CHAPTER 2

LITERATURE REVIEWS

1. Pathumma

Curcuma alismatifolia Gagnep., also known as the Siam tulip or Pathumma, is native to South-East Asia. A flowering stem of this species is reminiscent, at least for some distance, to a group of tulips (Bunya-atichart *et al.*, 2004). Pathumma is a member of the ginger family (*Zingiberaceae*), which is found wild in various habitats in eastern and southeastern Thailand ranging from near sea level up to 790 m above mean sea level. Owing to its increasing popularity, it is also subjected to rapid genetic erosion caused by over-collecting and land clearing. Since its introduction as an ornamental plant in early 1980, a large number of plants have been collected directly from the wild both for local consumption and export (Paisooksantivatana *et al.*, 2001). Moreover, the scientific documents revealed that *C. alismatifolia* leaves have an effective antidiarrhoeal and antioxidant properties as well as other *Curcuma* plants (Akter *et al.*, 2010).

The growing season of Pathumma is March through June with peak occurring during June through September. After flowering, the plants lie dormant from September to February where rhizome harvesting usually takes place. The Pathumma value for domestic market is lower than that of foreign market. The annual exported value of rhizomes is approximately 15-30 million baths. For more than a decade, the best markets have been European countries, particularly the Netherlands, the United States of America and Japan (Thongwai and Kunopakarn, 2007).

C. alismatifolia inflorescence flower have several apical bracts, which form cup-like structures. Most basal bracts are green, but the more distal and more numerous than the green ones are pink in native plant and in some cultivars such as *C. alismatifolia* cv. Chiang Mai Pink. The colored bracts mainly determine the attractiveness of the plant. Both types of bracts bear small axillary flower buds. Open flowers are small and have little color except the flag petal which are mostly blue (Bunya-atichart *et al*, 2004) (Figure 1, 2, 3).

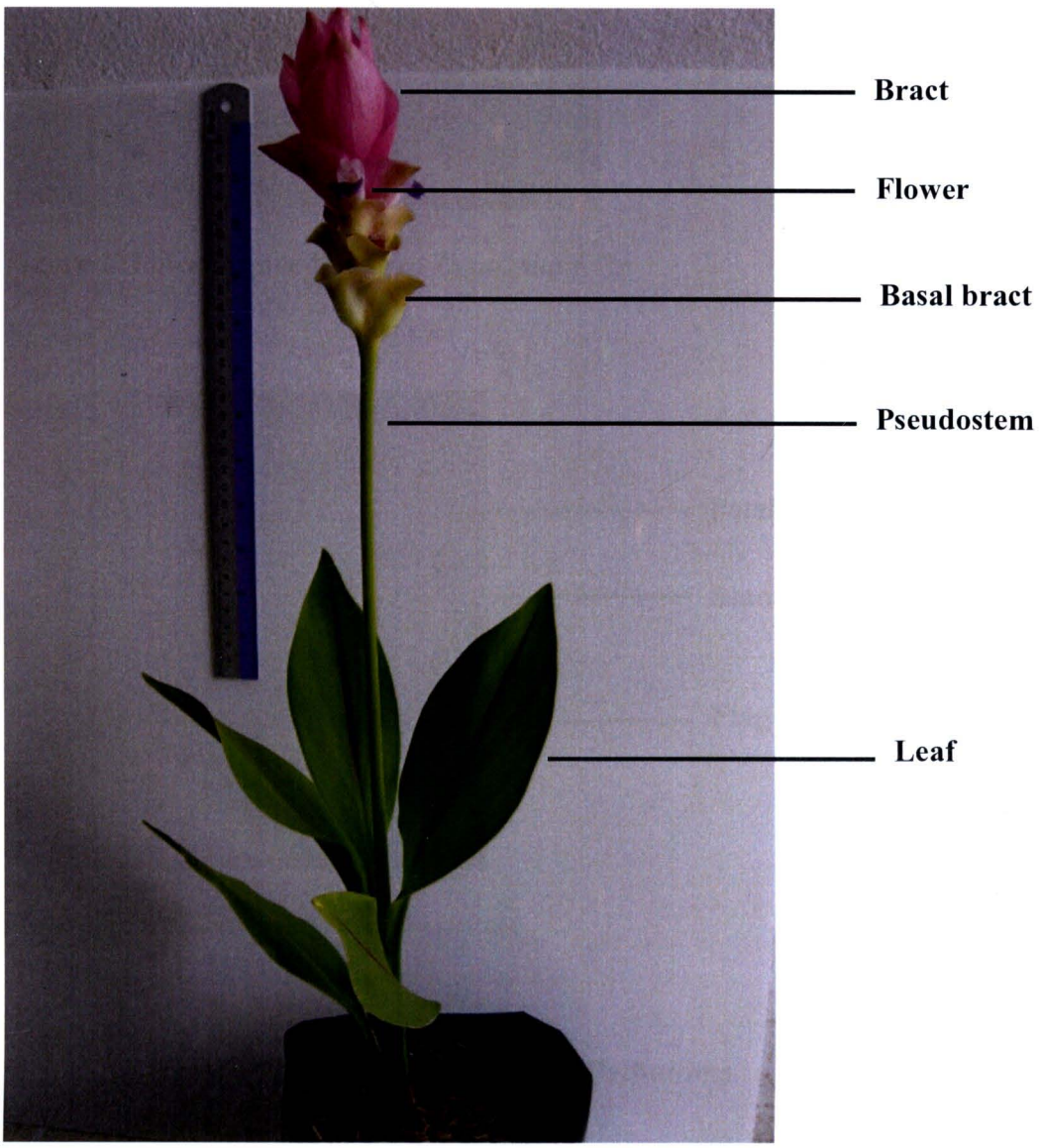


Figure 1 *C. alismatifolia* cv. Chiang Mai Pink

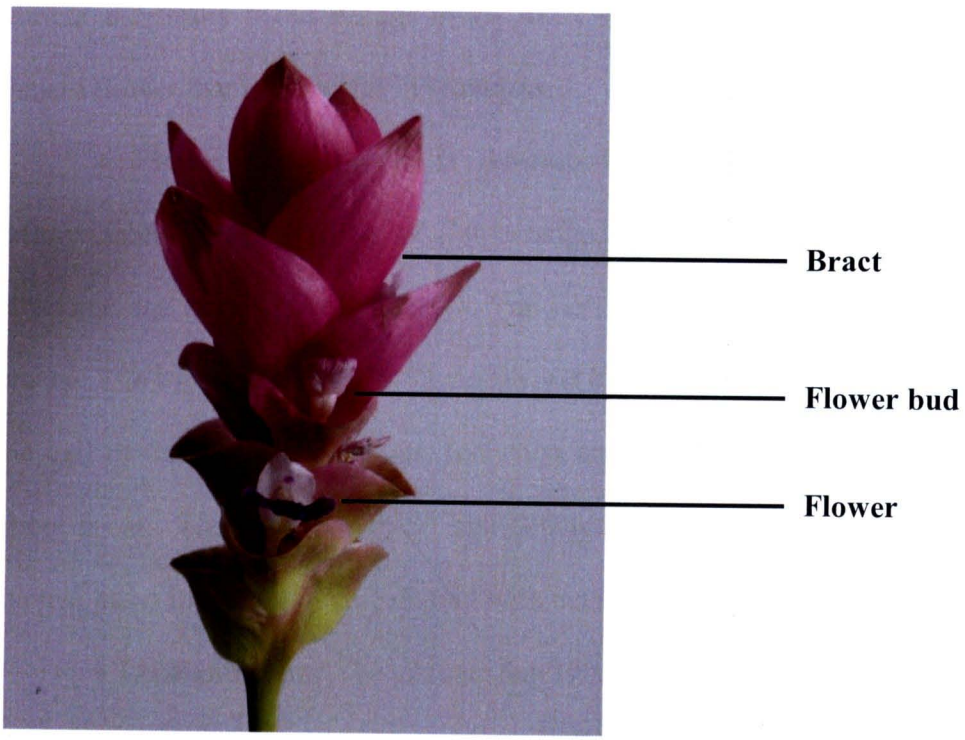


Figure 2 Inflorescence flower of *C. alismatifolia*

Figure 3 True flower of *C. alismatifolia*

1.1 The botanical characteristics of Pathumma

Pathumma belongs to the genus Curcuma which has an estimated 1,000 species (Brown, 2011). *Curcuma* is a perennial herb having a fleshy corm with

fibrous and fleshy ovoid storage roots (Akter *et al.*, 2010). Pathumma has an exotic shaped flower that is beautiful in blossoms.

- **Flower:** The flower is developed on the top of pseudostem. The inflorescence flower consists of fine-arranged bracts. The striking tubular-shaped blossoms are actually leafy bracts. The bottom bracts have 8-10 green and short bracts. The top bracts have 12-18 purple and big bracts. The true flower is located at the axil of the bottom bracts and some top bracts. The length of the true flower is about 6 cm. The petal is white. The flower of Pathumma is a perfect flower. The stamen has a filament which gathered with petal (Pathumma, 2011) (Figure 2, 3).

- **Leaf and stem:** The visible stem is actually pseudostem that consists of leaf sheath. The leaf sheath has function as petiole (stalk) and peduncle covering. The height of stem is about 50-60 cm. In the late of growth stage, the underground stem laterally expands and forms tuber. Pathumma is monocotyledon plant. Leaf has a green long blade or lamina with a light brown mid rib. The leaf margin is smooth. The leaf has 4-5 cm width and 30-35 cm length (Pathumma, 2011) (Figure 1).

Pathumma has rhizome and tuberous root for food storage (Figure 4). Plant stems may be adapted for functions other than support, transport and production of new growth. They may, for instance, serve as protective devices or as attachment organs for vines, carry on photosynthesis ort store food and water. Rhizomes are underground stems that grow horizontally and bear adventitious roots. They are usually light colored and burrow just below the ground surface. Tubers are the enlarged terminal portions of underground rhizomes. The eyes of a rhizome are actually lateral buds formed in the axil of small scale leaves at a node. The internodes

of the rhizome are short and the tuber body is filled with parenchyma cell containing starch, a storage of sugar (Rost *et al*, 2006).

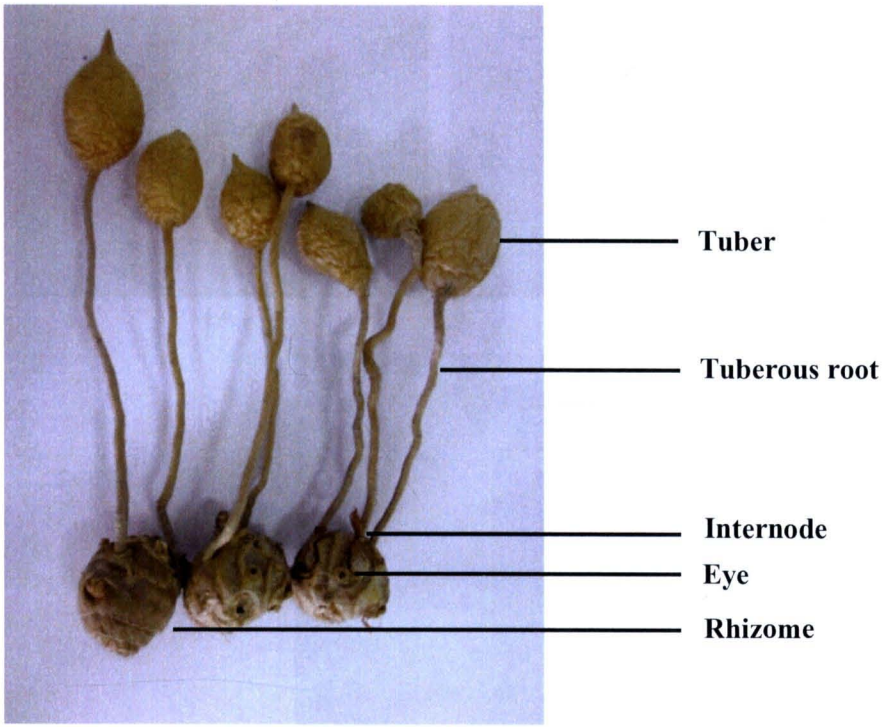


Figure 4 Morphology of rhizome of *Curcuma alismatifolia*

The genus *Curcuma* is devided into 2 groups including *Paracurcuma* or Pathumma group and *Eucurcuma* or Krajeaw group (Boontiang *et al*., 2009).

I. Paracurcuma: Pathumma is generally found in north and northeastern regions of Thailand, the Thailand-Laos and Thailand-Cambodia borders. The habitats of Pathumma are the prairie, mixed and Dipterocarp forests (Boontiang *et al*., 2009). The inflorescence flower of this plant has been developed from the top of pseudostem. The peduncle is tube-like shape. The color of true flower is purple or pale purple. The plants of this group are usually used for the cut flower, potted and ornamental plants such as *C. alismatifolia* cv. Chiang Mai Pink, cv. Chiang Mai White and *C. thorelli* cv. Chinag Mai Snow (Figure 5).

(A)

(C)

(B)

Available: http://www.tejastropicals.com/curcuma-alismatifolia-chiang-mai-white.html

Figure 5 Diversity of paracurcuma found in Thailand; (A) *C. alismatifolia* cv. Chiang Mai Pink, (B) *C. alismatifolia* cv. Chiang Mai White and (C) *C. thorelli* cv. Chiang Mai Snow

II. Eucurcuma: Plants in this group are distributed in all regions of Thailand. The species which grow in sunlight areas, have a thin leaf while the species which found in rain forest, have a thick leaf (Boontiang, 2009). The morphology of stem and flower is quite different from Pathumma that is the flower might develope from the underground rhizome before the growth of pseudostem or develope from pseudostem. In addition, the inflorescence flower is cylinder-like shaped and the true

flower is white or yellow. The representatives of this group are *C. roscoeana*, *C. aurantiaca*, *C. cordata* and *C. rhabdota* (Figure 6).

(A)

Available: http://www.ceapdesign.com.br/familias_botanicas/

(B)

Available: http://www.pacificbulbsociety.org/pbswiki/index.php/Curcuma

(C)

Available: http://blog.tourismthailand.org/EugeneTang/?p=4795

(D)

Available: http://www.pacificbulbsociety.org/pbswiki/files/Curcuma/

Figure 6 Diversity of Eucurcuma found in Thailand; (A) *C. roscoeana*, (B) *C. aurantiaca*, (C) *C. cordata* and (D) *C. rhabdota*

1.2 Pathumma plantation

Pathumma grows well in the fields that have direct sunlight and adequate water. In some areas, the watering is not necessary if rain water is high level. *Curcuma* will do dormant in winter, beginning in November, low moisture content will allow the leaves to die naturally. *Curcuma* plants require well-drained organic

soil mix and high level of humidity (about 50-70% relative humidity) (Brown, 2011). Besides, Pathumma can grow well in pot and under greenhouse environment.

2. Bacterial vascular wilt (Agrios, 1997)

Vascular wilts caused by bacteria affect mostly herbaceous plants such as several vegetables, field crops, ornamentals and tropical plants. The bacterial wilts are *Clavibacter* spp., *Erwinia* spp., *Pseudomonas* spp. and *Xanthomonas* spp.

Generally, vascular wilts occur when bacteria enter, multiply and move through the xylems vessels of the host plants. In the process, they interfere with the translocation of water and nutrients resulting in the drooping, wilting and death of the aboveground parts of the plants. In these respects, bacterial vascular wilts are similar to the fungal vascular wilts caused by *Fusarium*, *Verticillium* and *Ophiostoma*. In the fungal wilts, the fungi remain almost exclusively in the vascular tissues until the death of the plant whereas in the bacterial wilts, the bacteria often destroy part of cell walls of xylem vessels or cause them to rupture quite early in disease development. Subsequently, the bacteria spread and multiply in adjacent parenchyma tissues at various points along the vessels, kill and dissolve the infected cells, and cause the formation of pockets or cavities full of bacteria, gums and cellular debris. In some bacterial vascular wilts, for example, those of corn and sugarcane, once the bacteria reach the leaves, move out of the vascular bundles, spread throughout the intercellular spaces of the leaf and may ooze out through the stomata or cracks onto the leaf surface. Similarly, in some cases, as the bacterial wilt of carnation, the bacteria ooze to the surface of stems through cracks formed over the bacterial pockets or cavities.

More commonly, however, the wilt bacteria are confined primarily to the vascular elements and not the plant surface until the plant is killed by the disease eventually.

Bacterial vascular wilts can sometimes be determined by cutting an infected stem with a sharp razor blade and then separating the two parts slowly, in which case a thin bridge of a sticky substance can be seen between the cut surfaces while they are being separated. Better still, small pieces of infected stem, petiole or leaf can be placed in a drop of water and observe under a microscope, in which masses of bacteria will be seen flowing out from the cut ends of the vascular bundles.

The wilt bacteria will be overwinter in plant debris in the soil, seeds, vegetative propagative materials or insect vectors. They enter the plants through wounds that expose to open vascular elements, multiply and spread in the latter. They spread from plant to plant through the soil, handling and tools, direct contact of plants or insect vectors. Control of bacterial vascular wilt is difficult and depends primarily on the use of crop rotation, resistant varieties, bacterial free seed or other propagative material, removal of infected plant debris and proper sanitation.

2.1 *Ralstonia solanacearum*

Among several wilt disease causing Gram-negative plant pathogens, *R. solanacearum* has been intensively studied both in biochemical and genetical aspects, and has long been recognized as a model system for the analysis of pathogenicity (Salanoubat *et al.*, 2002). *Ralstonia* (formerly *Pseudomonas*) *solanacearum*, the causal agent of bacterial wilt, was ranked as one of the world's most important phytopathogenic bacteria prevalent in tropical, subtropical and some warm regions of the world. Moreover, it can also occur in cool temperate areas (Poussier *et al.*, 1999;

Wydra and Beri, 2006). The original habitat of this organism is probably the tropics, but its increasing occurrence in geographical regions with a prevailing temperate climate has resulted in speculations about its adaptation to these conditions, and its establishment (Schönfeld *et al.*, 2003). The bacteria can cause devastating problem in agriculture resulting in major losses to farmers. The species as a whole has a very broad host range and infects hundreds of species in many plant families. The majority of hosts are dicots with the major exception being bananas and plantains. Most economically important host plants are found in the Solanaceae or nightshade group including potato, tomato, tobacco, peanut, ginger and some ornamental plants (Olson, 2005). The species *R. solanacearum* is a complex taxonomic unit in which strains display an important diversity at different levels (physiological, serological, genetic characteristics and host range). In order to describe this intraspecific variability, several systems of classification have been proposed. Thus, the species was subdivided into five races according to its host range and into six biovars based on the utilization of three disaccharides including cellobiose, lactose and maltose, and oxidation of three hexose alcohols, dulcitol, mannitol and sorbitol (Poussier *et al.*, 1999).

2.1.1 Morphology of *R. solanacearum*

R. solanacearum is a Gram-negative rod, 0.5-1.5 μm in length, with a single polar flagellum. The positive staining reaction for poly-ß-hydroxybutyrate granules with Sudan Black B or Nile Blue distinguishes *R. solanacearum* from *Erwinia* species. In addition, *R. solanacearum* stains heavily at the poles with carbol fuchsin. Agar colonies are initially smooth, shining and opalescent, but become brown with age (OEPP/EPPO, 2004).

2.1.2 Pathogenesis of *R. solanacearum*

All races of *R. solacearum* can be transmitted through contaminated soil, irrigation water, equipment, or personnel. For example, it may be spread by transplanting and propagating infected plants, taking cuttings without disinfecting cutting implements between plants, pinching buds of plants without sanitizing, and especially by shared water irrigation systems. This bacterium can be spread in contaminated soil and on soiled shoes from contaminated areas. Infection occurs typically through the roots and wounding in root areas, a normal physiological process as rootlets grow. Bacteria are normally concentrated in the lower stem portions of the plant. The pathogen does not readily spread from plant-to-plant through the splashing of water, leaf-to leaf contact, or aerially (Floyd, 2004).

R. solanacearum enters the plant through wounds in the roots from cultivating equipment, nematodes, insects, and through cracks where secondary roots emerge. The bacteria reach the large xylem elements and are spread into the plant, where they multiply. Once established in the xylem vessels, the bacteria are able to enter the intercellular spaces of the parenchyma cells in the cortex and pith in various areas of the plant. Here, *R. solanacearum* is able to dissolve the cell walls and create slimy pockets of bacteria and cell debris. Production of highly polymerized polysaccharides increases the viscosity of the xylem, which results in plugging (Olson, 2005). Susceptible plants respond to these high bacterial populations by wilting, yellowing and dying.

R. solanacearum produces several known virulence factors, including extracellular polysaccharide (EPS), and a consortium of plant cell wall-degrading enzymes such as endoglucanase (EG) and polygalacturonase (PG) (Huang and Allen,

2000). Wilting is a result of vascular dysfunction caused by high bacterial cell densities (>$1x10^{10}$ cfu/g fresh weight) and the large amount of EPS these bacteria produce (Denny, 2000).

R. solanacearum can thrive in plant vascular systems without causing disease. Although latent infections are epidemiologically important, the traits required to establish and maintain bacterial populations in symptomless hosts are not understood (Huang and Allen, 2000). Controlling bacterial wilt is difficult because the pathogen has an extremely broad host range, and is able to survive in the soil in the absence of the host plant. Moreover, it can colonize host plants like members of the Solanaceae, and nonhost plants including many weeds, without producing visible symptoms (Dittapongpitch and Surat, 2003).

2.2 *Enterobacter*

Information regarding the ability of *Enterobacter* to function as a plant pathogen is limited, however, some species are known to induce plant diseases.

Bishop (1990) reported that *E. clocae* could cause internal decay of onions. The symptoms of bulbs were discolored (brown to black) and flaccid. This study considered that *E. cloacae* was an opportunistic pathogen of onions. Strains of *E. cloacae* were common components of the microflora in many environment including intestinal tracts of human, plant surfaces, sewage, water and soil.

Nishijima *et al.* (2004) reported that *E. cloacae* could cause rot disease in ginger rhizome. Beginning in 2001, they attempted to isolate and collect *R. solanacearum* from bacterial wilted-infected field plants grown on Hawaii but a facultative anaerobic, Gram negative and rod shaped bacterium was repeatedly

isolated along with the targeted bacteria. The bacteria were identified as *E. cloacae*. This study also revealed that rot symptoms, which usually occurred in the central cylinder of the rhizome, were characterized by yellowish-brown to brown discolored tissue and firm to spongy texture. Upon reinfecting into the host plants, ginger strains of *E. cloacae* produced basal stem and root rot, with foliar chlorosis and necrosis in tissue-cultured ginger plantlets, and discolored and spongy tissue in mature ginger rhizome slices and whole segments. Moreover, this study suggested that *E. cloacae* could exist as an endophyte of ginger rhizomes, and under conditions that are favorable for bacterial growth, or host susceptibility, including maturity of tissues, rhizome rot might occur. Rhizome quality might be impacted by the presence of *E. cloacae* under conditions such as high temperature, high relative humidity, and low oxygen atmosphere that might affect the development of decay, and such conditions should be avoided during postharvest handling and storage.

Masyahit *et al.* (2009) reported that *E. cloacae* could cause soft rot disease on Dragon fruit (*Hylocereus* spp.) in Malaysia Peninsular. The *in vitro* pathogenicity test resulted in yellowish to brownish soft watery symptoms on infected stem and fruit. This study indicated that disease intensity was significantly correlated with temperature and altitude of surveyed areas.

Wang *et al.* (2010) reported that *E. asburiae* and *Enterobacter* spp. could cause mulberry (*Morus alba*) wilt disease in China. Leaf wilt symptoms began on older leaves at the bottom of the plants and then spread to the younger leaves. Leaves of infected plant became withered and dry, turned dark brown and eventually dropped. Xylem tissues in these plants were moist and discolored with brown stripes. The causal agents were presumed to be *R. solanacearum*, however, this study

revealed that symptoms of mulberry wilt caused by *Enterobacter*. Symptoms caused by *Ralstonia* was characterized by flaccid wilted leaves without discoloration and defoliation which could be distinguished from wilt symptoms caused by *Enterobacter*. However, in both cases, vascular tissues become dark brown and neither wilt symptom showed soft rot.

2.3 *Pseudomonas*

Pseudomonads cause important diseases on a variety of crops and symptoms include cankers, leaf and stem spots, blight, soft rot and galls. Important pathogenicity and virulence factors are the type III secretion system, ice nucleation activity and the production of secondary metabolites such as phytotoxins, pectolytic enzymes, exopolysaccharides and hormone production. The most studied and economically important as a plant pathogen with more than 50 pathovars is *Pseudomonas syringae* (Catara, 2007). This species cause necrotic disease, blight and cankers in several plants particularly tomato. When the region of necrosis occurs in secondary-thickened stems, with infection of bark, cortex and underlying stem tissue, it is referred to as a canker. If this infection extends around the whole stem, the entire shoot shows quick wilting and drying (Agrios, 1997).

The information of bacterial wilt caused by *Pseudomonas* is scarce. *Pseudomonas corrugata* and *Ps. marginalis* could cause wilt disease in tomato (Kůdela *et al.*, 2010). Disease symptoms were observed that included external and internal dark brown lesions around the inoculation site, watering and collapse of pith and sometimes also vascular browning and wilting of leaves. Initially, affected plants showed daytime wilting near the top of stem, then merging into progressive leaf

wilting. Finally, the severely affected plants displayed sudden drying and dying, with or without yellowing. As the disease advanced, the whole plants collapsed. Stems cut showed watery browning of the cortex, light to dark browning of vascular tissues, discoloration of stems. The pith was watery, soft and rotted.

Ps. corrugata enters the host through wounds on the stem, collar and roots. Colonization of their cells in parenchymal tissues of tomato plants was consistently associated presumably with exopolysaccharide that embedded them to the cell wall surface. Stem colonization could also proceed towards xylem and epidermis where the bacteria exits in the form of bacterial ooze under conditions of high humidity. In addition, pathogenic pseudomonads and their metabolites were able to elicit plant defense reaction, hypersensitivity reaction, and toxic to plant cells such as lipodepsepeptides (LPDs) which caused chlorosis in tobacco leaves (Catara, 2007).

3. Chemicals for bacterial wilt disease control

From the farmer's observations and pathogen control research, the chemicals which are used in the agriculture include antibiotics and pesticides. Maneb, zineb, iprodione and carbendazim are favored for bacterial disease control.

3.1 Streptomycin

The medically important streptomycin has been used in crop protection as bacteriostatic and antifungal agents. Its principal use is to control bacterial diseases of stone and pome fruits, but it has also been used for early spraying of downy mildew on hops. It is soluble in water and is readily taken up by plants roots. Streptomycin is

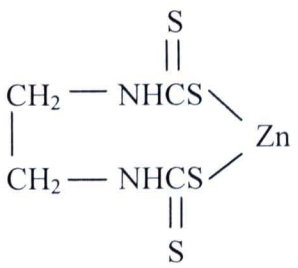
somewhat phytotoxic, causing chlorosis by interfering with the synthesis of chlorophyll (Hassall, 1982).

Mode of action: It probably attacks prokaryotes by attaching itself to ribosomes, one molecule of streptomycin uniting with one 30S ribosome subunit. It appears to prevent the ready movement of peptidyl tRNA from the P to A sites. Streptomycin has a much lower affinity for nonbacterial ribosomes than it has for those in prokaryotes. It does not affect protein synthesis in eukaryotes. Some yeasts and oomycetes are sensitive to streptomycin probably affecting mitochondrial protein synthesis (Hassall, 1982).

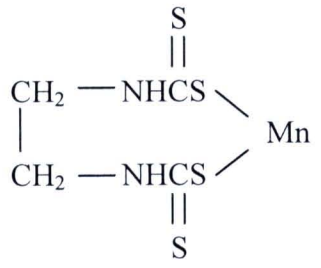
3.2 Maneb and Zineb

Maneb is a yellow with faint ador. It is a polymer of ethylene-(bis)dithiocarbamate units linked with manganese (figure 7). It is used in the control of manydiseases of fruits, vegetables, field crops and ornamentals (Karmin, 1997).

Zineb is a light-colored powder or crystal. It is a polymer of ethylene-(bis) thiocarbamate units linked with zinc (figure 7). It is used to prevent crop damage in the fields and protect harvested crop from deterioration during storage or transport (Karmin, 1997).



zineb



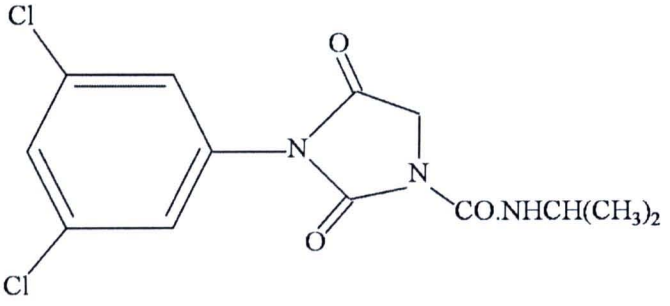
maneb

Figure 7 The chemical structure of zineb and maneb

Mode of action: Both maneb and zineb are dithiocarbamate that are the most used organic fungicides. These organic fungicides are usually far more effective than inorganic fungicides. Organic molecules are generally more compatible with fungal cells which are surrounded by walls and membranes in which a lipid layer is important in exchanging substances through the layer and preserving indispensable constituents within the cells (Hewitt, 1998).

3.3 Iprodione

Iprodione is a colorless and odorless crystal. The chemical name is 3-(3,5-dichlorophenyl)-N-(1-methylethyl) 2,4-dioxo-1-imidazoline-carboxamide (Figure 8). The compound is used in formulations with numerous other fungicides such as thiabendazole and carbendazim. Iprodione is a dicarboximide fungicides used to control a wide variety of crop diseases. It is used on vegetables, ornamentals, pome and stone fruits, root crops, cotton and sunflowers to control fungal pests. It may also be used as a post-harvest fungicides and as a seed treatment. Iprodione inhibits the germination of fungal spores and growth of fungal mycelium (Karmin, 1997).



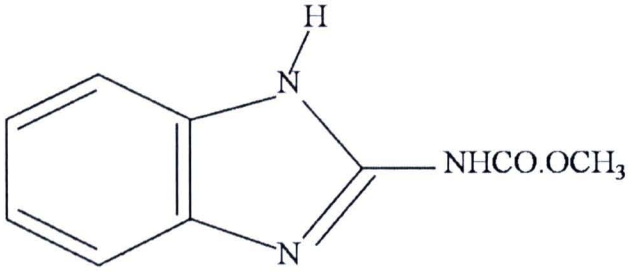
iprodione

Figure 8 The chemical structure of iprodione

Mode of action: The mode of action of this group is unknown but similarities in morphological changes of the test fungi treated with this chemical suggest a similar action. Protein kinases are probably relevant to the mode of action. Protein kinases are enzymes phosphorylating proteins by transferring a phosphate group from ATP to proteins (Hewitt, 1998).

3.4 Carbendazim

Carbendazim is a systemic benzimidazole fungicide that plays a very important role in plant disease control. Carbendazim is used to control a broad range of diseases on arable crops (cereals, oilseed rape), fruits, vegetables and ornamentals. It is also used in post-harvest food storage, and as a seed pre-planting treatment. It is frequently sold in combination with other fungicides, such as triazoles, dithiocarbamates and dicarboximides. The chemical name is methyl benzimidazol-2-yl carbamate (Figure 9). Carbendazim is grouped to class benzimidazoles that comprising of benomyl, carbendazim, thiophanate methyl, fuberidazole and thiabendazole. Benzimidazoles fungicide is one of the most important specific-site inhibiting and systemic fungicide group (Hutson and Miyamoto, 1998).



carbendazim

Figure 9 The chemical structure of carbendazim

Mode of action: The mode of action of the benzimidazoles is well researched and based on their effects on tubulin integrity. Microtubules are alternating helices of α- and β-tubulin which form an essential part of the cytoskeleton and are active in spindle formation and the segregation of chromosome in cell division. (Hewitt, 1998).

Although the chemicals have benefits to farmers but they caused poison accumulation in soil and resistance of microorganisms. Nowadays, the chemicals are concerned with the effects to human and environments. Several countries realize these effects thus the regulations to control pesticide use have been acted.

General considerations of chemical usage

A. Human health effects

In this century, people have increasingly concerned with poisonous chemicals, especially those that cause adverse effects after long periods of exposure. The average of human lifespan has increased tremendously due to cares and treatments for infectious diseases. This longer lifespan has made chronic, non infectious illness more common. Additionally, the industrial revolution has discovered many novel chemicals leading to the synthesis and widespread use of newly developed chemical compounds. This tremendous increase in both the quantity and variety of chemical uses has led to greater awareness of possible adverse health effects from industrial products (Karmin, 1997).

Pesticides can enter the human body through inhalation of aerosols, dust and vapor that contain pesticides; through oral exposure by consuming food and water; and through dermal exposure by direct contact of pesticides with skin. Pesticides are

sprayed onto food, especially fruits and vegetables, they secrete into soils and groundwater which can end up in drinking water, and sprayed pesticide can drift and pollute the air. The effects of pesticides on human health are more harmful based on the toxicity of the chemical and the length and magnitude of exposure. Farm workers and their families experience the greatest exposure to agricultural pesticides through direct contact with the chemicals. But every human contains a percentage of pesticides found in fat samples in their bodies. The chemicals can bioaccumulate in the body over time (Lorenz, 2009). In Thailand (2000-2009), Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health reported that the average of patients harmed from pesticides were 1,996 patients per year. The number of patients tends to be slowly increased every year due to the widespread use of pesticides. The patients, almost were farmers, were found in high level in the plant growing season (Kunphon, 2011).

B. Ecological and environmental effects

The release of pesticides into the environment may be followed by a very complex series of events that can transport the pesticide through the air or water, into the ground, or even into living organisms. Each pesticide has different route of distribution and extent of ion. It will depend on the formulation of the pesticide and when it is released. Despite this complexity, it is possible to identify situations that can pose concern and to try to minimize them. However, there are significant gaps in the knowledge of pesticide movement and fate in the environment, and so it is best to minimize unnecessary release of pesticides into the environment. The fewer pesticides that are unnecessarily released, the safer our environment will be (Karmin, 1997).

Adverse ecological effects from environment pollutants occur at all levels of biological organization, but most information about these effects has been obtained with single species. The effects can be global or local, temporary or permanent, short-lived (acute) or long-term (chronic). The most serious effects involve loss in production, changes in growth and loss of valuable species. These ecological losses in turn may be biologically, economically, aesthetically or socially important. Hence, ecological effects are of serious concern in regulating pollutants, and a variety of tests have been devised to help evaluate the potential for adverse ecological effects. For instances, the current interest is focused on the prevention of pesticide into surface water. It might be controlled by use of filter strips at the field edge that can absorb residue and is not allowed to water reservoir. Developing an understanding of how these tests and other information can be used to prevent environmental problems caused by pollutants (Karmin, 1997).

4. Biological control

Plant diseases need to be controlled to maintain the quality and abundance of crops produced around the world. Different approaches are used to prevent, mitigate or control plant diseases. Over the last 50 years, disease control has relied heavily on the use of chemical fungicides, bacteriocides and soil fumigants. However, there are now many problems associated with their continued deployment including increasing pressure to reduce chemical use in the environment in general, development of pesticide resistance in many pathogens, and decreasing availability of active ingredients through stricter registration and difficulty in finding novel active compounds. Additionally the spread of plant diseases in natural ecosystems may

preclude successful application of chemicals because the scale to which such applications might have to be applied. Consequently, the search for alternative non-chemical methods of disease control continues to gain significance. Indeed, for organic growers where chemical control measures are not permitted, these considerations have been paramount for many years (Oku, 1994; Pal and McSpadden Gardener, 2006; Walters, 2009).

The terms "biological control" or "biocontrol" have been used in different fields of biology. In plant pathology, the term applies to the use of organism antagonists to suppress diseases as well as the use of host-specific pathogens to control weed populations (Pal and McSpadden Gardener, 2006). The organism that suppresses the pest or pathogen is referred to the **biological control agents (BCAs)**. 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. 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.

Biologically based disease control measures have been used for many years. These include plant breeding for resistance, crop rotations, tillage systems and fertilizer practices that affect pathogens directly or alter microbial populations to inhibit pathogens, exploitation of disease suppressive soils and growing media, as well as environmental controls, particularly in the glasshouse. However, the greatest

interest has lied on the development of biological control agents (BCAs) especially that used as microbial inoculants mimicking the chemical products (Walters, 2009).

4.1 Mechanisms of biological control

Due to biological control can result from many different types of interactions between organisms, researchers have focused on characterization of mechanisms in different situations. General mechanisms include antibiosis, competition, parasitism, induced resistance and plant-growth promotion along with highly specialized mechanisms such as that associated with hypovirulence (Pal and McSpadden Gardener, 2006; Walters, 2009).

4.1.1 Production of antibiotics

Antibiotics are generally defined as low molecular weight organic compounds that are produced as secondary metabolites by microorganisms. Antibiotics may have a cidal (killing) or a static (inhibitory) effects on a range of microbes (Haggag and Mohamed, 2007). Different antibiotics have different mode of action on bacteria. These include the prevention of proper cell wall formation, the inhibition or interference with protein synthesis and membrane integrity, the disruption of plasma and/or outer membrane function, the inhibition or interference with DNA synthesis and the inhibition of synthesis of essential small molecules. 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 1.

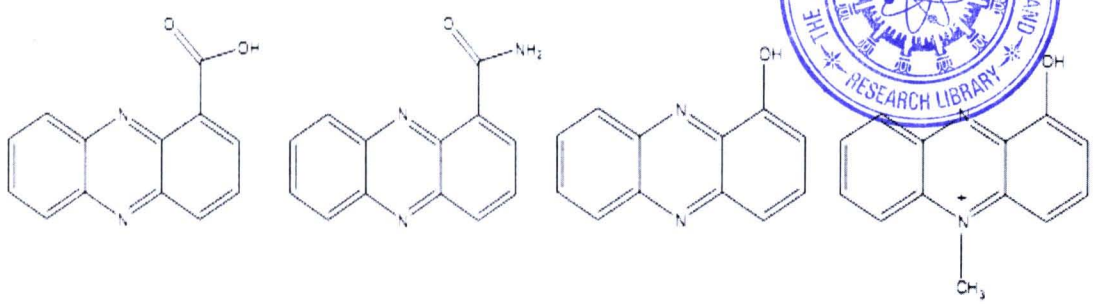
Table 1 Antibiotics produced by BCAs (Pal and McSpadden Gardener, 2006)

Antibitic	**Source**	**Target pathogen**	**Disease**
2,4-diacetyl-phloroglucinol	*Pseudomonas fluorescens* F113	*Pythium* spp.	Damping off
Bacillomycin D	*B. subtilis* AU195	*Aspergillus flavus*	Aflatoxin contamination
Bacillomycin, fengycin	*Bacillus amyloliquefaciens* FZB42	*Fusarium oxysporum*	Wilt
Xanthobaccin A	*Lysobacter* sp. strain SB-K88	*Aphanomyces cochlioides*	Damping off
Gliotoxin	*Trichoderma virens*	*Rhizoctonia solani*	Root rots
Herbicolin	*Pantoea agglomerans* C9-1	*Erwinia amylovora*	Fire blight
Iturin A	*B. subtilis* QST713	*Botrytis cinerea* and *Rhizoc. solani*	Damping off
Mycosubtilin	*B. subtilis* BBG100	*Pythium aphanidermatum*	Damping off
Phenazines	*Ps. fluorescens* 2-79 and 30-84	*Gaeumannomyces graminis* var. *tritici*	Take-all
Pyoluteorin, pyrrolnitrin	*Ps. fluorescens* Pf-5	*Pythium ultimum* and *Rhizoc. solani*	Damping off
Pyrrolnitrin, pseudane	*Burkholderia cepacia*	*Rhizoc. solani* and *Pyricularia oryzae*	Damping off and rice blast

Several biocontrol strains are known to produce multiple antibiotics which can suppress one or more pathogens. For example, *Bacillus cereus* UW85 is known to produce both zwittermycin and kanosamine (Milner *et al.*, 1996). The ability to produce multiple classes of antibiotics, that differentially inhibit different pathogens, is likely to enhance biological control. More recently, phenazine and 2,4-diacetyl-phloroglucinol producing *Pseudomonas putida* WCS358r, a genetically engineered strain displayed improved capacities to control plant diseases in field-grown wheat (Glandorf *et al.*, 2001).

Phenazine natural products are isolated as secondary metabolites primarily from *Pseudomonas*, *Streptomyces*, and a few other genera e.g. *Bacillus, Brevibacterium, Burkholderia* and *Vibrio* (Laursen and Nielsen, 2004; Rane *et al*, 2007; Ruangviriyachai, 2005). The biological properties of phenazine include antibiotic, antitumor, antimalaria and antiparasitic activities. These natural products are small, generally water-soluble and colored compounds. They are consisted of nitrogen-containing phenazine nucleus. They have an absorption spectra with two peaks in the UV range and at least one peak in the visible range that determines their colors. Phenazine compounds have been known as redox reagents and the broad-spectrum antibiotic. The role of phenazine pigments as antibiotics and virulence factors has been briefly reviewed recently. Almost all phenazines are broadly inhibitory to the growth of bacteria and fungi due to their ability to undergo cellular redox cycling in the presence of oxygen and reducing agents (including NADH and NADPH) and cause the accumulation of toxic superoxide and hydrogen peroxide (Mavrodi *et al*, 2010).

The diversity of phenazine derives from varied type and number of functional group attached to phenazine nucleus. More than 6,000 phenazine-containing compounds have been identified and reported during past century. Phenazine isolated from *Pseudomonas* spp. e.g. *Ps. aeruginosa*, *Ps. aureofaciens*, *Ps. fluorescens* and *Ps. chlororaphis* are mostly simple phenazines. Phenazine-1-carboxamide (PCN), phenazine-1-carboxylic acid (PCA), hydroxyphenazine and pyocyanin (Figure 10) were often isolated from these strains and studied on its biological control property (Ruangviriyachai, 2005). PCA has received wide attention for its potential application as a new form of biofungicide, with uses both in environmental protection and in the pharmaceutical industry (Zhou *et al*, 2010).



(a) (b) (c) (d)

Figure 10 Structure of some phenazine derivatives, phenazine-1-carboxylic acid (PCA)(a), phenazine-1-carboxamide (PCN)(b), 1-hydroxyphenazine(c) and pyocyanin (d) (Ruangviriyachai, 2005).

Fluorescent pseudomonads are ubiquitous soil-microorganisms and common inhabitants of the rhizosphere. They have emerged as the largest and potentially most promising group of plant growth-promoting rhizobacteria involved in the biocontrol

of plant diseases. Phenazine-1-carboxylic acid (PCA), produced by *Ps. fluorescens* and *Ps. aureofaciens*, plays an important role in the control of take-all disease of wheat caused by *Gaeumannomyces graminis* var. *tritici* (Tambong and Höfte, 2001). Phenazine-1-carboxamide (oxychlororaphin) produced by *Ps. chlororaphis* PCL 1391 can control tomato root rot caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Chin-A-Woeng *et al.*, 1998). Perneel *et al.* (2007) reported that phenazine producing *Pseudomonas* strains were effective in the biocontrol of cocoyam root rot caused by *Pythium myriotylum.*

4.1.2 Competition

From microbial perspective, soil and living plant surface 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 soil and waste products of other organisms such as insects. 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. 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 (Handelsman and Stabb, 1996; Pal and McSpadden Gardener, 2006)

Biocontrol based on competition, the most commonly cited example especially in soil and rhizosphere, involves iron, as its low bioavailability particularly in high pH soil is a factor limiting growth of microorganisms. Consequently, most microbes produce iron-chelating compounds termed siderophores to competitively acquire

ferric iron. Many siderophores produced by bacteria have a very high affinity for ferric iron. Their release sequesters the limited supply of iron making it unavailable to pathogens (Walters, 2009).

Microorganisms growing under aerobic conditions need iron for a variety of functions including reduction of oxygen for synthesis of ATP, reduction of ribotide precursors of DNA, for formation of heme, and for other essential purposes. A level of at least one micromolar iron is needed for optimum growth. These environmental restrictions and biological imperatives have required microorganisms to form specific molecules that can compete effectively with hydroxyl ion for the ferric state of iron, a nutrient which is abundant but essentially unavailable (Neilands, 1995). Under iron-limited conditions, many bacteria synthesize low molecular weight iron-chelating compounds known as siderophores (Krewulak and Vogel, 2008).

Siderophores (from the Greek: "iron carriers") are defined as ferric ion specific chelating agents elaborated by bacteria and fungi that produce under low iron environments. The role of these compounds is to scavenge iron from the environment and to make the mineral, which is almost always essential, available to the microbial cell (Neilands, 1995) by formation of soluble Fe^{3+} complexes that can be taken up by an active transport mechanism. The siderophore-Fe III complexes are typically taken up by the cell membrane of bacteria, where Fe is reduced and released from the siderophore into the cell (Figure 11) (Kalinowski *et al.*, 2000).

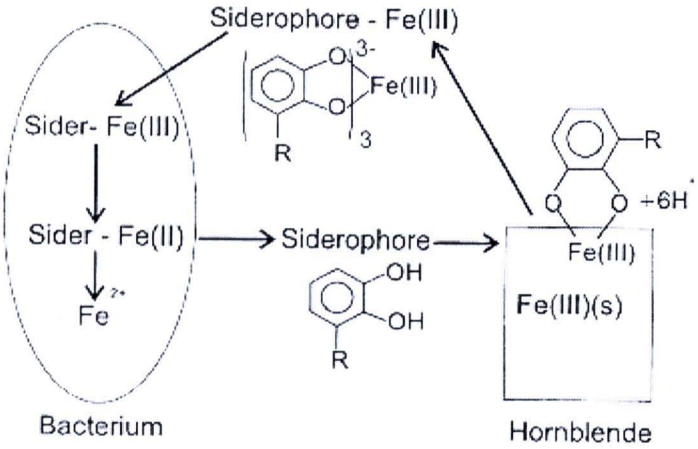


Figure 11 Schematic representation of the excretion of siderophore from a bacterium, complexation of the ligand to the Fe-containing mineral, and reabsorption of the Fe-siderophore complex into the bacterium (Kalinowski *et al.*, 2000)

Siderophores are low molecular weight molecules (300-2,000 daltons), water soluble and have the property to bind iron. Typically, microbial siderophores are classified as catecholates, hydroxamates, and α-carboxylates, depending on the chemical nature of their coordination sites with iron (Pérez-Miranda *et al.*, 2007). The two most common groups of siderophores which are produced from bacteria are the hydroxamates and the catecholates. The functional group in hydroxamates is hydroxamic acid, which is a carbonyl oxygen combined with an amino group. The catecholamide ligands have adjacent hydroxyl oxygens on an aromatic ring (Kalinowski *et al.*, 2000).

Siderophores play important roles in clinical and agricultural applications. They have applications in medicine for iron and aluminum overload therapy and antibiotics for better targeting. A siderophore from *Streptomyces pilosus*, desferrioxamine B, is marketed as the mesylate salt under the trade name Desferal and

advocated for removal of excess iron resulting from the supportive therapy for thalassemia. In agricultural applications, fluorescent pseudomonads form a line of siderophores comprised of a quinoline moiety, responsible for the fluorescence, and a peptide chain of variable length bearing hydroxamic acid and α-hydroxy acid functions. Capacity to form these pseudobactin or pyoverdine type siderophores has been associated with improved plant growth either through a direct effect on the plant, through control of noxious organisms in the soil, or via some other routes. In addition, some siderophore-producing fungi can be use for plant disease control (Neilands, 1995).

Bacillus and *Pseudomonas* have been reported to produce siderophores that could inhibit the growth of plant pathogens by competition for iron in soils (Meyer and Stintzi, 1998). Jagadeesh *et al.* (2001) demonstrated that siderophore from fluorescent *Pseudomonas* could promote the ability to inhibit wilt causing bacteria in tomato.

4.1.3 Parasitism and lytic enzymes

Parasitism and associated production of extracellular lytic enzymes has been thoroughly explored as a mode of action in biocontrol. This is a relatively simple phenomenon for bacteria where degradation of target cell walls is generally considered to reflect parasitism, and may range from simple attachment of bacterial cell to hyphae with minimal degradation, through biofilm formation to complete lysis and cell wall breakdown. The extracellular enzymes produced by bacterial BCAs include chitinase, protease and β-1,3-glucanase. While they may stress and/or lyse cell walls of living organisms, these enzymes generally act to decompose plant

residues and nonliving organic matter (Haggag and Mohamed, 2007; Pal and McSpadden Gardener, 2006; Walters, 2009).

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*). 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 parasitize powdery mildew pathogens (Kiss, 2003).

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. While it is clear that biocontrol microbes can release many different compounds into their surrounding environment, the types and amounts produced in natural systems in the presence and absence of plant disease have not been well documented and this remains a frontier for discovery (Pal and McSpadden Gardener, 2006).

4.1.4 Induced resistance

Some biocontrol agents induce a sustained change in plant, increasing its tolerance to infection by pathogen, a phenomenon known as induced resistance. Induced resistance defined as "the process of active resistance dependent on the host plant's physical or chemical barriers, active by biotic or abiotic agents (inducing agents)". Microorganisms play important role as inducers to stimulate the resistant reaction of host plants (Handelsman and Stabb, 1996; Walters, 2009).

Recently, phytopathologists have begun to characterize the determinants and pathways of induced resistance stimulated by biological control agents and other non-pathogenic microbes. The first pathway, termed systemic acquired resistance (SAR) is mediated by salicylic acid (SA), a compound which is frequently produced by following infection of the pathogen and typically leads to the expression of pathogenesis-related (PR) proteins of bacteria. 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. The second pathway, referred to as induced systemic resistance (ISR), is mediated by jasmonic acid (JA) and/or ethylene, which are produced by following applications of some nonpathogenic rhizobacteria.

A number of root-colonizing microbial strains 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. 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*). Many chemical elicitors of SAR and ISR may be produced by the PGPR strains upon inoculation including salicylic acid, siderophore, lipopolysaccharides, 2,3-butanediol and other volatile substances (Pal and McSpadden Gardener, 2006).

4.1.5 Plant-growth promotion

Both fungal and bacterial BCAs can exhibit the phenomenon of plant growth promotion. To some extent this may reflect the ability of BCAs to control well-known diseases caused by major pathogens or by reducing the deleterious effects of minor pathogens. In addition, many BCAs are able to promote plant growth in the absence of any pathogens, thereby exhibiting additional physiological activities (Walters, 2009).

Historically, most attention has been placed on root-colonizing bacteria that can enhance plant growth resulting in the term of plant-growth-promoting rhizobacteria (PGPR). Those PGPR BCA strains are typically *Bacillus* and *Pseudomonas* but numerous other species are known. Mechanism involved include N_2 fixation, solubilisation of nutrients such as phosphate, promotion of mycorrhizal and rhizobia function, regulating ethylene production in roots, release of phytohormones and decrease heavy metal toxicity. Root and seed colonization to various degrees are the key features for growth promotion with the significance of endophytic growth in this process gradually being recognized.

The fungal plant-growth promoters, *Trichoderma* spp. are probably the most well known. *Trichoderma* have the ability to solubilise phosphate and numerous other micronutrients, enhance efficiency of nitrogen use, increase root development and root hair formation, and exhibit ability to colonize roots (Walters, 2009).

4.1.6 Hypovirulence

A highly specialized mode of action concerns the use of hypovirulent isolates of fungal pathogens. Hypovirulent fungal isolates contain mycoviruses that instrinsically cause the fungal to be less fit. When hypovirulent isolates are

introduced into plant tissues infected with a virulent pathogen, the viruses can be transmitted via hyphal anastamoses, spreading the viral infection, and decreasing disease. The classical example of this process is hypovirulent isolates of *Cryphonectria parasitica*, containing unencapsidated ds-RNA viruses of family *Hypoviridae* which have been used to control chestnut blight in Eurobe. Hypovirus infection is persistant and non-lytic, and is associated with inability to effectively penetrate the host plant, reduced sexual sporulation, female infertility and reduced pigmentation (Walters, 2009).

4.2 Inoculant production

Maximizing the potential for successfully developing and deploying a biocontrol product begins with a carefully crafted microbial screening procedure, proceeds with developing mass production protocols that optimize product quantity and quality, and ends with devising a product formulation that preserves shelf-life, aids product delivery and enhances bioactivity. Microbial selection procedures that require prospective biocontrol agents to possess both efficacy and amenability to increase the likelihood of selecting agents with enhanced commercial development potential. Scale-up of biomass production procedures must optimize product quantity without compromise of product efficacy or amenability to stabilization and formulation (Schisler *et al.*, 2004).

The term "bacterial inoculants" is used as a formulation containing one or more beneficial bacterial strains (or species) in an easy-to-use and economical carrier material, either organic, inorganic or synthesized from defined molecules. The inoculant is the means of bacterial transport from the factory to the living plant. The

desired effects of the inoculant on plant growth can include nitrogen fixation in legumes, biocontrol of soil-borne diseases, enhancement of mineral uptake, weathering of soil minerals and nutritional or hormonal effects. Bacterial inoculants may require lengthy and expensive registration procedures in some countries (Bashan, 1998).

Numerous recent studies show a promising trend in the field of inoculants technology. "Mixed inoculants" (combinations of microorganisms) that interact synergistically are currently being devised. Microbial studies performed without plant indicate that some mixtures allow the bacteria to interact with each other synergistically, providing nutrients, removing inhibitory products and stimulating each other through physical or biochemical activities that may enhance some beneficial aspects of their physiology, like nitrogen fixation (Bashan, 1998). Enhancing biocontrol activity by using mixtures of antagonists may have advantages for instance, it may broaden the spectrum of activity, enhance the efficacy and reliability of the biocontrol, and more importantly, and it may allow the combination of various traits without employment of genetic engineering (Nandakumar *et al.*, 2001).

"Formulation" is the crucial issue for inoculants containing an effective bacterial strain and can determine the success or failure of a biological agent. Formulation is the industrial "art" of converting a promising laboratory-proven bacterium into a commercial field product. Chemical formulations of agroproducts set high standards for long shelf life, ease of use and resistance to abuse by the farmers. Microbial inoculant formulations are expected to match the above characteristics and overcome two major problems for living organisms including the

loss of viability during short storage in the grower's warehouse in which developing countries usually lack refrigeration and the long shelf-life and stability over the range of -5 to 30°C within the marketing distribution systems. Products lacking this range of temperature tolerance will be unacceptable in the agricultural market (Bashan, 1998).

4.2.1 Four basic dispersal forms of inoculants (Bashan, 1998).

A. Powders This form is used as a seed coating before planting. The smaller the particle size, the better inoculant will adhere to the seeds. Standard size vary from 0.075-0.25 nm and the amount of inoculants used is around 200-300 g/ha. These inoculants are the most common both in developed and developing countries.

B. Slurries This inoculant is based on powder-type inoculants suspend in liquid (usually water). The suspension is directly applied to the furrow or, alternatively, the seeds are dipped just prior to sowing.

C. Granules These inoculants are applied directly to the furrow together with the seeds. Size ranges from 0.35-1.18 mm. Rhizobial inoculants is used at a rate of 5-30 kg/ha. These inoculants are popular and have been successfully commercialized since 1975. Granular forms can be used in macro sizes, 1-3 mm in diameter, or in micro sizes, 100-200 μm.

D. Liquids These inoculants use broth cultures or liquid formulations, mainly in water, but also in mineral or organic oils. The seeds are either dipped into the inoculant before sowing or evenly sprayed with the liquid inoculant. After drying, the seeds are sown. This method ensures even

coverage of the seeds without loss of inoculums when dried. For biocontrol agents of leaf diseases, the inoculant can be diluted in water and sprayed for better coverage of the leaves. Alternatively, the suspension can be sprayed directly into the furrow or on the seeds before sowing. The in-furrow inoculant provides a larger amount of bacteria to the plant than seed inoculation.

The use of each type of inoculant depends upon market availability, cost and the needs of a particular crop under specific environmental conditions. For example, the granular form is better than powder inoculants for rhizobia under stressful planting conditions, but since more amounts is required, it is costlier.

4.2.2 Methods of inoculation (Bashan, 1998).

A. Seed inoculation is the most popular method worldwide, as long as the farmer is willing to take the extra step of mixing the inoculants with the seeds immediately before sowing. Microbial inoculants can be applied during three possible stages. First stage, plant seeds are coated with bacterial inoculums for proper time before plantation. Second stage, the bacterial inoculums can be used "on site" as a seed application just before sowing or inoculants are use delivery directly onto the seeds in the furrow. Last, the inoculants are applied after seedlings emerge.

B. Soil inoculation is an alternative to seed inoculation. It is more convenient for the farmer than seed inoculation, but is sometimes not as effective. It is also more expensive because more inoculant is required. Soil inoculants can be done either with peat-based granules or with microgranulated forms of inert

materials; sand, calcium carbonate or marble powder. These materials have been previously mixed with the inoculums in the factory or can be mixed with the seeds by the farmer prior to sowing. The technique uses a specific granular applicator which makes use of insecticide applicators that farmers already have.

4.2.3 Commercial biocontrol products

Over the past fifty years, academic research has led to the development of a small but vital commercial sector that produces a number of biocontrol products. Most of the commercial production of biological control agents is handled by relatively small companies, such as Agraquest, BioWorks, Novozymes, Prophyta and Kemira Agro. Occasionally, such companies are absorbed by or act as subsidiaries of multi-billion dollar agrochemical companies, such as Bayer, Monsanto, Syngenta, and Sumitomo. However, significant expansion is expected over the next 10 years due to increasing petroleum prices, the expanded demand for organic food and increased demand for "safer" pesticides in agriculture, forestry and urban landscapes (Pal and McSpadden Gardener, 2006).

Fungal products are dominated by *Trichoderma* spp. which are also easy to produce, generally have a low toxicity and can sporulate well. Bacterial products are dominated by *Bacillus* species reflecting their ease of growth and production of long-live spores mentioned earlier (Walters, 2009). Additionally, biocontrol strains of fluorescent pseudomonads are developed for commercial products but these strains have a disadvantage from an application due to they generally lose viability when stored for several weeks. Examples of commercially available biocontrol bacteria

include *B. subtilis* GB03 (Kodiak™; Gustafson™), MBI600 (Subtilex™; Becker™; Underwood™) and OST713 (Serenade™; AgraQuest™), *B. licheniformis* SB3086 (EcoGuard™; Novozymes™), a mixture of *B. subtilis* GB122 and *B. licheniformis* GB99 (BioYield™; Gustafson™) and a few strains of *Ps. fluorescens*, *Ps. putida* and *Ps. chlororaphis* (Cedomon™; BioAgri™).

There are several reports demonstrated that *Bacillus* and *Pseudomonas* could be potential biocontrol agents against plant pathogens including bacteria, fungi and nematodes.

Ps. fluorescens CV6 isolated from cucumber rhizosphere in Varamin, Iran, could be as potential biological control agents against *Phytophthora drechsleri*, causal agent of cucumber root rot. BCAs were able to produce siderophore, idole-3-acetic acid (IAA), hydrogen cyanide, protease and phosphatase (Maleki *et al.*, 2010)

Endophytic bacteria isolated from internal tissues of cotton were potential control agents against vascular wilt of cotton caused by *Fusarium oxysporum* f.sp. *vasinfectum*. These bacteria including *B. pumilus* and *Ps. putida* could reduce disease severity when tested bacteria survived in cotton stems for up to 28 days (Chen *et al.*, 1995).

Bacterial wilt caused by *Ralstonia solanacearum* in potato could be controlled by rhizosphere bacterial isolates including *B. subtilis*, *Paenibacillus macerans* and *Ps. fluorescens*. In greenhouse studies, antagonists reduced wilt incidence by 60-80% and increased plant height by 35-45% (Aliye *et al.*, 2008)

B. megaterium could be as potential biological control agents against rice root-knot nematode (*Meloidogyne graminicola*). Treatment with *B. megaterium* resulted

in a greater than 40% reduction in nematode penetration and gall formation compared with non-treated rice roots. The study showed that the exposure of *M. graminicola* eggs to secondary metabolites of *B. megaterium* reduced hatching by over 60% compared with eggs not exposed to the bacteria (Padgham and Sikora, 2007).

Moreover, *Bacillus* sp. and *B. megaterium* could reduce anthracnose, Cercospora leaf spot and root rot of chilli spur pepper under field conditions. Moreover, it was found that a mixture of *Bacillus* sp. and *B. megaterium* effectively reduced the severity of anthracnose on seed by 41% which were significantly as compared with *Bacillus* sp. or *B. megaterium* only (Plodpai *et al.*, 2008).

Ps. chlororaphis (syn. *Ps. aureofaciens*) could inhibit growth of *Drechslera teres* and *Tilletia caries* in spring barley and winter wheat. Antagonistic strain showed good biocontrol activity (>70% disease reduction) in field tests. This isolate could be stored as a suspension in a refrigerator, frozen or applied to seeds for at least one month without loosing its disease controlling ability (Hökeberg *et al.*, 1997).

Trichoderma harzianum and *B. amyloliquefaciens* could be used as potential biological control agents (BCAs) against *Sclerotinia sclerotiorum*. The antogonists protected over 80% of tomato, squash and eggplant seedlings inoculated with *S. sclerotiorum*. The efficacy of *T. harzianum* and *B. amyloliquefaciens* compared with two commercial products, PlantShield and SoilGard, for controlling of *S. sclerotiorum* was similar or slightly lower depending on the crop plant (Abdallah *et al.*, 2008).

B. licheniformis could be BCA to control the grey mold of tomato caused by *Botrytis cinerea*. Both artificial infection experiments in a greenhouse and natural infection experiments revealed that the wettable powder formulation N1E, consisted of corn starch and olive oil, significantly reduced disease severity on tomato plants

and flowers. The disease control value of N1E on tomato plants was 90.5% as compared to the 77% conferred by a chemical fungicide, the mixture of carbendazim and diethofencarb (1:1) (Lee *et al.*, 2006).

The mixture of PGPR strains use as talc-based formulation significantly reduced the sheath blight incidence in rice under field conditions, compared to the respective individual strains. The disease reduction was 29.2% for single strains and 45.1% for mixtures. In addition to disease suppression, treatment with mixture of PGPR strains increased the yield by 25.9% and 17.7% for a single strain. The mixture consisting of *Ps. fluorescens* PF1 plus FP7 was the most effective in reducing the disease and in promoting plant growth and grain yield (Nandakumar *et al.*, 2001).

Mixtures of PGPR can elicit induced systemic resistance to fungal, bacterial, and viral diseases in the four hosts tested. The specific diseases and hosts tested included: bacterial wilt of tomato caused by *R. solanacearum*, anthracnose of long cayenne pepper caused by *Colletotrichum gloeosporioides*, damping off of green kuang futsoi caused by *Rhizoc. solani* and cucumber mosaic virus (CMV) on cucumber. The results indicated that four mixtures of PGPR and one individual strain treatment significantly reduced the severity of all four diseases compared to the non-bacterized control (Jetiyanon and Kloepper, 2002).

Moreover, three strains of PGPR, *Serratia* J2, fluorescent *Pseudomonas* J3 and *Bacillus* BB11 had ability to control bacterial wilt of tomato caused by *R. solanacearum*. In filed experiments, the disease was reduced up to 90% while the yield increased up to 200%. In addition, Yield increased with stored formulation ranging from 35-67% (Guo *et al.*, 2004).