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RESEARCH ARTICLE

Biological control by microorganisms and ionizing radiation

Sahar M. Ouda

Department of Plant Research, Nuclear research Center, Atomic Energy Authority, Cairo, Egypt

Biotechnology Department, Faculty of Science and Education (Girls), Al-Khurmah, Taif University, KSA

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Corresponding Author
Sahar M. Ouda
dr.saharmouda@yahoo.com

Abstract

Biological control is the suppression of damaging activities of one organism by one or more other organisms. They are many environmental benefits of biological control including safety for humans and other non target organisms, reduction of pesticide residues in food, increased activity of most other natural enemies, and increased biodiversity in managed ecosystems, their advantages are numerous. Biological control of plant pathogens was applied and it can result from many different types of interactions between organisms. In addition, in the field of biological control of insects, there are varieties of the bacteria, fungi and viruses are currently used for control of a broad range of crop and forestry pests and larvae of several blood-sucking pests of humans and domestic animals. Nematodes in soil are subject to infections by bacteria and fungi, this creates the possibility of using soil microorganisms to control plant-parasitic nematodes. In the field of biological control of human diseases, food borne *Salmonella* infections are a major public health concern worldwide, bacteriophages offer highly specific and effective biocontrol of such pathogens. Also, chronic gut diseases can arise if pathogens in the gut flora begin to grow at high levels. However, some species are beneficial because they can repress the activities of the harmful types; this has led to the development of foods (**Probiotics)** that serve to increase numbers of the beneficial type. In the field biological control of plant viruses, viruses cannot be directly controlled by chemical application, and the major means of control (depending on the disease) include: Chemical or biological control of the vector (the organism transmitting the disease). The potential of using living organisms, like insects, fungi, and bacteria were tested as biological control agents for weed management, and there are a novel approach offered by living organisms as agents for biological weed control, this weed management tool is evolving as an alternative to herbicides. Recently, nuclear techniques have a significant role to play in facilitating the use and increasing the cost-effectiveness and safety of biological control agents, nuclear techniques can improve the efficiency of biological control by decreasing of the cost of production of bioagent, increasing host suitability and stimulation of biological process.

1. Biological control and environmental benefits

The terms “biological control” and its abbreviated synonym “biocontrol” have been used in different fields of biology, most notably entomology and plant pathology. In entomology, it has been used to describe the use of live predatory insects, entomopathogenic nematodes, microbial pathogens to suppress populations of different pest insects. In plant pathology, the term applies to the use of microbial antagonists to suppress diseases as well as the use of host specific pathogens to control weed populations. In both

fields, the organism that suppresses the pest or pathogen is referred to as the **biological control agent (BCA).** More broadly, the term biological control also has been applied to the use of the natural products extracted or fermented from various sources. These formulations may be very simple mixtures of natural ingredients with specific activities or complex mixtures with multiple effects on the host as well as the target pest or pathogen. And, while such inputs may mimic the activities of living organisms, nonliving inputs should more properly be referred to as biopesticides or biofertilizers, depending on the primary benefit provided to the host plant. The various definitions offered in the scientific literature have sometimes caused confusion and controversy. For example, members of the U.S. National Research Council took into account modern biotechnological developments and referred to biological control as "the use of natural or modified organisms, genes, or gene products, to reduce the effects of undesirable organisms and to favor desirable organisms such as crops, beneficial insects, and microorganisms", but this definition spurred much subsequent debate and it was frequently considered too broad by many scientists who worked in the field (US Congress, 1995). Because the term biological control can refer to a spectrum of ideas, it is important to stipulate the breadth of the term when it is applied to the review of any particular work. Published definitions of biocontrol differ depending on the target of suppression; number, type and source of biological agents; and the degree and timing of human intervention. Most broadly, biological control is the suppression of damaging activities of one organism by one or more other organisms, often referred to as natural enemies. With regards to plant diseases, suppression can be accomplished in many ways. If growers' activities are considered relevant, cultural practices such as the use of rotations and planting of disease resistant cultivars (whether naturally selected or genetically engineered) would be included in the definition. Because the plant host responds to numerous biological factors, both pathogenic and non-pathogenic, induced host resistance might be considered a form of biological control. More narrowly, **biological control refers to the purposeful utilization of introduced or resident living organisms, other than disease resistant host plants, to suppress the activities and populations of one or more plant pathogens.** This may involve the use of microbial inoculants to suppress a single type or class of plant diseases. Or, this may involve managing soils to promote the combined activities of native soil- and plant-associated organisms that contribute to general suppression. Most narrowly, biological control refers to the suppression of a single pathogen (or pest), by a single antagonist, in a single cropping system. Most specialists in the field would concur with one of the narrower definitions presented above. In this review, biological control will be narrowly defined as highlighted above in bold (Pal and McSpadden, 2006).

2. Biological control and application fields:

1. <u>Biological control of Plant Diseases (microbial pathogen)</u>:

Plant diseases need to be controlled to maintain the quality and abundance of food, feed, and fiber produced by growers around the world. Different approaches may be used to prevent, mitigate or control plant diseases. Beyond good agronomic and horticultural practices, growers often rely heavily on chemical fertilizers and pesticides. Such inputs to agriculture have contributed significantly to the spectacular improvements in crop productivity and quality over the past 100 years. However, the environmental pollution caused by excessive use and misuse of agrochemicals, as well as fear-mongering by some opponents of pesticides, has led to considerable changes in people's attitudes towards the use of pesticides in agriculture. Today, there are strict regulations on chemical pesticide use, and there is political pressure to remove the most hazardous chemicals from the market. Additionally, the spread of plant diseases in natural ecosystems may preclude successful application of chemicals, because of the scale to which such applications might have to be applied. Consequently, some pest management researchers have focused their efforts on developing alternative inputs to synthetic chemicals for controlling pests and diseases. Among these alternatives are those referred to as biological controls (Pal and McSpadden, 2006).

a. Biological control agents:

Bacteria as plant diseases biological control agent:

Bacteria belonging to the genus pseudomonas from various habitats are often necessary due to their importance in a diverse range of microbiological phenomena. P.fluorescens is considered as biological control agent against various root diseases (Ursula et al., 2000). The antagonistic activity of P.fluorescens on phytopthogenic fungi. P.fluorescens on cross streaking with the fungal pathogens revealed that it has been producing some inhibitory compound that suppresses the fungal growth. Similar results were obtained

for P.fluorescens inhibiting the growth of plant pathogens (Brion and Genevieve., 1999 and Goud & Muralikrishnan, 2009). P. ultimum, a pathogen on cotton causing damping off is suppressed by P.fluorescens by releasing of phenazine 1- carboxylic acid (Wilson et al., 2000). P.oryzae causing rice blast was inhibited by P.fluorescens in vitro and this inhibitory compound was also developed (Vidhyasekaran et al., 1997). The introduction of beneficial microorganisms for the biological control of soil borne plant pathogens has considerable potential in agriculture (Weller et al., 2007). Bacteria introduced on potato seed pieces, cotton, wheat and other species (Ganeshan and Manoj, 2005) have increased plant growth or reduced severity of root diseases. Dry weight of fungal growth under different concentrations of crude antibiotic was taken as a measure of effect of crude antibiotic on phytopathogenic fungi. In Pythium ultimum the dry weight reduced from 0.40mg to 0.31mg (50mcg/ml),0.21(100mcg/ml) and 0.10(150mcg/ml). As the concentration of crude antibiotic was increased the dry weight of fungus decreased. It could be reasoned out that the antifungal compounds present in the culture filtrate have inhibited the growth of the fungi. Similar trend of results were obtained with M.phaseolina and P.oryzae . Purified compounds showed inhibitory activity towards P.ultimum (Zhengyu et al., 2004). P.fluorescens produces a variety of antibiotics such as phenazine 1- carboxylic acid (Zhengyu et al., 2004), 2,4-diacetyl phloroglucinol(Weller et al., 2007; Ramesh et al., 2002) etc. These antibiotics alone or in combination with some siderophores inhibit the growth of phytopathogenic fungi. As the concentration of the compound increased the zone of clearance increased. Pure compounds like phenazine 1-carboxylic acid controlled take all disease (Zhengyu et al., 2004). This suggests that P.fluorescens produces a broad spectrum antifungal compound, which inhibits a variety of plant Pathogenic fungi and inhibits P.ultimum more when compared to M.phaseolina and P.oryzae. Its wide antagonistic activity against several phytopathogens in vitro shows its potential to be used as a broad spectrum biocontrol agent (Fuente et al., 2004). As agricultural practices become more sustainable, there is an increasing need for ecologically sound methods of disease control. Biological control, which exploits the natural antagonistic activity of certain root-colonizing bacteria against fungal pathogens, is one such approach. Biological control agents often perform inadequately under field conditions, however, and this has impeded acceptance of the technology as an alternative to chemical pesticides. Soil pseudomonads possess a variety of promising properties which make them better biocontrol agents (Goud & Muralikrishnan, 2009).

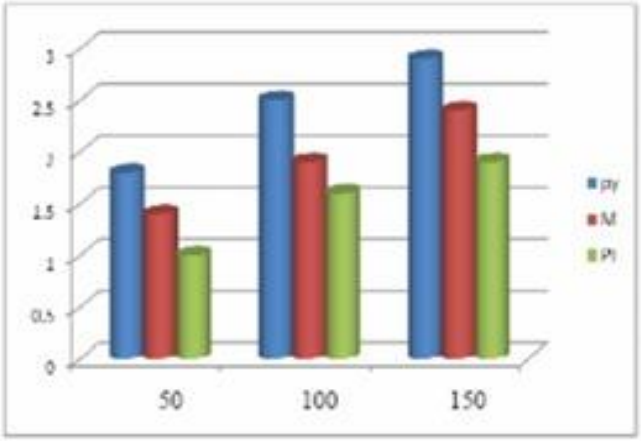

Figure (1): Percentage of inhibition exhibited by p.fluorescens as observed in pour plate method; PY- P.ultimum, M- M.phaseolina, PI- P.oryzae (Goud & Muralikrishnan, 2009)

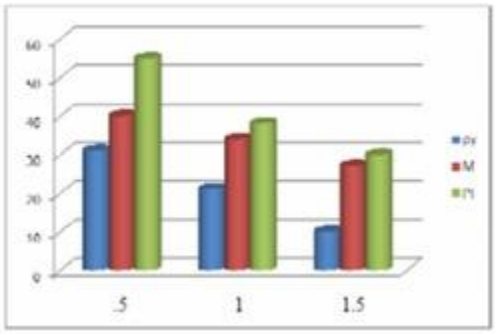

Figure (2): Measurement of fungal biomass (expressed in mg) as observed in flask culture method; PY- P.ultimum, M- M.phaseolina, PI- P.oryzae (Goud & Muralikrishnan, 2009)

Fungi as plant diseases biological control agent

Currently, the role of BCAs (Biological Control Agents) is a well established fact and has become increasingly crucial, and in several cases, complementary or even replacing the chemicacounter parts where antagonistic fungi play an important part. Fungal based BCAs have gained wide acceptance next to bacteria (mainly, Bacillus thuringiensis), primarily because of their broader spectrum in terms of disease control and yield. Trichoderma spp. has been the cynosure of many researchers who have been contributing to biological control pursuit through use of fungi (Verma et al,2007). Fungi of the genus Trichoderma are important biocontrol agents (BCAs) of several soil borne phytopathogens (Benitez, et al, 2004). The secondary metabolites involvement in biocontrol has been recently reviewed (Reino,et al, 2008) Trichoderma use different mechanisms for the control of phytopathogens which include mycoparasitism, competition for space and nutrients, secretion of antibiotics and fungal cell wall degrading enzymes (Harman,et al,2004) . In addition, Trichoderma could have a stimulatory effect on plant growth (Naseby et al, 2000) as a result of modification of soil conditions. Trichoderma harzianum is an efficient biocontrol agent that is commercially produced to prevent development of several soil born pathogenic fungi. Different mechanisms have been suggested as being responsible for their biocontrol activity, which include competition for space and nutrients, secretion of chitinolytic enzymes, mycoparasitism and production of inhibitory compounds (Ghahfarokhi and Goltapeh . 2010). Arbuscular Mycorrhizal Fungi (AMF) is the major components of the rhizosphere of most plant plays an important role in decreasing plant disease incidence. Several AMF species have been found to control soil borne pathogens such as species of Aphanomyces, Cylindrocladium, Fusarium, Macrophomina, Pythium, Rhizoctonia, Sclerotiniumand Verticillium. Under green house conditions Glomus fascicuatum and Gigaspora margarita were shown to decrease root rot diseases caused by Fusarium oxysporum in asparagus and Glomus clarum was shown to decrease root necroses due to Rhictonia solani in cowpea. The AM fungus Glomus mosseae was shown to systemically reduce take-all disease infection caused by Gaeumannomyces graminis var. tritici in barley (Al-Askar and Rashad, 2010). Filamentous fungi of the genus Trichoderma have long been recognized as agents for the biocontrol of plant diseases. Trichoderma spp. can directly affect mycelia or survival of other fungi through production of toxic secondary metabolites, formation of specialized structures, and secretion of cell wall degrading enzymes (Sarrocco et al, 2006) . This mycoparasitic activity of Trichoderma spp. against phytopathogenic fungi and oomycetes due to lytic activity of cell wall-degrading enzymes has been widely studied. In addition to mycoparasitism, other mechanisms have been proposed to account for biocontrol of plant disease by Trichoderma spp., including the induction of resistance in the host plant and competition for nutrients and potential infection sites (Harman, et al., 2004). All of these mechanisms have been shown to be employed effectively by Trichoderma virens. This biocontrol agent has been recognized as an aggressive mycoparasite capable of competing ecologically when colonizing potential sites of infection. Different strains have been shown to induce phytoalexin production and systemic resistance. T. virens produces secondary metabolites, including gliotoxin, gliovirin, and peptaibols with known antimicrobial activities that have been shown to act synergistically with lytic enzymes to enhance the destruction of host cell walls(Djonovic, et al.,. 2006) .The performance of endophyte Piriformospora indica in different substrata under greenhouse and practical field conditions is studied53. Roots of winter wheat

were colonized efficiently, and biomass was particularly increased on poor substrata. In greenhouse experiments, symptoms of severity of a typical leaf (Blumeria graminis f. sp. tritici), stem base (Pseudocercosporella herpotrichoides), and root (Fusarium culmorum) pathogen was reduced significantly. However, in field experiments, symptoms caused by the leaf pathogen did not differ in Piriformospora indica-colonized compared with control plants. In the field, severity of Pseudocercosporella herpotrichoides disease was significantly reduced in plants colonized by the endophyte. Increased numbers of sheath layers and hydrogen peroxide concentrations after B. graminis attack were detected in Piriformospora indicacolonized plants, suggesting that root colonization causes induction of systemic resistance or priming of the host plant. Although the endophyte is not well suited for growth at Central European temperature conditions, it remains to be shown whether P. indica is more suitable for tropical or subtropical farming (Serfling, et al., 2007).

B. Mechanisms of interaction between bioagent- pathogen in biological control of plant diseases:

Because biological control can result from many different types of interactions between organisms, researchers have focused on characterizing the mechanisms operating in different experimental situations. In all cases, pathogens are antagonized by the presence and activities of other organisms that they encounter. Here, we assert that the different mechanisms of antagonism occur across a spectrum of directionality related to the amount of interspecies contact and specificity of the interactions (Table 1). Direct antagonism results from physical contact and/or a high-degree of selectivity for the pathogen by the mechanism(s) expressed by the biocontrol agents , BCAs (Pal and McSpadden, 2006).

The most effective BCAs studied to date appear to antagonize pathogens using multiple mechanisms. For instance, pseudomonads known to produce the antibiotic 2,4-diacetylphloroglucinol (DAPG) may also induce host defenses (Iavicoli et al. 2003). Additionally, DAPG-producers can aggressively colonize roots, a trait that might further contribute to their ability to suppress pathogen activity in the rhizosphere of wheat through competition for organic nutrients (Raaijmakers, et al., 2002).

Hyperparasites and predation:

In hyperparasitism, the pathogen is directly attacked by a specific BCA that kills it or its propagules. In general, there are four major classes of hyperparasites: obligate bacterial pathogens, hypoviruses, facultative parasites, and predators. Pasteuria penetrans is an obligate bacterial pathogen of root-knot nematodes that has been used as a BCA. Hypoviruses are hyperparasites. A classical example is the virus that infects Cryphonectria parasitica, a fungus causing chestnut blight, which causes hypovirulence, a reduction in disease-producing capacity of the pathogen. The phenomenon has controlled the chestnut blight in many places (Milgroom and Cortesi 2004). However, the interaction of virus, fungus, tree, and environment determines the success or failure of hypovirulence. There are several fungal parasites of plant pathogens, including those that attack sclerotia (e.g. Coniothyrium minitans) while others attack living hyphae (e.g. Pythium oligandrum). And, a single fungal pathogen can be attacked by multiple hyperparasites. For example, Acremonium alternatum, Acrodontium crateriforme, Ampelomyces quisqualis, Cladosporium oxysporum, and Gliocladium virens are just a few of the fungi that have the capacity to parasitize powdery mildew pathogens (Kiss 2003). Other hyperparasites attack plant-pathogenic nematodes during different stages of their life cycles (e.g. Paecilomyces lilacinus and Dactylella oviparasitica). In contrast to hyperparasitism, **microbial predation** is more general and pathogen non-specific and generally provides less predictable levels of disease control. Some BCAs exhibit predatory behavior under nutrient-limited conditions. However, such activity generally is not expressed under typical growing conditions. For example, some species of Trichoderma produce a range of enzymes that are directed against cell walls of fungi. However, when fresh bark is used in composts, Trichoderma spp. do not directly attack the plant pathogen, Rhizoctonia solani. But in decomposing bark, the concentration of readily available cellulose decreases and this activates the chitinase genes of Trichoderma spp., which in turn produce chitinase to parasitize R. solani (Benhamou and Chet 1997).

Antibiotic-mediated suppression:

Antibiotics are microbial toxins that can, at low concentrations, poison or kill other microorganisms. Most microbes produce and secrete one or more compounds with antibiotic activity. In some instances, antibiotics produced by microorganisms have been shown to be particularly effective at suppressing plant pathogens and the diseases they cause. Some examples of antibiotics reported to be involved in plant pathogen suppression are listed in Table 2. In all cases, the antibiotics have been shown to be particularly effective at suppressing growth of the target pathogen in vitro and/or in situ. To be effective, antibiotics

Table 1. Types of interspecies antagonisms leading to biological control of plant pathogens.

Type	Mechanism	Examples
Direct antagonism	Hyperparasitism/ predation	Lytic/some nonlytic mycoviruses Ampelomyces quisqualis Lysobacter enzymogenes Pasteuria penetrans Trichoderma virens
Mixed-path antagonism	Antibiotics	2,4-diacetylphloroglucinol Phenazines Cyclic lipopeptides
	Lytic enzymes	Chitinases Glucanases Proteases
	Unregulated waste products	Ammonia Carbon dioxide Hydrogen cyanide
	Physical/chemical interference	Blockage of soil pores Germination signals consumption Molecular cross-talk confuse
Indirect antagonism	Competition	Exudates/leachates consumption
	Induction of host resistance	Contact with fungal cell walls Detection of pathogen-associated, molecular patterns Phytohormone-mediated induction

must be produced in sufficient quantities near the pathogen to result in a biocontrol effect (Pal and McSpadden, 2006). In situ production of antibiotics by several different biocontrol agents has been measured (Thomashow et al. 2002); however, the effective quantities are difficult to estimate because of the small quantities produced relative to the other, less toxic, organic compounds present in the phytosphere. And while methods have been developed to ascertain when and where biocontrol agents may produce antibiotics (Notz et al. 2001), detecting expression in the infection court is difficult because of the heterogenous distribution of plant-associated microbes and the potential sites of infection. The ability to produce multiple antibiotics probably helps to suppress diverse microbial competitors, some of which are likely to be plant pathogens. The ability to produce multiple classes of antibiotics, that differentially inhibit different pathogens, is likely to enhance biological control. More recently, Pseudomonas putida WCS358r strains genetically engineered to produce phenazine and DAPG displayed improved capacities to suppress plant diseases in field-grown wheat (Glandorf et al. 2001).

Lytic enzymes and other byproducts of microbial life:

Diverse microorganisms secrete and excrete other metabolites that can interfere with pathogen growth and/or activities. Many microorganisms produce and release **lytic enzymes** that can hydrolyze a wide variety of polymeric compounds, including chitin, proteins, cellulose, hemicellulose, and DNA. Expression and secretion of these enzymes by different microbes can sometimes result in the suppression of plant pathogen activities directly.

For example, a b-1,3-glucanase contributes significantly to biocontrol activities of Lysobacter enzymogenes strain C3 (Palumbo et al. 2005). While they may stress and/or lyse cell walls of living

organisms, these enzymes generally act to decompose plant residues and nonliving organic matter. Currently, it is unclear how much of the lytic enzyme activity that can be detected in the natural environment represents specific responses to microbe-microbe interactions. It seems more likely that such activities are largely indicative of the need to degrade complex polymers in order to obtain carbon nutrition. Nonetheless, microbes that show preference for colonizing and lysing plant pathogens might be classified as biocontrol agents. Lysobacter and Myxobacteria are known to produce copious amounts of lytic enzymes, and some isolates have been shown to be effective at suppressing fungal plant pathogens (Bull et al. 2002). So, the lines between competition, hyperparasitism, and antibiosis are generally blurred.

Furthermore, some products of lytic enzyme activity may contribute to indirect disease suppression. For example, oligosaccharides derived from fungal cell walls are known to be potent inducers of plant host defenses. Interestingly, Lysobacter enzymogenes strain C3 has been shown to induce plant host resistance to disease (Kilic-Ekici and Yuen 2003), though the precise activities leading to this induction are not entirely clear. The quantitative contribution of any and all of the above compounds to disease suppression is likely to be dependent on the composition and carbon to nitrogen ratio of the soil organic matter that serves as a food source for microbial populations in the soil and rhizosphere. However, such activities can be manipulated so as to result in greater disease suppression. For example, in postharvest disease control, addition of chitosan can stimulate microbial degradation of pathogens similar to that of an applied hyperparasite (Benhamou 2004). Chitosan is a non-toxic and biodegradable polymer of beta-1,4 glucosamine produced from chitin by alkaline deacylation. Amendment of plant growth substratum with chitosan suppressed the root rot caused by Fusarium oxysporum f. sp. radicis-lycopersici in tomato (Lafontaine and Benhamou 1996). Although the exact mechanism of action of chitosan is not fully understood, it has been observed that treatment with chitosan increased resistance to pathogens.

Table 2. Some of antibiotics produced by BCAs:

Antibiotic	Source	Target pathogen	Disease	Reference
Bacillomycin D	**Bacillus subtilis AU195**	**Aspergillus flavus**	**Aflatoxin contamination**	**Moyne et al. (2001)**
Bacillomycin, fengycin	**Bacillus amyloliquefaciens FZB42**	**Fusarium oxysporum**	**Wilt**	**Koumoutsi et al. (2004)**
Xanthobaccin A	**Lysobacter sp. strain SB-K88**	**Aphanomyces cochlioides**	**Damping off**	**Islam et al. (2005)**
Gliotoxin	**Trichoderma virens**	**Rhizoctonia solani**	**Root rots**	**Wilhite et al. (2001)**
Mycosubtilin	**B. Subtilis BBG100**	**Pythium aphanidermatum**	**Damping off**	**Leclere et al. (2005)**

Competition:

From a microbial perspective, soils and living plant surfaces are frequently nutrient limited environments. To successfully colonize the phytosphere, a microbe must effectively compete for the available nutrients. On plant surfaces, host-supplied nutrients include exudates, leachates, or senesced tissue. Additionally, nutrients can be obtained from waste products of other organisms such as insects (e.g. aphid honeydew on leaf surface) and the soil. While difficult to prove directly, much indirect evidence suggests that **competition** between pathogens and non-pathogens for nutrient resources is important for limiting disease incidence and severity. In general, soilborne pathogens, such as species of Fusarium and Pythium, that infect through mycelial contact are more susceptible to competition from other soil- and plant-associated microbes than those pathogens that germinate directly on plant surfaces and infect through appressoria and infection pegs. The most abundant nonpathogenic plant-associated microbes are generally thought to protect the plant by rapid colonization and thereby exhausting the limited available substrates so that none are available for pathogens to grow. For example, effective catabolism of nutrients in the spermosphere has been identified as a mechanism contributing to the suppression of Pythium ultimum by Enterobacter cloacae (van Dijk and Nelson 2000, Kageyama and Nelson 2003).

Induction of host resistance:

Plants actively respond to a variety of environmental stimuli, including gravity, light, temperature, physical stress, water and nutrient availability. Plants also respond to a variety of chemical stimuli produced by soil- and plant-associated microbes. Such stimuli can either induce or condition plant host defenses through biochemical changes that enhance resistance against
subsequent infection by a variety of pathogens. **Induction of host defenses** can be local and/or systemic in nature, depending on the type, source, and amount of stimuli. Recently, phytopathologists have begun to characterize the determinants and pathways of induced resistance stimulated by biological control agents and other non-pathogenic microbes (Table 3).

The first of these pathways, termed systemic acquired resistance (SAR), is mediated by salicylic acid (SA), a compound which is frequently produced following pathogen infection and typically leads to the expression of pathogenesis-related (PR) proteins. These PR proteins include a variety
of enzymes some of which may act directly to lyse invading cells, reinforce cell wall boundaries to resist infections, or induce localized cell death. A second phenotype, first referred to as induced systemic resistance (ISR), is mediated by jasmonic acid (JA) and/or ethylene, which are produced following applications of some nonpathogenic rhizobacteria. Interestingly, the SA- and JA- dependent defense pathways can be mutually antagonistic, and some bacterial pathogens take advantage of this to overcome the SAR. For example, pathogenic strains of Pseudomonas
syringae produce coronatine, which is similar to JA, to overcome the SA-mediated pathway (He et al. 2004). Because the various host-resistance pathways can be activated to varying degrees by different microbes and insect feeding, it is plausible that multiple stimuli are constantly being received and processed by the plant. Thus, the magnitude and duration of host defense induction will likely vary over time. Only if induction can be controlled, i.e. by overwhelming or synergistically interacting with endogenous signals, will host resistance be increased. A number of strains of root-colonizing microbes have been identified as potential elicitors of plant host defenses. Some biocontrol strains of Pseudomonas sp. and Trichoderma sp. are known to strongly induce plant host defenses (Harman 2004). In several instances, inoculations with plant-growth-promoting rhizobacteria (PGPR) were effective in controlling multiple diseases caused by different pathogens, including anthracnose (Colletotrichum lagenarium), angular leaf spot (Pseudomonas syringae pv. lachrymans and bacterial wilt (Erwinia tracheiphila). A number of chemical elicitors of SAR and ISR may be produced by the PGPR strains upon inoculation, including salicylic acid, siderophore, lipopolysaccharides, and 2,3-butanediol, and other volatile substances Ongena et al. 2004, Ryu et al. 2004). Again, there may be multiple functions to such molecules blurring the lines between direct and indirect antagonisms. More generally, a substantial number of microbial products have been identified as elicitors of host defenses, indicating that host defenses are likely stimulated continually over the course of a plant's lifecycle. Excluding the components directly related to pathogenesis, these inducers include lipopolysaccharides and flagellin from Gram-negative bacteria; cold shock proteins of diverse bacteria; transglutaminase, elicitins, and β-glucans in Oomycetes; invertase in yeast; chitin and ergosterol in all fungi; and xylanase in Trichoderma (Numberger et al. 2004). These data suggest that plants would detect the composition of their plant-associated microbial communities and respond to changes in the abundance, types, and localization of many different signals. The importance of

such interactions is indicated by the fact that further induction of host resistance pathways, by chemical and microbiological inducers, is not always effective at improving plant health or productivity in the field (Vallad and Goodman 2004).

Table 3. Bacterial determinants and types of host resistance induced by biocontrol agents.

Bacterial strain	Plant species	Bacterial determinant	Reference
Bacillus mycoides strain Bac J	Sugar beet	Peroxidase, chitinase and β-1,3-glucanase	Bargabus et al. (2002)
Bacillus pumilus 203-6	Sugar beet	Peroxidase, chitinase and β-1,3-glucanase	Bargabus et al. (2004)
Pseudomonas putida strains	Arabidopsis	Lipopolysaccharide	Meziane et al. (2005)
Serratia marcescens 90-166	Cucumber	Siderophore	Press et al. (2001)

2. Biological control of insects:

Several researchers experimented with the use of fungi as microbial control agents in the late 19th century. However, it was not until the development of the bacterium Bacillus thuringiensis Berliner that the use of microbes for the control of insects became widespread. Today a variety of entomopathogens are used for the control of invertebrate pests in glasshouse and row crops, orchards, ornamentals, range, turf and lawn, stored products, and forestry and for the abatement of pest and vector insects of veterinary and medical importance (Lacey and Kaya, 2000). Entomopathogenic organisms used for microbial control include bacteria, viruses, fungi, protozoa, and nematodes. The comparison of entomopathogens with conventional chemical pesticides is usually solely from the perspective of their efficacy and cost. When environmental benefits including safety for humans and other non target organisms, reduction of pesticide residues in food, increased activity of most other natural enemies, and increased biodiversity in managed ecosystems are taken into account, their advantages are numerous. They also offer some distinct advantages over arthropod biocontrol agents in that most can be applied with conventional equipment and many can be produced with artificial media and stored for extended periods of time. Like arthropod natural enemies, many entomopathogens are specific to certain species or groups of insect pests and some have the potential to provide long-term control. There are also some disadvantages, mostly linked with their persistence, speed of kill, specificity (too broad or too narrow host range), and cost relative to conventional chemical insecticides. Strategies for the use of entomopathogenic organisms for insect control are basically the same as that for other biological control agents (Lacey, et al., 2001).

Bacteria as insect biological control agent

Today a number of isolates of the bacterium are commercially produced with activity against Lepidoptera, Coleoptera, and Diptera (Lacey, et al., 2001). Isolates that are active against chewing lice, plant-parasitic nematodes, and other pests have also been discovered. As of 1998 about 200 B. thuringiensis- based products were registered in the United States alone (Schnepf et al., 1998).

Most of the insecticidal activity of B. thuringiensis is associated with the proteinaceous toxins located in parasporal inclusion bodies, also known as parasporal crystals. They are produced at the time of sporulation and account for up to 30% of the total protein content of the bacterium (Agaisse and Lereclus, 1995). Collectively, the toxins found in parasporal crystals are referred to as d-endotoxins. B. thuringiensis insecticidal proteins are highly specific insect gut toxins with a superior safety record in regard to their effects on nontarget organisms (Glare and O'Callaghan, 2000; Lacey and Siegel, 2000) including vertebrates (Lacey and Siegel, 2000). Their mode of action is thought to involve a cascade of events leading to insect death within several hours following ingestion (Powell et al., 1995). Cry1 proteins, which are active primarily against larval lepidopteran pests, have been the most extensively studied B. thuringiensis insecticidal proteins with respect to their structure and mode of action (Knowles, 1994). The Cry1 proteins (protoxins) which are found in the crystal are biologically inactive. Following ingestion and solubilization in the alkaline midgut, cleavage by gut proteases produces a smaller 60- to 65-kDa activated protein that recognizes specific binding sites at the brush border membrane surface of the epithelial columnar cells lining the gut lumen. The next steps are pore formation, membrane transport disruption, and cell lysis leading ultimately to insect death (Schnepf et al. (1998).

Several strategies have been proposed for resistance management, these include the use of high dosage, seed mixtures (transgenics and nontransformed cultivars), and toxin mixtures, and the rotation or alternation of B. thuringiensis toxins (Gelernter, 1997; Schnepf et al., 1998). Plant-colonizing bacteria including Pseudomonas fluorescens Migula, P. cepacia (Burkholder) Palleroni and Holmes, Rhizobium leguminosarum Jordan, and Azosporillium spp. have also been used to produce and deliver B. thuringiensis insecticidal proteins (Udayasuryian et al., 1995; Schnepf et al., 1998). Specific delivery systems based on the hosts developing in aquatic habitats have also been proposed to control mosquito larvae (Porter et al., 1993). These include the cyanobacterium Agmellenum quadruplicatum (Stevens et al., 1994), Synechococcus sp. (Soltes- Rak et al., 1993), and Caulobacter crescentus Poindexter (Thanabalu et al., 1992).

Varieties of the bacterium are currently used for control of a broad range of crop and forestry pests and larvae of several blood-sucking pests of humans and domestic animals (Charles et al., 2000; Glare and O'Callaghan, 2000). Application of B. thuringiensis to agroecosystems and aquatic environments allows survival of beneficial insects and natural enemies of targeted insects. In agroecosystems it is used against several species of lepidopteran, coleopteran, and some dipteran pests in food and fiber crops. Its use in forestry has increased relative to other interventions, including chemical pesticides (Evans, 1997). B. thuringiensis subsp. israelensis de Barjac (Bti) is used exclusively or in combination with other interventions for the control of larvae of dozens of species of medically important and pestiferous black flies and mosquitoes around the world (Skovmand et al., 2000). Other species of bacteria are used on a much smaller scale for insect control. These include Paenibacillus (5Bacillus) popilliae (Dutky) and related species and Serratia entomophila Grimont for control of white grubs (Scarabaeidae) and Bacillus sphaericus Neide for control of mosquito larvae (Lacey et al., 2001)

Fungi as insect biological control agent

Some 700 species of entomopathogenic fungi have been reported, but only 10 of these have been or are currently being developed for insect control (Hajek et al., 2000). A broad range of obligate parasitism to opportunistic pathogens that can survive saprophytically in the absence of living hosts, have been documented for the entomopathogenic fungi. In most species of entomopathogenic fungi, access to the host is through the cuticle and may involve complex biochemical interactions between the host and the fungus before germination, penetration, growth, and reproduction of the fungus can occur (Lacy et al., 2001). Fungi Imperfecti (Deuteromycotina: Hyphomycetes), on the other hand, have simpler life cycles and lack sexual reproduction, and many have considerably broader insect host ranges. Many entomopathogenic fungi, especially those in the Entomophthorales, are responsible for epizootics that often successfully regulate pest insect populations. Several species offer good potential for production on inexpensive artificial media and have good shelf lives. Entomopathogenic Hyphomycetes have been investigated for use against a broad range of insect pests, including whiteflies, aphids, thrips, termites, grasshoppers and locusts, beetles, and others (Lacey et al., 1996; Keller et al., 1997; Goettel et al., 2000). Commercial products based on Beauveria bassiana (Balsamo) Vuillemin, Metarhizium anisopliae (Metschnikoff) Sorokin, Verticillium lecanii (Zimmermann) Viegas, and Paecilomyces fumosoroseus (Wize) Brown and Smith and experimental isolates of Metarhizium flavoviride Gams and Rozsypal, Nomuraea rileyi (Farlow) Samson, and Aschersonia aleyrodis Webber are currently in use or under

development. Several commercial sources of entomopathogenic Hyphomycetes are listed (Lacey,et al., 2001). In conjunction with inundative applications, the endophytic nature of B. bassiana in corn offers the potential of season-long control of Ostrinia nubilalis (Hu¨ bner) and has a suppressing effect on overwintering larvae (Anderson and Lewis, 1991). Despite their somewhat broader host range, the Hyphomycetes still provide a degree of selectivity (Goettel et al., 1990). A complex set of interacting processes, both environmental and biotic, is necessary for or inhibitory to development of epizootics caused by entomopathogenic fungi. These include sensitivity to solar radiation; microbial antagonists; host behavior, physiological condition, and age; pathogen vigor and age; presence of pesticides; and appropriate temperature, humidity, and inoculum thresholds (Lacey et al., 2001). To take full advantage of the epizootic potential of fungi we need to understand not only the determinants that are critical for fungal virulence and infection but also the techniques to exert control over them through optimization of culture methods, formulation, environmental manipulation, and genetic engineering. Successful use of entomopathogenic fungi as microbial control agents will ultimately depend on the use of the right propagule, formulated in an optimal manner and applied at an appropriate dosage and time. Timing will depend on the presence of susceptible host stages, favorable environmental conditions (Lacey and Shapiro, 2003. Quite a number of insects can be controlled with fungi , these include the cabbage loopers (photo2) in which the body cavity becomes overwhelmed with spores (photo 2), images from 1-12 were provided from Angelfire website**.** One of the most puzzling problems in insect control has been the **control of mosquitoes**. Mosquitoes have long been of concern to people because their bites are painful and they transmit some of our most important diseases. Coelomomyces and Culicinomyces are known to affect mosquito populations, and have been studied extensively. There are, however, many other fungi that infect and kill mosquitoes at the larval and/or adult stage. The discovery, in 1977, of the selective mosquito-pathogenic bacterium Bacillus thuringiensis Berliner israelensis (Bti) curtailed widespread interest in the search for other suitable biological control agents. In recent years interest in mosquito-killing fungi is reviving, mainly due to continuous and increasing levels of insecticide resistance and increasing global risk of mosquito-borne diseases. The potential of many fungi as mosquito control agents, only a handful have been commercialized and are marketed for use in abatement programs. We argue that entomopathogenic fungi, both new and existing ones with renewed/improved efficacies may contribute to an expansion of the limited arsenal of effective mosquito control tools, and that they may contribute in a significant and sustainable manner to the control of vector-borne diseases such as malaria, dengue and filariasis (Ernst-Jan Scholte, et al.,2004).

Baculoviruses as insect biological control agent

The information on the potential of viruses as microbial control agents is somewhat deficient. More than 400 insect species, mostly in the Lepidoptera and Hymenoptera, have been reported as hosts for baculoviruses (Sun and Peng, 2007). Baculoviruses occur widely among Lepidoptera, and in some species of forest and agricultural insects, they cause epizootics in outbreak populations, their mode of action, epizootiology, and use for control of pest insects in forestry and agroecosystems were summarized by Vail et al. 1999 & Jenny and Judith, 2003).

The baculovirus virions are enveloped rod-shaped nucleocapsids containing circular, supercoiled, doublestranded DNA. The virions of GVs are individually occluded in a protein matrix (granulin). In the NPVs, singly enveloped (SNPV) or multiply enveloped (MNPV) virions are occluded in a protein matrix (polyhedrin). After ingestion by the host, the occlusion bodies, or polyhedra, are dissolved in the alkaline environment of the host insect's midgut. The liberated virions enter the gut epithelial cells and replicate in the nuclei. Nonoccluded virus particles that are budded from the gut cells into the hemocoel invade other tissues (fat body, tracheal matrix, hypodermis, etc.) within the host. Virus particles that are occluded within polyhedra are generally the infective inoculum for subsequent hosts. Some transmission of baculovirus virions may be facilitated by predators and ovipositing parasitoids via mechanical transmission (Jehle et al., 2006)

Photo: 2. The control of cabbage loopers with the fungus Noumorea rileyi.

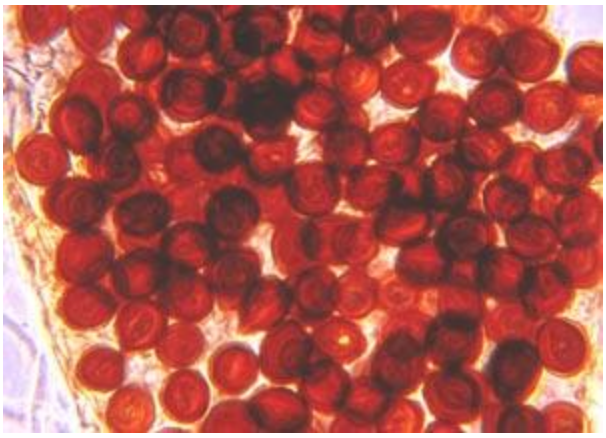

Photo: 3. Insect cavity filled with fungal spores.

Photo: 4. An insect larva infected with a species of Cordyceps.

Photo: 5. Insect larvae with several perithecial stroma.

species of Cordyceps that infects the larvae of many beetles and moths (photo 4), even those deeply embedded in the soil (photo 5);

Photo: 6. A weevil highly infected with Stilbella.

Photo: 7. Colonies of Paecilomyces variotii growing out of a beetle larva.

species of Zoophora on flies; Stilbella on weevils (photo 6), Hirsutella on the larva of a citrus mite, Paecilomyces on beetle larvae (photo 7), and species of Beauvaria (photo 8) that will infect a large number of insects.

Photo: 8. Beauvaria bassiana infecting a weevils.

Photo: 9. Noumorea rileyi infecting a soybean looper.

In Florida, species of Aschersonia commonly infects citrus white flies and Noumorea on soybean looper (photo:9).

Photo: 10. A beetle larva infected with a species of Metarrhizium.

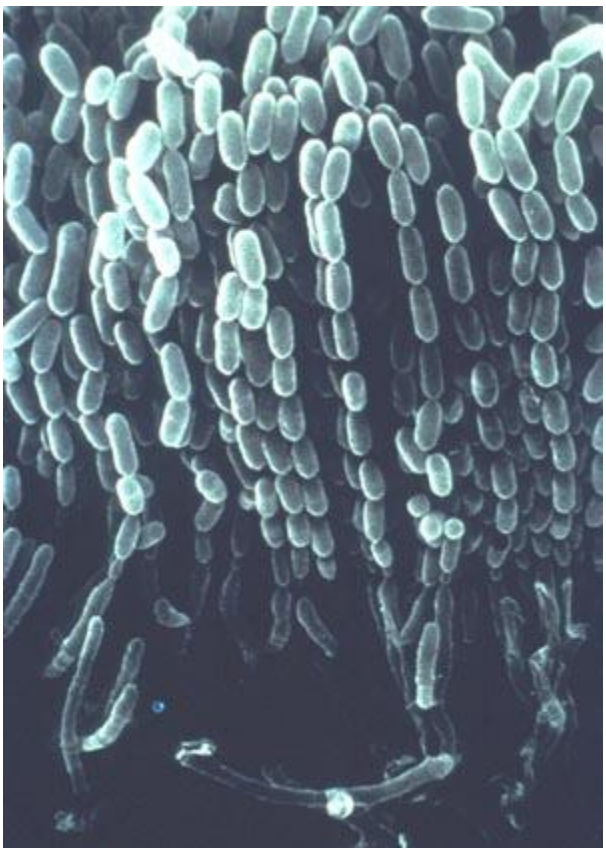

Photo: 11. Chains of conidia characteristic of Metarrhizium

Species of Metarrhizium infects a number of insects (photo 10), forming long chains of spores (photo 11).

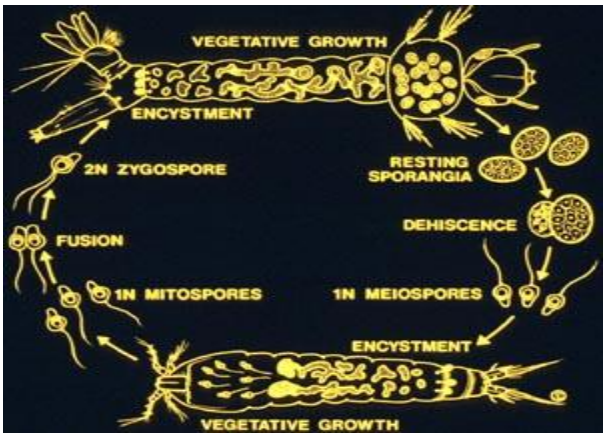

Photo: 12. The life cycle of Coelomomyces psorophora in mosquitoes (top) and copepods (below).

The efficacy, specificity, and production of secondary inoculum make baculoviruses attractive alternatives to broad-spectrum insecticides and ideal components due to their lack of untoward effects on beneficial insects including other biological control organism (Sun and Peng, 2007).

Some of the drawbacks of the use of entomopathogenic viruses are their relatively slow action compared to that of chemical insecticides, sensitivity to UV light, and the requirement for living systems for production. During the time following initial infection, insects continue to feed until the latter stages of infection. Fortunately, the genomes of AcMNPV and other baculoviruses are amenable to genetic manipulation and improvement with recombinant technology (Inceoglu et al. 2006).

In developing countries, where the cost of imported insecticides is high and that of labor is lower, in vivo production could provide both a viable means of producing large quantities of virus and a source of employment. The use of baculoviruses for insect control is expected to increase in the coming years, particularly in developing countries and for the control of insects in high-value crops grown on small acreages (Lacey, et al., 2001)**.**

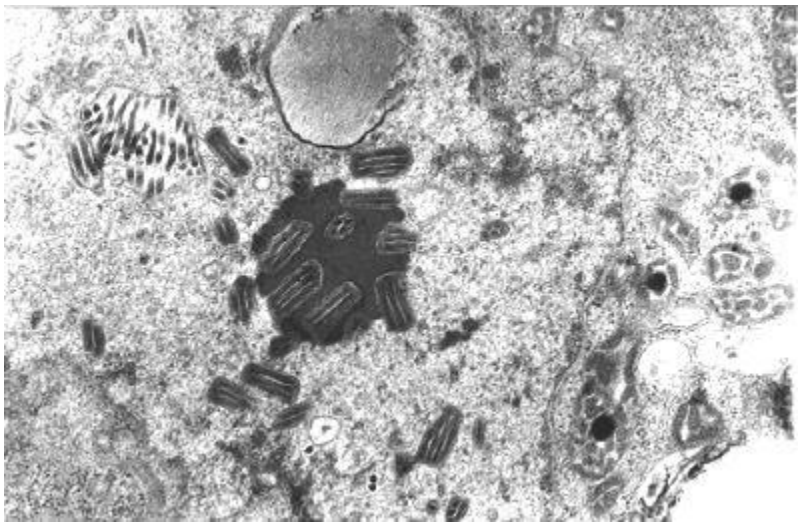

Photo.13. This high magnification electron micrograph shows a thin section of an insect cell infected with the baculovirus AcMNPV. A portion of the nucleus containing enveloped virions, also referred to as occlusion derived virions

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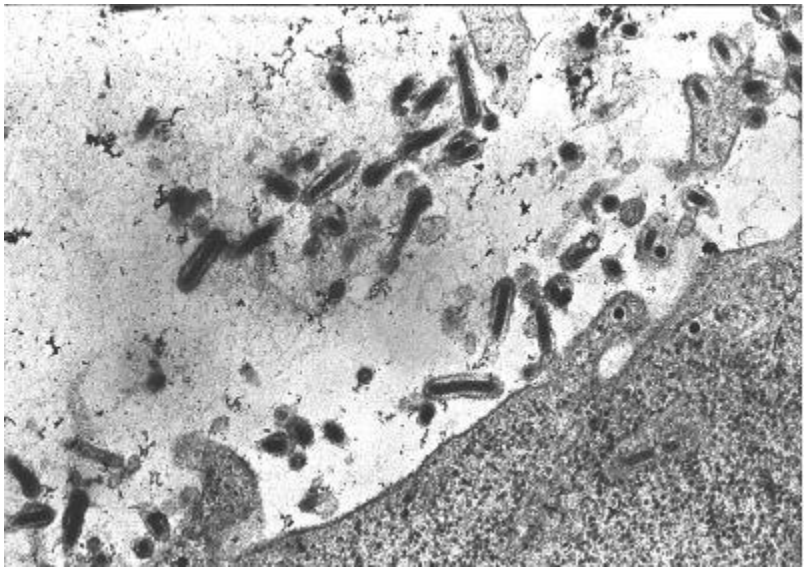

Photo 14. This electron micrograph shows a thin section of an insect cell infected with the baculovirus AcMNPV. A portion of the cell cytoplasm is seen in the bottom right hand corner. Many enveloped extracellular virions (also referred to as budded virions - BV) have budded through the cytoplasmic membrane and are visible outside the cell.

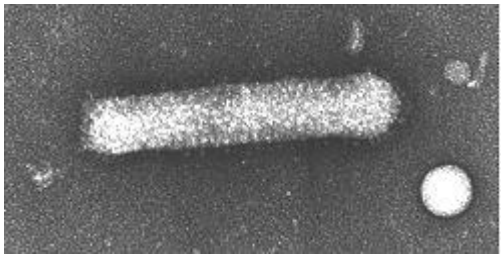

Photo.15. This high magnification electron micrograph shows a negatively-stained baculovirus virion (BV) (approximately 60 x 330 nm). Note the asymetric capsid structure and the presence of an envelope with surface projections (peplomers), images 13,14,15 were provided from (http:/ /meds. queensu. Ca/ carstens/ baculovirus / baculovirus.html).

3. Biological control of Nematodes:

a. Biological control of plant parasitic nematodes:

Nematodes in soil are subject to infections by bacteria and fungi. This creates the possibility of using soil microorganisms to control plant-parasitic nematodes, bacteria are numerically the most abundant organisms in soil, and some of them, for example members of the genera Pasteuria, Pseudomonas and Bacillus (Meyer, 2003), have shown great potential for the biological control of nematodes. Extensive investigations have been conducted over the last twenty years to assess their potential to control plant-parasitic nematodes. These research efforts have found that nematophagous bacteria are distributed broadly, possess diverse modes of action, and have broad host ranges. A variety of nematophagous bacterial groups have been isolated from soil, host-plant tissues, and nematodes and their eggs and cysts (Kerry, 2000; Meyer, 2003). They affect nematodes by a variety of modes: for example parasitizing; producing toxins, antibiotics, or enzymes; interfering with nematode– plant-host recognition; competing for nutrients; inducing systemic resistance of plants; and promoting plant health (Siddiqui & Mahmood, 1999). These bacteria have a wide range of suppressive activities on different nematode species, including free-living and predatory nematodes as well as animal- and plant-parasitic nematodes (Siddiqui & Mahmood, 1999). They form a network with complex interactions among bacteria, nematodes, plants and the environment to control populations of plant-parasitic nematodes in natural conditions (Kerry, 2000).

Nematophagous bacteria and their modes of action against nematodes

- Parasitic bacteria -- Pasteuria

Members of the genus Pasteuria are obligate, mycelial, endospore-forming bacterial parasites of plant-parasitic nematodes (Bekal et al., 2001). A number of bacterial species in this genus have shown great potential as biocontrol agents against plant parasitic nematodes. They occur worldwide and have been reported from at least 51 countries (Siddiqui & Mahmood, 1999). Members of the genus have been reported to infect 323 nematode species belonging to 116 genera, including both plant-parasitic nematodes and free-living nematodes (Chen & Dickson, 1998). Attachment of the spores to the nematode cuticle is the first step in the infection process (Davies et al., 2000). However, spores of individual Pasteuria populations do not adhere to or recognize all species of nematode. The spores of each Pasteuria species usually have a narrow host range. For example, Pas. penetrans infects Meloidogyne spp., Pas. Thornei infects Pratylenchus spp., and Pas. nishizawae infects the genera Heterodera and Globodera (Gives et al., 1999; Atibalentja et al., 2000).

Opportunistic parasitic bacteria

In fact, most nematophagous bacteria, except for obligate parasitic bacteria, usually live a saprophytic life, targeting nematodes as one possible nutrient resource. They are, however, also able to penetrate the cuticle barrier to infect and kill a nematode host in some conditions. They are described as opportunistic parasitic

As a pathogen, Br. Laterosporus has been demonstrated to have a very wide spectrum of biological activities. So far, it has been reported that four nematode species (three parasitic nematodes, namely Heterodera glycines, Trichostrongylus colubriformis and Bursaphelenchus xylophilus, and thesaprophytic nematode Panagrellus redivius) could be killed by various B. laterosporus isolates (Oliveira et al., 2004; Huang et al., 2005).

After attaching to the epidermis of the host body, Br. laterosporus can propagate rapidly and form a single clone in the epidermis of the nematode cuticle. The growth of a clone can result in a circular hole shaped by the continuous degradation and digestion of host cuticle and tissue (Fig. 16). Finally, bacteria enter the body of the host, and digest all the host tissue as nutrients for pathogenic growth (Huang et al., 2005). During bacterial infection, the degradation of all the nematode cuticle components around the holes suggests the involvement of hydrolytic enzymes (Decraemer et al., 2003; Huang et al., 2005). Histopathological observations and molecular biological analyses have demonstrated that major pathogenic activity could be attributed to an extracellular alkaline serine protease, designated BLG4 (Huang et al., 2005; Tian et al., 2007).

Rhizobacteria

Rhizobacteria have also been studied for the biological control of plant-parasitic nematodes, aerobic endospore-forming bacteria (AEFB) (mainly Bacillus spp.) and Pseudomonas spp. are among the dominant populations in the rhizosphere that are able to antagonize nematodes ((Baoyu, et al.,2007).). Numerous Bacillus strains can suppress pests and pathogens of plants and promote plant growth. Some

species are pathogens of nematodes (Li et al., 2005). The most thoroughly studied is probably Ba. subtilis (Lin et al., 2001; Siddiqui, 2002). In addition, a number of studies have reported direct antagonism by other Bacillus spp. towards plant-parasitic nematode species belonging to the genera Meloidogyne, Heterodera and Rotylenchulus (Meyer, 2003 and Li et al., 2005). Rhizosphere Pseudomonas strains also exhibit diverse pathogenic mechanisms upon interaction with nematodes (Andreogloua et al., 2003; Siddiqui et al., 2005). The mechanisms employed by some Pseudomonas strains to reduce the plant parasitic nematode population have been studied. These mechanisms include the production of antibiotics and the induction of systemic resistance (Siddiqui & Shaukat, 2002, 2003). Other rhizobacteria reported to show antagonistic effects against nematodes include members of the genera Actinomycetes, Agrobacterium, Arthrobacter, Alcaligenes, Aureobacterium, Azotobacter, Beijerinckia, Burkholderia, Chromobacterium Clavibacter, Clostridium, Comamonas, Corynebacterium, Curtobacterium, Desulforibtio, Enterobacter, Flavobacterium, Gluconobacter, Hydrogenophaga, Klebsiella, Methylobacterium, Phyllobacterium, Phingobacterium, Rhizobium, Serratia, Stenotrotrophomonas and Variovorax (Baoyu, et al.,2007).

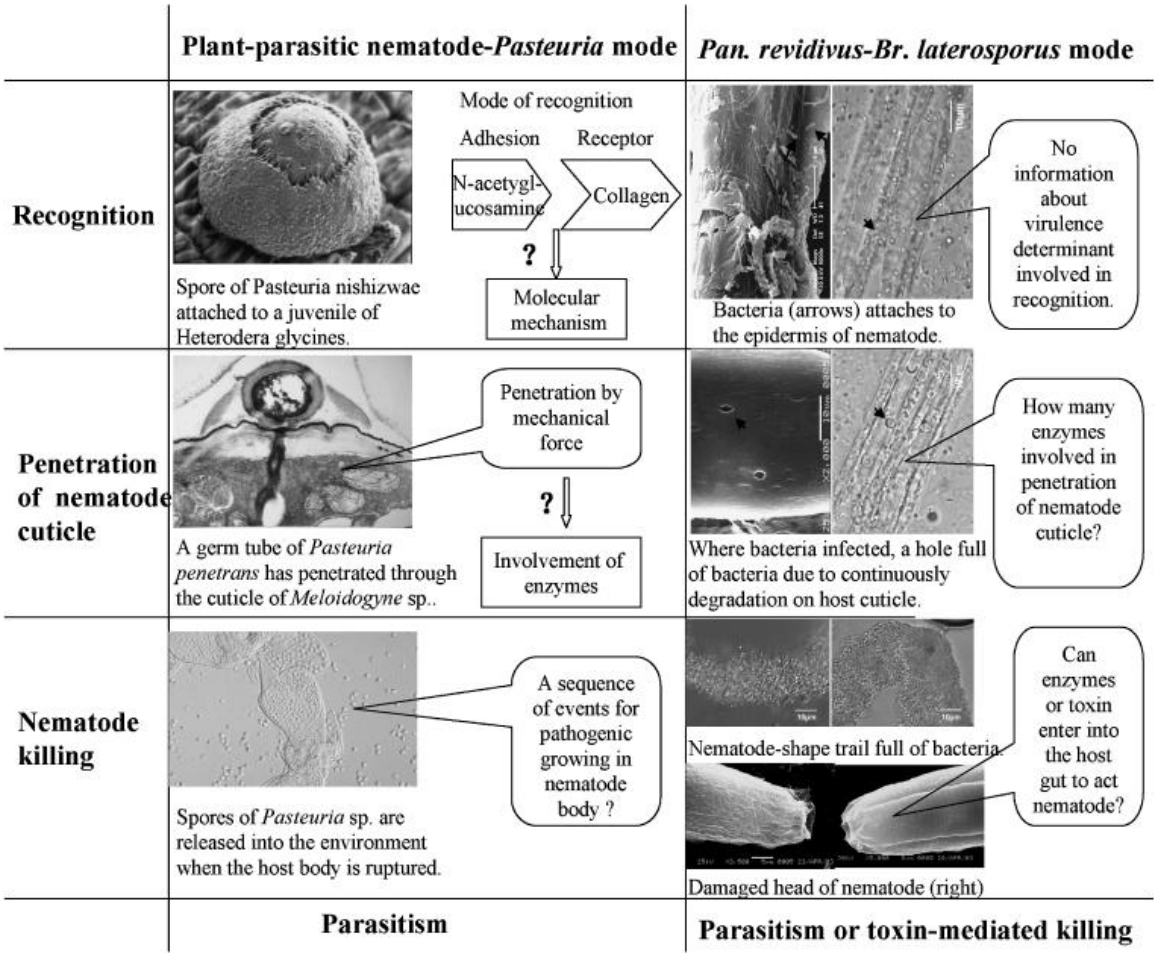

photo. 16. Pathogenic mechanisms of typical bacterium–nematode interaction models (Meloidogyne incognita–Pasteuria penetrans; Panagrellusredivius–Brevibacillus laterosporus) (Morton et al., 2004; Huang et al., 2005).

protein-forming bacteria

Bacillus thuringiensis (Bt) produces one or more parasporal crystal inclusions (d-endotoxins), which are known to be toxic to a wide range of insect species in the orders Lepidoptera (butterflies and moths), Diptera (flies and mosquitoes), Coleoptera (beetles and weevils) and Hymenoptera (wasps and bees), (Maagd et al., 2001). Some Cry proteins are also toxic to other invertebrates such as nematodes, mites and

protozoans (Feitelson et al., 1992). To date, there are six Cry proteins (Cry5, Cry6, Cry12, Cry13, Cry14, Cry21) known to be toxic to larvae of a number of free-living or parasitic nematodes (Wei et al., 2003; Kotze et al., 2005).

Nematophagous fungi and their modes of action against nematodes:

Since long, scientists have been using nematode-trapping fungi for the control of plant parasitic nematodes (Kumar and Singh, 2010;Kumar, 2007; Singh et al., 2007;). Furthermore, it has been observed that the application of organic manures in combination with nematode-trapping stimulates the bioefficacy of these nematode-trapping fungi and consequently, lower the population of root-knot nematodes. (Kumar and Singh, 2006, 2010; Kumar, 2007; Wachira et al., 2009). However, the fundamental mechanisms behind above facts were not cleared at that time and are not fully explained today. Dactylaria brochopaga is a nematophagous fungus, which dramatically captures and kills saprophytic and parasitic nematodes in vivo and in vitro by producing three celled trapping rings. D. brochopaga is a common fungus in agricultural soils, decaying plant materials and old decayed root-galls (Kumar et al., 2010; Kumar and Singh, 2010; ; Singh et al., 2007; Saadabi, 2006;). The bioefficacy of this fungus in reducing the population of M. graminicola was described by Singh et al. (2004) and recently, in reducing the population of M. incognita by Kumar and Singh (2010). The use of fungi for biocontrol of nematodes, **myconematocide**, has been limited. Some success has been experienced, however, with infesting seedlings or soil with species of a nematophagous fungus, Dactyella. Species of Dactyella and Arthrobotrys are well known as **nematode trapping** fungi. They have peculiar nets **(photo.17)**, constricting rings **(photo. 18)**, knobs **(photo. 19)** that trap nematodes. Once the hyphae trap a nematode, it will invade the body cavity, resulting in death. Aquatic nematodes are susceptible to attack by species of the aquatic oomycete Lagenidium (Angelfire website).

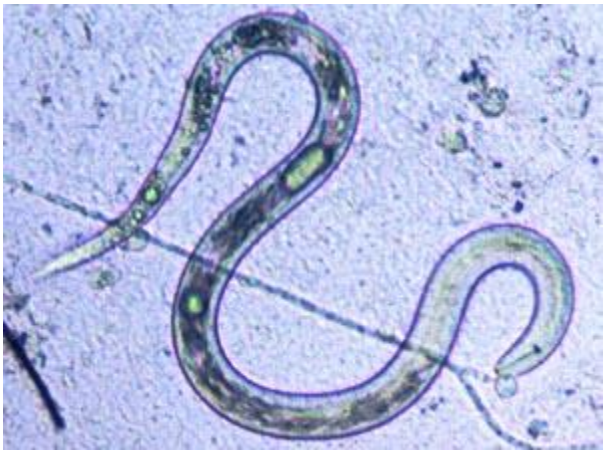

photo. 17. The nematophagus fungus Arthrobotrys candida..

Photo. 18. Formation of a net-like trap by Arthrobotrys oligospora.

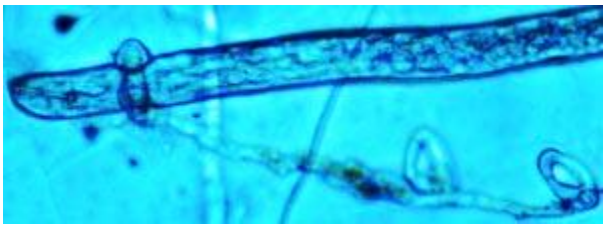

Photo. 19. Arthrobotrys brocophaga developing constricting ring traps.

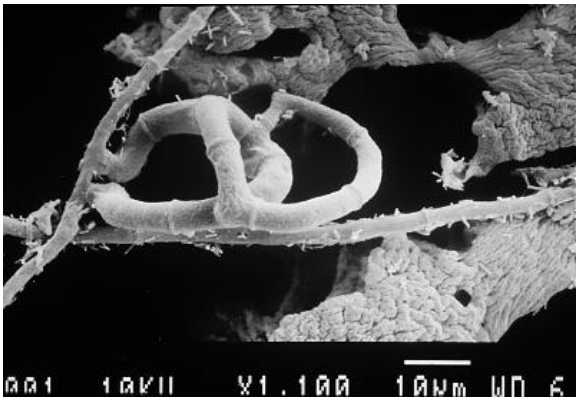

Photo .20. Fungal species which trap nematodes do so with adhesive areas along their vegetative hyphae, or with trapping devices which grow along these hyphae and snare nematodes in rings or net-like devices (Thomas and Alexandra, 1999)

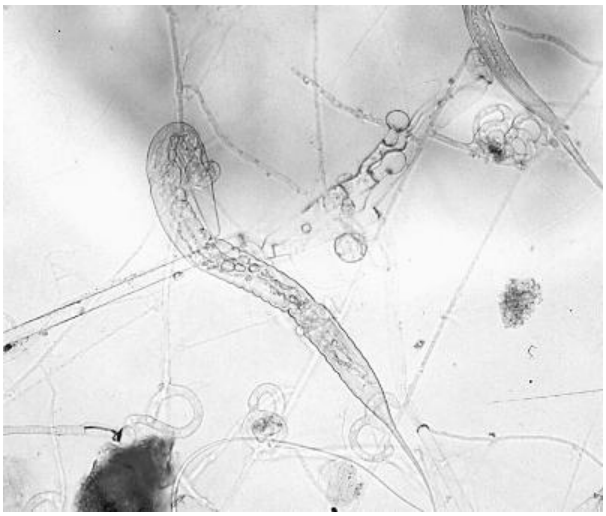

Photo. 21.Nematodes are trapped, fungal hyphae penetrate the worms cuticle and grow within the nematode(Thomas and Alexandra, 1999) .

b. Biological control of animal parasitic nematodes

The philosophy behind biological control is to utilise one or more of the natural enemies of the nematodes, making it possible to reduce the infection on pasture to a level where grazing animals can avoid both clinical and subclinical effects of the parasitic nematodes. The important requirement is the presence of the fungi in the faecal pats where the development of the pre-parasitic larvae takes place. Therefore, to be effective, the fungi should pass through the gastrointestinal tract of the host without loss of viability. The fungi, Duddingtonia flagrans and Verticillium chlamydosporium, which can be isolated from organic environment of India produces thick walled chlamydospores, the stage responsible for their survival during passage through the gut of ruminants following oral administration. The results had indicated survival of the fungus during gastrointestinal transit in grazing animals and successful reduction of numbers of parasitic nematode larvae on pasture. The dose of fungal spores to be given to an animal and the time of administration for effective parasite control has been standardized. The fungus behaves in density dependent manner and appears to be environment-friendly. The challenge lies ahead in its field application (De and Sanyal. 2009). Fungi that exhibit anti-nematode properties have been known for a long time. They consist of a great variety of species characterized by their ability to capture and exploit nematodes either as the main source of nutrients or supplementary to a saprophytic existence. They are divided into three major groups based on their morphology and types of nematode-destroying apparatus (Barron, 1997).

Predacious Fungi They produce specialized nematode-trapping structures (adhesive knobs, networks, rings etc.) on the mycelium, the trapping activity of the fungus was influenced by the motility of the infective larvae & there is no specificity for the parasitic species (Nansen et al. 1996). Unfortunately various trials performed to test A. oligospora mycelium and conidia failed due to the destruction of these structures in the GI tract of the host animals. A high dose (between 470 & 680 gm of fungal material on millet) of one of the three different fungal species (A. musiformis, A. tortur, Dactylaria candida) was fed to housed lambs, harboring a mono infection of either H. contortus or O. circumcincta. This subsequently led to survival of A. tortur through the GI tract at a level high enough to significantly reduce the number of H. contortus in faecal cultures (Gronvold et al. 1993). The other line of research is with Duddingtonia flagrans. This predacious fungus produces three dimensional, sticky networks on its growing hyphae. It also produces an abundance of intercalary thick walled resting spores, chlamydospores. This fungus is relatively slow growing and as with other predacious fungi growth is strongly influenced by temperature (Fernandez et al. 1999). Many other species of predacious fungi are fast growing but the spores of these fungi are much more sensitive to the stress of the GI tract than that of the chlamydospores of D. flagrans. In plot trials D. flagrans have shown good reduction of free living larval stages of parasitic nematodes of horses .These field trials showed that daily feeding of fungal spores to grazing animals for 3-4 months prevents build-up of dangerous levels of infective larvae on the pasture (De and Sanyal. 2009). In a world in which sheep producers are facing increasing problems due to the rapid spread of anthelmintic resistance, the battle against gastrointestinal parasitic nematodes is a difficult one. One of the potential new tools for integrated control strategies is biological control by means of the nematode-destroying microfungus Duddingtonia flagrans. This fungus forms sticky traps that catch developing larval stages of parasitic nematodes in the fecal environment. When resting spores (chlamydospores) of this fungus are fed daily to grazing animals for a period of time, the pasture infectivity and thus, the worm burden of grazing animals are lowered, especially in young lambs (Larsen , 2006). In an Australian study Knox and Faedo (2001) found that sheep feed supplement containing D. flagrans chlamydospores had lower egg counts and improved live weight gains compared to untreated animals.

4. Biological control of human diseases (microbial human pthogens):

Biological control of food borne pathogens:

Currently our work is focused on the use of bacteriophages to control the pathogens salmonella, Escherichia coli O157:H7, Campylobacter jejuni, Listeria monocytogenes and Bacillus cereus (Hudson etal., 2006; 2009 and Bigwood et al., 2009) . Food borne Salmonella infections are a major public health

concern worldwide. Bacteriophages offer highly specific and effective biocontrol of such pathogens. We evaluated the broad host range, virulent phage FO1-E2 for reduction of Salmonella Typhimurium in different RTE foods.. Phage particles retained their infectivity, although they were readily immobilized by the food matrix, resulting in loss of their ability to diffuse and infect target cells. At the end of the incubation period, phage-resistant Salmonella strains appeared which, however, were not able to compensate for the initial killing effect. Altogether, our data show that virulent phages such as FO1-E2 offer an effective biocontrol measure for Salmonella in foods (Susanne Guenther et al., 2012).

Table 4 .Examples of chronic gut diseases thought to be related to micro-organisms and their activities (Glenn, 2002).

Name of disease	Comments
Ulcerative colitis (UC)	Confined to the colon, where most microbial activity in the body occurs. An example of an inflammatory bowel disease. UC cannot be induced in animal models lacking a gut flora. Purported aetiological link with sulphate-reducing bacteria which produce toxic sulfides and have the ability to invade colonocytes
Crohn's disease (CD)	Another form of inflammatory bowel disease. Can affect any area of the gastrointestinal tract from mouth to anus.Microbial involvement is less convincing than for UC, but mycobacteria have been implicated in CD
Bowel cancer	Second most common form of cancer in the West, responsible for 1 in 5 fatalities in the USA. Certain components of the gut flora can produce known carcinogens, e.g. nitrosamines, heterocyclic amines
Irritable bowel syndrome	Estimated to affect 20 % of the UK population. Related to stress but also to gut 'dysfunction'. Often occurs after antibiotic intake and has been linked to excessive carriage of Candida spp.
Pseudomembranous colitis	Caused by the proliferation of Clostridium difficile within the flora. Invariably occurs after exposure to antibiotics, intestinalis (PCI) whereby the normal suppressant effect of gut bacteria against C. difficile is compromised
Pneumatosis cystoides	Characterized by gas-filled cysts in the bowel lining. PCI is thought to be due to a flaw in the metabolism of gasintestinalis produced during the normal fermentation process.
Type B gastritis; Peptic ulcer; Stomach carcinoma	All believed to be linked with the carriage of Helicobacter pylori, a common gastric isolate.

Biological control of gut microbial flora:

By definition, probiotics are live human bacteria when fed in either pills, tablets, or foods that benefit the host. The clinical study on the use of a probiotic therapy in ulcerative colitis was reported by (**Floch, 2010)**

This microflora plays an important role in the digestive process and, without its activities, life would be extremely uncomfortable, if not impossible. The typical 'function' of the large intestine is often thought to be water absorption and the storage, then excretion, of waste material. However, because of the metabolic capacity of the gut flora (which ferments about 100 g of food each day), the hindgut is probably the most

active organ in the body. It has a significant impact on health and well-being. Usually, we live in close harmony with these bacteria, but sometimes the process can go wrong. For example, chronic gut diseases can arise if pathogens in the gut flora begin to grow at high levels. However, some species are beneficial because they can repress the activities of the harmful types. This has led to the development of foods that serve to increase numbers of the latter. **Probiotics** include live micro-organisms in the food, while **prebiotics** are carbohydrates which have selective effects that enhance the growth of the 'beneficial' flora already in the gut. Everyone probably has probiotic microbes within their gut flora, mostly in the large intestine. The most common types are Lactobacillus or Bifidobacterium species, although other lactic-acid-excreting bacteria are also thought to be useful. Apart from in the breast-fed infant (whose gut flora is dominated by bifidobacteria), indigenous probiotics are probably not present at sufficiently high levels. Hence, diet can be used to boost natural populations. One good analogy is the higher incidence of infection seen in bottlefed compared to breast-fed infants, the former having lower probiotic numbers in the gut. **Steer, et al., 2000).**

5. Biological control of viruses:

Most viruses are restricted to a particular type of host. Some infect bacteria, and are known as bacteriophages, whereas others are known that infect algae, protozoa, fungi (mycoviruses), invertebrates, vertebrates or vascular plants. However, some viruses that are transmitted between vertebrate or plant hosts by feeding insects (vectors) can replicate within both their host and their vector. Viruses cause many diseases of international importance. Amongst the human viruses, smallpox, polio, influenza, hepatitis, human immunodeficiency virus (HIV-AIDS), measles and the SARS corona virus are particularly well known. While antibiotics can be very effective against diseases caused by bacteria, these treatments are ineffective against viruses and most control measures rely on vaccines (antibodies raised against some component of the virus) or relief of the symptoms to encourage the body's own defense system (Hans, 1996). Viruses also cause many important plant diseases and are responsible for huge losses in crop production and quality in all parts of the world. Infected plants may show a range of symptoms depending on the disease but often there is leaf yellowing (either of the whole leaf or in a pattern of stripes or blotches), leaf distortion (e.g. curling) and/or other growth distortions (e.g. stunting of the whole plant, abnormalities in flower or fruit formation), Peypelut, et al., (2004) and Krause-Sakate, et al., (2002 & 2004). Plant viruses cannot be directly controlled by chemical application. The major means of control (depending on the disease) include: Chemical or biological control of the vector (the organism transmitting the disease, often an insect). Most plant viruses are therefore transmitted by a vector organism (insects, nematodes & mites) that feeds on the plant or (in some diseases) are introduced through wounds made (Medina,et al., 1998; Nault, 1997 and Martelli et al.,(2000). Cucumber mosaic virus (CMV) occurs naturally on very wide range of plant species, including cultivated crops (e.g. tomato, pepper, cucurbits) and weeds, which serve as reservoirs of infection for banana. Therefore management of CMV infection banana, regardless of which virus strain is involved, is based primarily on eliminating or reducing external sources of infection (i.e. weeds) and secondarily on controlling aphid vector populations. In the majority of situations effective control of CMV can be achieved by weed control to eliminate adventitious plant species which serve as reservoirs for both virus and aphid vectors, Geering et al., (2001) and Hu et al., (1995)..

Photo(22):Yellow vein-banding symptoms on grapevine caused by Grapevine fanleaf virus.

Fruit distortion on eggplant fruit caused by Tomato bushy stunt virus. A healthy fruit is shown on the left.

photo (23): shows the green peach aphid Myzus persicae, the vector of many plant viruses, including Potato virus Y.

Photo.24 :Micrutalis malleifera, the treehopper vector of Tomato pseudo-curly top virus.

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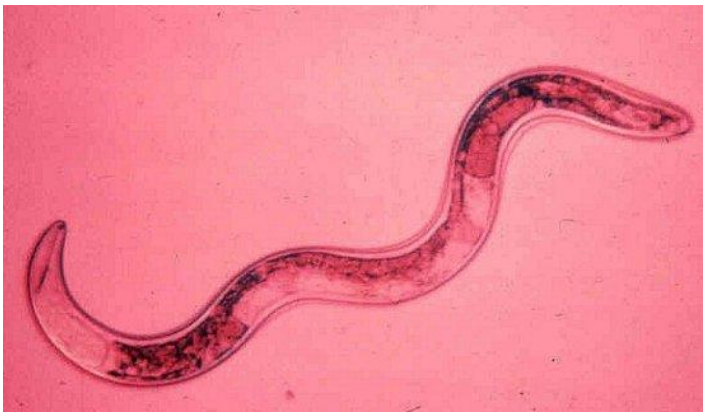

Photo (25): an adult female of Paratrichodorus pachydermus, the vector of Tobacco rattle virus

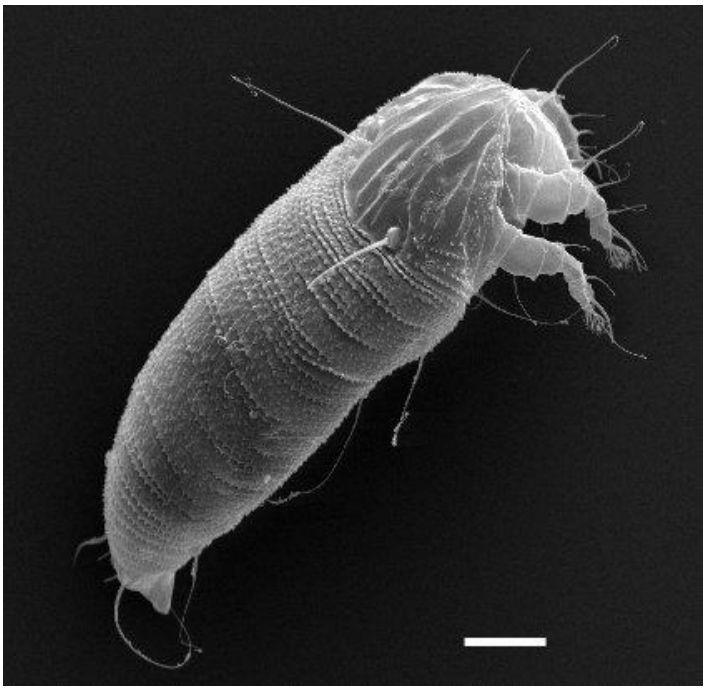

Photo (26):Aceria tosichella, the vector of Wheat streak mosaic virus

6. Biological control of Weeds and Noxious Plants:

There are about 30,000 species of plants that are considered to be weeds and are directly or indirectly noxious to humans and their domesticated animals. About 1,600 of these can cause serious crop losses, with many crops having several competing weeds and costing close to $14 billion dollars in losses and control of these pests. In order to control weeds and prevent these losses, agriculturalists have turned increasingly to the use of herbicides. As we are well aware many of the herbicides have gotten into our drinking water and lakes and have the potential of causing serious health problems (Greathead, 2000; Boyetchko et. al. (2009) and Smith et al., (2009).

Traditional agricultural practices have generally focused on herbicides, mechanical, and cultural methods as the main tools for weed management. Although these methods have served crop production well, it is important to recognize that there are scientists around the world, testing the potential of using living organisms, like insects, fungi, and bacteria, as biological control agents for weed management. Awareness of the need for increased environmental stewardship, combined with the expanding organic food industry, is stimulating the need for new technologies to assist with weed control. This article showed a novel approach offered by living organisms as agents for biological weed control and described how this weed management tool is evolving as an alternative to herbicides, Boyetchko, et al., (2009).

Deleterious rhizosphere inhabiting bacteria (DRB) have potential to suppress plant growth. This study focused on the isolation of DRB with potential for development as commercial products for weed control (Flores-Vargas and, 2006). Also, Bailey et al. (2000) showed that, further evaluations with fungal (Colletotrichum, and Fusarium isolates) and bacterial agents Pseudomonas syringae pv. Tagetis for biological control of Canada thistle included delivery and application of inoculum to roots and/or shoots to facilitate entry, infection and efficacy (i.e. inoculum level, placement, deposition, water volume) and the evaluation of suitable formulations (e.g. granules, surfactants).

Until recently, phytopathogenic bacteria have not been considered potential biological weed control candidates because they lack the ability to penetrate intact plants. This deficiency can be overcome by providing entry wounds or using surfactants. Spray application of Pseudomonas syringae pv. tagetis in aqueous buffer with a surfactant produced severe disease in Canada thistle, common ragweed, Jerusalem artichoke, sunflower, and certain other members of the Compositae under field conditions. Spray application of the bacterium without surfactant was ineffective on all reported hosts. Xanthomonas campestris pv. poannua controlled annual bluegrass when applied by spray during mowing. The bacterium entered through mowing injuries, causing lethal, systemic wilt. Efficacy of these bacterial bioherbicides and of future biocontrol strategies employing bacteria is dependent on facilitated host penetration (David, et al., 1996).

Mycoherbicides have advantages over chemical herbicides in that they can be more host-specific, preparation costs will be less expensive, and human health hazards can be eliminated. Numerous fungi have been tested for weed control. **Skeleton weed** that invades crop and pasture lands has been shown, under greenhouse conditions and field tests to be controlled by species of the rust fungus Puccinia **chondrillina** (Coombs, et al., 2004).

A new mycoherbicide that utilizes Colletotrichum gloeosporoides to control **jointvetch** where in rice fields, chemical control presents a real problem. . Jointvetch can be a major problem to rice farmers because in the harvesting of rice, the jointvetch seeds, that are similar in size, contaminate the rice and lowers its market value (photo. 28), Usha Sarma (2006).

Waterhyacinth was first introduced into Florida in the 1890s. Within the past century, it has spread over almost ½ million acres of waterways. It is a beautiful plant and was very likely spread by people who put them in the gold-fish ponds, fountains, and other bodies of water from which it escaped. It was soon determined that it was highly infected with a species of Cercospora (renamed C. piaropi). Studies were done on waterhyacinth where the results were very promising (Photo.33). While good biocontrol was

achieved, difficulties were experienced in the application of inoculum and the survival ability of waterhyacinth. Charudattan et al 1995.

Photo.27. prolific growth of the rust Puccinia.

Photo 28. Black jointvetch seeds can heavily contaminate rice grains at harvest.

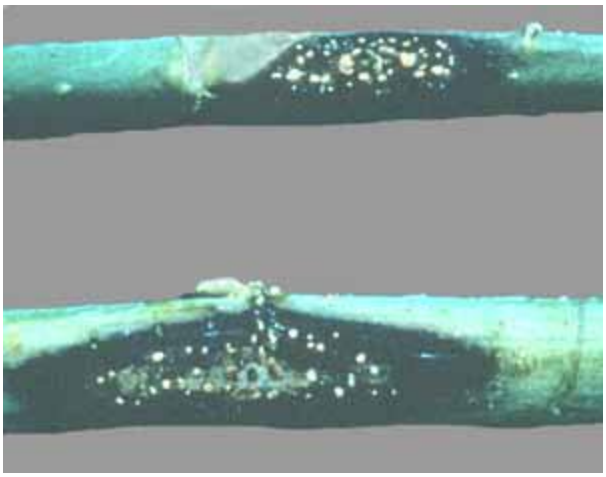

Photo 29. Jointvetch stems infected with Colletotrichum gloeosporoid

Fig. 31. Luxuriant growth of waterhyacinth in freshwater lakes and streams.

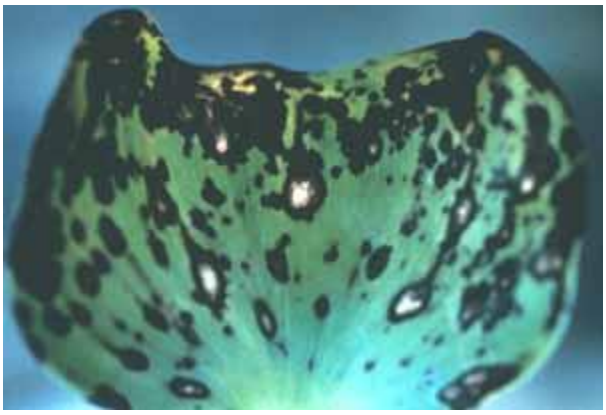

Fig. 32. A waterhyacinth leaf heavily infected with Cercospora piaropi.

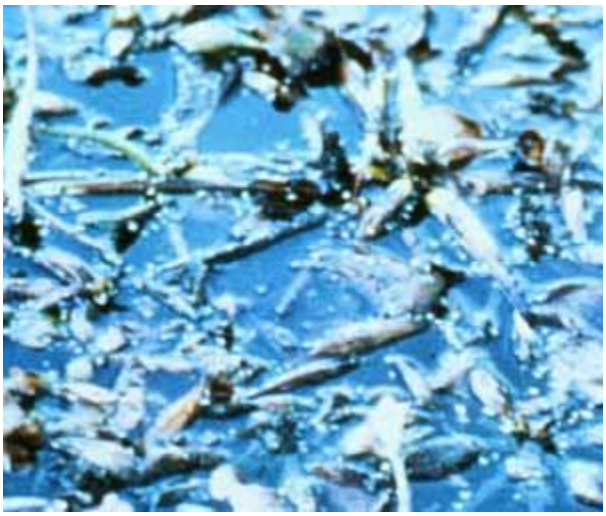

Fig. 33. Effective control of waterhyacinth with Cercospora.

II. Ionizing radiation and biological control:

Many pests, including arthropods and weeds, adversely affect agricultural production, and pre- and post-harvest losses of the order of 30_40% are common. biological control offers one of the most promising, environmentally sound, and sustainable tools for control of arthropod pests and weeds (van Lenteren, et al., 2006; van Driesche, et al.,2008). Public support for biological control as one of the preferred methods of managing non-indigenous and indigenous pests is increasing in many countries. There appear to be significant opportunities for increasing the use and cost-effectiveness of the application of classical and augmentative biological control through nuclear techniques for the production, shipping and release of biological control agents (Hendrichs et al., 2009).

Nuclear techniques are already applied in certain areas of entomology (Bakri, Heather, Hendrichs, and Ferris 2005a) and include the use of radiation sources for (1) studying sperm precedence, parasitoid_host interaction studies, etc., (2) post-harvest disinfestation for quarantine or phytosanitary security in support of agricultural international trade (IDIDAS 2004), and (3) insect sterilization as part of the application of the Sterile Insect Technique (SIT) (Dyck, Hendrichs, and Robinson 2005), where exposure to carefully selected irradiation doses of gamma or X-rays maximizes the induction of dominant lethal mutations in germ cells of pest insects, while minimizing other physiological changes (Bakri, et al., 2005b).

In classical biological control, non-indigenous biological control agents, usually selected from the suite of parasitoids, predators and diseases that co-evolved with the pest, are introduced into the target area. One of the key concerns in this approach is the host specificity and host range of the introduced biological control agents (Louda, et al., 2003). In view of the growing awareness and concern, countries and their respective national plant protection organizations are increasingly implementing stringent environmental risk assessment methods in order to screen potential biological control agents before release (van Lenteren et al. 2006). In cases where doubts remain about very promising natural enemies of weeds or insect pests, the release of such biological control agents that have been radiation-sterilized would enable a more definite and safe assessment of host specificity under natural conditions without any risk of permanent establishment (Hendrichs et al., 2009).

There are several ways in which nuclear techniques can improve the efficiency of augmentative biological control. For example, the cost of production may be decreased by simplifying the rearing process, increasing host suitability and shelf life, improving diets and dealing with disease and contamination (Greany and Carpenter 1999).

The potential applications of nuclear techniques to increase the cost-effectiveness, trade and safety in the use of biological control agents of agricultural insect pests were recoreded in many resreashes. The resutes focused on the six major areas. The main research results in these major areas are described below, and a summary of the main findings is presented in Table 5 (Hendrichs et al. 2009).

1. Suppressing host immune reactions

Exposure to radiation has been shown to suppress host immune system responses and it can also make older instars of irradiated larvae suitable for parasitoid development and thus increase rearing efficiency and parasitoid quality (Hendrichs et al., 2009). G. mellonella larvae irradiated with 65 Gy were found to be suitable for parasitization by Venturia canescens (Gravenhorst), thus facilitating the use of G. mellonella as a potential factitious host for the rearing of this biological control agent (Genchev, et al., 2007). Behavioural and physiological interactions between hosts and parasitoids are complex, often difficult to study, and not well understood in terms of improving rearing efficiency. Certain physiological processes in the host (e.g., defence mechanisms, hormone metabolism) can be selectively modified by radiation, thereby facilitating the study of particular host-parasitoid interactions (Hendrichs et al., 2009). Radiation can likewise be used to modify or terminate certain parasitoid processes that affect host physiology and behaviour, e.g., by sterilizing the wasps or parasitoid eggs (Bai, et al., 2003).

2. **Expanding the time window when a host is suitable for parasitization**

Normal host development limits the time window when a host is suitable for parasitization and it is known that radiation can delay normal host development and thus may extend the time window for host parasitization or modify the internal host environment to the benefit of the biological control agent. This was assessed for parasitoids of the Mediterranean flour moth Ephestia kuehniella (Zeller), the house fly Musca domestica (L.), the Indian meal moth Plodia interpunctella (Hu¨bner) and S. cerealella (Hamed et al. 2009; Zapater, et al., 2009).

3. Allowing for storage and stockpiling of hosts or prey

Certain insect species, radiation can be used to arrest development and thus allow for storage and stockpiling of hosts or prey. Studies on the Mediterranean flour moth E. kuehniella, M. domestica, S. cerealella, and the cotton leafworm or tobacco cutworm Spodoptera litura showed that irradiation caused a prolongation in the development of host stages suitable for parasitization, thus facilitating the use of these hosts under mass-rearing conditions (Seth,et al., 2009; Zapater et al. 2009). Host eggs of E. kuehniella irradiated at 200 Gy could be stored at 48C for up to 30 days without any quantitative or qualitative loss in the production of Trichogramma evanescens (Westwood) and for up to 60 days with only a minor decrease in quality (Tunc¸et al., 2009b). Parasitoids in diapause could be stored inside irradiated host eggs for a period of 50 days without adverse effect on emergence, and irradiation of eggs did not affect acceptance by parasitoids (Tunc¸bilek et al. 2009b).

4. Reproductive stimulation by use of very low dose radiation

The controversial phenomenon known as ‘radiation hormesis, refers to the use of very low dose radiation to stimulate biological processes (Luckey 1991). Two of the studies related to this application, (Wang, et al., 2009) report noting a stimulation of reproduction and parasitization parameters in the parasitoids Habrobracon hebetor (Say), Trichogramma chilonis Ishii and V. canescens after exposure to very low doses of radiation, an intriguing discovery warranting further investigation.

5. Facilitating handling, shipment, trade and release

The continued development and emergence of non-parasitised fertile hosts, as well as of unused prey (pest) insects during mass-production of biological control agents often requires additional handling steps. In the case of fruit flies such as the West Indian fruit fly Anastrepha obliqua (Macquart), the sapote fruit fly Anastrepha serpentina (Wiedemann), and A. ludens, irradiation of larvae is used routinely in the mass-production of tens of millions of parasitoids of these pest fruit flies (Cancino et al. 2009b).

6. Avoiding the shipment of fertile pest individuals

Irradiation of house fly pupae was shown to be very beneficial for the commercial shipment of house fly pupal parasitoids, allowing early shipment of recently parasitised pupae while ensuring that clean shipments were not contaminated with unparasitised pupae that would emerge later with the customers (Zapater et al. 2009). In another example, fruit fly parasitoids have been sent from Mexico to South America after being reared on irradiated A. ludens, which is a quarantine pest in this region (J. Cancino, personal communication). Furthermore, the feasibility of inoculative and augmentative releases of entomopathogenic nematodes within sterilized hosts was proposed to establish a safe mode of transport and dispersion without concern for the inadvertent release of uninfected fertile hosts (Hendrichs et al. 2009).

7. Shipping sterilized hosts or prey in the absence of biological control agents

Needs and opportunities exist for some commercial biological control companies to ship mass produced sterile hosts/prey in the absence of natural enemies for redistribution, both within and between countries, for use as host/prey at smaller rearing facilities. This alternative can be implemented in order to gain efficiencies in the production of biological control agents or to standardize the use of strains of host/prey material to ensure product quality (Steinberg and Cayol 2009).

8. Supplementing hosts in the field for survival or early build-up of biological control agents

Radiation can be used to produce sterile host insects or host insects generating sterile F1 individuals to be released as hosts for the biological control agents without increasing the risk that the released host insects will become pests themselves (Hendrichs et al. 2009).

Irradiated eggs, as well as sterile F1 eggs and larvae resulting from irradiated parents of the gypsy moth, L. dispar, were distributed in a natural forest and found to be acceptable and suitable as hosts for a number of parasitoid species. Most importantly, the parasitoids did not differentiate, under these natural conditions, between sterile F1 larvae and untreated larvae (Zubrik and Novotny 2009).

9. Integrating SIT or F1 sterility and biological control

The release of sterile or semi-sterile insects together with biological control agents has been known to have synergistic effects for population suppression when applied simultaneously (Bloem, et al., 1998). This synergy results from the sterile insects impacting on the adult stage, while the biological control agents target mostly the immature stages, including reproducing on the F1 offspring in inherited sterility releases.

The compatibility of the application of entomopathogenic nematodes with F1 sterility for population suppression of S. litura was demonstrated in laboratory experiments. Various feasible modes of integration of these two bio-rational strategies have been proposed (Seth et al. 2009). Another system under development for integrating augmentative parasitoid releases with the SIT is the release of Diglyphus isaea (Walker), the parasitoid of celery miner fly Liriomyza bryoniae (Kaltenbach), a serious pest of vegetables and ornamentals, together with sterile males of L. bryoniae for application in greenhouses (Kaspi and Parella 2008; Steinberg and Cayol 2009). Developing the SIT against biocontrol agents that have become pest insects themselves is another application linking nuclear techniques with biological control agents. One case is the cactus moth Cactoblastis cactorum (Berg), a textbook example of very effective classical biological control of introduced cactus Opuntia spp., which has invaded the south-eastern USA, and where its westward expansion is being contained by the integrated application of SIT to protect native Opuntiabased ecosystems in the south-western USA and Mexico (Tate, et al., 2007).

10. Reproductively inactivating hosts as sentinels in the field

The exploration for, and collection of new exotic biological control agents and the monitoring of field populations of native biological control agents are sometimes complicated by the fact that hosts are rare or difficult to locate. Reproductively sterilized host insects may be placed in the field in strategic locations as sentinels to aid in these efforts (Jordao-Paranhos, et al., 2003). Several approaches were evaluated including the use of (1) irradiated eggs of S. cerealella, a factitious host of Trichogramma, to monitor effects of seasonal environmental conditions on the establishment of released Trichogramma in

Table.5. Listing of some of the studies of nuclear applications conducted in conjunction withthe FAO/IAEA Coordinated Research Project to improve the cost-effectiveness, trade and safety of biological control of agricultural insect pests using nuclear techniques.

Constraints addressed	Pest species	Biological control agent	References
Suppressing host immune reactions	Lymantria dispar (L.)	Microsporidia	Hoch et al. (2009b
Expanding the period of host suitability	Anastrepha spp.	Various parasitoids	Cancino et al. (2009a,b)
Extending storage and stockpiling time for hosts or prey	Spodoptera litura (F.)	Steinernema glaseri (Steiner)	Seth et al. (2009)
Stimulation effects of low dose radiation	Helicoverpa armigera (Hu¨bner)	Trichogramma chilonis Ishii	Wang et al. (2009)
Utilisation of by-products from insect mass rearing facilities	Ceratitis capitata (Wiedemann)	Diachasmimorpha longicaudata	Viscarret et al. (2006
Avoiding unnecessary handling and sorting steps before shipment	Spodoptera litura (F.)	Steinernema glaseri (Steiner	Seth and Barik (2009
Avoiding the shipment and release of fertile pest individuals	Musca domestica (L.)	Spalangia endius Walker	Zapater et al. (2009)
Shipping sterilized hosts or prey in the absence of biological control agents	Ceratitis capitata (Wiedemann)	------	Steinberg and Cayol (2009)
Synergising biological control agents and F1 sterility	Helicoverpa armigera (Hu¨bner)	Trichogramma chilonis Ishii	Wang et al. (2009
Using SIT against biological control agents that have become a pest	Exorista sorbillans (Wiedemann	------	Hasan et al. (2009
Building up natural enemies in advance of pest populations	Lymantria dispar (L.)	Various	Zubrik and Novotny (2009
Monitoring natural enemies in the field	Helicoverpa armigera (Hu¨bner)	Trichogramma chilonis Ishii	Wang et al. (2009
Screening classical biological control agents in the field		Episimus unguiculus Clarke	Moeri (2007); Moeri et al. (2009

sugarcane fields (Fatima et al. 2009), (2) sterile F1 larvae from irradiated L. dispar for monitoring the density and type of parasitoids and pathogens in forests (Zubrik and Novotny 2009), (3) reproductively inactivated larvae (400 and 600 Gy) of E. kuehniella and P. interpunctella to monitor the density of V. canescens and Habrobracon hebetor (Say) in warehouses and mills (Celmer 2006), and (4) sterilized M. domestica pupae in traps to monitor wild populations of pteromalid parasitoids in the field and under conditions of livestock production (Zapater, et al., 2009)

11. Screening classical biological control agents under field conditions

Classical biological control has resulted in many significant successes, but also many cases of direct and indirect non-target impacts have been documented (Henneman and Memott 2001). Also, inundative biological control can result in environmental problems (van Lenteren et al. 2003), which has fostered growing concerns about the need to preserve biodiversity and natural ecosystems. Therefore, the importation of exotic biological control agents, particularly insect herbivores of invasive plants, is becoming increasingly difficult due to concerns over the possibility that imported species may shift hosts and become pests of crops or protected species. In some cases, despite careful selection (Briese 2006 and van Lenteren et al. 2006) and extensive prerelease studies under quarantine conditions, the release of promising biological control agents is ultimately rejected because of remaining doubts about their host specificity. In such situations, exotic biological control agents may be sterilized using radiation so that they can be released and studied under actual field conditions without the risk of establishing permanent breeding populations in space and time. The use of sterilized individuals allows further assessment and confirmation of oviposition behaviour and host (acceptability) associations. Also, the use of F1 sterile larvae of exotic herbivores being considered for introduction and release against plant pests would allow field-testing of larval feeding preferences and the ability of these larvae to develop and survive on related weeds, crops and other native plants that are of concern (Carpenter et al., 2001). A model system that includes Opuntia spp. and C. cactorum has been developed to study the host range of an exotic herbivore. Radiation biology studies revealed that the optimum dose at which females are sterilized and males remain partially fertile and produce sterile progeny is 200 Gy (Carpenter et al. 2001). Whole plant and single cladode host preference tests demonstrated that C. cactorum females mated with males irradiated at 200 Gy exhibited normal oviposition preferences and can be used safely under field conditions to predict the host range, as well as to study possible interactions with natural enemies (Hight et al. 2009). Another system under evaluation involves the exotic herbivore Episimus unguiculus Clarke (E. utilis Zimmerman), which is currently in quarantine in Florida, for the eventual biological control of the Brazilian pepper tree Schinus terebinthifolius Raddi (Moeri 2007; Moeri, et al., 2009).

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