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BIOLOGICAL CONTROL MECHANISMS AGAINST PLANT-BASED PATHOGENS

[1]Rao, A. P., [1]Agbo, B. E., [1]Ikpoh, I. S., [2]Udoekong, N. S. and [1]Etuk, H. A.
[1]Microbiology Department, faculty of Biological Sciences, University of Calabar,
P. M. B. 1115, Calabar, Nigeria.
[2]Department of Science and Technology, Akwa Ibom State Polytechnic, Ikot Osurua,
Ikot Ekpene, Nigeria.
Correspondence: beagbo@unical.edu.ng, Tel.:+234(0)7031127120

ABSTRACT

Antagonistic bacteria are bacteria that have the potential to control plant diseases, they are referred to as biological control agents. Bacterial antagonist carries out their biocontrol activities through various mechanisms such as: antibiosis, diacetyl phloroglucinol, pyoluteorin, phenazine and pyrollnitrin production. Other mechanisms include the production of lytic enzymes, production of volatile compound (hydrogen cyanide), production of antifungal metabolites (class of cyclic lipo-peptides) and competition for siderophore. Many soil microorganisms are antagonistic to plant-based pathogens, they secrete potent enzymes which destroy other organisms by digesting their cell walls and degrading cellular materials which serve as food for the inhibitor organism. Plant pathology, biological control refers to the intentional use or introduction of resistant resident microorganisms to stop the activities and microbial population of one or more plant disease causing agent. Examples of soil bacteria with antagonistic activities are *Pseudomonas spp., Bacillus spp., Escherichia spp., Serratia spp., Staphylococcus spp.* and *Streptomyces spp*. These antagonize by competing with plant pathogens for food sources, producing metabolites that slows down the growth of the pathogens and physically eliminating the pathogens from the plant thereby occupying their space. Application of antagonists can be done by the use or introduction of organic materials that contain natural microbial population such as application of bio-fertilizer and compost during pre-harvest period.

Keywords: Plant pathogen, biological control agent and antagonistic activities.

1. INTRODUCTION

Antagonistic bacteria used to control plant diseases are referred to as biological control agents (Gheorghe *et al.*, 2008). Most of these bacteria are facultative anaerobes and are non-pathogenic to humans. Some sxample of soil bacteria with antagonistic activities include *Pseudomonas spp., Bacillus spp., Escherichia coli, Serratia spp., Staphyloccocus spp., Streptococcus spp. and Streptomyces.*

Bacterial antagonists produce a wide variety of antibiotics which confer a competitive advantage and microbial fitness for their survival in most environments (Paulsen *et al.*, 2005). Due to their ability to produce variable metabolites and to utilize several organic compounds, most *pseudomonads* are not specific for one pathogen but have a wide host range and suppress several pathogens (Raajamker *et al.*, 2010).
Mechanisms of biological control by bacterial antagonist are: antibiosis, diacytyl phloroglurinol, pyluteonin, phenazine and pyrollnitrin, production of lytic enzymes, production of volatile compound-hydrogen cyanide and competition for siderophore. Antibiosis refers to the inhibition or destruction of the pathogen by metabolic products produced during growth of the antagonist (Siddique *et al.*, 2005). Diacetyl-phloroglucinol (DAPG) is synthesized by several plant associated fluorescent Pseudomonads and it plays a key role in the suppression of a wide variety of soil-borne pathogens. Among the various extracellular metabolites produced, DAPG is of prime importance in plant protection (Mushtaq *et al.*, 2010).
Antagonist bacterial strains are also known to produce phenazines and pyrrolnitrin (Prn) which are broad spectrum antibiotics to kill the phyto-pathogens in soil (Dharni *et al.*, 2012). Soil bacteria with antagonistic potentials prevent the proliferation of soil pathogens and facilitate growth through the production and secretion of siderophores (Motta *et al.*, 2004). Siderophores bind most of the available iron thereby effectively

preventing any fungal pathogen in the immediate vicinity from proliferating due to lack of iron siderophores which is easily available to antagonist bacteria (Khan and Ahmad, 2006).

2. ANTAGONISTIC ACTIVITY OF SOIL BACTERIA

Soil possesses a vast diverse microbial population including both bacterial and fungal community (Islam & Toyotak, 2004). Many soil micro-organisms are antagonistic, they secret potent enzymes which destroys other cells by digesting their cell walls, and degrading cellular materials which serve as nutrient for the inhibitor organism (Garbeva and Van-Elsas, 2004).

Every soil has natural potential to suppress the activity of plant pathogens to some degree due to the presence and activity of soil micro-organisms. This phenomenon is termed general suppression or non-specific antagonism or biological buffering (Weller *et al.*, 2002). Biological buffering is assumed to be related to the total microbial population (biomass) in a given soil, which competes with pathogen for available resources or causes direct form of antagonism.

Most of these bacteria used for antagonistic activities are facultative anaerobes and are non-pathogenic to humans. They are bio-control agents that are used to control plant diseases (Gheorghe *et al.*, 2008). Example of soil bacteria with antagonistic activities are *Pseudomonas spp.*, *Bacillus spp.*, *Escherichia coli*, *Serratia spp.*, *Staphyloccocus spp.*, *Streptococcus spp. and Streptomyces.*

2.1 Antagonistic Activity of *Pseudomonads*

The genus *Pseudomonas* comprises of relatively large and important group of gram negative, non-spore forming, motile, rod bacteria (Saravaan *et al.*, 2004). They are the best studied soil borne bacterial group. Some members of this genus are characterized by production of diffusible and insoluble pigments. *Pseudomonads* are well known for their ability to degrade compounds which are difficult to utilize by other organisms (Khan and Ahmad, 2006).

Most *pseudomonads* are not specific for one pathogen but have a wide host range and suppress several pathogens, because of their ability to produce variable metabolites and to utilize several organic compounds (Raajamker *et al.*, 2010). They also produce a wide variety of antibiotics which confer a competitive advantage and microbial fitness for their survival in most environments (Paulsen *et al.*, 2005).

Pseudomonas spp. are ubiquitously present in the soil and has been found to be suppressive to different plant pathogens including *Fusarium Solani*, and *Aspergillus niger*. They have been used has effective bio-control agents over the years. This bacteria are fast growing, good colonizers and possess appropriate ecological rhizosphere competence (Akbtar *et al.*, 2010; Rao *et al.*, 2017).

Pseudomonas strain with antifungal activities have been detected in plant rhizosphere (Weller *et al.*, 2002) and found to control blister blight disease in tea (Saravaan *et al.*, 2004). Besides tea, fluorescent pseudomonads have been used successfully against pathogens of other crops including rice (Akbtar *et al.*, 2014); wheat (Kim *et al.*, 1997); tomatoes (Islam and Toyotak, 2004); soyabean (Mushag *et al.*, 2010) and maize (Palumbo *et al.*, 2007). Haas and Defago, (2005) demonstrated that co-inoculation of Pseudomonas and fungal pathogen successfully reduced the disease of pea plant. *Pseudomonas chlororaphis* strain MFI, drastically reduced attacks by *Fusarium oxysporum* species on tomatoes plantlets, in seed assays and green house trials. In another study the same strain *P. chlororaphis* strain MFI was able to completely inhibit the mycelial growth and conidium germination of *Aspergillus niger* (Khan and Ahmad, 2006).

2.2 Antagonistic Activity of *Bacillius*

Bacillius spp are particularly gaining recognition as safe biocontrol agents in a variety of crops specifically as seed protectants and antifungal agent (Stein, 2005). Moreover they are spore formers which impact a natural formulation advantage over other soil micro-organisms used for this purpose (Kim *et al.*, 1997). It has been found that members of this genus have successfully controlled plant disease in a wide variety of crop including: rice (Akbtar *et al.*, 2014); Wheat (Nuorozian *et al.*, 2006); tomatoes (Islam and Toyotak, 2004) and cucumber (Haas & Keel, 2003). *Bacillus subtilis* AFJ showed better suppression of crown rot of groundnut caused by *Asperigillus niger* and *Fusarium* wilt of pea caused by *Fusarium udium*.

In a similar study *Bacillus amyloliquefaciens* isolates inhibited growth and production of mycelia and sclerotia of *Seclerotina scleroties*. In an *in-vitro* study, protected tomatoes and eggplant seedlings inoculated with *Sclerotina sclerotinum* were offered over 80% protection from pathogenic attacks; this was comparable to commercial products (Al Abdullah, 2005).

Kim and Chung, (2004) showed that *Bacillus spp*. inhibited mycelia growth and significantly reduced incidence of grey mould and powdery mildew in cucumber and straw. The bacterium is applied extensively in the field of agriculture as a pesticide for the control of pests, *Bacillus thuringiensis* produces the Cry crystal toxin that attacks the gut of pests and kill them. These Cry toxins are good agricultural tools for growing plants (Kalu, 2012).

2.3 Other Bacteria with Antagonistic Potential

Diverse groups of Rhizobacteria apart from the most widely studied genera *Pseudomonas* and *Bacillus* has also proved their efficacy as powerful biocontrol agents. Within the last decade a large array of soil bacteria including specie belonging to the genera *Ascospirillum*, *Ascobacter*, *Klebbsiella*, *Enterobacter*, *Alcaligens*, *Arthrobacter*, *Burkholderia*, *Serratia*, have been evaluated for their antagonistic effect on fungi (Bloemberge and Lugteneberg, 2001). Among their overall beneficial effects are their ability to deplete the immediate environment of available nutrients such as iron and to elude various metabolites thereby promoting plant growth (Weller *et al.*, 2002).

3. MECHANISM OF BIOCONTROL BY BACTERIAL ANTAGONIST

The terms plant pathology biological control refers to the intentional use of introduced or resistant resident microorganisms to suppress the activities and microbial population of one or more plant pathogens (Heydari and Pessarakli, 2010).

Micro-organisms that exhibit preference in colonizing and suppressing plant pathogen might be classified or named as bio-control agents (Heydari and Pessarakli, 2010). Plant growth promoting bacteria (PGPB) are rhizobacteria [bacteria specie found in narrow zones of soil specifically influenced by the root system] that suppress plant pathogens by producing various types of inhibitory substances or by increasing the natural resistance of the plant (Gheorghe *et al.*, 2008) or by displacing out competing the pathogens such bio-control PGPBs have the capacity to rapidly colonize the rhizosphere, and compete with deleterious micro-organisms as well as soil borne pathogens at the root surface (Stockwell *et al.*, 2002). Some of these modes of action used by biocontrol plant growth promoting bacteria are as follows.

3.1 Antibiosis

This refers to the inhibition or destruction of the pathogen by metabolic products produced during growth of an antagonist. The products include volatile compounds, toxic compounds and antibiotics which are all deleterious to growth or metabolic activities of other micro-organisms at low concentrations (Siddique *et al.*, 2005). Mechanism of antagonist activities of *Pseudomonads* on *Fusarium oxysporium* [causative agent of fire blight in orchards] was attributed to antibiosis (Stockwell *et al*., 2002). Antibiosis involves the production of volatile and non-volatile compound which act as antibiotics or antifungal agent.

In general antibiotics are toxins produced by microbial cells that can at low concentration, poison or kill other micro-organism (Heydari and Pessarakli, 2010; Yadav *et al.*, 2010). Researches have shown that some antibiotics produced by micro-organisms are effective against plant pathogens (Islam *et al.*, 2005). Heydari and Pessarakli, (2010) provided comprehensive lists of antibiotics that have antifungal potentials among them are: 2, 4-dacetyl phloroglucinol (2, 4 DAPG); pyrrolnitrin (PRN), pyoluteorin (PL7) and different derivative of phenazine (PHZ).

3.1.1 Diacetyl Phloroglucinol (DAPG)

Diacetyl phloroglucinol (DAPG) is a Polypeptide compound which has received a particular attention because of its broad spectrum antifungal properties (Haas and Keel, 2003). 2, 4- DAPG is synthesized by several plant associated fluorescent P*seudomonads* and it plays a key role in the suppression of a wide variety of soil borne pathogens (Ramette *et al.*, 2003). 2, 4- DAPG inhibits zoospores produced by *Pythium spp*. (Mushtaq *et al.*, 2010). 2, 4- DAPG producing Pseudomonads are of worldwide origin and are commonly found in the rhizosphere of the soil (Yeaman and Yount, 2003). Among the various extracellular metabolites produced DAPG is of prime importance in plant protection. The polypeptide antibiotics DAPG is a phenolic molecule synthesized by condensation of three molecules of acetyl coenzymeA with one molecule of malonyl coenzymeA to produce the precursor monoacetyl phloroglucinol which is subsequently transacetylated to generate *phl* gene utilizing a *chs*-type enzyme. Biosynthetic locus of DAPG is highly conserved it comprises the biosynthetic genes PhlACBD. Mutation in the biosynthetic gene cluster of DAPG reduced bio-control activity of antagonistic bacteria (Paulin *et al.*, 2009).

3.1.2 Pyoluteorin

Pyluteorin (PLT) is a phenolic polypeptide with resorcinol ring. The ring is linked to bichlorinated pyrrole. Biosynthesis of pyrrole moiety is unknown. It was first isolated from *Pseudomonas aeruginosa*. This is a chlorinated polyketide antibiotic just like 2, 4- DAPG, it is produced by several species of bacteria. Its inhibitory activity against oomycetes fungi including plant pathogen P*ythium ultinum* (Srivastava and Shalni, 2008) has been well documented.

3.1.3 Phenazine and Pyrollnitrin

Phenazines are also antibiotics with broad spectrum activity. They comprise of a large family of over 100 compounds in cyclic rings, and nitrogen containing brightly coloured pigments (Lugtenberg *et al.*, 2002). Currently more than 50 naturally occurring phenazine compounds have been described and are exclusively produced by bacteria such as P*seudomonas*, *Bacillus*, S*treptosmyces* and *Norcadia*. Phenazine and its derivatives are well known for their antifungal properties (Chi-Awoeng *et al*., 2003). Phenazine are redox active compound and thus the mechanism for their action is assumed to be due to their ability to engage redox recycling in the presence of various reducing agents and molecular oxygen resulting in the accumulation of toxic superoxide ion and hydrogen peroxide (H_2O_2) which are harmful to the cell or can lead to death of the cell (Mavrodi *et al*., 2001).

Pyrollnitrin is a secondary metabolite which is synthesized from tryptophan. It has a strong antifungal activity (Dharni *et al*., 2012). A narrow range of gram negative bacteria such as *Pseudomonas*, *Serratia*, *Bukholderia* and *Enterobacter* has been found to produce pyrrolnitrin.

Antagonist bacteria strains can produce phenazines and pyrrolnitrin (Prn) which are broad spectrum antibiotics to kill phyto-pathogens in the soil. Researchers have studied a rhizospheric soil isolate of *Pseudomonas fluorescens* strain producing both phenazine-1-carboxylic acid (PCA) and Prn. In order to study the contribution of these antibiotics, the *phzD* and *prnC* genes involved in phenazine-1-carboxylic acid (PCA) and pyrrolnitrin (Prn) biosynthesis, were disrupted in a site-specific manner using a group II intron-based Targetron gene-knockout system, and gene disruption followed by allelic exchange through homologous recombination, respectively. The resulting knockout strains Psdphz122s-34 and PsdprnC::gen did not produce PCA and Prn, respectively (Singh *et al*., 2014). By combining the two strategies, a Psdphz122s-34prnC::gen double mutant could be generated. Identification and lack of PCA production was achieved using HPLC/APCI-MS analysis and TLC detection for both the antibiotics in these mutants. Loss of antifungal activity by the phyto-pathogenic fungus *Fusarium oxysporum* has been observed using *in-vitro* growth assays on plates or growth chamber experiments with tomato seedling on an artificial substrate. Based on the characterization of these gene knockout mutants, researchers proposed that PCA and Prn have a major role in antifungal activity of fluorescence *Pseudomonas* strain PSD (Baker, 2006; Singh *et al*., 2014).

3.2 Production of Lytic Enzymes

Many active micro-organisms can produce many other metabolites that interfere with pathogen's growth and activities. Among these metabolites are lytic enzymes that can break down polymeric compounds including, protein, DNA, cellulose, hemicelluloses and chitin (Heydari and Pessarakli, 2010). Some examples of lytic enzymes include metabolites that directly suppress plant pathogens for example control of *Sclerotium spp.* by *Serratia mascescens* appeared to be mediated by chitinase expression (Heydari and Pessarakli, 2010). Other microbial by-products also contribute to pathogen suppression. Antagonistic activities of these metabolites indicated the need to degrade complex polymer in order to obtain carbon nutrient for example *Myxobacter* that produce lytic enzyme have been shown to be effective against some plant pathogens (Heydari and Pessarakli, 2010)

3.3 Production of Volatile Compound: Hydrogen Cyanide

Hydrogen cyanide is reported to be one of the antifungal secondary metabolites produced by bacterial antagonist (Jeffrey *et al.*, 2006). Hydrogen cyanide blocks the cytochrome oxidase pathway effectively and is highly toxic to all aerobic micro-organism at pico-molar concentrations (Ramette *el al.*, 2003; Heydari and Pessarakli, 2010). Florescent *Pseudomonas* has been reported to produce HCN to suppress some root pathogens (Mavrodi *et al.*, 2001).

3.4 Production of Antifungal Metabolites: Class of Cyclic Lipopeptides

The production of antifungal metabolites belonging to the class of cyclic lipopeptides such as viscosinamide and tensin has also been reported (Bloemberge and Lugteneberg, 2001). Recent advances in the understanding of genetics and regulation of synthesis of bacterial metabolites especially antibiotics have contributed significantly to the advancement of plant protection (Nielson & Sorenson, 2003; Edeghor *et al.*, 2016). Lipo-peptides are none ribosomally-synthesize peptide synthetase (NRPS). NRPS are giant enzymes composed of molecules that house repeated set of ribosomal domain which select, activate and couple amino acids drawn from a pool of nearly 500 potential building blocks (Walsh *et al.*, 2013; Chowdhury *et al.*, 2015). Plant protective activity of antagonist has been associated with the capability to secret a wide array of antibiotic compounds against fungal growth in isolated culture under laboratory conditions (Velilleli *et al.*, 2014; Chowdhury *et al.*, 2015). A recent research performed with six strains of *Bacillus* belonging to the *B. amyloliquefaciens* species correlated earlier findings that antifungal activity is linked with the ability to produce cyclic lipo-peptide. Remarkably, production of iturin and fengygrin in *B. amyloliquefaciens* was enhanced in the presence of certain phyto-pathogens (Cawoy *et al.*, 2014; Chowdhury *et al.*, 2015). This is in line with recent findings that non-ribosomal synthesis of antifungal and antibacterial compounds including bacillibactin is stimulated in the presence of plant pathogens under laboratory conditions (Li *et al.*, 2014; Chowdhury *et al.*, 2015).

3.5 Competition for Siderophore

Siderophores are iron-binding proteins of low molecular mass (400-100 Daltons) secreted by some soil microorganisms. Bacterial antagonists vigorously compete with the pathogen for nutrient and space. Antagonists of this group are often better acclimatized to adverse environmental conditions than the pathogens (Janisiewiez and Peterson, 2004). Since the antagonist has the ability to effectively utilize nutrients at low concentrations, it exhibits rapid growth and survives on the surface of the substrate or infection site under temperature, pH or osmotic conditions which are unfavorable for the growth of the pathogens (Garbeva and Van-Elsas, 2004). Such an antagonist will stop or slow down the growth but not kill the pathogen.

Iron is one of the most abundant minerals on earth yet in the soil it is unavailable for direct assimilation by plants or micro-organisms. This is because ferric iron (Fe^{+3}), the common form of iron in nature is sparingly soluble. Therefore the amount of soluble iron in the soil barely supports microbial growth. Soil microorganisms secrete siderophore which are iron binding proteins of low molecular mass (400-100 Daltons) (Balhara *et al.*, 2014). They bind Fe^{+3} with a very high affinity. Most aerobic and facultative anaerobic micro-organisms produce Fe^{+3} chelating siderophores which bind and transport ferric iron back to the microbial cells where it is taken up by cellular receptors. Soil bacteria with antagonistic potentials prevent the proliferation of soil pathogens and facilitate growth through the production and secretion of such siderophores (Motta *et al.*, 2004). Siderophores bind most of the available iron thereby effectively preventing any fungal pathogen in the immediate vicinity from proliferating due to lack of iron siderophores which is easily available to antagonist bacteria (Khan and Ahmad, 2006). The major types of siderophores produced by antagonistic bacteria are pyoverdin, pyochelin and salicyclic acid (Balhara *et al.,* 2014).

Numerous studies indicate that Pseudomonads are among the efficient competitors for Ferric iron (Fe^{+3}). The most common siderophore in these species are called pyoverdins or pseudobactin (Melent'ev *et al.,* 2006). They are general peptide siderophores constituting quinoline chromophore (mioties responsible for colouration), a peptide chain and a dicarboxylic acid connected to the chromophore (Ramette *et al.,* 2003). The characteristic fluorescent pigments of fluorescent are due to pyoverdin. Pyoverdin produced *in-situ* may chelate iron and make iron available for pathogens.

3.6 Induction of Systemic Resistance (ISR)

Under normal conditions, all plants possess active defense mechanism against pathogen's attack which sometimes fail upon infection by virulent pathogens. This happens as a result of pathogen's suppression of the resistance reaction (Haas and Defago, 2005). If however defense mechanism are triggered by stimulus before infection by the pathogen the disease can be minimized i.e. the plants will have enhanced defensive capacity. This systemic protection of plant by an inducing agent when applied to a single part of the plant is known as the induced systemic resistance (Lugtenbeg *et al.,* 2002; Weller *et al.,* 2002; Cawoy *et al.,* 2014). Certain bacteria turn on or trigger a phenomenon known as ISR phenotypically similar to systemic acquired resistance (SAR). SAR evolves when plants successfully activate their defense mechanism in response to primary infection by a pathogen, notably when the latter induces a hypersensitive reaction through which it becomes limited in a local necrotic lesion of brown, desiccated tissue (Dharni *et al.,* 2012). Like SAR, ISR is effective against different types of pathogens but differs from SAR in that the inducing bacterial antagonist does not cause visible symptoms on the host plant (Chin-Awoeng *et al., 2003*). Antagonist-elicited ISR was first observed on carnation (*Dianthus caryophillus*) with reduced susceptibility to wilt caused by *Fusarium sp.* (Kim & Chung, 2004) and on cucumber (*Cucumis sativus*) with reduced susceptibility to foliar disease caused by *Colletotrichum orbiculare* (Walsh *et al.,* 2013). Manifestation of ISR is dependent on the combination of host plant and bacterial strain (Stein, 2005). Most reports of antagonist-mediated ISR involve free-living rhizobacterial strains, but endophytic bacteria have also been observed to have ISR activity. For example, ISR was triggered by *P. fluorescens* strain EP1 against red rot caused by *Colletotrichum falcatum* on sugarcane (Mandeel, 2005), *Burkholderia phytofirmans* PsJN against *Boitrytis cinerea* on grapevine (Sinha and Sexana, 2002) and *Verticllium dahliae* on tomato (Baker, 2006), *P. denitrificans* and *P. putida* against *Ceratocystis fagacearum* on oak (Cawoy *et al.,* 2014). *P. fluorescens* against *F. oxysporum* has also been reported.

4. APPLICATION OF ANTAGONISTS

Indigenous micro-organisms present in plant's ecosystem can help minimize disease potential or disease damage only if they are allowed to grow vigorously (Heydari and Pessarakli, 2010; Velivelli *et al.,* 2014). They carry out these tasks by competing with the pathogens for food sources, producing metabolites that inhibit the growth of the pathogens and physically eliminating the pathogens from the plant thereby occupying their space (Heydari and Pessarakli, 2010). Micro-organisms that are not indigenously present in plant environment can be introduced in an attempt to control disease (Heydari and Pessarakli, 2010; Velivelli *et al.,* 2014). This can be done through various methods. Application of antagonist can be carried out by the application of organic materials that contain natural microbial population such as application of bio-fertilizers and compost during the pre-harvest period. Also post-harvest application of fungicides has also been adopted (Heydari and Pessarakli, 2010).

4.1 Bio-fertilizers: Organic Fertilizers with Microbial Supplement

Fertilizers supply essential plant nutrients, mainly nitrogen potassium phosphorous. These fertilizers increase the yield of crops but they cause several health hazards. Due to several health hazards, consumer preferences shift towards the use of organic food grown without the use of any chemical. In recent years, bio-fertilizers have emerged as an important component of biological nitrogen fixation. Bio-fertilizers are low cost renewable source of nutrient that supplements chemical fertilizers. Organic fertilizers with microbial supplement have gained importance due to its bio-control abilities (Asikong *et al.*, 2013). Organisms used for bio-fertilizers are bacteria of the genera *Bacillus*, *Pseudomonas*, *Lactobacillus*, photosynthetic bacteria and all nitrogen fixing bacteria.

The product must be applied before disease development as they are preventive not curative (Heydari and Pessarakli, 2010; Velivelli *et al.*, 2014). Natural organic fertilizers nitrogen and potassium are used as nutrient for bacterial antagonist and not for any secondary effect on plant.

4.2 Use of Compost for Biocontrol

Addition of compost organic matter to plants rhizosphere helps in the suppression or inhibition of soil borne fungal disease (Nielson and Sorenson, 2003; Heydari and Pessaraki, 2010). The concentration of antagonistic micro-organism in compost amended soils is very high but greatly reduced in soils before amendment (Nielson & Sorenson, 2003). As a result predictive disease suppressive models have been developed based on the composition and concentration of microbial biomass (Heydari and Pessarakli, 2010). The disease suppressive effect of composts depends on the diversity and activity of micro flora inhabiting compost, composting substrates and environment during its production and curing; rate of its application, stability and maturity of compost. Composts made from substrates with high content of lignin, cellulose, tannins and waxes can sustain their disease suppressive effects for a longer period, compared to those from food wastes, manures and bio-solids having more readily degradable materials. Fresh organic matter does not support bio-control activity, even when inoculated with the best strains. High concentrations of glucose and amino acids in fresh crop residues repress the production of enzymes required for antagonism. Composts must be stabilized well enough and colonized to a degree that micro-biostasis prevails. Excessively humified organic matter as peat, cannot support the activity of bio-control agents. Organic matter with properties in between these two extreme degrees of decomposition levels support biological control. Members of the genera *Bacillus*, *Enterobacter*, *Pseudomonas* and *Flavobacterium*, *Streptomyces* are the major microflora in composts.

5. APPLICATION OF BIOFUNGICIDES

The concept of bio-fungicides is based upon observations of natural processes where beneficial microorganisms, usually isolated from soil, hinder the activity of plant pathogens. Bio-control microorganisms are free-living fungi, bacteria, or actinomycetes that are active in root, soil, and foliar environments (Hayes, 2013). Researches have tested the efficacy of biocontrol-active micro-organisms on post-harvest fungal pathogens which cause losses to fruits and vegetables (Janisiewiez and Korten, 2002; Heydari and Pessarakli, 2010). Spray application of bacteria antagonist have resulted in significant reduction in infection caused by some fungal pathogens during storage (Heydari and Pessarakli, 2010). In post-harvest environment, it is often possible to control humidity and temperature in addition, the post-harvest environment is an artificial ecological island separated from the buffering or cushioning effect of natural microbial ecosystem (Velilleli *et al.*, 2014). Such conditions favour the use of introduced antagonist for bio-control.

A lot of antagonist bacteria have been discovered to exercise control over a number of different post-harvest pathogens of fruits: pome fruits and citrus fruits. Patent have been issued or are still undecided on a number of antagonistic bacteria (Janisiewiez & Korten, 2002).

6. EFFICACY OF BIOCONTROL INOCULANTS: RHIZOSPHERE COMPETENCE AND NON-TARGET EFFECTS

Application of microbial antagonists as inoculants to suppress plant pathogens is very appealing and it's potential for sustainable agriculture its potential for sustainable agriculture has been reviewed by some authors (Haas and Defago, 2005). Nonetheless, for many microbial antagonists, lack of correlation between *in-vitro* antagonistic activity and field performance as well as inconsistent and inefficient bio-control in diverse field situations have been reported as the main drawback of this approach (Bloemberge and Lugteneberg, 2001). Consequently, only few strains have been commercialized as bio-pesticides and bio-fertilizers.

Bio-control of pathogens is a multi-trait phenomenon which success relies on many factors. Some of the factors are external such as edaphic and climatic conditions but the main factor is the inherent traits of the biocontrol strains, this include sufficient production of required metabolites or efficient root colonization (Rhizosphere competence) (Weller *et al.*, 2002). Colonization of a large part of the root system by biocontrol agent is required for efficient suppression of the invading pathogen because it is believed to function as the delivery system for the cells producing the anti-fungal metabolites (Lugtenberg *et al.*, 2002). The expression of genes involved in biosynthesis of most extracellular metabolites is cell density dependent, such that the production of the metabolites is enhanced above a certain population threshold (Haas & Defago, 2005). Furthermore, Most bacterial antagonists produce secondary metabolites with antifungal activity and their effects are not necessary restricted to root pathogens for example 2,4-DAGs and PLT have been reported to affect other soil micro-organism including some useful bacteria (Balhara *et al.*, 2014). It is therefore important to note that application of bacteria antagonist can influence the structure of microbial community within the agricultural ecosystem.

7. SUMMARY AND CONCLUSION

Soil harbours diverse microbial population including both bacterial and fungal communities. Most of the soil microorganisms are antagonistic; they secrete potent enzymes which destroy other cells by digesting their cell walls, and degrading cellular materials which serve as nutrient for the inhibitor organism. Antagonistic bacteria can be used to control plant diseases and are referred to as biological control agents. Examples of soil bacteria with antagonistic activities are *Pseudomonas spp.*, *Bacillus spp.*, *Escherichia coli*, *Serratia spp.*, *Staphylococcus spp.*, *Streotococcus spp*. and *Streptomyces*.

Pseudomonads are the best studied soil borne bacterial group. They are well known for their ability to degrade compounds which are difficult to utilize by other organisms. They produce wide varieties of antibiotics which confers a competitive advantage and microbial fitness for their survival in most environments.

Bacillus spp. is equally gaining recognition as safe bio-control agents in a variety of crops especially as seed protectants and antifungal agents. Diverse groups of *Rhizobacteria* apart from the most widely studied genera, *Pseudomonas* and *Bacillus*, have also proved efficacious as powerful biocontrol agents. Within the last decade, a large array of soil bacteria including species belonging to genera *Ascopirillum*, *Ascobacter*, *Klebsiella*, *Enterobacter*, *Alcaligens*, *Arthrobacter*, *Burkholderia* and *Serratia* have been evaluated for their antagonistic effect on fungi. Among the overall beneficial effects are their ability to deplete the immediate environment of available nutrients such as iron and to elude various metabolites thereby promoting plant growth.

The mechanism of bio-control by bacterial antagonist is antibiosis-which is the inhibition or destruction of the pathogen by metabolic products produced during growth of an antagonist. Antibiosis involves the production of volatile and non-volatile compounds which act as antibiotics or antifungal agents. Antibiotics are toxins produced by microbes that can poison or kill other microorganism at low concentration. Another mechanism of action of antagonistic bacteria is by the production of other metabolites that can interpose with pathogen's growth and activities. Among these metabolites are lytic enzymes that break down polymeric compounds including chitin, protein, DNA, hemicelluloses and cellulose. Other mechanisms are through the production of volatile compounds like hydrogen cyanide, production of antifungal metabolites (class of cyclic lipo-peptides). The outstanding mechanism is competition for siderophore, bacterial antagonist vigorously compete with the pathogen for nutrients and space. Antagonist of this group is often better acclimatized to adverse environmental conditions than the pathogens.

Antagonists accomplish their task by competing with the pathogen for food sources, production of metabolites that stops the growth of the pathogen and physically eliminating the pathogens from the plant thereby occupying their space. Application of antagonist can be achieved by the use of organic materials such as bio-fertilizer and compost that contain natural microbial population during pre-harvest period.

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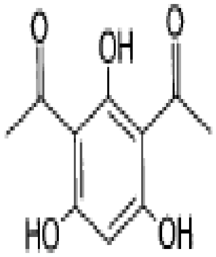
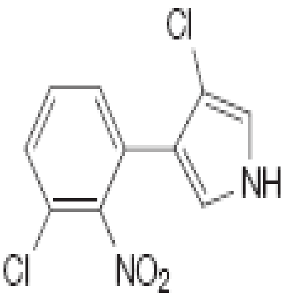
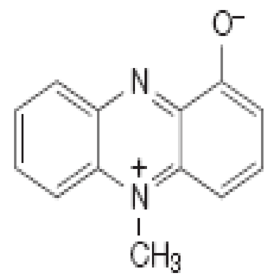
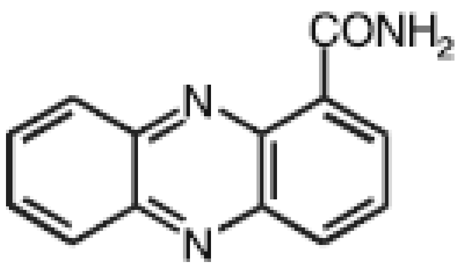
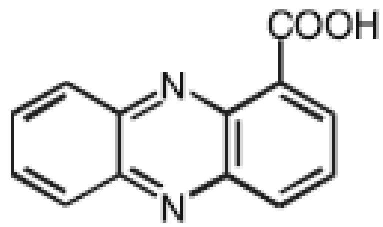






2, 4 DAPG pyrrolnitrin pyocyanin





Phenazine-1-carboxamine (PCN) phenazine-1-caboxylic acid (PCA)

Fig. 1: Structures of some Phenazine and Pyrollnitrin compounds

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