohi and Tandon, 2014). However, it is known to possess phytotoxic properties when enriched in soil to control pest which was documented in a study, where neem bark and leaves inhibited germination and growth of various crops such as alfalfa, carrot, bean, rice, radish, and sesame along with various weeds thus, demonstrating allelopathic properties (Xuan et al., 2004). Neem bark extract based dyed fabric was recently shown to also exhibit anti-lepidopteran efficacy which was more significant in comparison to neem leaf extracts due to the presence of higher azadirachtin, cyanogenic glucosides, and nimbin content (Ahmad et al., 2015). Therapeutically, this component of neem tree is known to exhibit anti-ulcer and anti-secretory properties that are used to control gastric hypersecretion and gastroduodenal ulcers (Bandyopadhyay et al., 2004).

NEEM ACTIVE PESTICIDAL COMPONENTS

Neem parts constituting leaf, seed, bark, flower, and oil possess a multiplicity of components that are responsible for its multiple pesticidal activities.

Azadirachtin

The main component of neem oil, leaves, flowers, and fruits with insecticidal properties is Azadirachtin. It constitutes 0.1–0.3 % of neem seeds and was first isolated from *A. indica* by Morgan et al. at Keele University, England (Schmutterer, 1985). It is a complex tetranortriterpenoid limonoid with repellent and pesticidal properties. Biosynthesis of triterpenoids from *A. indica* initiates with azadirone and a C-ring opening, which culminates in Azadirachtin formation. Azadirachtin, along with other related triterpenoids such as Azadirachtin B, salannin and nimbin, are the active ingredients in neem plant based bioinsecticides and they act by disrupting the growth and development of insects and by deterring their feeding. It is considered as a botanical pesticide with exceptional growth regulating and biocidal efficacy along with deterrent effects on the ovipositing and feeding of insects (Morgan, 2009). An attempt to evaluate the exact molecular mechanism of insecticidal activity of azadirachtin on *Monochamus alternatus*, a pine sawyer beetle, has indicated enrichment of differentially expressed genes (DEGs) in 50 pathways. 920 and 9984 unique genes were found to be up and down regulated significantly. Such detailed gene profiling to assess the azadirachtin internalization with *M. alternatus*, can promote the development of efficient azadirachtin derived herbal pesticides (Lin et al., 2016).

Mechanism of Action

Azadirachtin is structurally similar to the insect hormones known as "ecdysones" which are responsible for metamorphosis in insects. The feeding behavior in insects is dependent on the neural inputs received from the chemical sensors of the insects, for example, the taste receptors in the mouthparts, tarsi and oral cavity. These sensors integrate a "sensory code" that is delivered to the central nervous system. Manifestation of antifeedancy by azadirachtin occurs through the stimulation of deterrent cells in these chemoreceptors and by blocking the feeding stimulation in insects by firing the "sugar" receptor cells (Jennifer Mordue Luntz et al., 1998).

In addition to antifeedancy, azadirachtin injection also leads to physiological effects in the insect's midgut, which causes a reduction in the post-ingestive digestive efficiency. This reduction in efficiency is known as "secondary" antifeedancy and is due to disturbances in the hormonal as well as physiological systems. These disturbances include hindrance in the food movement through the insect's midgut and inhibition in production of digestive enzymes (Schmutterer, 1985). An early study conducted by Nisbet et al. (1996) highlighted this antifeedant feature of azadirachtin. It was established that a concentration of 50–100 ppm of azadirachtin caused an insecticidal effect however, it has a high potential to harm beneficial insects as well. Therefore, a low concentration was tested which concluded that a concentration of only 5 ppm of azadirachtin can dramatically decrease the fecundity in aphids within 48 h of feeding. Further, a diet containing more than 10 ppm azadirachtin led to the production of non-viable nymphs. Thus, it can be concluded that even with a low concentration of azadirachtin, it cannot cause an immediate antifeedancy. Secondary antifeedancy effect as well as a sterilant effect can rapidly manifest themselves and aid in providing crop protection by reducing the pest population without harming non-target or natural predator populations (Nisbet et al., 1996).

Azadirachtin interferes with the growth and molting process of insects. Its ingestion leads to abnormal molts, growth reduction and increased mortalities. Azadirachtin interferes with the synthesis of an "ecdysteroid" hormone, which is responsible for the molting in insects. Indirectly, azadirachtin affects the neurosecretory system in insects by blocking the release of morphogenetic peptide hormones such as prothoracicotropic hormones that control the prothoracic glands and allatostatins, which in turn control the corpora allata (responsible for secreting juvenile hormones). Molting hormones from prothoracic glands are responsible for controlling the formation of new cuticle, and play a central role in ecdysis. The formation of juvenile

stages during each molt is controlled by the juvenile hormone from the corpora allata (Nisbet, 2000). Disruption in these events by azadirachtin, leads to various sterility and molting defects. Moreover, cellular uptake of azadirachtin inhibits both cell division as well as protein synthesis thus, causing midgut cell necrosis and flaccid paralysis of muscles (Nisbet, 2000). Neem products influence fecundity in female insects in a dose-dependent manner. Azadirachtin prevents oviposition by inhibiting oogenesis and synthesis of ovarian ecdysteroid. In males, azadirachtin acts by interrupting the meiotic process responsible for sperm production (Linton et al., 1997).

Nimbolide

Two main active ingredients; Nimbolide B and Nimbic acid B also demonstrate herbicidal activity of neem. Their allelopathic and phytotoxic activity was observed in a study where they inhibited the growth of lettuce, crabgrass, alfalfa, jungle rice, and barnyard grass. The allelopathic activity increased with an increase in the concentration of active compounds, but the intensity varied with different species of weed (Kato-Noguchi et al., 2014).

Salannin

Salannin is an active component of neem with insect-growth regulating and antifeedancy activity. Salannin deters feeding, increases the larval stage duration and causes delayed molt, leading to decreased pupal weight that results in larval and pupal mortality. This has been demonstrated in an early study on *Oxya fuscovittata* where salannin caused delayed molting and nymphal mortality (Govindachari et al., 1996). The bioactivity observed was more prominent in azadirachtin as compared to salannin, however, a combination of azadirachtin with salannin and nimbin can provide insect growth-regulating activity with increased efficacy. Biological activity of salannin has also been assessed in the tobacco armyworm *Spodoptera litura* and gram pod borer *Helicoverpa armigera*. All three components of salannin, including salannol, salannin, and 3-O-acetyl salannol, exhibited strong antifeedant activity. Nutritional assays were performed to analyse the antifeedant feature of the component and a significant reduction in the growth and development of larvae fed with the neem compounds was observed, indicating feeding deterrence in insects (Koul et al., 2004). This study also supported the use of multiple active components, as they augmented the bioactivity and confirmed that the formulations had a variety of growth inhibitory, antifeedant, and toxic effects. Contrary to this, a recent study was conducted, that attempted to increase the potent variability of salannin as an insecticide molecule. The objective was to convert salannin into two metabolites N-(2-hydroxyethyl)-a,b-unsaturated-g-lactam salanninactam and g-hydroxybutenolide salanninolideb. This conversion was enabled by transforming the C-7 furan moiety using a fungal strain *Cunninghamella echinulate* (Haldar et al., 2014). This transformation of complex natural molecules into novel metabolites can be exploited for potential benefits as it can limit the use of multiple compounds in insecticidal formulations. However, the detailed mechanisms of insecticidal action of these transformed salannins are still not known.

PROCEDURES FOR THE EXTRACTION OF NEEM FUNCTIONAL INGREDIENTS

All parts of the neem tree contain bioactive compounds, however the active ingredients of neem either have low solubility in water but complete solubility in organic solvents such as alcohols, hydrocarbons, ethers, or ketones, or are highly concentrated. Thus, they need to be extracted, which can be undertaken using the following methods (**Figure 3**).

Water Extraction

One of the simplest techniques of extraction is through crushing and grinding of kernels, followed by subsequent extraction with water. Neem seeds are usually kept overnight, and the crude suspension obtained is then filtered and used as a sprayable emulsion. However, since the active ingredients have less solubility in water, the process requires a large amount of water. One study evaluated the antifecundity effect using aqueous extracts of neem seed kernels on the development of *Bactrocera dorsalis* and *Bactrocera cucurditaei* when fed as a water source, which was then compared with pure azadirachtin. Crude extract was prepared by grinding the neem seed kernels into a fine powder, followed by subsequent extraction with distilled water using a laboratory blender. The crude extract was then filtered and dried. Results indicated that when compared to aqueous neem seed extract, pure azadirachtin had a greater effect on fertility and fecundity. The post-embryonic effect of aqueous neem seed extract was confirmed for the first time in this study, thus identifying a cheap, safe and renewable alternative to synthetic pesticides (Singh, 2003).

Hexane Extraction

Hexane extraction involves grating and steeping the neem seed kernels in the solvent hexane, resulting in the removal of oil. This oil is useful in killing the eggs of many insects such as the larvae of mosquitoes and leafhoppers that are otherwise difficult to control. Purification of azadirachtin essentially involves its enrichment through solvent extraction. Briefly, the neem seed kernels are suspended in hexane with continuous stirring and are also filtered. This concentrates the neem oil containing limonoids. This same method is applied to isolate azadirachtin from neem seeds using methanol, following which it is partitioned with hexane in order to remove any other non-polar compounds present (Sinha et al., 1999). Precipitation induced by hexane is an important pre-concentration step for the extraction and separation of neem oil limonoids. Hexane is employed because it is a very non-polar solvent. When added into neem oil, it forms a new more non-polar hexane-oil phase as compared to the oil phase, thus reducing the solubility and leading to the precipitation of the more polar limonoids from the neem oil. This method results in the formation of fine powder containing azadirachtin and other components (Melwita and Ju, 2010).

Alcohol Extraction

The most direct method for the extraction of neem constituents in concentrated form, is through alcohol extraction, as limonoids

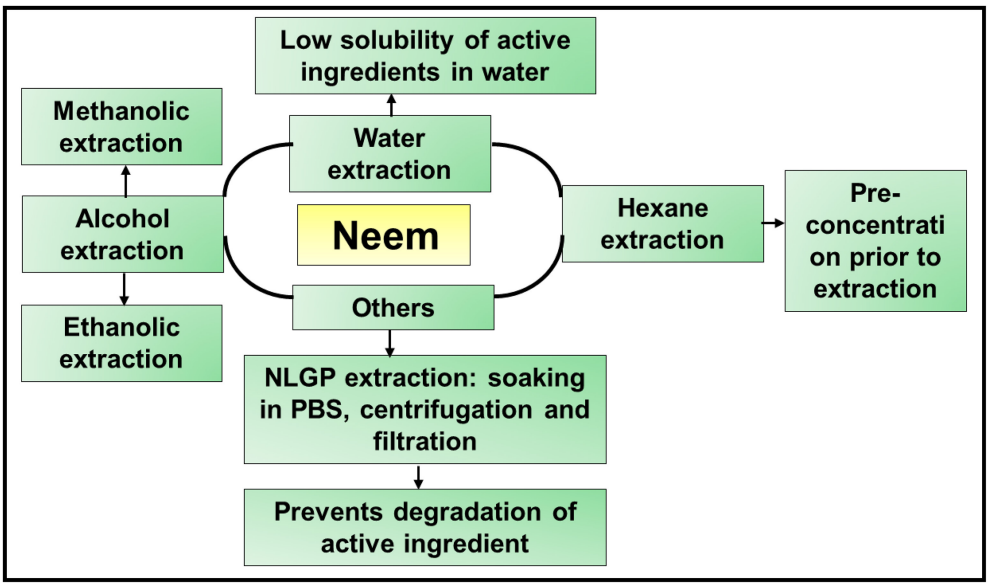

FIGURE 3 | Schematic representation of various extraction procedures used to extract the active ingredients from neem leaf, bark, and seeds. Three major extraction procedures are employed to extract various active components of neem: water, alcohol, and hexane extractions. These techniques are used either alone or in combination to yield the limonoids. However, extraction of NLGP from neem leaves involves a different procedure, which includes soaking the neem leaf powder overnight, followed by a series of centrifugation steps and finally filtration to yield the purified protein.

have very high solubility in alcohol solvents. During the process, kernels are grated and steeped in ethanol or methanol. The yield obtained is 50 times more concentrated than the yield obtained through water extraction. In one study, methanolic neem leaf extracts were studied for their anti-inflammatory potential. A simple extraction procedure was applied to yield green crude extract. Briefly, dried neem leaves were ground and then dissolved in methanol with continuous shaking. Solvent was then evaporated to dryness, resulting in a green crude extract (Schumacher et al., 2011). Another study employed hexane and ethanol as two solvents in a 1:5 ratio for extraction from neem seeds and evaluated the effects of temperature, type of solvent and particle size on the kinetic and thermodynamic parameters of extraction. An increase in the temperature of extraction resulted in higher oil yield, but a lower oil quality. The extraction process was endothermic, spontaneous and irreversible (Liauw et al., 2008). The resulting concentrated active ingredients, obtained through the above mentioned extraction techniques, can then be modified into dust, granules, emulsifiable concentrates and wettable powders for more sophisticated use (Liauw et al., 2008).

Extraction of Neem Leaf Glycoprotein (NLGP)

NLGP is a component of neem with immunomodulatory properties (Baral et al., 2010). The extraction of this active ingredient was first described by Baral and Chattopadhyay (2004). Briefly, the active component of neem leaf is isolated by soaking neem leaf powder obtained by shed drying and pulverizing neem leaves, in phosphate buffered saline (PBS), followed by a series of centrifugation and filtration steps. It is a simple yet very common technique used for the extraction of NLGP, and the extract obtained can then be analyzed for its endotoxin, protein, and carbohydrate concentration (Baral and Chattopadhyay, 2004; Chakraborty et al., 2008; Goswami et al., 2014). The anti-cancer potential of neem leaf components has been the attention of numerous alternative therapies, as its multiple active ingredients offers anti-mutagenic (Arumugam et al., 2014), anti-proliferative (Sharma et al., 2014; Patel et al., 2016) and anti-inflammatory properties (Sarker et al., 2014). Baral et al. reported that NLGP prevents the growth of murine Ehrlich carcinoma (EC) as well as B16 melanoma in mice, by inducing lymphocytosis and by stimulating the increase of cluster of differentiation (CD)4^+ and $CD8^+$. Thus, it was concluded that it inhibits tumor growth through immune activation (Baral and Chattopadhyay, 2004). In line with this, it has been observed that NLGP causes activation of natural killer (NK) cells and NK-T cells and stimulates the secretion of interferon gamma (IFNγ) and Tumor necrosis factor alpha (TNFα) leading to tumor cell cytotoxicity (Haque and Baral, 2006). The analysis of mechanism involved in NLGP mediated tumor restriction revealed the secretion of IFNγ within the NLGP treated tumor microenvironment. Further, low expressions of FasR(+) cells was observed within the CD8(+)T cells. Collectively, it has been suggested that NLGP enhances the optimal functioning of T cells inhibiting the tumor growth (Barik et al., 2013). This therapy stimulates the activation of NK/NKT cells along with initiating Th1-type immune response and thus, maintains normal immune homeostasis in immunosuppressed hosts through upregulation of type 1 response (Mandal-Ghosh et al., 2007; Bose et al.,

2009). It also helps in the maturation of myeloid and mouse bone marrow derived dendritic cells providing efficient antigen presentation as well as co-stimulation of effector T cells (Goswami et al., 2010) indicating it's potential as a candidate for vaccine tool toward cancer immunotherapy. Recently, it was also established that a combination of NLGP with sarcoma antigen (SarAg) vaccination demonstrates anti-tumor immunity which had high superiority as compared to the SarAg vaccination alone since, the inoculation of the vaccination presented disease free survival until 60 days (Ghosh et al., 2016).

NEEM BASED NANO-BIOPESTICIDES

In agricultural practices, herb-based insecticides have the disadvantage of getting degraded when exposed to sunlight, due to low shelf life. Moreover, the active ingredients of neem cause non-specific toxicity. Aqueous extracts of neem leaves have shown toxicity to *Oreochromis niloticus*, by causing telangectiasis, bend in secondary lamellae, pyknosis, secondary lamellae shortening, and necrosis. Therefore, it is imperative to consider eco-toxicological properties of active ingredients of bio-pesticides (Alim and Matter, 2015). To overcome this, nano-biotechnology offers great potential, as it involves the production of unique nanoformulations that have the ability to improve the physiochemical stability, degradability, and effectiveness of natural products (Perlatti et al., 2013). These nanocapsules provide slow, controlled and cyclic assembly. It facilitates sustained release of the active compounds that can be controlled at the site of action thus, minimizing non-target toxic effects. Additionally, they prevent the loss of volatile components, thus augmenting the stability of the phytochemicals (Duran and Marcato, 2013). During the past decade, this "controlled release" nanotechnology has gained increasing attention which has been summarized in **Table 3** including the previous studies that have successfully encapsulated neem functional ingredients to increase their efficacy. Neem active ingredients predominantly, Azadirachtin can be loaded to both organic nanoparticles (Feng and Peng, 2012) as well as inorganic nanoparticles (Choudhury et al., 2016). Neem leaves comprise of reducing phytochemicals which can be used for the biosynthesis of silver NPs (Shankar et al., 2004). Such NPs capped with neem leaf extracts can act as excellent biopesticide delivery tools for efficient insecticidal activity. Furthermore, neem oil can be loaded onto silica based NPs. These preparations in a study demonstrated significant reduction in *Tuta absoluta*, a tomato leafminer. They presented no significant difference in their insecticidal efficacy when compared with a chemical pesticide, imidacloprid (El-Samahy et al., 2014). In another study, nanoemulsions of neem oil extracted from the seeds of the plant was developed in order to retard the high degradability of neem based biopesticides. A significant reduction of the storage pest *Zabrotes subfasciatus* validated the efficacy of nanoscale carriers in providing stability to the biopesticidal ingredient along with providing controlled release. Such nanoemulsion preparation also presented high UV stability (da costa et al., 2014). On the other hand, loading of neem oil on polymeric nanocarriers, Poly (ε-caprolactone) (PCL) and β-cyclodextrin to control *Bemisia tabaci*, although indicated effective in causing insecticidal activity the efficacy observed was less when compared to the commercial neem oil (Carvalho et al., 2012). Neem seed cake offers potential efficacy as a nano-biofertilizer. Preparation of slow releasing nanostructures containing neem cake stimulates the germination of rhizobacteria along with delivering nutrients to plant (Celsia and Mala, 2014; Mala et al., 2016). Nevertheless, the numerous benefits of NPs in agrochemical delivery have paved way to a new era of biopesticides. This technology provides several benefits including slow release characteristics, enhanced stability of functional ingredients, use of small dose, limited loss by degradation and leaching, ease in handling, transportation and in masking of odor.

FUTURE PERSPECTIVE

Recently emerging issues regarding the increasing prevalence of pest resistance has prompted the adoption of alternative

TABLE 3 | List of nanocarriers used to encapsulate neem active components and their potential agricultural applications.

Neem component	Active ingredient	Carrier	Nanoparticle size	Potential application	Reference
Neem	Azadirachtin	Carboxymethyl chitosan with ricinoleic acid (R-CM-chitosan)	200–500 nm	Botanical pesticide	Feng and Peng, 2012
Neem seed kernels	Azadirachtin	Nanoemulsion	1–5 μm	Efficient as a pesticide causing high mortality against a storage pest *Zabrotes subfasciatus*	da costa et al., 2014
Neem oil	Azadirachtin	β-cyclodextrin and PCL	PCL: 4 μm β-cyclodextrin: 83.2 nm	Exhibits high efficacy against nymphs and eggs of *Bemisia tabaci* infecting soybean	Carvalho et al., 2012
Neem seed kernel: -Neem extract -Neem oil	Azadirachtin	PCL	230–245 nm	Exhibits 100% larval mortality against *Plutella xylostella*	Forim et al., 2013
Neem oil	Azadirachtin	Silica NPs	20 nm	Exhibits significant insecticidal effect against *Tuta absoluta*	El-Samahy et al., 2014
Neem leaves	Azadirachtin	Silver NPs	100 nm	Neem coated silver nanoparticles exhibited strong anti-fungal properties (against *Aspergillus terreus*)	Choudhury et al., 2016

strategies with special emphasis on integrated pest managements. Neem is an ideal alternative candidate as a natural non-synthetic plant pesticide. Over the years, numerous research has validated its pesticidal activity. It is a cost effective and eco-friendly alternative to the commercial chemically synthesized pesticides. However, owing to its instability to ultraviolet light and limitation of less efficiency as compared to its synthetic counterparts (Barnby et al., 1989), it is vital to develop a novel and efficient strategy to replace toxic chemically synthesized pesticides. This can be achieved by utilizing the past knowledge of neem phytocomponents with pesticidal activity and integrating it with current innovative strategies to develop a unique and effective pest management tool. Amalgamation of nanoscience incorporating organic nanocapsules to provide a dual benefit of controlled delivery of the functional ingredients as well as biodegradable and non-toxic carriers can act as a turning point of modern agriculture. In line with this, inorganic nanoparticles, due to their small size and ease in surface modifications (Joany et al., 2015) can also support the upcoming sustainable agricultural practices. Addition of such nanoformulations can not only act as anti-feedant, ovicidal, sterilant, and morphological and physiological defects in insects but also as a herbal fertilizer. The property of slow release of active ingredients in the soil, conditions the soil and provides nutrients that promote the growth of plants (Mala et al., 2016), which can revolutionize the industry of botanical fertilizers. However, integration of a targeted approach to prevent the side-effects on non-target and ecologically important organisms is an important aspect which still needs to be addressed.

Additionally, this meliaceae plant has unique agro-medicinal properties. Since, parallel to its efficacy as a bio-pesticide it also instills immunomodulatory (Goswami et al., 2016), anti-cancer (Sironmani, 2016), anti-microbial (Verma and Mehata, 2016), and wound healing (Bhagavathy and Kancharla, 2016) properties, which can pave way to an interdisciplinary approach by integrating the attributes of this plant to provide multiple benefits in agriculture as well as in biomedicine.

CONCLUSIONS

The environmental risks associated with the continuous use of synthetic pesticides have prompted the use of plant based insecticidal components that provide selective toxicity to insects with minimum off target effects. The use of botanical pesticides offers eco-friendly pest control strategy to aid the agricultural practices. Among the various herbs, neem plant based insecticides has been the most accepted bio-pesticides, due to the presence of multiple limonoids in neem plant extracts and oil that not only provides a sustainable pest control mechanism but also prevents plant disease resistance, from various synthetic insecticides. Additionally, the efficacy of these pesticidal ingredients of neem can be augmented by encapsulating them in nanocarriers that facilitates in providing sustained and control release of phytochemicals along with site targeted delivery thus, increasing the productivity and yield of crops.

AUTHOR CONTRIBUTIONS

The primary manuscript was written by SC and JK. Substantial comments and editing was provided by RK, AS, DC, CB, RS, and JK to provide an improved draft. All authors have read and approved the manuscript for publication.

FUNDING

The authors would like to thank the Australia-India Strategic Research Fund (AISRF), National Health and Medical Research Council (NHMRC) and Indian Council of Agricultural Research (ICAR) Ph.D. fellowship ID-29-1/2009-EQR/Edn (Pt.III), for financial support.

ACKNOWLEDGMENTS

The authors would like to thank the Australia–India Strategic Research Fund (AISRF), National Health and Medical Research Council (NHMRC), and Indian Council of Agricultural Research (ICAR) for financial support. The authors declare no conflict of interest.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Organic Farming :: Compost

Composting

Composting - an overview

Composting is the natural process of 'rotting' or decomposition of organic matter by microorganisms under controlled conditions. Raw organic materials such as crop residues, animal wastes, food garbage, some municipal wastes and suitable industrial wastes, enhance their suitability for application to the soil as a fertilizing resource, after having undergone composting.

A mass of rotted organic matter made from waste is called compost. The compost made from farm waste like sugarcane trash, paddy straw, weeds and other plants and other waste is called farm compost. The average nutrient contents of farm compost are 0.5 per cent N, 0.15 per cent P2O5and 0.5 per cent K2O. The nutrient value of farm compost can be increased by application of superphosphate or rock phosphate at 10 to 15 kg/t of raw material at the initial stage of filling the compost pit. The compost made from town refuses like night soil, street sweepings and dustbin refuse is called town compost. It contains 1.4 per cent N, 1.00 per cent P2O5 and 1.4 per cent K2O.

Farm compost is made by placing farm wastes in trenches of suitable size, say, 4.5 m to 5.0 m long, 1.5m to 2.0 m wide and 1.0 m to 2.0 m deep. Farm waste is placed in the trenches layer by layer. Each layer is well moistened by sprinkling cow dung slurry or water. Trenches are filled up to a height of 0.5 m above the ground. The compost is ready for application within five to six months.

Composting is essentially a microbiological decomposition of organic residues collected from rural area (rural compost) or urban area (urban compost).

Methods of composting

In **Coimbatore method**, composting is done in pits of different sizes depending on the waste material available. A layer of waste materials is first laid in the pit. It is moistened with a suspension of 5-10 kg cow dung in 2.5 to 5.0 l of water and 0.5 to 1.0 kg fine bone meal sprinkled over it uniformly. Similar layers are laid one over the other till the material rises 0.75 m above the ground level. It is finally plastered with wet mud and left undisturbed for 8 to 10 weeks. Plaster is then removed, material moistened with water, given a turning and made into a rectangular heap under a shade. It is left undisturbed till its use.

In the **Indore method** of composting, organic wastes are spread in the cattle shed to serve as bedding. Urine soaked material along with dung is removed every day and formed into a layer of about 15 cm thick at suitable sites. Urine soaked earth, scraped from cattle sheds is mixed with water and sprinkled over the layer of wastes twice or thrice a day. Layering process continued for about a fortnight. A thin layer of well decomposed compost is sprinkled over top and the heap given a turning and reformed. Old compost acts as inoculum for decomposing the material. The heap is left undisturbed for about a month. Then it is thoroughly moistened and given a turning. The compost is ready for application in another month.

In the **Bangalore method** of composting, dry waste material of 25 cm thick is spread in a pit and a thick suspension of cow dung in water is sprinkled over for moistening. A thin layer of dry waste is laid over the moistened layer. The pit is filled alternately with dry layers of material and cow dung suspension till it rises 0.5 m above ground level. It is left exposed without covering for 15 days. It is given a turning, plastered with wet mud and left undisturbed for about 5 months or till required.

In Coimbatore method, there is anaerobic decomposition to start with, following by aerobic fermentation. It is the reverse in Bangalore method. The Bangalore compost is not so thoroughly decomposed as the Indore compost or even as much as the Coimbatore compost, but it is bulkiest.

Compost is a rich source of organic matter. Soil organic matter plays an important role in sustaining soil fertility, and hence in sustainable agricultural production. In addition to being a source of plant nutrient, it improves the physico-chemical and biological properties of the soil. As a result of these improvements, the soil:

(i) becomes more resistant to stresses such as drought, diseases and toxicity;
(ii) helps the crop in improved uptake of plant nutrients; and
(iii) possesses an active nutrient cycling capacity because of vigorous microbial activity.

These advantages manifest themselves in reduced cropping risks, higher yields and lower outlays on inorganic fertilizers for farmers.

Dung and urine produced by animals per day

Animal	Urine (ml / kg live wt)	Quantity of dung (Kg) per day
Horse	3-18	9-18
Cattle	17-45	18-30
Buffaloes	20-45	25-40
Sheep and goats	10-40	1-2.5
Pigs	5-30	3-5
Poultry	-	2.5-3.5

Nutritive value of animal solid and liquid excreta

Animal	Dung (mg/g)			Urine (%)		
	N	P	K	N	P	K
Cattle	20-45	4-10	7-25	1.21	0.01	1.35
Sheep and goat	20-45	4-11	20-29	1.47	0.05	1.96
Pig	20-45	6-12	15-48	0.38	0.1	0.99
Poultry	28-62	9-26	8-29	-	-	-

Why composting is necessary?

- The rejected biological materials contain complex chemical compounds such as lignin, cellulose, hemicellulose, polysaccharides, proteins, lipids etc.
- These complex materials cannot be used as such as resource materials.
- The complex materials should be converted into simple inorganic element as available nutrient.
- The material put into soil without conversion will undergo conversion inside the soil.

- This conversion process take away all energy and available nutrients from the soil affecting the crop.
- Hence conversion period is mandatory.

Advantages of Composting

- Volume reduction of waste.
- Final weight of compost is very less.
- Composting temperature kill pathogen, weed seeds and seeds.
- Matured compost comes into equilibrium with the soil.
- During composting number of wastes from several sources are blended together.
- Excellent soil conditioner
- Saleable product
- Improves manure handling
- Redues the risk of pollution
- Pathogen reduction
- Additional revenue.
- Suppress plant diseases and pests.
- Reduce or eliminate the need for chemical fertilizers.
- Promote higher yields of agricultural crops.
- Facilitate reforestation, wetlands restoration, and habitat revitalization efforts by amending contaminated, compacted, and marginal soils.
- Cost-effectively remediate soils contaminated by hazardous waste.
- Remove solids, oil, grease, and heavy metals from stormwater runoff.
- Capture and destroy 99.6 percent of industrial volatile organic chemicals (VOCs) in contaminated air.
- Provide cost savings of at least 50 percent over conventional soil, water, and air pollution remediation technologies, where applicable.

Drawbacks of Using Composts

Agricultural use of composts remains low for several reasons:

- The product is weighty and bulky, making it expensive to transport.
- The nutrient value of compost is low compared with that of chemical fertilizers, and the rate of nutrient release is slow so that it cannot usually meet the nutrient requirement of crops in a short time, thus resulting in some nutrient deficiency
- The nutrient composition of compost is highly variable compared to chemical fertilizers.
- Agricultural users might have concerns regarding potential levels of heavy metals and other possible contaminants in compost, particularly mixed municipal solid wastes. The potential for contamination becomes an important issue when compost is used on food crops.
- Long-term and/or heavy application of composts to agricultural soils has been found to result in salt, nutrient, or heavy metal accumulation and may adversely affect plant growth, soil organisms, water quality, and animal and human health

Composting organic materials with high lignin content - lime treatment

- By adding organic wastes such as sawdust, wood shavings, coir pith, pine needles, and dry fallen leaves, while preparing organic waste mixtures for composting, one can ensure that the compost produced contains sufficient and long-lasting humus. However, gardeners often find that where they use lignin-rich plant materials, the compost does not ripen rapidly. A technique for making good compost from hard plant materials involves mixing lime in a ratio of 5 kg per 1000 kg of waste material. Lime can be applied as dry powder or after mixing with a sufficient quantity of water. Treatment with lime enhances the process of decomposition of hard materials.
- Liming can enhance the humification process in plant residues by enhancing microbial population and activity and by weakening lignin structure. It also improves the humus quality by changing the ratio of humic to fulvic acids and decreases the amount of bitumen, which interferes with the decomposition process. Instead of lime, powdered phosphate rock can be used in a ratio of 20 kg per 1 000 kg of organic waste. Phosphate rock contains a lot of lime. The phosphates and micronutrients contained in phosphate rock make composts rich in plant nutrients.

Composting weeds

- This method has been developed for composting weeds such as parthenium, water hyacinth (*Eichornia crassipes*), cyperus (*Cyperus rotundus*) and cynodon (*Cynodon dactylon*).

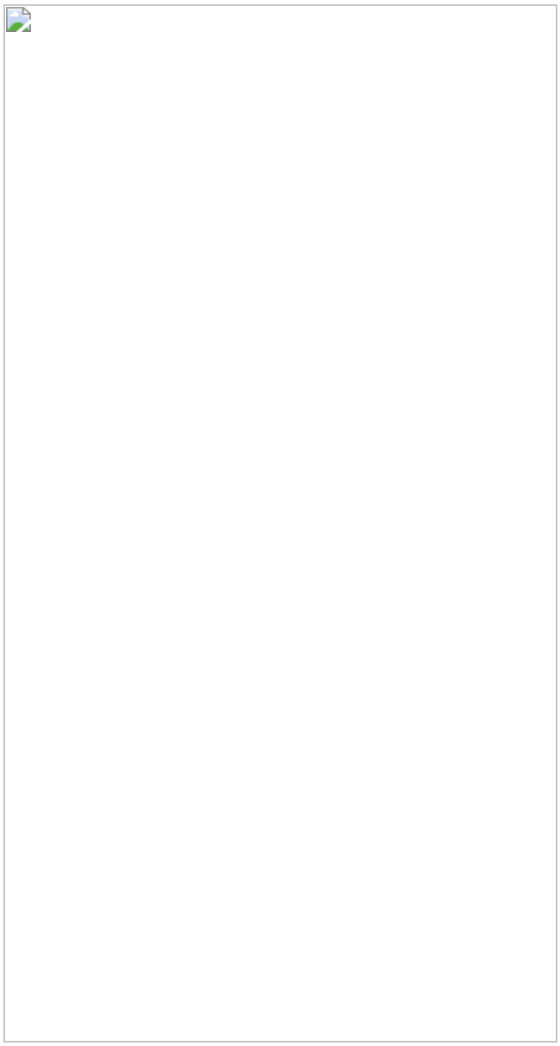
Materials Required

- 250 g of *Trichoderma viride* and *Pleurotus sajor-caju* consortia, and 5 kg of urea. An elevated shaded place is selected, or a thatched shed is erected. An area of 500 cm × 150 cm is marked out. The material to be composted is cut to 10-15 cm in size. About 100 kg of cut material is spread over the marked area. About 50 g of microbial consortia is sprinkled over this layer. About 100 kg of weeds are spread on this layer. One kilogram of urea is sprinkled uniformly over the layer. This process is repeated until the level rises to 1 m. Water is sprinkled as necessary to maintain a moisture level of 50-60 percent. Thereafter, the surface of the heap is covered with a thin layer of soil. The pile requires a thorough turning on the twenty-first day. The compost is ready in about 40 days.

Compost enrichment

Farm compost is poor in P content (0.4-0.8 percent). Addition of P makes the compost more balanced, and supplies nutrient to micro-organisms for their multiplication and faster decomposition. The addition of P also reduces N losses. Compost can be enriched by:

- Application of superphosphate, bonemeal or phosphate rock: 1 kg of superphosphate or bonemeal is applied over each layer of animal dung. Low-grade phosphate rock can also be used for this purpose.
- Use of animal bones: these can be broken into small pieces, boiled with wood ash leachate or lime water and drained, and the residue applied to the pits. This procedure of boiling bones facilitates their disintegration. Even the addition of raw bones, broken into small pieces and added to the pit, improves the nutrient value of compost significantly.
- Wood ash waste can also be added to increase the K content of compost.
- Addition of N-fixing and P-solubilizing cultures (IARI, 1989): The quality of compost can be further improved by the secondary inoculation of *Azotobacter*, *Azospirillum lipoferum*, and *Azospirillum brasilence* (N-fixers); and *Bacillus megaterium* or *Pseudomonas* sp. (P solubilizers). These organisms, in the form of culture broth or water suspension of biofertilizer products, can be sprinkled when the decomposing material is turned after one month. By this time, the temperature of the compost has also stabilized at about 35 °C. As a result of this inoculation, the N content of straw compost can be increased by up to 2 percent. In addition to improving N content and the availability of other plant nutrients, these additions help to reduce the composting time considerably.



The Benefits of Using Composts to Agriculture

Compost has been considered as a valuable soil amendment for centuries. Most people are aware that using composts is an effective way to increase healthy plant production, help save money, reduce the use of chemical fertilizers, and conserve natural resources. Compost provides a stable organic matter that improves the physical, chemical, and biological properties of soils, thereby enhancing soil quality and crop production. When correctly applied, compost has the following beneficial effects on soil properties, thus creating suitable conditions for root development and consequently promoting higher yield and higher quality of crops.

Improves the Physical Properties of Soils

- Reduces the soil bulk density and improves the soil structure directly by loosening heavy soils with organic matter, and indirectly by means of aggregate-stabilizing humus contained in composts. Incorporating composts into compacted soils improves root penetration and turf establishment.
- Increases the water-holding capacity of the soil directly by binding water to organic matter, and indirectly by improving the soil structure, thus improving the absorption and movement of water into the soil. Therefore, water requirement and irrigation will be reduced.
- Protects the surface soil from water and wind erosion by reducing the soil-dispersion action of beating raindrops, increasing infiltration, reducing water runoff, and increasing surface wetness. Preventing erosion is essential for protecting waterways and maintaining the quality and productivity of the soil.
- Helps bind the soil particles into crumbs by the fungi or actinomycetes mycelia contained in the compost and stimulated in the soil by its application, generally increasing the stability of the soil against wind and water erosion.
- Improves soil aeration and thus supplies enough oxygen to the roots and escapes excess carbon dioxide from the root space.
- Increases the soil temperature directly by its dark color, which increases heat absorption by the soil, and indirectly by the improved soil structure.
- Helps moderate soil temperature and prevents rapid fluctuations of soil temperature, hence, providing a better environment for root growth. This is especially true of compost used as a surface mulch.

Enhances the Chemical Properties of Soils

- Enables soils to hold more plant nutrients and increases the cation exchange capacity (CEC), anion exchange capacity (AEC), and buffering capacity of soils for longer periods of time after composts are applied to soils. This is important mainly for soils containing little clay and organic matter.
- Builds up nutrients in the soil. Composts contain the major nutrients required by all plants [N,P,K, calcium (Ca), magnesium(Mg), and S] plus essential micronutrients or trace elements, such as copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), boron (B), and molybdenum (Mb).
- The nutrients from mature composts are released to the plants slowly and steadily. The benefits will last for more than one season.
- Stabilizes the volatile nitrogen of raw materials into large protein particles during composting, thereby reducing N losses.
- Provides active agents, such as growth substances, which may be beneficial mainly to germinating plants.
- Adds organic matter and humus to regenerate poor soils.
- Buffers the soil against rapid changes due to acidity, alkalinity, salinity, pesticides, and toxic heavy metals.

Improves the Biological Properties of Soils

- Supplies food and encourages the growth of beneficial microorganisms and earthworms.
- Helps suppress certain plant diseases, soil borne diseases, and parasites.
- Research has shown that composts can help control plant diseases (e.g. Pythium root rot, Rhizoctonia root rot, chili wilt, and parasitic nematode) and reduce crop losses. A major California fruit and vegetable grower was able to cut pesticide use by 80% after three years of compost applications as part of an organic matter

management system. Research has also indicated that some composts, particularly those prepared from tree barks, release chemicals that inhibit some plant pathogens. Disease control with compost has been attributed to four possible mechanisms:

- 1) successful competition for nutrients by beneficial microorganisms;
  2) antibiotic production by beneficial microorganisms;
  3) successful predation against pathogens by beneficial microorganisms;
  4) activation of disease-resistant genes in plants by composts; and
  5) high temperatures that result from composting kill pathogens.
- Reduces and kills weed seeds by a combination of factors including the heat of the compost pile, rotting, and premature germination.

Economic and Social Benefits of Composting

The economic and social benefits of composting include the following:

- Brings higher prices for organically grown crops.
- Composting can offer several potential economic benefits to communities:
- Extends current landfill longevity and delays the construction of a more expensive replacement landfill or incinerator.
- Reduces or avoids landfill or combustor tipping fees, and reduces waste disposal fees and long-distance transportation costs.
- Offers environmental benefits from reduced landfill and combustion use.
- Creates new jobs for citizens.
- Produces marketable products and a less-cost alternative to standard landfill cover, artificial soil amendments, and conventional bioremediation techniques.
- Provides a source of plant nutrients and improves soil fertility; results in significant cost savings by reducing the need for water, pesticides, fungicides, herbicides, and nematodes.
- Used as an alternative to natural topsoil in new construction, landscape renovations, and container gardens. Using composts in these types of applications is not only less expensive than purchasing topsoil, but it can also often produce better results when establishing a healthy vegetative cover.
- Used as mulch for trees, orchards, landscapes, lawns, gardens, and makes an excellent potting mix. Placed over the roots of plants, compost mulch conserves water and stabilizes soil temperatures. In addition, it keeps plants healthy by controlling weeds, providing a slow release of nutrients, and preventing soil loss through erosion.

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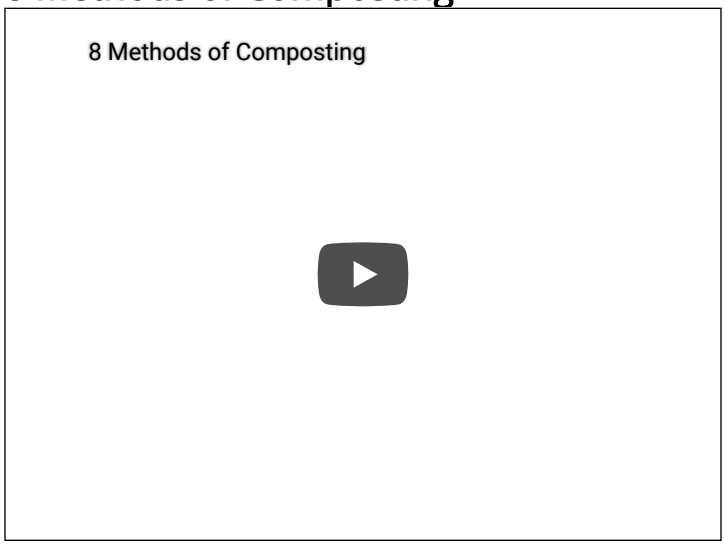
8 METHODS OF COMPOSTING

8 Methods of Composting



8 Methods of Composting is what I consider to be the most commonly used methods of composting for a business or home environment.

If you have read 7 Composting Methods then this article is an update to that article. I added Mechanical Composting as I think this is another very viable method of composting that like all composters have its own advantages and disadvantages.

For a deeper understanding of composting register to receive the monthly newsletter and you will be emailed the white paper by Bob's James on Composting Principles which discuss the mechanics of composting.

The other information in this article talks about the different methods of composting.

Everybody has different needs so at any given point in time one or more of these methods might suit your current living conditions and you might at some point change the way you compost many times throughout your lifetime.

What you once found useful might become obsolete as your needs and environment change so it's a good idea to have an understanding of the pros and cons of each system. However, what might be a pro for you may be a con for someone else. You just need to work out what is best for you.

They all work in varying degrees for different purposes, some more efficiently than others and some are just simply, different. You may have tried some of these methods, are happy with your method, are looking for something to compliment your system or are looking for a change.

So I hope this information sheds some light on factors you may not have considered when you last chose your composter or if you are now choosing a new composter.

Composting Methods

Traditional backyard composting is typically achieved by:

1. Open air composting (hot composting)
2. Direct Composting (in-ground composting)

More Recent methods of composting are:

3. Tumbler Composting (A form of hot composting)
4. Worm Farm Composting (Vermicomposting)
5. EMO Composting (Bacteria composting)
6. Combination Composting (Compot Composting)
7. Commercial Composting
8. Mechanical Composting

Elements generally required in most systems in order to produce compost.

Air	Compost needs to be aerated or it creates an anaerobic environment for bacteria which produces unpleasant odours and attracts vermin
Water	Essential to keep the compost moist
Vegetable Matter	Essential to obtain organically rich compost
Worms	Digest decomposed matter and release worm castings that provide plants with the nutrients they need for growth
Carbon-nitrogen mix (brown and green waste)	Essential to create the right temperature for creating compost from green waste and to kill seeds and disease
Bacteria (EMO's)	Will decompose the food before the worms eat it
Soldier Flies	Not essential but devours waste food quicker than worms or bacteria
Other Beneficial Bugs	Cockroaches and other insects that help in the decomposition process (including maggots if putting meat in a compost pile – not recommended for most composters except the Compot.

1. Open Air Composting

Open Air Composting is traditionally a pile of green and brown matter in your backyard.

More often than not it is a bay constructed of anything you can get your hands on that is cheap and easy to put together.

Or you might have a couple of bins upturned sitting on the ground like the Gedye bin you can buy in a shop.

Wire cages are also used inlaid with piping around the edges to hold water and capture heat.

This can then be used for hot water systems in sustainability situations.

Open Air Composting is generally considered to be a **Hot Composting** method. Some people often call it a **Cold Composting** when smaller quantities of waste are used because it does not build up the same amount of heat.

To me, **Cold Composting** still produces heat and therefore is not technically cold composting.

Perhaps one could call it **Warm Composting** as the only way you could completely cold compost something is to let it rot in the fridge. And we all know that smell in the fridge.

Pros and Cons of Bay Composting (https://www.directcompostsolutions.com/pros-cons-open-air-composting/)

2. Direct Composting

Direct Compost is simply digging a hole or trench in the ground and burying your scraps.

It is also probably the oldest and most effective method of composting, but like all other methods of composting it too has its limitations. The main one being that it takes a long time to decompose unless you chop everything up.

You can only bury fruit and veg or you run the risk of it being dug up by all sorts of garden critters from birds to vermin. And you have to keep digging holes.

It does, however, produce an abundance of worms that then help to nourish your garden and improve your soil.

Pros and Cons of Direct Composting (https://www.directcompostsolutions.com/pros-cons-direct-trench-composting/)

3. Tumbler Composting

Tumbler Composting comes in many shapes and sizes of single to double units that you may purchase commercially from your local hardware store.

For many people, this is a great system if you are relatively strong and keen to turn it every day or every few days.

For others, it is hard work especially if you are getting on in years. But you can get some mechanized ones that make turning easier.

You often need two of these systems so you can let one sit for a few months to fully decompose before you empty it. While this is happening you fill the other one up.

This can be a good system if you have a large amount of green and brown waste to dispose of and have the space to fit this system.

If you are only filling it with green and brown waste then a bay system would be just as good though you may have to watch out for snakes and rats nesting in the warm compost.

Pros and Cons of Tumbler Composting (https://www.directcompostsolutions.com/pros-cons-tumbler-composting-method/)

4. Worm Farm Composting

Worm Farm Composting for many is the most common and preferred choice of composting because of their capabilities to grow worms, produce compost and compost tea and keep rats out of your compost.

The worms produce castings concentrated with nutrients lower in nitrogen compared to other composting methods.

Worm farms can be utilized even if you have no garden.

I think everyone has tried at some point in time to make their own worm farm with varying degrees of success using anything they can find that is cheap.

Do not house them in metal containers as copper leaches out, which is toxic to your worms.

I personally have tried foam containers only to find the worm juice eats out the foam so they leak everywhere.

Unless you have them on the ground somewhere so the nutrients can go directly into the soil you end up with a big mess.

If you use plastic containers you can collect the juice but then you have to add a tap to drain it off or some way of rotating the containers to collect the worm tea.

They need to be kept out of the sun, frost, and rain, and somewhere that's not too cold either.

Worms are temperamental little critters and will try and escape their containers if the conditions are not right and they are not happy.

It is said that you should use local worms for your area. I personally have no experience with this so you would have to try worms from other areas to know for sure if they will survive.

Local Worm Types

a. South Australia Red Worms (Lumbricus rubellus) and Tiger worms (Eisenia fetida) under ideal conditions are said to rapidly reproduce 8 to 1500 worms
b. The Tropics use Pontoscolex corethrunus or Pheretima group, commonly found in gardens
c. Fishing worms are apparently not good for composting.

If you can be bothered (according to Bob) you need to test the pH of each batch as some may be are more acidic than others.

But who has time for this or could be bothered.

That's why I love the Compot because the local worms in your garden will come and you don't need to add worms unless you have really bad soil.

Pros and Cons of Worm Farm Composting (https://www.directcompostsolutions.com/pros-cons-worm-farm-composting/)

5. EMO Composting

EMO Composting or Effective MicroOrganisms is a system generally used for indoor composting but can be used by anyone who likes this method of composting.

The most common product using EMO's is the Bokashi but other indoor systems can use it plus there are some systems that us carbon filter in the lid as well to filter odors.

Generally speaking, you need two of these, so while one is sitting the other is being filled.

You can collect juice to use in your garden.

But you cannot put everything from your kitchen is the Bokashi System.

You can buy the EMO online through many sites selling the Bokashi System.

You can use the EMO's in other systems if you so desire to aid the composting process.

Pros and Cons of EMO Composting (https://www.directcompostsolutions.com/pros-cons-emo-composting/)

6. Combination Composting

Combination Composting or **Compot Composting** is a combination method of open-air composting, direct composting, vermicomposting, and EMO composting.

All the elements of composting are used and will suit most household circumstances.
For some people, it too has its challenges. But for me, the challenges are less and the rewards are better.
You can compost **'ALL' your kitchen waste** and not just 'some' of it.

So ultimately you have over 50% less waste each week to put in your council bin.

Just Fill…Forget…Refill…when ready and give it a good clean out once a year.

It is faster and requires less work than most other composters.

And it nourishes your soil with all your own waste.

To me, it is the easiest composter I have ever used.

Pros and Cons of Compot Composting (https://www.directcompostsolutions.com/pros-cons-combined-compot-composting/)

7. Commercial Composting

Commercial Composting is different to backyard composting and uses different materials.
The Compost is made in long rows using such materials as, sawdust, pine bark, sand plus ferrous sulphate and maybe some sulphate of ammonia all mixed together.
It is usually turned every 3 to 4 days and is generally ready in 6 weeks for bagging.
There is not much nutrient value in the cheap commercial compost.
But there are small independent commercial compost companies that produce a better quality product, than the large commercial compost companies. They are however more expensive.

Some producers such as McLeod's Agriculture are certified organic as well.
The old saying "you get what you pay for" certainly applies to commercial compost.

The cheaper commercial compost is a good filler for raised garden beds or to backfill a Compot in clay or sandy soil.

Or it can be used to mix with composted soil to fill a pot plant perhaps.
If you are buying commercial grade compost to grow things it is best to buy a high-quality propagation mix.

Pros and Cons of Commercial Composting (https://www.directcompostsolutions.com/pros-cons-commercial-composting/)

8. Mechanical Composting

Mechanical Composting is an efficient method of composting that uses electricity to create the heat required and rotation of the contents required to produce semi-composted waste literally within a 24 hour period.

This system suits restaurants, hotels, motels, hospitals, schools, kindergartens and any large institution creating large amounts of waste from many people. It is a manageable in-house system instead of sending your waste off to council tips. You do however need to further compost the waste so need someone to collect the leftover contents for further composting in a garden bed or bay composting system.

There are also small systems that suit some people for their private residence but they can be quite expensive and will, of course, cost you ongoing electricity. Like all composters they to come with some pros and cons, but they do produce fast semi-composted soil.

Pros and Cons of Mechanical Composting (https://www.directcompostsolutions.com/pros-and-cons-of-mechanical-composting/)

In closing...

8 methods of composting is a guide to a number of composting methods that you might want to consider using in your home or business.

Some are similar, some are the same, some work better as a combination and some are just different. Either way composting is still the best thing you can do for your business, your garden, and the environment.

Much of the damaging effects to the environment comes from the methane produced in large council tips. Methane is worse than co2 for the environment. Keeping your waste out of the council tips reduces methane waste and ultimately helps the environment.

If you have time to grow your own veggies and utilize your compost then that is an added bonus.

Ideally, we all should play our part in reducing council waste.

How you do it is up to you, but whatever method you choose – doing something is better than nothing.

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Types of Composting and Understanding the Process

On this page

Composting Basics

Note

You may also consider contacting a compostable waste hauler about designing your compost plan.

There are five main areas that must be "controlled" during composting.

Feedstock and Nutrient Balance

Composting, or controlled decomposition, requires a proper balance of "green" organic materials and "brown" organic materials. "Green" organic material includes grass clippings, food scraps, and manure, which contain large amounts of nitrogen. "Brown" organic materials includes dry leaves, wood chips, and

branches, which contain large amounts of carbon but little nitrogen. Obtaining the right nutrient mix requires experimentation and patience. It is part of the art and science of composting.

Particle Size

Grinding, chipping, and shredding materials increases the surface area on which microorganisms can feed. Smaller particles also produce a more homogeneous compost mixture and improve pile insulation to help maintain optimum temperatures (see below). If the particles are too small, however, they might prevent air from flowing freely through the pile.

Moisture Content

Microorganisms living in a compost pile need enough moisture to survive. Water is the key element that helps transports substances within the compost pile and makes the nutrients in organic material accessible to the microbes. Organic material contains some moisture in varying amounts, but moisture also might come in the form of rainfall or intentional watering.

Oxygen Flow

Turning the pile, placing the pile on a series of pipes, or including bulking agents such as wood chips and shredded newspaper all help aerate the pile. Aerating the pile allows decomposition to occur at a faster rate than anaerobic conditions. Care must be taken, however, not to provide too much oxygen, which can dry out the pile and impede the composting process.

Temperature

Microorganisms require a certain temperature range for optimal activity. Certain temperatures promote rapid composting and destroy pathogens and weed seeds. Microbial activity can raise the temperature of the pile's core to at least 140° F. If the temperature does not increase, anaerobic conditions (i.e., rotting) occur. Controlling the previous four factors can bring about the proper temperature.

Onsite Composting

Organizations that are going to compost small amounts of wasted food can compost onsite. Composting can significantly reduce the amount of wasted food that is thrown away. Yard trimmings and small quantities of food scraps can be composted onsite. Animal products and large quantities of food scraps are not appropriate for onsite composting.

Learn how to create your own compost pile

Things to Think About

- The climate and seasons changes will not have a big effect on onsite composting. Small adjustments can be made when changes happen such as when the rainy season approaches.
- Food scraps need to be handled properly so they don't cause odors or attract unwanted insects or animals.
- Onsite composting takes very little time or equipment. Education is the key. Local communities might hold composting demonstrations and seminars to encourage homeowners or businesses to compost on their own properties.
- Creating compost can take up to two years, but manual turning can speed up the process to between three to six months.
- Compost, however, should not be used as potting soil for houseplants because of the presence of weed and grass seeds.
- You can leave grass clippings on the lawn-known as "grasscycling." These cuttings will decompose naturally and return some nutrients back to the soil, similar to composting.
- You can put leaves aside and use them as mulch around trees and scrubs to retain moisture.

Vermicomposting

Red worms in bins feed on food scraps, yard trimmings, and other organic matter to create compost. The worms break down this material into high quality compost called castings. Worm bins are easy to construct and are also available for purchase. One pound of mature worms (approximately 800-1,000 worms) can eat up to half a pound of organic material per day. The bins can be sized to match the volume of food scraps that will be turned into castings.

It typically takes three to four months to produce usable castings. The castings can be used as potting soil. The other byproduct of vermicomposting known as "worm tea" is used as a high-quality liquid fertilizer for houseplants or gardens.

Note

Night-crawlers and field worms found in gardens are not appropriate for vermiculture.

What Can Be Composted - Vermiculture?

- Food scraps
- Paper
- Yard trimmings such as grass and plants

Things to Think About

- Ideal for apartment dwellers or small offices.
- Schools can use vermiculture to teach children conservation and recycling.
- It is important to keep the worms alive and healthy by providing the proper conditions and sufficient food.
- Prepare bedding, bury garbage, and separate worms from their castings.
- Worms are sensitive to changes in climate.
    - Extreme temperatures and direct sunlight are not healthy for the worms.
    - The best temperatures for vermicomposting range from 55° F to 77° F.
    - In hot, arid areas, the bin should be placed under the shade.
    - Vermicomposting indoors can avoid many of these problems.

Aerated (Turned) Windrow Composting

Aerated or turned windrow composting is suited for large volumes such as that generated by entire communities and collected by local governments, and high volume food-processing businesses (e.g., restaurants, cafeterias, packing plants). It will yield significant amounts of compost, which might require assistance to market the end-product. Local governments may want to make the compost available to residents for a low or no cost.

This type of composting involves forming organic waste into rows of long piles called “windrows” and aerating them periodically by either manually or mechanically turning the piles. The ideal pile height is between four and eight feet with a width of 14 to 16 feet. This size pile is large enough to generate enough heat and maintain temperatures. It is small enough to allow oxygen flow to the windrow's core.

Large volumes of diverse wastes such as yard trimmings, grease, liquids, and animal byproducts (such as fish and poultry wastes) can be composted through this method.

Things to Think About

- Windrow composting often requires large tracts of land, sturdy equipment, a continual supply of labor to maintain and operate the facility, and patience to experiment with various materials mixtures and turning frequencies.
- In a warm, arid climate, windrows are sometimes covered or placed under a shelter to prevent water from evaporating.
- In rainy seasons, the shapes of the pile can be adjusted so that water runs off the top of the pile rather than being absorbed into the pile.
- Windrow composting can work in cold climates. Often the outside of the pile might freeze, but in its core, a windrow can reach 140° F.
- Leachate is liquid released during the composting process. This can contaminate local ground water and surface-water supplies. It should be collected and treated.
- Windrow composting is a large-scale operation and might be subject to regulatory enforcement, zoning, and siting requirements. Compost should be tested in a laboratory for bacterial and heavy metal content.

- Odors also need to be controlled. The public should be informed of the operation and have a method to address any complaints about animals or bad odors.

Aerated Static Pile Composting

Aerated static pile composting produces compost relatively quickly (within three to six months). It is suitable for a relatively homogenous mix of organic waste and work well for larger quantity generators of yard trimmings and compostable municipal solid waste (e.g., food scraps, paper products), such as local governments, landscapers, or farms. This method, however, does not work well for composting animal byproducts or grease from food processing industries.

In aerated static pile composting, organic waste mixed in a large pile. To aerate the pile, layers of loosely piled bulking agents (e.g., wood chips, shredded newspaper) are added so that air can pass from the bottom to the top of the pile. The piles also can be placed over a network of pipes that deliver air into or draw air out of the pile. Air blowers might be activated by a timer or a temperature sensors.

Things to Think about

- In a warm, arid climate, it may be necessary to cover the pile or place it under a shelter to prevent water from evaporating.
- In the cold, the core of the pile will retain its warm temperature. Aeration might be more difficult because passive air flowing is used rather than active turning. Placing the aerated static piles indoors with proper ventilation is also sometimes an option.
- Since there is no physical turning, this method requires careful monitoring to ensure that the outside of the pile heats up as much as the core.
- Applying a thick layer of finished compost over the pile may help alleviate any odors. If the air blower draws air out of the pile, filtering the air through a biofilter made from finished compost will also reduce any of the odors.
- This method may require significant cost and technical assistance to purchase, install, and maintain equipment such as blowers, pipes, sensors, and fans.
- Having a controlled supply of air allows construction of large piles, which require less land than the windrow method.

In-Vessel Composting

In-vessel composting can process large amounts of waste without taking up as much space as the windrow method and it can accommodate virtually any type of organic waste (e.g., meat, animal manure, biosolids, food scraps). This method involves feeding organic materials into a drum, silo, concrete-lined trench, or similar equipment. This allows good control of the environmental conditions such

as temperature, moisture, and airflow. The material is mechanically turned or mixed to make sure the material is aerated. The size of the vessel can vary in size and capacity.

This method produces compost in just a few weeks. It takes a few more weeks or months until it is ready to use because the microbial activity needs to balance and the pile needs to cool.

Things to Think About

- Some are small enough to fit in a school or restaurant kitchen.
- Some are very large, similar to the size of school bus. Large food processing plants often use these.
- Careful control, often electronically, of the climate allows year-round use of this method.
- Use in extremely cold weather is possible with insulation or indoor use.
- Very little odor or leachate is produced.
- This method is expensive and may require technical expertise to operate it properly.
- Uses much less land and manual labor than windrow composting.

LAST UPDATED ON AUGUST 29, 2016

Navigation

Production of Various Bio-Fertilizers | Microbiology

Article Shared by Ashwathy V

In this article we will discuss about the production of various bio-fertilizers.

1. Production of Bacterial Bio-Fertilizer:

With day-by-day increasing the population, especially in developing countries like India, the stress on agriculture is also increasing continuously. With the development, the land area under farming is not increasing but is further decreasing, this has posed extra burden on the agriculture. Therefore, the land available for agriculture should be economically utilized and maximum results be obtained.

Enhancing soil fertility by using organic fertilizers.

Most of our agricultural lands are deprived of either one mineral or the other. These minerals are essential for the growth and development of plants. One of the nutrients for any type of plant is nitrogen. Nitrogen is a major element required by the plant for growth and development. The nitrogen is provided in the form of chemical fertilizer.

Such chemical fertilizers pose health hazards and pollution problem in soil besides these are quite expensive, bringing the cost of production much higher.

Therefore, bio-fertilizers are being recommended in place of chemical fertilizers. Bio-fertilizers are the formulations of living microorganisms which are able to fix atmospheric nitrogen in the available form for plants (nitrate form) either by living freely in the soil or associated symbiotically with plants.

Although nitrogen fixers are present in the soil, enrichment of soil with effective microbial strains is much beneficial for the crop yields. Use of composite bio-fertilizers can increase soil fertility.

It has been proved that bio-fertilizers are cost effective, cheap and renewable source to supplement the chemical fertilizers:

(i) History:

In 1895, Nobbe and Hiltner applied for patents in England and the United States for a legume inoculant that was later marketed as Nitragin. Nitragin was produced on gelatin and agar nutrient media.

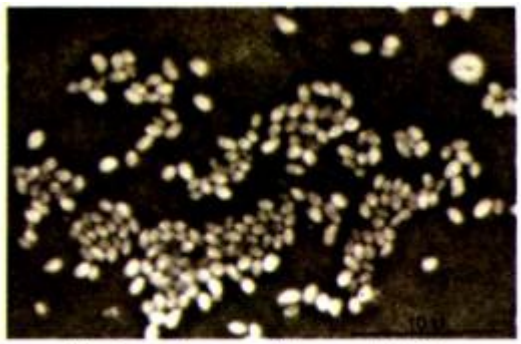
However, agar based inoculants were soon replaced by peat-based ones because in agar based inoculants, mortality was very high during the dry phase. In India, the production of bio-fertilizers on commercial scale was started only during late 1960's when yellow seeded soybean was introduced for the first time.

Recognition of Indian peat as suitable carrier for production of bio-fertilizer in 1969 further augmented, the growth of bio-fertilizer industry in India.

The performance of Indian peat-based bio-fertilizer at Indian Agricultural Research Institute, New Delhi, was found to be comparable to that obtained with imported 'Nitragin' bio-fertilizer from the U.S.A. Since then, the process of development of bio-fertilizer, specially of rhizobial bio-fertilizer for various crops in India has made a tremendous success.

(ii) Production of Bio-Fertilizer:

In order to meet the food requirements of ever increasing population, the nitrogen fertilizer requirement for crop production by 2000 A.D. was estimated to be about 11.4×10^6 tonnes. Biological nitrogen fixation can be the key to fill up this gap because of high cost and several other demerits of chemical fertilizers.



Nitrobacter, a rod shaped nitrifying bacteria, involved in the production of biofertilizer.

For production of a good and efficient bio-fertilizer, first of all an efficient nitrogen fixing strain is required, then its inoculum

(the form in which the strain is to be applied in fields) is produced.

Packing, storing and maintenance are other aspects of bio-fertilizer production. While producing bio-fertilizers the standards laid down by BIS have also to be kept in mind for making the product authentic. Commercial production of bacteria, involved in the production of bio-fertilizer is shown in Fig. 34.1.

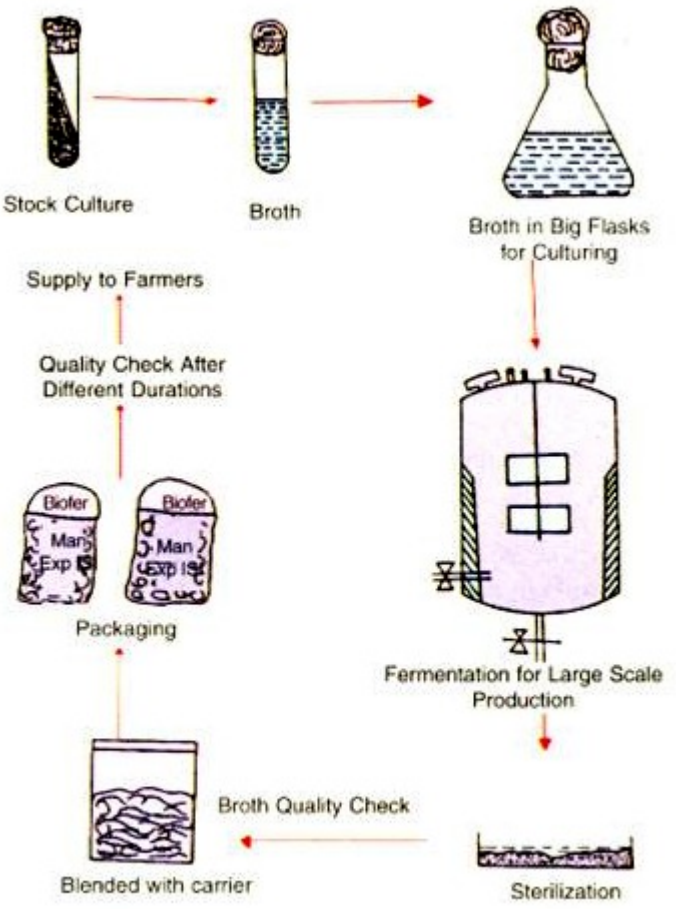


Fig. 34.1 : Commercial production of biofertilizer.

(iii) Criteria for Strain Selection:

The efficient nitrogen fixing strain is evolved or selected in laboratory, maintained and

multiplied on nutritionally rich artificial medium before inoculating the seed or soil. In soil, the strain has to survive and multiply to compete for infection site on roots against hostile environment in soil.

(iv) Steps for Preparing Bio-Fertilizer:

The isolated strain is inoculated in small flasks containing suitable medium for inoculum production. The volume of the starter culture should be a minimum of 1% to obtain atleast 1×10^9 cells/ml. Now the culture obtained is added to the carrier for inoculant (bio-fertilizer) preparation.

Carriers carry the nitrogen fixing organisms to the fields. In some cases carrier is first sterilised and then inoculated, while in other cases it is first inoculated and then sterilised by UV irradiation. The inoculum is now packed with 10^9-10^{10} viable cells per gram. Final moisture content should be around 40-60%. For large scale production of inoculum, culture fermenters are used.

(a) Seed Pelleting:

Direct seed coating with the gum arable or sugary syrup and useful nitrogen fixing strains especially the coating of rhizobia over specific host legume seeds are another method for obtaining fruitful results. As

before, first of all the inoculum is prepared of the desired strain and then the seeds are inoculated by using either direct coating method or slurry method. Immediately after seed coating, $CaCO_3$ is added to sticky seeds.

The practice of seed inoculation dates back to 1896 when Voecher used this technique. In many soils the nodule bacteria are absent or are not adequate in either number or quality to meet the nitrogen requirements of the plants. Under these conditions, it is necessary to inoculate seeds or seedlings with highly effective rhizobia.

(b) Inoculant Carriers:

Most inoculants are the mixture of the broth culture and a finely milled, neutralized carrier material. Carrier is a substance having properties such as, non-toxicity, good moisture absorption capacity, free of lump forming material, easy to sterilize, inexpensive, easily available and good buffering capacity, so that it can prolong and maintain the growth of nitrogen fixing microorganisms which it is carrying.

The most frequently used carrier for inoculant production is peat. However, peat is not available in certain countries such as India. A wide range of substitutes e.g.

lignite, coal, charcoal, bagasse, filter mud, vermiculite, polyacrylamide, mineral soils, vegetable oils, etc. have been tested as alternative carriers.

Carrier processing e.g. mining, drying and milling are the most capital intensive aspects of inoculant (bio-fertiliser) production. First of all the carrier like peat is mined, drained and cleared off stones, roots, etc. Then, it is shredded and dried.

The peat is then passed through heavy mills. Material with a particle size of 10-40 μm is collected for seed coating. Peat with particle size of 500-1500 μm is used for soil implant inoculant. Carriers have to be neutralised by adding precipitated calcium carbonate (pH 6.5-7.0). After this, the carriers are sterilized for use as inoculants.

(c) Quality Standards for Inoculants:

Like every product, the bio-fertilizers should also follow certain standards. The inoculant should be carrier-based and should contain a minimum of 10^8 viable cells per gram of carrier on dry mass basis within 15 days of manufacture, and 10^7 within 15 days before the expiry date marked on the packet when the inoculant is stored at 25-30°C. The inoculant should have a maximum expiry

period of 12 months from date of manufacture. The inoculant should not have any contaminant.

The contamination is one of the biggest problems faced by the bio-fertilizer industry. The pH of inoculant should be between 6.0 and 7.5. Each packet containing the bio-fertiliser should be marked with the information's e.g, name of product, leguminous crop for which intended, name and address of manufacture, type of carrier, batch or code number, date of manufacture, date of expiry, net quantity meant for net area and storage instructions. Each packet should also be marked with ISI (BIS) certification mark.

The inoculant (bio-fertilizer) should be stored in a cool place away from direct heat preferably at a temperature of 15°C. The bio inoculant should be packed in 50-75 μ low density polyethylene packets.

Two main methods of inoculation are currently being used (a) seed inoculation and (b) soil inoculation. The soil inoculation is done by delivering the inoculant directly into the sowing furrow with the seeds. Seed inoculation by pelleting or coating the seed with inoculant is the most popular methods.

(v) Green Manuring:

Green manuring is defined as a **"farming practice where a leguminous plant which has derived enough benefits from its association with appropriate species of Rhizobium is ploughed into the field soil and then a non -legume is sown and allowed to get benefitted from the already present nitrogen fixer".**

The practice of green manuring began from time immemorial from several century B.C. in India and China. During the course of time, availability of chemical fertilizers decreased the significance of green manuring. In recent years, due to hike in price of chemical fertilizers, the practice of green manuring is reemphasized.

Some of the cultivated legumes and annual legumes such as Crotolaria juncea, C. striata, Cassia mimosoides, Cyamopsis pamas, Glycine wightii, Indigofera linifolia, Sesbania rostrata, Leucaena leucocephala, etc. contribute nitrogen.

In addition to nitrogen, green manuring provides organic matter, phosphorus, potassium besides minimising the pathogenic organisms in soil. The reclamation of "usar lands" can also be done by green manuring.

In India besides a large number of private and semi-Government organisations, the National Bio-fertilizer Development Centre sponsored by the Ministry of Agriculture and the establishment of National Centres for blue green algal collections at IARI, New Delhi, the Department of Biotechnology, Govt. of India, Ministry of Science & Technology are the major developments that reflect our concern to harness bio-fertilizers in our agricultural economy.

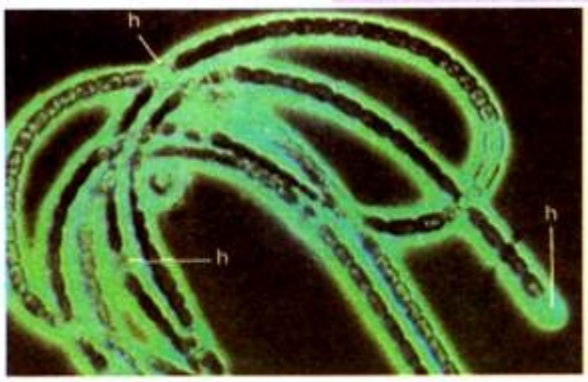
2. Algal and Other Bio-Fertilizers:

Biological nitrogen fertilizers play a vital role to solve the problems of soil fertility, soil productivity and environmental quality. Anabaena azollae, a cyanobacterium lives in symbiotic association with the free floating water fern Azolla.

The symbiotic system Azolla-Anabaena complex is known to contribute 40-60 kg N ha^{-1} per rice crop. Anabaena azolle can grow photo autotrophically and fixes atmospheric nitrogen. The nitrogen fixing cyanobacteria such as A. azollae and variabilis when immobilized in polyurethane foam and sugar cane waste have significantly increased the nitrogen fixing activity and ammonia secretion.

The inoculation of cyanobacteria in nee crop significantly influenced the growth of rice crop by secretion of ammonia in flood water. The use of neem cake coupled with the inculation of Azolla greatly increased the nitrogen utilization efficiency in rice crop.

Besides Anabaena, other nitrogen fixing cyanobacteria like Aulosira, Calothrix, Hapalosiphon, Scytonema, Tolypothrix and Westiellopsis have been held responsible for the spontaneous fertility of the tropic rice fields.



The nitrogen fixing cyanobacteria *Anabaena azollae* with heterocysts (h).

In addition to contributing N, the cyanobacteria add organic matter, secrete growth promoting substance like auxins and vitamins, mobilise insoluble phosphate and improve physical and chemical nature of the soil. Algalization has been shown to ameliorate the saline- alkali soils, help in the formation of soil aggregates, reduce soil compaction, and narrow C: N ratio.

These organisms enable the crop to utilize more of the applied nutrients leading to increased fertilizer utilising efficiency of crop plant. Most of the cyanobacteria act as supplements to fertilizer N contributing up to 30 kg N ha^{-1} $season^{-1}$. The increase in the crop yield varies between 5-25 percent.

(i) Mass Production of Cyanobacterial Biofertilizers:

For outdoor cultivation of cyanobacterial biofertilizers, the regional specific strain should be used. In such practices, a mixture of 5 or 6 regionally acclimatized strains of cyanobacteria e.g. species of Anabaena, Aulosira, Cylindrospermum, Gloeotrichia, Nostoc, Plectonema, Tolypothrix etc. are generally used as starter inoculum.

The following methods are used for mass cultivation:

(a) Cemented tank method,

(b) Shallow metal troughs method,

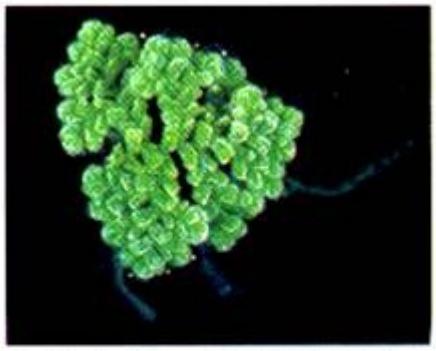
(c) Polythene lined pit method and

(d) Field method.

The polythene lined method is most suitable for small and marginal farmers for the preparation of bio-fertilizer. In this method,

small pits are prepared in field and lined with thick polythene sheets.

The mass cultivation of cyanobacteria is done by using any of the above four methods; the steps are given below:



Azolla-Anabaena Symbiosis: *Azolla*, a water fern.

(a) Prepare the cemented tank, shallow trays of iron sheets or polythene lined pits in an open area. Width of tanks or pits should not be more than 1.5 m. This will facilitate the proper handling of culture.

(b) Transfer 2-3 kg soil and add 100 g superphosphate. Water the pit to about 10 cm height. Mix lime to adjust the pH. Add 2 ml of insecticide to protect the culture from mosquitoes. Mix well and allow to settle down soil particles.

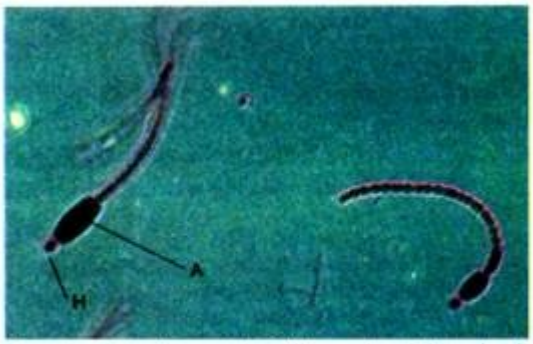
(c) When water becomes clear, sprinkle 100 g starter culture on the surface of water.

(d) When temperature remains around 35-40°C during summer, optimum growth of

cyanobacteria is achieved. The water level is always maintained about 10 cm during the period.

(e) After drying, the algal mass (mat) is separated from the soil that forms flakes. During summer about 1 kg pure algal mat per m^2 area is produced. It is collected, powdered, and packed in polythene bag and supplied to the farmers after sealing the packets.

(f) The algal flakes can be used as starter inoculum again.



Terminal heterocysts (H) and subterminal akinetes (A) of *Cylindrospermum*, a cyanobacterium involved in mass production of biofertilizers.

(ii) Mass Cultivation of Azolla:

The aquatic heterosporus fern contains endophytic cyanobacterium, Anabaena azollae in its leaf cavity. There are number of species of Azolla, namely A. caroliniana, A, filiculoides, A. maxicana, A. nilotica, A. pinnata and A. rubra which are used as biofertilizer especially for paddy. For mass cultivation of Azolla, microplots (20 m^2) are

prepared in nurseries in which sufficient water (5-10 cm) is added.

For profuse growth of Azolla, 4-20 kg P_2O_5/ha is also amended. Optimum pH (8.0) and temperature (14-30°C) should be maintained. Finally, microplots are inoculated with fresh Azolla (0.5 to 0.4 kg/m^2). An insecticide (Furadon) is used to check the insect's attack. After 3 weeks, the mat of Azolla is ready for harvest and the same microplot is inoculated with fresh Azolla to repeat the cultivation.

Azolla mat is harvested and dried to use as green manure.

There are two methods for its application in field:

(a) Incorporation of Azolla in soil prior to rice cultivation, and

(b) Transplantation of rice followed by water draining and incorporation of Azolla.

However, reports from the IRRI, Manila (Philippines) revealed that growing of Azolla in rice field before rice transplantation increased the yield equivalent to that obtained from 30 kg/ha nitrogen as urea or ammonium phosphate.

3. Endophytic Nitrogen Fixers:

Recently, several non-leguminous and particularly graminaceous species such as rice, wheat and forage grasses have registered tremendous interest in nitrogen fixation. Isolation of a number of diazotrophic bacteria such as Azospirillum, Herbaspirillum and Acetobacter is reported.

The term endophyte refers to microorganisms (bacteria and fungi) that colonize root interior of plants and live most of their life inside the plant tissue. Splitting the term endophyte into facultative and obligate was suggested to distinguish, respectively, strains that are able to colonize both the surface and root interior and to survive well in soil from those that do not survive well in soil but colonize the root interior and aerial parts.

(i) Facultative Endophytic Diazotrophs:

This group is composed of Azospirillum spp. and considered important with non-legume plants. Although A. lipoferum was the first species of the genus isolated by Tarrand (1978). A. brasilense among all the seven known species is the best characterized at physiological and molecular levels.

(ii) Obligate Endophytic Diazotrophs:

This group includes Acetobacter diazotrophicus (syn. Gluconacetobacter diazotrophicus) a nitrogen fixing bacterium clustered in the alpha sub-class of the proteobacteria, Azoarcus spp., Herbaspirillum spp. and a partially identified Burkholderia sp. are clustered in the beta sub-class of the proteobacteria.

(iii) Other Bacteria:

Alcaligens, a diazotrophic member of this genus has been consistently isolated from the rhizosphere of wet rice land. Burkholderia, the other bacterium appears to have potential as rice inoculant. In the case of Klebsiella, substituted nitrogen fixation has been observed in rice inoculated with K. oxytoca or any other Klebsiella spp. that are considered as endophytes.

The diazotrophic nature of some members of the genus Pseudomonas is still a matter of debate. Nevertheless, several bacteria within it are clearly diazotrophic such as Pseudomonas diazotrophicus, P. flurorescens, P. saccharophila and P. stutzeri. Recently, several researchers have attempted to construct an artificial association between rhizobia and rice particularly with Azorhizobium caulinodans.

(a) Isolation and Identification of Endophytes:

For isolation and identification of natural diazotrophs from plant samples, root or stem, washed with sterile water, surface sterilized with 70% ethanol for 5 minutes and with sodium hypochlorite (2-5%) for 30 second, washed several times using sterile water. Sterilization of root and stem will be verified by rolling them on BMS agar plates.

Then homogenize the sample in a mortar and pestle in sterile phosphate buffer, saline 1% sugar solution and serially diluted and 0.1 ml sample transfer into vials containing 5-8 ml of respective semisolid media for the targeted bacterium with respective C sources with an initial pH of 6.0.

The number of diazotrophic populations is determined by the most probable number methods using a McCrady table. Vials with veil pellicles reaching the surface after incubation at 30°C with or without gas production and with positive reaction for acetylene reduction activity, show the presence of good endophytes.

(b) Applications in Agriculture:

Obligate endophytes have an enormous potential for use because of their ability to

colonize the entire plant interior and establish themselves niches protected from oxygen or other inhibitory factors; thus their potential to fix nitrogen can be expressed maximally.

Recent studies in Brazil showed that the sugarcane varieties fix up to 80% nitrogen. It has been reported that wetland rice receives some nitrogen by endodiazotrophs. Tropical pasture grasses such as Brachiaria, Digitaria, Panicum and Paspalum spp. fix nitrogen.

4. Bio-Fertilizers aiding Phosphorus Nutrition:

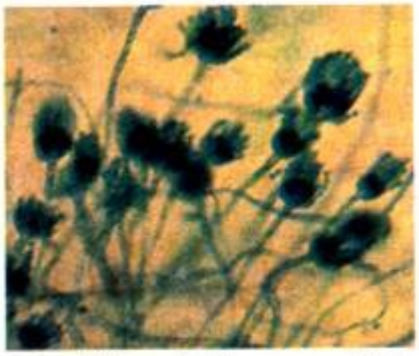
Tropical soils are deficient in phosphorus. Further most of the microorganisms solubilize P and thus make it available for plant growth. It is estimated that in most tropical soils, 75% super phosphate applied is fixed and only 25% is available for plant growth.

There are some fungi such as Aspergillus awamori, Penicillium digitatum, etc. and bacteria like Bacillus polymyxa, Pseudomonas striata, etc. that solubilize unavailable form of P to available form. India has 250 mt of rock phosphate deposits. The cheaper source of rock phosphate like Mussoorie rock phosphate

and Udaipur rock phosphate available in our country can be used along with phosphate solubilising microorganisms (Table 34.1).

Vesicular-arbuscular mycorrhizal (VAM) fungi colonize roots of several crop plants. They are zygomycetous fungi belonging to the genera Glomus, Gigaspora, Acaulospora, Sclercystis, etc.

These are obligate symbionts and cannot be cultured on synthetic media. They help plant growth through improved phosphorus nutrition and protect the roots against pathogens. Nearly 25-30% of phosphate fertilizer can be saved through inoculation with efficient VAM fungi as reported by Bagyaraj (1992).



Penicillium solubilizes unavailable form of P to available form.

5. Production of Mycorrhizal Bio-Fertilizer:

Methods of inoculum production of mycorrhizal fungi differ with respects to

their nature, depending upon types i.e., ectomycorrhizal or endomycorrhizal.

(i) Ectomycorrhizal Fungi:

In this case, the basidiospores, chopped sporocarps, sclerotia, pure mycelial culture, fragmented mycorrhizal roots or soil from mycorhizosphere region can be used as inoculum. The inoculum is mixed with nursery soil and seeds are sown thereafter.

Institute for mycorrhizal Research and Development, USA and Abbot Laboratories, USA have developed a mycelial inoculum of Pisolithus tinctorius in a mycelial vermiculite-peat moss substrate with trade name 'MycoRhiz' which is commercially available on large quantities (Table 34.2).

(ii) VA Mycorrhizal Fungi:

VA mycorrhiza can be produced on a large scale by pot culture technique. This requires the host plant mycorrhizal fungi and natural soil. The host plants which support large scale production of inoculum are sudan grass, strawberry, sorghum, maize, onion, citrus, etc.

The starter inoculum of VAM can be isolated from soil by wet sieving and decantation technique. VAM spores are surface sterilised and brought to the pot culture. Commonly

used pot substrates are sand: soil (1:1, w/w) with a little amount of moisture.

There are two methods of using the inoculum:

(a) Using a dried spore-root-soil to plants by placing the inoculum several centimetres below the seeds or seedlings,

(b) Using a mixture of soil- roots, and spores in soil pellets and spores are adhered to seed surface with adhesive.

Commercially available pot culture of VA mycorrhizal hosts grown under aseptic conditions can provide effective inoculum. Various types of VAM inocula are currently produced by Native Plants, Inc (NPI), Salt Lake City.

In India, Tata Energy Research Institute (TERI), New Delhi and Forest Research Institute, Dehradun have established mycorrhizae banks. Inocula of these can be procured as needed and used in horticulture and forestry programmes.

Table 34.1 : Some major nitrogen-fixing microorganisms and beneficiaries plants.

Name of microorganisms	*Name of crop plants which receive benefits*
Rhizobium spp. living symbiotically in root nodules	All grain legumes (pulses), some oil yielding (soybean, ground nut), some fodder legumes (*e.g.* clover),
Nostoc, *Anabaena*, *Aulosira* and others (free living blue green algae)	Rice
Anabaena azollae living symbiotically with the waterfern,	Rice *Azolla* spp.
Azotobacter chroococcum (free living bacterium)	Rice, maize, cotton and others
Frankia spp. (actinomycete) living symbiotically in nonlegume root nodules	*Alnus*, *Casuarina* and others
Azospirillum spp. (associate symbiont)	Maize, sorghum, pearl-millet, finger millet and others
Bacillus polymyxa, *Clostridium* spp. *Rhodospirillum* spp.	non-specific hosts

Table 34.2 : Commercially available biofertilizers and their manufacturers, beneficiary crop and associated microorganism

Product	*Manufacturer's Name*	*Microbe used*	*Beneficial crop.*
NitraginTM	Nitragin Sales Corpn. Wisconsin, 53209	*Rhizobium*	Soybean
Rhizocote	Coated seed Ltd., Nelson, New Zealand	*Rhizobium*	Legumes
Nodosit	Union Chemiques S.A. Belzium	*Rhizobium*	Legumes
Rhizonit	Phylaxia Allami Budapest, Hungary	*Rhizobium*	Legumes
Nitrazina	Wytwornia Walcz. Poland	*Azotobacter*	Cereals & vegetables
N-germ	Laboratoire de Microbiologie France	*BGA*	Rice
Tropical Inoculants	Tropical Inoculants Brisbane, Queensland	*Azotobacter*	Rice and wheat
Nodulaid	Agricultural Lab. New South Wales, UK	*Rhizobium*	Legumes
Azotobacterin	Tashkent laboratories Moscow	*Azotobacter*	Vegetables, cereals
Nodion	Indian Organic Chems. ltd. Mahew Mahal, Bombay	*Rhizobium*	Legumes
Azoteeka	Bacifil, 25 Nawal Kishore Rd. Lucknow	*Azotobacter*	Cereals
Agro-teeka	National Fertilizers & Chemicals 11, Ind. Area-II, Ramdarbar, Chandigarh	*Azotobacter*	Wheat, rice, maize, tea, Sugarcane potato
Rhizoteeka	Microbes India, 87, Lenin Savabe, Calcutta	*Rhizobium*	Legumes
Nitrogeron	Root Nodne Pvt. Ltd. Australia	*Rhizobium*	Legume

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