Evaluation of Biological Control Strategies Against A Range Of Plant Pathogens

THESIS

By

Ankit Thakur

Submitted to

Agricultural University of Athens

75, Iera Odos Street, 11855

Athens (Greece)

in

partial fulfilment of the requirement for the degree of

Master of Science in Crop Protection and Environment

(Laboratory of Plant Pathology) 2016

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CERTIFICATE

This is to certify that the thesis entitled "**Evaluation of Biological Control Strategies Against A Range of Plant Pathogens**" submitted by Ankit Thakur to the Agricultural University of Athens, Greece in partial fulfillment of the requirements of the degree of **Master of Science in Crop Protection and Environment** has been approved by the Advisory Committee after the thesis presentation of the student.

……	**……**
Dr. Sotirios E. Tjamos	**Dr. Polymnia Antoniou**
(Major Advisor)	(Member)

……

Dr. Epaminondas J. Paplomatas

Director of Laboratory of Plant Pathology

(Chairperson of the Advisory Committee)

List of tables and figures

Department of Crop Science, Laboratory of Plant Pathology, Agricultural University of Athens 75, Iera Odos Street, 11855, Athens (Greece)

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Abstract

The present investigation entitled "Evaluation of Biological Control Strategies against a Range of Plant Pathogens" was undertaken during the summer (April) of 2015 and stretched for one year. The experiments were primarily focused on observing the biocontrol potential of *Paenibacillus alvei* (strain *K165*) against *Cucumber mosaic virus*, *Potato virus Y* and *Tomato spotted wilt virus* in tomato (*Solanum lycopersicum*) plants; and the use of natural zeolite (from Kimolos island) in suppressing the soil borne plant pathogens, namely, *Rhizoctonia solani*, *Pythium ultimum* and *Scleortinia sclerotiorum* in lettuce (*Lactuca sativa*) plants. The observations made were based on severity of the phenotypic symptoms monitored in lettuce and tomato plants. The examination of tomato plants for the virus presence was done using enzyme linked immune sorbent assay (ELISA) test. There was no significant resistance offered by biocontrol bacterium K165 against the different viruses tested. Although the plants infected with virus did not show extreme visual viral symptoms but still the ELISA test confirmed the presence of virus. Zeolite was able to confer partial resistance in the lettuce plants against *Sclerotinia sclerotiorum*. Different mutants (*mpk3, mpk6, bam*) of *Arabidopsis thaliana* were also tested for their resistance against *Cucumber mosaic virus*. *BAM* mutants showed partial resistance and less viral load when tested through ELISA.

Περίληψη

Η αντιμετώπιση των εδαφογενών παθογόνων που προκαλούν σήψεις ριζών στα φυτά αποτελεί σημαντική πρόκληση στις μέρες μας τόσο λόγω της φύσης των συγκεκριμένων μυκήτων που δυσκολεύει την αντιμετώπισή τους όσο και λόγω της αυξημένης ζήτησης προϊόντων που έχουν παραχθεί με φιλικές προς το περιβάλλον μεθόδους. Στην παρούσα μελέτη ερευνήθηκε η αποτελεσματικότητα της εφαρμογής ζεόλιθου στο υπόστρωμα των φυτών σε συγκέντρωση 20% ή στο σπορείο, στην αντιμετώπιση των μυκήτων *Rhizoctonia solani*, *Sclerotinia sclerotiorum* και του ωομύκητα *Pythium ultimum*. Πρόκειται για ένα φυσικό ορυκτό που χρησιμοποιείται συχνά ως βελτιωτικό εδάφους λόγω της μεγάλης του ικανότητας ανταλλαγής κατιόντων και της εξαιρετικά μεγάλης απορροφητικότητας του που τον καθιστούν ιδανικό για αργή αποδέσμευση των θρεπτικών στοιχείων και νερού. Η χρήση του ζεόλιθου για αντιμετώπιση των παθογόνων *R. solani* και *P. ultimum* φαίνεται αναποτελεσματική. Αντιθέτως στην περίπτωση του μύκητα *S. sclerotiorum* υπήρξε σημαντική μείωση της έντασης των συμπτωμάτων. Επιπλέον η έκφραση του γονιδίου *PR1*, δείκτη ενεργοποίησης της άμυνας που εξαρτάται από το σαλικυλικό οξύ, βρέθηκε ελαφρώς αυξημένη στα μολυσμένα με τον ωομύκητα *P. ultimum* φυτά στα οποία εφαρμόστηκε ο ζεόλιθος σε σχέση με τα μη μολυσμένα φυτά αλλά και σε σχέση με τους μάρτυρες. Παρόμοια πορεία ακολουθεί και η έκφραση του γονιδίου *LOX*, δείκτη ενεργοποίησης της άμυνας που εξαρτάται από το ιασμονικό οξύ/αιθυλένιο, αλλά μόνο στην εφαρμογή 20% ζεόλιθος, γεγονός που δείχνει ότι πιθανώς η εφαρμογή του ζεόλιθου μόνο στον σπόρο δεν ήταν αρκετή για να αυξήσει την έκφραση του γονιδίου. Σε κάθε περίπτωση κρίνεται απαραίτητη η περεταίρω έρευνα για εξαγωγή σαφέστερων συμπερασμάτων.

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Introduction

The majority of the world's living domain is made of plants such as trees, grasses, flowers and so on. Directly or indirectly plants also make the food on which humans and all animals survive. In the pecking order of living beings, plants are the sole life forms that can transform the light energy from sun into stored, usable chemical energy as carbohydrates, proteins and fats. All animals, including humans, depend on these plant substances for survival. Plants whether cultivated or wild, grow and produce well as long as the soil provides them with sufficient nutrients and moisture, sufficient light reaches their leaves, and the temperature remains within a certain "normal" range.

It is accepted that a plant is healthy, or normal, when it can carry out its physiological functions to the best of its genetic potential. The meristematic (cambium) cells of a healthy plant divide and differentiate as needed, and different types of specialized cells absorb water and nutrients from soil; translocate these to all plant parts; carry on photosynthesis, translocate, metabolize or store the photosynthetic products; and produce seed or other reproductive organs for survival and multiplication. When the ability of the cells of a plant or a plant part to carry out one or more of these essential functions is interfered with, by either a pathogenic organism or an adverse environmental factor, the activities of the cells are disrupted, altered or inhibited, the cells malfunction die, and the plant becomes diseased. At first the malady is restrained to one or a few cells and is invisible. Soon, however, the reaction becomes more widespread and affected plant parts develop changes that are visible to the naked eye. These visible changes are the symptoms of the disease.

The agents that cause disease in plants include pathogenic microorganisms, such as viruses, bacteria, fungi, protozoa, and nematodes, and unfavourable environmental conditions, such as lack or excess of nutrients, moisture, and light, and the presence of toxic chemicals in air or soil. Plants also suffer competition from other, unwanted plants (weeds), and, of course, they are often damaged by the attacks of insects. Once the plant is diseased the whole physiology of the plant disorients and consequently it weakens and finally dies. Plant diseases, by their presence, prevent the cultivation and growth of food plants in some areas; or food plants may be

cultivated and grown but plant diseases may attack them, destroy parts or all of the plants, and reduce much of their produce, i.e., food, before they can be harvested or consumed (G.N.Agriose).

The precinct of agriculture has always shaped the human civilizations from times immemorial. The cultivations of crops was the primary occupation in the human settings in the ancient times and still continues to be. This history of agriculture dates back to some 10,000 years ago and since then the plant diseases have been a problem for the mankind. The diseases of plants are of immense importance to human beings as they damage the plants, plant products on which humans depend for food, clothing, furniture, the environment, and, in many cases, housing. For countries where food is plentiful, plant diseases are significant primarily because they cause economic loss to growers. Plant diseases, however, also result in increased prices of products to consumers; they sometimes cause direct and severe pathological effects on humans and animals that eat diseased plant products; and, in trying to control the diseases, people release billions of pounds of toxic pesticides that pollute the water and the environment (G.N.Agriose). Plant disease can inflict much more serious damage on a larger scale. The often-cited potato blight epidemic of the 1840s in Europe is an example of the magnitude of devastation caused by a plant disease. This disease caused by the Oomycete pathogen *Phytophthora infestans* , decimated crops across Europe, and in Ireland, led to the death of some one million people and the emigration of several million more (Large, 1940; Strange, 2003). Incredibly, today, more than 170 years later, potato blight still poses a major problem for potato growers across the globe. Also, in the Great Bengal Famine of 1943, the fungal pathogen *Cochilobolus miyabeanus* devastated rice crops and led to the starvation and death of estimated 2 million people in India (Schumann, 1991). The examples quoted above are few from the many, negative impacts that mass destruction of the crops by the diseases has caused on the human beings.

It is estimated that all sorts of crop enemies, i.e., microbial pathogens, insects, weeds together destroy 31% to 42% crop production annually. The losses are usually lesser in the developed nations and higher in the developing economies. It has been estimated that of the 36.5% average of total Losses, 14.1% are caused by diseases, 10.2% by insects, and 12.2% by weeds. Considering that 14.1% of the crops are lost to plant diseases alone, the total annual worldwide crop loss from

plant diseases is about 220 million US dollars. To these should be added 6-12% losses of crops after harvest, which are particularly high in developing tropical countries where training and resources such as refrigeration are generally lacking. Also, these losses do not include losses caused by environmental factors such as freezes, droughts, air pollutants, nutrient deficiencies, and toxicities (G.N.Agrios).

In the last 100 years, the control of plant diseases and other plant pests has depended increasingly on the extensive use of toxic chemicals. Controlling plant disease often necessitates the application of such toxic chemicals not only on plants and plant products that we consume, but also into the soil, where many pathogenic microorganisms live and attack the plant roots. Many of these chemicals have been shown to be toxic to nontarget microorganisms and animals and may be toxic to humans. The short- and long-term costs of environmental contamination on human health and welfare caused by our efforts to control plant diseases (and other pests) are difficult to estimate. Since the first fungicide, sulphur, was used to control the powdery mildew of grapes, production of most crops has depended on fungicides to avoid disease losses. Genetic resistance of crops towards diseases has been in many cases short lived, and GMOs and have only limited success for disease control and acceptability. With more intensive cropping new diseases have arisen which are devastating if not controlled, such as Asian rust of soybean. In addition, new races and more aggressive pathotypes of diseases have arisen (Gisi, Ulrich, Chet, I; Gulino, Maria Lodovica 2009). All these changes require a rapid development of disease management techniques which are in harmony with the environment.

It has been known for many years that the world population is increasing. Statistics from the United Nations (UNFPA, 2006) predict the world population to grow from the current 5 billion to 8.9 billion by the year 2050, with clear consequences on the quantity of food that need to be produced to avoid widespread hunger(Gisi, Ulrich, Chet, I; Gulino, Maria Lodovica 2009). But the need for measures to control plant diseases limits the amount of land available for cultivation each year, limits the kinds of crops that can be grown in fields that are already contaminated with certain microorganisms, and annually necessitates the use of millions of kilograms of pesticides for treating seeds, fumigating soils, spraying plants, or the postharvest treatment of the fruits. Such control measures not only add to the cost of food production, some of them, e.g., crop rotation, necessarily limit the amount

of food that can be produced, whereas others add toxic chemicals to the environment (G.N.Agrios). Therefore, plant diseases management should be done balancing all the factors involved so that environment is contaminated to the lowest and the quantity of the food produce should not decline.

There are lots approaches used to control the plant diseases. The first line of defence is the exclusion of pathogen through plant quarantine, and, for example, the use of pathogen-free propogating material. The next line of defence is to exclude, eliminate or reduce pathogen inoculums. This can be achieved in various ways, including cultural control, use of host plant resistance and chemical control (Dale Walters, 2008). The pathogens can evolve rapidly to overcome host resistance without apparent loss of fitness (Bronson & Elingboe, 1986; Brown, 2003). The fungicides quickly took a large share of the cereal fungicide market because of their effectiveness against important cereal pathogens. Although the risk of resistance developing was predicted to be moderate, the first signs of resistance were reported within two years in wheat powdery mildew pathogen, *Blumeria gaminis* f. sp. *tritici*, in northern Germany (Leadbeater, 2005). Moreover, in a recent study, 4200 isolates of *Alternaria solani* were collected in the five year period from 2002 to 2006 from 11 potato producing states in United States. Of these isolates 96% exhibited reduced sensitivity to Quninone outside Inhibitor (QoI) group of fungicides and/or had F129L mutation in the cytochrome *b* gene (Pasche & Gudmestad, 2008).

Hence the continued ability of the pathogens to overcome host resistance genes and to develop resistance to chemicals seriously erodes our ability to provide effective, lasting disease control on important crops. These problems, combined with the withdrawal of active substances from the market and increasing public concern with the effects of pesticides on the environment creates a huge challenge for agriculturists.

Plant's defense strategies against pathogens

Each plant species is affected by approximately 100 different kinds of fungi, bacteria, mollicutes, viruses, and nematodes. Frequently, a single plant is attacked by hundreds, thousands, and, in leafspot diseases of large trees, probably hundreds of thousands of individuals of a single kind of pathogen. Although such plants may suffer damage to a lesser or greater extent, many survive all these attacks and, not

uncommonly, manage to grow well and to produce appreciable yields (Agrios, 2009). Humans depend almost exclusively on plants for food, and plants provide many important non-food products including wood, dyes, textiles, medicines, cosmetics, soaps, rubber, plastics, inks, and industrial chemicals. Understanding how plants defend themselves from pathogens and herbivores is essential in order to protect our food supply and develop highly disease-resistant plant species (Brian C. Freeman and Gwyn Beattie, 2008). In general, plants defend themselves against pathogens by a combination of weapons from two arsenals: (1) structural characteristics that act as physical barriers and inhibit the pathogen from gaining entrance and spreading through the plant and (2) biochemical reactions that take place in the cells and tissues of the plant and produce substances that are either toxic to the pathogen or create conditions that inhibit growth of the pathogen in the plant. The combinations of structural characteristics and biochemical reactions employed in the defense of plants are different in different host–pathogen systems. In addition, even within the same host and pathogen, the combinations vary with the age of the plant, the kind of plant organ and tissue attacked, the nutritional condition of the plant, and the weather conditions (J.R. Aist, 1976).Whatever is resistance that is conferred as a plant defense mechanism, against a pathogen or against an abiotic agent, it is controlled directly or indirectly by the genetic material (genes) of the host plant and of the attacking pathogen.

Broadly defined, disease is any physiological abnormality or significant disruption in the "normal" health of a plant. Disease can be caused by living (biotic) agents, including fungi and bacteria, or by environmental (abiotic) factors such as nutrient deficiency, drought, lack of oxygen, excessive temperature, ultraviolet radiation, or pollution. In order to protect themselves from damage, plants have developed a wide variety of constitutive and inducible defenses. Constitutive (continuous) defenses include many preformed barriers such as cell walls, waxy epidermal cuticles, and bark. These substances not only protect the plant from invasion, they also give the plant strength and rigidity. In addition to preformed barriers, virtually all living plant cells have the ability to detect invading pathogens and respond with inducible defenses including the production of toxic chemicals, pathogen-degrading enzymes, and deliberate cell suicide. Plants often wait until pathogens are detected before producing toxic chemicals or defense-related proteins because of the high energy costs and nutrient requirements associated with their production and maintenance (Bindschedler L.V., J. Dewdney, K.A. Blee, J.M. Stone, T. Asai, J. Plotnikov, C. Denoux, T. Hayes, C. Gerrish, D.R. Davies, F.M. Ausubel, and G.P. Bolwell. 2006).

Many pathogens establish intimate connections with their hosts in order to suppress plant defenses and promote the release of nutrients. Pathogens that keep their host alive and feed on living plant tissue are called biotrophs. Examples of

biotrophic pathogens include the powdery mildew fungus *Blumeria graminis* and the bacterial rice pathogen *Xanthomonas oryzae*. Other pathogens resort to brute force like thieves who blast open a bank vault with explosives. These pathogens often produce toxins or tissue-degrading enzymes that overwhelm plant defenses and promote the quick release of nutrients. These pathogens are called necrotrophs, and examples include the gray mold fungus *Botrytis cinerea* and the bacterial soft-rot pathogen*Erwinia carotovora*. Some pathogens are biotrophic during the early stages of infection but become necrotrophic during the latter stages of disease. These pathogens are called hemibiotrophs and include the fungus *Magnaporthe grisea,* the causative agent of rice blast disease (Broekaert, W.F., B.P.A. Cammue, M.F.C. DeBolle, K. Thevissen, G. W. DeSamblanx, and R.W. Osborne. 1997).
Most biotrophic and hemibiotrophic pathogens can only cause disease on a relatively small group of host plants because of the slightly different set of specialized genes and molecular mechanisms required for each host-pathogen interaction. The host range refers to the plant species on which a pathogen is capable of causing disease. For example, brome mosaic virus (BMV) infects grasses such as barley but not legumes. A plant species that does not show disease when infected with a pathogen is referred to as a non-host plant species for that pathogen. Organisms that do not cause disease on any plant species, such as the saprophytic bacterial species *Pseudomonas putida*, are referred to as non-pathogens (Broekaert, W.F., F.R. Terras, B.P. Cammue, and R.W. Osborn. 1995).

When a pathogen is capable of causing disease on a particular host species, two outcomes are possible: A compatible response is an interaction that results in disease, while an incompatible response is an interaction that results in little or no disease at all. Although a particular plant species may be a susceptible host for a particular pathogen, some individuals may harbor genes that help recognize the presence of the pathogen and activate defenses. For example, some tomato cultivars show disease when infected with the bacterial pathogen *Pseudomonas syringae* (a compatible response), but others (cultivar Rio Grande, for example) are capable of recognizing the bacteria and limiting disease via resistance (an incompatible response). Disease resistance exists as a continuum of responses ranging from immunity (the complete lack of any disease symptoms) to highly resistant (some disease symptoms) to highly susceptible (significant disease symptoms) (Dow, M., M.A. Newman, and E. von Roepenack. 2000).

Plants have developed multiple layers of sophisticated surveillance mechanisms that recognize potentially dangerous pathogens and rapidly respond before those organisms have a chance to cause serious damage. These surveillance systems are

linked to specific pre-programmed defense responses. Basal resistance, also called innate immunity, is the first line of pre-formed and inducible defenses that protect plants against entire groups of pathogens. Basal resistance can be triggered when plant cells recognize microbe-associated molecular patterns (MAMPs) including specific proteins, lipopolysaccharides, and cell wall components commonly found in microbes. The result is that living plant cells become fortified against attack. Non-pathogens as well as pathogens are capable of triggering basal resistance in plants due to the widespread presence of these molecular components in their cells (Heath, M.C. 2000).

Pathogens have developed counter measures that are able to suppress basal resistance in certain plant species. If a pathogen is capable of suppressing basal defense, plants may respond with another line of defense: the hypersensitive response (HR). The HR is characterized by deliberate plant cell suicide at the site of infection. Although drastic compared to basal resistance, the HR may limit pathogen access to water and nutrients by sacrificing a few cells in order to save the rest of the plant. The HR is typically more pathogen-specific than basal resistance and is often triggered when gene products in the plant cell recognize the presence of specific disease-causing effectormolecules introduced into the host by the pathogen. Bacteria, fungi, viruses, and microscopic worms called nematodes are capable of inducing the HR in plants (Heil, M., and R.M. Bostock. 2002.).

Once the hypersensitive response has been triggered, plant tissues may become highly resistant to a broad range of pathogens for an extended period of time. This phenomenon is called systemic acquired resistance (SAR) and represents a heightened state of readiness in which plant resources are mobilized in case of further attack. Researchers have learned to artificially trigger SAR by spraying plants with chemicals called plant activators. These substances are gaining favor in the agricultural community because they are much less toxic to humans and wildlife than fungicides or antibiotics, and their protective effects can last much longer (Brian C. Freeman and Gwyn A. Beattie, 2005).

In addition to the hypersensitive response, plants can defend themselves against viruses by a variety of mechanisms including a sophisticated genetic defense system called RNA silencing. Many viruses produce double-stranded RNA or DNA during replication in a host cell. Plants can recognize these foreign molecules and respond by digesting the genetic strands into useless fragments and halting the infection. Plants that are infected with viruses will often exhibit chlorosis and mottling, but disease symptoms may eventually disappear if RNA

silencing is successful, a process called recovery. In addition, the plant may retain a template of the digested genetic strand that can be used to quickly respond to future attack by similar viruses, a process analogous to the memory of vertebrate immune systems (Brian C. Freeman and Gwyn A. Beattie, 2005).

All plant tissues contain pre-formed structural barriers that help limit pathogen attachment, invasion and infection. The cell wall is a major line of defense against fungal and bacterial pathogens. It provides an excellent structural barrier that also incorporates a wide variety of chemical defenses that can be rapidly activated when the cell detects the presence of potential pathogens. All plant cells have a primary cell wall, which provides structural support and is essential for turgor pressure, and many also form a secondary cell wall that develops inside of the primary cell wall after the cell stops growing. The primary cell wall consists mostly of cellulose, a complex polysaccharide consisting of thousands of glucose monomers linked together to form long polymer chains. These chains are bundled into fibers called microfibrils, which give strength and flexibility to the wall. The cell wall may also contain two groups of branched polysaccharides: cross-linking glycans and pectins. Cross-linking glycans include hemicellulose fibers that give the wall strength via cross-linkages with cellulose. Pectins form hydrated gels that help "cement" neighboring cells together and regulate the water content of the wall. Soft-rot pathogens often target pectins for digestion using specialized enzymes that cause cells to break apart: these organisms are extremely common, and anyone who has seen fruits or vegetables become brown and "mushy" have seen these pathogens in action (Brian C. Freeman).

Cell walls contain proteins and enzymes that actively work to reshape the wall during cell growth yet thicken and strengthen the wall during induced defense. When a plant cell detects the presence of a potential pathogen, enzymes catalyze an oxidative burst that produces highly reactive oxygen molecules capable of damaging the cells of invading organisms. Reactive oxygen molecules also help strengthen the cell wall by catalyzing cross-linkages between cell wall polymers, and they serve as a signal to neighboring cells that an attack is underway. Plant cells also respond to microbial attack by rapidly synthesizing and depositing callose between the cell wall and cell membrane adjacent to the invading pathogen. Callose deposits, called papillae, are polysaccharide polymers that impede cellular penetration at the site of infection, and these are often produced as part of the induced basal defense response (Brian C. Freeman and Gwyn A. Beattie).

Some plant cells are highly specialized for plant defense. Idioblasts ("crazy cells") help protect plants against herbivory because they contain toxic chemicals or

sharp crystals that tear the mouthparts of insects and mammals as they feed. There are many classes of idioblasts including pigmented cells, sclereids, crystalliferous cells, and silica cells. Pigmented cells often contain bitter-tasting tannins that make plant parts undesirable as a food source. Young red wines often contain high levels of tannins that give wine a sharp, biting taste. Sclereids are irregularly-shaped cells with thick secondary walls that are difficult to chew: the rough texture of pear fruit (*Pyrus* spp.) is caused by thousands of sclereid stone cells that can abrasively wear down the teeth of feeding animals. Stinging nettles (*Urtica dioica*) produce stinging cells shaped like hypodermic needles that break off when disturbed and inject highly irritating toxins into herbivore tissues. Some stinging cells contain prostaglandins, hormones that amplify pain receptors in vertebrate animals and increase the sensation of pain. Crystalliferous cells contain crystals of calcium oxalate that may tear herbivore mouthparts when chewed and can be toxic if ingested. Members of the genera *Philodendron* and *Dieffenbachia* are very common tropical house plants that contain large amounts of these cells. Humans and pets who chew the leaves of these plants may experience a burning sensation in the mouth and throat that is often accompanied by swelling, choking, and an inability to speak. For these reasons, species of Dieffenbachia are commonly called dumb cane. Grasses and sedges contain rows of silica cells in their epidermal layers which give strength and rigidity to the growing leaf blades and deter feeding by chewing insects (Mc Spadden).

Monoterpenoids and sesquiterpenoids are the primary components of essential oils, which are highly volatile compounds that contribute to the fragrance (essence) of plants that produce them. Essential oils often function as insect toxins and many protect against fungal or bacterial attack. Mint plants (*Mentha* spp.) produce large quantities of the monoterpenoids menthol and menthone which are produced and stored in glandular trichomes on the epidermis. Pyrethrins are monoterpenoid esters produced by chrysanthemum plants that act as insect neurotoxins. Many commercially available insecticides are actually synthetic analogues of pyrethrins, called pyrethroids, including the insecticides permethrin and cypermethrin. Pine tree resin contains large quantities of the monoterpenoids alpha- and beta-pinene, which are potent insect repellents; these compounds give the organic solvent turpentine its characteristic sharp odor.

Members of the nightshade family (*Solanaceae*) produce many important alkaloid compounds. Nicotine is an alkaloid that is produced in the roots of tobacco plants (*Nicotiana tabacum*) and transported to leaves where it is stored in vacuoles. It is released when herbivores graze on the leaves and break open the vacuoles. Atropine is a neurotoxin and cardiac stimulant produced by the deadly

nightshade plant (*Atropa belladonna*). Although it is toxic in large quantities, it has been used medicinally by humans in small amounts as a pupil dilator and antidote for some nerve gas poisonings. Capsaicin and related capsaicinoids produced by members of the genus *Capsicum* are the active components of chili peppers and produce their characteristic burning sensation in hot, spicy foods (Ainst, 1998).

Defensins are small cysteine-rich proteins that display broad anti-microbial activity and were first isolated from the endosperm of barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*). They are widely distributed and may be present in most plants. Defensins are best characterized in seeds, but can be found in virtually all types of plant tissues including leaves, pods, tubers, fruit, roots, bark, and floral tissues. They exhibit a wide range of biological activities that serve to inhibit the growth of many fungi and bacteria. Some defensins also inhibit digestive proteins in herbivores. The precise mechanisms employed by plant defensins to inhibit fungi and bacteria are still being characterized, but they appear to act upon molecular targets in the plasma membrane of pathogens. These defensins may inhibit pre-existing ion channels or form new membrane pores that disrupt cellular ion balance.

Hydrolytic enzymes are produced by some plants in response to pathogens and often accumulate in extracellular spaces where they degrade the cell walls of pathogenic fungi. Chitinases are enzymes that catalyze the degradation of chitin, a polymer with a backbone similar to cellulose that is present in the cell walls of true fungi. Glucanases are enzymes that catalyze the degradation of glycosidic linkages in glucans, a class of polymers similar to cellulose that is present in the cell walls of many oomycetes (water molds). In vitro analysis has verified the anti-fungal properties of these compounds, and transgenic plants expressing high levels of these enzymes exhibit increased resistance to a wide range of both foliar and root pathogens. Lysozymes are hydrolytic enzymes that are capable of degrading bacterial cell walls (Brian C. Freeman and Gwyn A. Beattie).

Fungal pathogens

Fungi are small, generally microscopic, eukaryotic, usually filamentous, branched, spore- bearing organisms that lack chlorophyll. Fungi have cell walls that contain chitin and glucans (but no cellulose) as the skeletal components. These are embedded in a matrix of polysaccharides and glycoproteins. A group of fungal

like organisms, the Oomycota, usually referred to as oomycetes, until about 1990 were considered to be true fungi. The vast majoirity of oomycetes have cell walls composed of glucans with a bit of cellulose but no chitin. The Oomycota now belong to the kingdom Chromista (rather than Fungi) but continue to be believed as fungi because of same disease causing mechanisms (Agrios, 2009). Out of 100,000 known fungal species, most of them are saprophytic i.e., they live on dead organic matter which they help to decompose. More than 10,000 species of fungi can cause diseases in plants. Today all known cultivated plants are attacked by fungi causing unwanted deviations in their normal physiology. Obligate fungus (biotrophs) can only grow in association with the host plants, multiplying on them using their machinery. Whilst, non obligate fungus require host plants only for a part of their life cycle as they can also use dead organic matter for the extension and continuation of their forms.

Most fungi have a filamentous body called a mycelium which branches out in all directions. The individual branches of a mycelium are called hyphae which are generally uniform in thickness. In some fungi the mycelium consists of many cells containing one or two nuclei per cell. Some lower fungi lack true mycelium and produce instead a system of strands of glossy dissimilar and continuously varying diameter called a rhizomycelium, whilst some protozoans produce a naked, amoeboid, multinucleate body called plasmodium (G.C. Ainsworth).

Pythium ultimum

Oomycetes produce oospores as their resting spores and zoospores or zoosporangia as their asexual spores. The most important plant pathogenic oomycetes belong to two orders, namely *Saprolegniales* and *Perenosparales*. The latter contains several of the most important genera of plant pathogens known; these are *Pythium* and *Phytophthora*, each consisting of many very important plant pathogenic species, and several genera causing downy mildews (Agrios, 2009).

Pyhtium species are one of the most common and most important causes of seed rot, seedling damping off, and root rot of all type of cultivated plants, and also of soft rot of fleshy fruits in contact with the soil (W.A. Campbell).

Damping off diseases of seedlings occur round the globe in valleys and forest soils, in tropical and temperate countries, and in every greenhouse. The disease

affects the seeds, seedlings and roots of all solanaceous crops; however the greatest damage is done to the seeds and seedling roots during germination either before or after emergence. It is quite frequent that the seedlings die in their seedbeds or very soon after transplanting. Older plants are seldom killed by the pathogen. Young seedlings can be attacked before mergence at any point on the plant, from which the infection spreads rapidly, the invaded cells collapse, and the seedling is often overrun by the oomycete and dies, which is known as pre emergence damping-off (G.M. Waterhouse, 1968). The sporangium is a major survival structure in soil, even though oospores are produced. This is especially true for asexual strains of *P. ultimum.* Sporangia are exogenously dormant propagules that will germinate in response to stimulants from the seeds of host plants. This response occurs within the first few hours of seed germination, resulting in seed infection within the first 24 h after sowing. The molecules in seed exudates eliciting these rapid responses are largely unknown but are believed to consist of long-chain unsaturated fatty acids. *Enterobacter cloacae* is a common plant-associated rhizobacterium effective in controlling *Pythium* diseases. Other biological control agents such as *Pseudomonas* and *Bacillus* species suppress *Pythium* diseases largely through the biosynthesis of antibiotics or other *Pythium*-inhibitory substances. However, no antibiotic production or parasitism has been found in *E. cloacae,* even though *E. cloacae* attaches to the hyphae of *P. ultimum* colonizing seed surfaces. The control of *Pythium* dampingoff by *E. cloacae* can be attributed to its ability to reduce or eliminate responses of sporangia to germinating seeds by metabolizing long-chain fatty acids, mainly linoleic acid, released from the seed during germination. *E. cloacae* protected cucumber, cotton, and ryegrass from damping-off but were ineffective in protecting the seeds of snap bean, lima bean, soybean, corn, and pea. Results indicated that this response was regulated by the exudation patterns of seed exudate carbohydrates, since *E. cloacae* was effective only on those plants whose exudates contained low levels of carbohydrates. Additional experiments in which different mono-, di-, and trisaccharides were added with *E. cloacae* to the spermosphere of cucumber revealed that some sugars (e.g., D-galactose, D-glucose, sucrose, and _-methyl-D-glucoside) significantly reduced the efficacy of biological control of *Pythium* damping-off, whereas other sugars (e.g., 3-*O*-methyl-D-glucose, D-trehalose, L-glucose, and L-sorbose) did not. In culture, D-galactose, Dglucose, sucrose, and _-methyl-D-glucoside were capable of supporting abundant growth of *E. cloacae*, whereas 3-*O*-methyl- D-glucose, D-trehalose, L-glucose, and L-sorbose supported no growth of *E. cloaca.* none of the sugars tested increased the colonization or infection of seeds by *P. ultimum*, suggesting that the differential responses observed were due to direct effects on *E. cloacae*. Because of the importance of

seed exudates in controlling the spermosphere environment, it is hypothesized that seed exudates may affect the ability of *E. cloacae* to inactivate sporangium germination stimulants and thus affect disease control (Koji Kageyama and Eric B. Nelson, 2003).

Most alfalfa seed is treated with the fungicide mefenoxam (Apron XL) for control of soil borne seedling diseases caused by *Phytophthora medicaginis* and *Pythium* spp. However, Apron XL is not active against *Aphanomyces euteiches*, the causal agent of Aphanomyces root rot (ARR), an important component of the alfalfa seedling root rot complex. Moreover, Apron XL-treated seed cannot be used in organic production systems. A seed coating using aluminosilicate (natural zeolite) at a rate of 0.33 g of zeolite per gram of alfalfa seed was tested as an alfalfa seed treatment. Inoculated growth chamber trials were conducted to determine the percentage of seedlings protected from Phytophthora root rot (PRR) and ARR. The mineral seed coating resulted in significantly greater control of PRR, with a mean of 89% healthy seedlings (disease score of 1 or 2 on a 1-to-5 scale) compared with the Apron XL treatment, with a mean of 38% healthy seedlings, or the control treatment, with 15% healthy seedlings. The mineral seed coating also resulted in significantly greater protection against ARR, with 67% healthy seedlings compared with 3 and 2% healthy seedlings with the Apron XL and control treatments, respectively. The coated seed were used for in vitro assays with *Pythium ultimum* and *P.paroecandrum* to test for protection from seed rot and damping off. The mineral seed coating resulted in a significantly greater percentage of healthy seedlings compared with the Apron XL and control treatments. In growth chamber assays with naturally infested field soils with a range of disease pressure, the mineral seed coating resulted in a similar or greater percentage of healthy plants than the Apron XL treatment. The mineral coating had no effect on in vitro growth of *Sinorhizobium meliloti*, and nodule numbers were similar on roots from mineral-coated and untreated seed. These experiments indicate that the zeolite seed coating is a promising means of controlling seedling diseases in alfalfa production systems (Deborah A. Samac).

Rhizoctonia solani

Basidiomycetes cause serious plant losses by primarily attacking the roots and lower stems of plants. Some of these fungi, e.g., *Rhizoctonia* (teleomorph: Thanatophorus) and Sclerotium (teleomorph: Aethalium), attack primarily herbaceous plants. The above mentioned fungi are soil inhabitant basidiomycetes and they can cause serious diseases on many hosts by affecting the roots, stems,

tubers, corms, and other plant parts that develop in or on the ground. These two fungi were known as sterile fungi because for many years they were thought to produce only sclerotia and to be incapable of producing spores of any kind, either sexual or asexual. The two were distinguished from each other by the characteristic of their mycelium and y the fact that *Rhizoctonia sclerotia* have a uniform texture throughout, where as *Scleortium* sclerotia are internally differentiated in to three areas. It is now known that at least some species within these two genera produce basidiospores as their sexual spores and, therefore they are Basidiomycetes (N.A. Anderson, 1982).

The disease symptoms of *Rhizoctonia* may differ from on different crops, with the stage of growth at which plant becomes infected, with the prevalent environmental conditions. The most common symptoms on most plants are roots rot, stem rot or stem canker of growing and grown plants. However, on some hosts, *Rhizoctonia* also causes rotting of storage organs and foliage blights or spots, especially of foliage near to the ground (G.w. Brushl, 1975). Rhizoctonia spp. represent a large, diverse, and complex group of fungi. Mycelial cells of *R. solani*, contain several nuclei (multinucleate), which form a colorless mycelium at young age but it turns yellow with age. The perfect stage of multinucleate *R. solani* is *Thanatophorus*. It is evident that *Rhizoctonia solani* and other species are "collective species" consisting of several more or less related strains which are distinguished from one another as anastomosis occurs only between the isolates of the same anastomosis group. Although the various anastomosis groups are not entirely host specific, they show certain fairly well defined tendencies: isolates of anastomosis group 1 (AG1) cause seed and hypocotyls rot and aerial (sheath) and web blights of many plant species; isolates of AG 2 cause canker of root crops, wire stem on crucifers, and brown patch on trufgrasses; isolates of AG 3 affect mostly potato, causing stem cankers and stolon lesions; and isolates of AG 4 cause seed or hypocotyls rot on almost all angiosperms, and stem lesions on soil lines in almost all legumes. As of 2008, 13 more anastomosis groups are known within R. solani (Blazier, 2009) Recognition of these groups and their greater or lesser host specificity has help in various advancements in plant breeding and biocontol strategies (Agrios, 2009). The strain 2 which also belongs to AG 1-1C does not shows lectin activity (M. Hamshou, 2007). The control is done by the application of pentachlornitrobenzene (PCNB).

Sclerotinia sclerotiorum

Sclerotinia sclerotiorum is a ubiquitous necrotrophic pathogen that attacks a wide

range of cultivated and wild plant species including canola (oilseed rape), mustard, alfalfa, soybean, field-bean, lentil, field pea, and sunflower. It results in damage of the plant tissue, followed by cell death and soft rot or white mould of the crop. Initially the pathogen was first reported to infect sunflower during 1861. It caused root rot, stem rot and head rot in sunflower. *S. sclerotiorum* infect 64 plant families, 225 genera and in total it affects 383 plant species. But, subsequent survey during 1994 reflected a further increase in the host range of the pathogen. Pathogen was able to infect 408 plant species pertaining to 75 families and 278 genera and most of them belong to Dicotyledonae subclass of Angiospermae. It causes head rot of sunflower, leaf blight of canola, pod rot of dry bean , blossom blight of alfalfa and lettuce drop. Most of the plants susceptible to the necrotrophic pathogen belong to Solanaceae, Cruciferae, Umbelliferae, Compositae, Chenopodiaceae and Leguminosae. Flax, resistant to *S. scelrotiorum* became susceptible during the year 2000 in Manitoba and Saskatchewan. Increase in host range of *S. sclerotiorum* narrows down the opportunity for disease management using either crop rotation or resistant varieties. This warrants for the development of eco-friendly management strategies for controlling the infection of white mold pathogen in different crop plants (W. G. Dilantha Fernando, S. Nakkeeran and Yilan Zhang, 2004).

Sclerotinia sclerotiorum is omnipresent and has a very wide host range and causes economic losses in crops such as oilseeds, pulses, forage legumes, vegetables and ornamentals. There was severe yield loss due to the infection of *Sclerotinia* in vegetables such as lettuce, celery, potato and cabbage. Average crop loss of drybean due to *S. sclerotiorum* was 30%, with individual field loss of 92% in Nebraska. Yield loss of soybean in United States, Brazil, China, Argentina, India, Canada, Paraguay, Indonesia, Italy and Bolivia by *Heterodera glycines*, *Septoria glycines*, *Macrophomina phaseolina* and *Sclerotinia sclerotiorum* in 1998 was 28.5 × 106 t, valued at U.S. $6.29 × 109. An annual loss of $ 15 million was realized by the sunflower producers in United States due to Sclerotinia infection. In canola (Brassica rapa and Brassica napus), and soybean (Glycine max) the disease manifests itself as stem rot, resulting in crop lodging and severe yield losses. Sclerotia of S. sclerotiorum remain viable in soil for many years. It imbibes moisture from moist soil and leads to germination of the sclerotia. Sclerotia germinate to produce apothecia (Carpogenic germination) or directly produce mycelium (Myceliogenic germination). Apothecia develop most rapidly when soils are saturated and temperatures are in the range of 10 to 20°C. Fungal infection and mycelial growth is maximized in the presence of free water on the plant surfaces. Apothecia liberate ascospores into the air and land on the petals.

Infection was initiated via the senescing petals that serve as an initial source of nutrients for the germination of ascospores landing on petals. Upon establishment the fungus deploys two main pathogenicity determinants, the secretion of oxalic acid and a battery of acidic lytic enzymes released by the advancing mycelium]. Stems and petioles are infected, vascular tissues are disrupted, and stems, pods, or leaves beyond the site of infection die. As nutrients are exhausted, fungal mycelia aggregate into sclerotia that form both inside and outside the plant stem. These sclerotia then fall to the ground and over winter for years. During the favorable environmental conditions resting structures germinate and initiate the disease cycle again (Purdy, L.H. 1979, Phytopathology, 69, 875-880).

Management of sclerotinia with chemical fungicides though remains successful; accumulation of pesticide residues in the edible parts threatens the scope for export of the commodities to other countries. Most of the conventional methods are not effective in management of S. sclerotiorum. In the midst of these obstacles, the antagonistic fungi *Coniothyrium minitans* has been commercialized for management of white mold fungus in both agricultural and horticultural crops.

But the efficacy of biocontrol by *C. minitans* is not consistent. On the contrary, recent research activities on the usage of bacterial biocontrol agents for the management of S. sclerotiorum reflects that Pseudomonas chlororaphis (PA23), Bacillus amyloliquefaciens (BS6) and Pantoea agglomerans exert multiple mode of action and lead to the suppression of carpogenic germination and mycelial growth through the production of volatile and non volatile antimicrobial antibiotics. Moreover PA23 and BS6 triggers induced resistance via the production of defense related gene products. P. agglomerans degrades oxalic acid through the production of oxalate oxidase. Strains PA23 and BS6 protected canola crop from infection of stem rot fungus under field conditions. Since, mass multiplication of bacteria remains easier than fungal biocontrol agents, above-mentioned promising strains would pave the way for the management of S. sclerotiorum in both agricultural and horticultural crops. Development of consortial formulations with multiple modes of action will lead to the genesis of suitable bacterial biocontrol agents for controlling S. sclerotiorum in different cropping systems (W. G. Dilantha Fernando, S. Nakkeeran and Yilan Zhang, 2004).

Augmentation of soil with either organic or inorganic compounds leads to the proliferation of microbes that have the potential to suppress the germination of sclerotial structures present in the soil. Soil application of compost inhibited carpogenic germination of *S. sclerotiorum* and reduced Sclerotinia infection in carrot. Microbes in the compost might be responsible for the inhibition of sclerotial germination. Soil solarization is a method followed in different parts of

the world for the management of soilborne pathogens. Amending soil with S-H, CF-5 mixtures promoted the growth of *Trichoderma* sp, soil-borne bacteria and actinomycetes. It controlled carpogenic germination of *S. sclerotiorum*. Solarization of soil with transparent thick plastic sheets of 60 micron thickness over two months period under field conditions reduced the incidence of lettuce drop caused by *S. sclerotiorum* by 76%. Timely sowing, field sanitation, burning of stubble deep plowing followed by crop rotation with irrigated rice cultivars and seed treatment with benomyl (0.1%) was found effective in managing Sclerotinia rot in Indian mustard (W. G. Dilantha Fernando, S. Nakkeeran and Yilan Zhang, 2004).

Plant and viral pathogens interactions

Viruses are obligate, intracellular parasites that replicate in intimate association with a host cell. They do not separate themselves from the host cell with impermeable or semi-permeable membranes or walls, and they must exploit the translational machinery of the host cell to synthesize viral proteins (Hull, 2002). This extricable relationship between the host and virus confronts plants with challenges that are different in important respects to those presented by cellular pathogens, such as fungi, oomycetes and bacteria, which to a greater extent can live independently of the host. The considerations have almost certainly influenced the co-evolution of the plants and the viruses that infect them in ways that are not seen in other plant-pathogen relationships (John P. Carr, 2009). Most of the plant viruses are positive sense single stranded (ss) in their genomic orientation. Replication of these viruses requires RNA- dependent RNA polymerase activity provided by the replicase complex formed by recruitment of virus- and host- encoded proteins, with the virus providing the catalytic moiety (Buck, 1996; O' Kelly and Kao, 1998). Replication proceeds via synthesis of a negative strand complementary RNA to act as a template for positive sense RNA and for some viruses as the template for sub genomic mRNA synthesis (Buck, 1996). Replicase complexes assemble on intracellular membranes, which become modified to provide an optional environment for production of viral genomic RNA and mRNA. Membrane modification also minimizes exposure to host surveillance and resistance mechanisms, particularly those based on RNA silencing (Scwartz et al., 2004). Geminiviruses are the DNA viruses and a characteristic symptom of their infection is triggering to host cell division. Since

most plant cells are fully differentiated and contain little or no DNA polymerase, resumption of the cell cycle results in increased DNA polymerase activity, which is required by the virus for replication of geminivirus DNA (Guttirez, 2002).

It is assumed that plant viruses evolve rapidly because they have small genomes, short generation times and because RNA viruses in particular are replicated by error prone RdRp enzyme complexes (Lecoq et al., 2004). Additionally DNA and RNA viruses exhibit recombination, and reassortment can take place in mixed infection of viruses with multi component genomes. These factors provide the variability necessary for evolution to take place, but they do not explain why some viral strains or isolates come to predominate within a host cell, host plant or host population while others disappear altogether (Rossinck, 2006). The genetic make up of plant viruses can remain stable even in the place of selection pressure exerted by the plant R genes (Mac Donald, 2003).

Recovery of plant from viral infections

Recovery of plant from viral infection was reported as early as 1928. In tobacco infected with tobacco ringspot virus, the lower, initially infected leaves exhibited necrotic patches, but the upper leaves were asymptomatic and resistant to secondary infection (Wingard, 1928). Plants expressing transgenes from TEV or potato virus Y (PVY) recovered from infection with the respective virus, despite being initially susceptible (Lindboo et al., 1993). The resistance against TEV in TEV derived transgenic plants was specific as it did not protect against a closely related virus. It was later demonstrated that non-transgenic plants demonstrate a similar recovery CaMv and the nepovirus tobacco black ring virus (TBRV) infection, becoming resistant to secondary infection (Ratcliff et al., 1997). As in pathogen-derived resistance against TEV and PVY, recovery occurred via a mechanism operating at the transcript level and this was shown to be due to RNA silencing. The TBRV infected plants were also highly resistant to a recombinant PVX strain harboring TBRV sequences, indicating that RNA silencing might mediate cross protection between viral strains that have sequence homology (Mathew Lewsey, Peter Palukaitis, John P. Carr, 2009).

***Tomato Spotted Wilt Virus(Bunyaviridae*):-**

Tospoviruses constitute the only genus of plant-infecting viruses in the family *Bunyaviridae*; however, these viruses share many molecular characteristics typical of other members of this virus family. They have an enveloped virion containing the viral genome which is distributed among three RNA segments that replicate in a manner consistent with that of other negative strand viruses. All three segments have highly conserved, complementary termini resulting in a pan-handle structure and genes with functions similar to those of viruses in other genera are located in similar locations on the genome. However, the genome organization is distinct from the other genera. The small (S) and middle (M) segments each encode two genes in opposite or ambisense polarity. Classification of a Tospovirus population as a distinct species (virus) is based upon the similarity of sequence between the nucleocapsid genes of the respective viruses. This is in contrast to the system used to differentiate viruses in other genera which traditionally relied on serological neutralization of infectivity or other biological properties (hemagluttination) mediated by the glycoproteins. Tospovirus isolates with greater than 90% nucleotide similarity in the nucleocapsid gene are classified as
isolates of the same species (virus). Serologically related isolates with 80–90% sequence identity are subjectively classified as strains or as distinct species depending on other criteria. Isolates with less than 80% identity are classified as distinct species (M Tsompana and J W Moyer, 2003).

Diseases now known to be caused by tomato spotted wilt virus (TSWV) were first reported in 1915 and were shown to be of viral etiology by 1930. This taxon of plant viruses was categorized as a monotypic virus group consisting of a single virus (TSWV) until the report of impatiens necrotic spot virus (INSV) in 1991.

Thus, most of the characteristics which define the genus Tospovirus were obtained through investigation of TSWV even after the discovery of additional viruses in the genus. Biological investigations beginning in the 1940s revealed a virus that had an unusually large host range and occurred in nature as a complex mixture of phenotypicn isolates. However, it was one of the least stable viruses and most difficult plant viruses to mechanically transmit. Although the enveloped virions were observed in the 1960s, molecular characterization and elucidation of the genome organization were not completed until the early 1990s. The virus was shown to be vectored by thrips in the 1930s and later transmitted in a persistent manner. Thrips were demonstrated to be a host for replication of the virus and that replication was required for transmission in the early 1990s. Later it was recognized that limited, localized replication may occur in thrips that does not result in the thrips becoming viruliferous. Members of other genus of tospovirus are pathogenically important for humans and animals, only TSWV, is important pathogen for plants thereby it is also referred as type member of the genus and

also helped in coining the genus name.

Bean, lettuce, peanut, potato, tomato, pepper and tobacco are the crops that are mainly infected by TSWV, and it, systemically attacks the crops that it infects. Infection in the early stages of plant growth causes the most damage that may include severe stunting of the entire plant which often results in death. TSWV epidemics in peanut, pepper, tobacco, and, tomato in Southeastern United States caused major economic losses and forced shifts in production practices. Losses due to TSWV outbreaks in peanut were estimated at more than 100 million US dollars alone in Georgia in the USA (H.R. Pappu, 2008).

Potato Virus Y* (PVY) *Poty viridae

Potato virus Y was first recognized in 1931 as an aphid transmitted member within a group of viruses associated with potato degeneration, a disorder since the 18th century. PVY is the type species of the genus *potyvirus,* one of the six genera of the family *potyviridae.* PVY is naturally spread by vegetatively propogated material and by aphids in numerous species in a non persistant manner. Transmission by contact has also been reported. PVY has a wide host range and is highly variable with some host specificity. Genome sequences are reliable tools for detection and strain differentiation are available. Bioassays and serology have been largely developed. PVY is one of the most damaging plant pathogens causing significant losses in four main crops around the world: potato, pepper, tomato and tobacco. In surveys of viruses with worldwide economic importance, PVY was listed in top five viruses affecting the field- grown vegetables. PVY was also found responsible for damages in peanuts in Europe and in eggplant crops in India. Efficient control strategies depending on the crop have been developed. However none of them seems capable to take into account PVY evolution and to suppress risks of new epidemics (C.Kerlam, B. Moury, 2008).

***Cucumber mosaic virus* (*Bromoviridae*)**

Cucumber mosaic virus (CMV) was first described as a disease of cucurbits in 1916 by Doolittle in Michigan and Jagger in New York. The virus can infect a large number of indicator plant species and has been isolated from over 500 naturally infected species. Cross-protection was used in the 1930s to discriminate isolates of CMV with differences in phenotypes or host range (strains). CMV was

not purified reliably until the middle 1960s. Later serology and hybridization technology were used to detect and differentiate two major subgroups of CMV. The nucleotide sequence and the genome organization of one strain of each CMV subgroup were determined between 1984 and 1990, while biologically active cDNA clones of several CMV strains were developed in the early 1990s. The major functions of each of the five encoded proteins have been assigned, although each protein is also involved in other host–virus relationships. CMV isolates have a worldwide distribution, having been reported from both temperate and tropical regions. Most reported isolates belong to subgroup I. Subgroup II isolates are found more frequently in cooler areas or seasons of temperate regions. This has been associated with lower temperature optima for in planta virus accumulation shown for the few isolates characterized for this property. Most isolates in subgroup IB have been reported from East Asia, which is presumed to be the origin of this subgroup. Subgroup IB isolates also have been reported from other areas, for example, the Mediterranean region, California, Brazil, and Australia. Those in the Mediterranean could have been introduced recently from East Asia.

Isolates of CMV are heterogeneous in symptoms, host range, transmission, serology, physicochemical properties, and nucleotide sequence of the genomic RNAs. On the basis of different criteria (e.g., serological typing, peptide mapping of the coat protein, sequence similarity of their genomic RNA) CMV isolates can be classified into two major subgroups, now named subgroup I and subgroup II. The percentage identity in the nucleotide sequence between pairs of isolates belonging to each of these subgroups ranges from 69% to 77%, depending on the pair of isolates and the RNA segment compared, dissimilarity being highest for RNA2. Nucleotide sequence identity among isolates within a subgroup is above 88% for subgroup I and above 96% for subgroup II, indicating a higher heterogeneity of subgroup I. Analysis of the open reading frames (ORFs) and 50 non coding regions of RNA3 of subgroup I isolates shows a group of closely related isolates forming a monophyletic cluster, named subgroup IA; the rest of subgroup I isolates are included in the nonmonophyletic group IB. Analyses of RNA2 show that subgroups IA and IB constitute monophyletic groups, while analyses of RNA1 show no clear division into groups IA and IB. Hence, the different genomic segments have followed different evolutionary histories. Cross-protection occurs between strains from all subgroups. Isolates of subgroup I and II can be distinguished using monoclonal antibodies, and isolates from subgroup IA, IB, and II can be distinguished by reverse transcriptase polymerase chain reaction (RT-PCR). CMV isolates differ from isolates of the other two cucumovirus species, Tomato aspermy virus (TAV) and Peanut stunt virus, having only 50–67% nucleotide sequence identity, depending on the RNA and isolates being

compared. The host range of the collective isolates of CMV is over 1300 species in more than 500 genera of over 100 families, with new hosts reported each year. Some recently described strains from new hosts have lost the ability to infect many of the typical hosts of CMV. This may be a general feature for adaptation to unusual hosts. CMV infects most of the major horticultural crops as well as many weed species; the latter act as reservoirs for the virus. Infection of various indicator plants was used to differentiate CMV from other viruses, since unlike most other viruses of cucurbitaceous or solanaceous hosts, CMV could infect representative species of both families. These include cucumber (Cucumis sativus), tomato (Solanum lycopersicum), and tobacco (Nicotiana tabacum), all systemic hosts ofCMV, as well as cowpea (Vigna unguiculata) and Chenopodium quinoa, which limit CMV infection to the inoculated leaves, although there are legume strains that will infect cowpea systemically. Most isolates of CMVare best propagated in squash (marrow) (Cucurbita pepo), tobacco, N. clevelandii, or N. glutinosa (F Garcea Arenal; P.Palukaitis, 2008).

Tobacco rattle virus

Tobacco rattle virus (TRV, genus *Tobravirus*), first described on tobacco in the 1930s, is now seldom reported on tobacco crops. Symptoms of rattle on tobacco are systemic necrotic flecks and line patterns, and death or stunting of shoots. It was known as Mauche, ratel, Streifen und Kra¨uselkrankheit in Germany, its geographic origin. Distribution today is limited to Europe, Japan, New Zealand, and North America. The host range of TRV is very wide, as it infects over 400 species of both dicots and monocots, many of which are weeds and other wild plants. TRV causes important diseases of potato, pepper, and various ornamentals. Biological vectors of TRV are nematodes in the genera Paratrichodorus and Trichodorus (Trichodoridae). *Nicotiana clevelandii* is recommended as a propagative host for TRV, and as a trap plant for testing nematode transmission characteristics. Transmissibility is mediated by read-through proteins encoded on RNA-2 of the bipartite, ssRNA genome which extend from the surface of rigid rod-shaped particles of various sizes. This region of RNA-2 has been exploited for expression of introduced genes, and the virus is now widely used as a virus-induced gene silencing (VIGS) vector for genetic experiments.

Biological control of pathogens :strategies and implementations

Over the last 50 years, there has been the extensive use of chemicals in agriculture that has resulted in deterioration of environmental systems and the assets of agriculture. It was in 1962, when through her book "Silent Spring", Rachel Carson exposed the indiscriminate use of chemicals and pesticides, the world came to know about the losses that have occurred to biological systems due to chemicals. The world research then focused on an alternative approach to confront the plant pathogens. The first used eco friendly approaches to protect the agricultural systems from diseases were 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 wells as environmental controls, particularly in the glasshouse. However, the greatest interest has been in the development of biological control agents (BCAs) used as microbial inoculants, mimicking the use of chemical pesticides. Many aspects of the understanding of the BCAs have been understood extensively in the last 10 years (John M. Whipps, 2001, 2004).

Since the first international symposium on soil –borne plant pathogens in Berkeley in 1963, the increasing use of potentially hazardous fungicides in agriculture has been the cause of growing worldwide concern (Ilan Chet).

The terms "biological control" and its abbreviated synonym "biocontrol" have been used in different fields of biology, most notably entomology and plant pathology. In entomology, it has been used to describe the use of live predatory insects, entomopathogenic nematodes, or microbial pathogens to suppress populations of different pest insects. In plant pathology, the term applies to the use of microbial antagonists to suppress diseases as well as the use of host-specific pathogens to control weed populations. In both fields, the organism that suppresses the pest or pathogen is referred to as the biological control agent (BCA). More broadly, the term biological control also has been applied to the use of the natural products extracted or fermented from various sources. These formulations may be very simple mixtures of natural ingredients with specific activities or complex mixtures with multiple effects on the host as well as the target pest or pathogen. And, while such inputs may mimic the activities of living organisms, non-living inputs should more properly be referred to as biopesticides or bio fertilizers, depending on the primary benefit provided to the host plant

(K.K.Pal, Brian McSpadden Gardener, 2006).

From the plant's perspective, biological control can be considered a net positive result arising from a variety of specific and non-specific interactions. Using the spectrum of Odum's concepts, we can begin to classify and functionally delineate the diverse components of ecosystems that contribute to biocontrol. Mutualism is an association between two or more species where both species derive benefit. Sometimes, it is an obligatory lifelong interaction involving close physical and biochemical contact, such as those between plants and mycorrhizal fungi. However, they are generally facultative and opportunistic. For example, bacteria in the genus Rhizobium can reproduce either in the soil or, to a much greater degree, through their mutualistic association with legume plants. These types of mutualism can contribute to biological control, by fortifying the plant with improved nutrition and/or by stimulating host defenses. Protocooperation is a form of mutualism, but the organisms involved do not depend exclusively on each other for survival. Many of the microbes isolated and classified as BCAs can be considered facultative mutualists involved in protocooperation, because survival rarely depends on any specific host and disease suppression will vary depending on the prevailing environmental conditions. Further down the spectrum, commensalism is a symbiotic interaction between two living organisms, where one organism benefits and the other is neither harmed nor benefited. Most plant-associated microbes are assumed to be commensals with regards to the host plant, because their presence, individually or in total, rarely results in overtly positive or negative consequences to the plant. And, while their presence may present a variety of challenges to an infecting pathogen, an absence of measurable decrease in pathogen infection or disease severity is indicative of commensal interactions. Neutralism describes the biological interactions when the population density of one species has absolutely no effect whatsoever on the other. Related to biological control, an inability to associate the population dynamics of pathogen with that of another organism would indicate neutralism. In contrast, antagonism between organisms results in a negative outcome for one or both. Competition within and between species results in decreased growth, activity and/or fecundity of the interacting organisms. Biocontrol can occur when non-pathogens compete with pathogens for nutrients in and around the host plant. Direct interactions that benefit one population at the expense of another also affect our understanding of

biological control. Parasitism is a symbiosis in which two phylogenetically unrelated organisms coexist over a prolonged period of time. In this type of association, one organism, usually the physically smaller of the two (called the parasite) benefits and the other (called the host) is harmed to some measurable extent. The activities of various hyperparasites, i.e., those agents that parasitize plant pathogens, can result in biocontrol. And, interestingly, host infection and parasitism by relatively avirulent pathogens may lead to biocontrol of more virulent pathogens through the stimulation of host defense systems. Lastly, predation refers to the hunting and killing of one organism by another for consumption and sustenance. While the term predator typically refer to animals that feed at higher trophic levels in the macroscopic world, it has also been applied to the actions of microbes, e.g. protists, and mesofauna, e.g. fungal feeding nematodes and microarthropods, that consume pathogen biomass for sustenance. Biological control can result in varying degrees from all of these types of interactions, depending on the environmental context within which they occur. Significant biological control, as defined above, most generally arises from manipulating mutualisms between microbes and their plant hosts or from manipulating antagonisms between microbes and pathogens.

Direct antagonism results from physical contact and/or a high-degree of selectivity for the pathogen by the mechanism(s) expressed by the BCA(s). In such a scheme, hyperparasitism by obligate parasites of a plant pathogen would be considered the most direct type of antagonism because the activities of no other organism would be required to exert a suppressive effect. In contrast, indirect antagonisms result from activities that do not involve sensing or targeting a pathogen by the BCA(s). Stimulation of plant host defense pathways by non-pathogenic BCAs is the most indirect form of antagonism.

In hyperparasitism, the pathogen is directly attacked by a specific BCA that kills it or its propagules. In general, there are four major classes of hyperparasites: obligate bacterial pathogens, hypoviruses, facultative parasites, and predators. *Pasteuria penetrans* is an obligate bacterial pathogen of root-knot nematodes that has been used as a BCA. Hypoviruses are hyperparasites. A classical example is the virus that infects *Cryphonectria parasitica*, a fungus causing chestnut blight, which causes hypovirulence, a reduction in disease-producing capacity of the pathogen. The phenomenon has controlled the chestnut

blight in many places (Milgroom and Cortesi 2004). However, the interaction of virus, fungus, tree, and environment determines the success or failure of hypovirulence. There are several fungal parasites of plant pathogens, including those that attack sclerotia (e.g. *Coniothyrium minitans*) while others attack living hyphae (e.g. *Pythium oligandrum*). And, a single fungal pathogen can be attacked by multiple hyperparasites. *Trichoderma* produce a range of enzymes that are directed against cell walls of fungi. However, when fresh bark is used in composts, *Trichoderma* spp. do not directly attack the plant pathogen, *Rhizoctonia solani*. But in decomposing bark, the concentration of readily available cellulose decreases and this activates the chitinase genes of *Trichoderma* spp., which in turn produce chitinase to parasitize *R. solani* (Benhamou and Chet 1997).

The antibiotics have been shown to be particularly effective at suppressing growth of the target pathogen *in vitro* and/or *in situ*. To be effective, antibiotics must be produced in sufficient quantities near the pathogen to result in a biocontrol effect. *In situ* production of antibiotics by several different biocontrol agents has been measured (Thomashow et al. 2002); however, the effective quantities are difficult to estimate because of the small quantities produced relative to the other, less toxic, organic compounds present in the phytosphere. And while methods have been developed to ascertain when and where biocontrol agents may produce antibiotics (Notz et al. 2001), detecting expression in the infection court is difficult because of the heterogenous distribution of plant-associated microbes and the potential sites of infection. In a few cases, the relative importance of antibiotic production by biocontrol bacteria has been demonstrated, where one or more genes responsible for biosynthesis of the antibiotics have been manipulated. For example, mutant strains incapable of producing phenazines (Thomashow and Weller 1988) or phloroglucinols (Keel et al. 1992, Fenton et al. 1992) have been shown to be equally capable of colonizing the rhizosphere but much less capable of suppressing soilborne root diseases than the corresponding wild-type and complemented mutant strains. Several biocontrol strains are known to produce multiple antibiotics which can suppress one or more pathogens. For example, Bacillus cereus strain UW85 is known to produce both zwittermycin (Silo-Suh et al. 1994) and kanosamine (Milner et al. 1996). The ability to produce multiple antibiotics probably helps to suppress diverse microbial competitors, some of which are likely to be plant pathogens. The ability to produce multiple classes of antibiotics, that differentially inhibit different pathogens, is likely to enhance biological control. More recently, *Pseudomonas putida* WCS358r strains genetically engineered to produce phenazine and DAPG displayed improved capacities to suppress plant diseases in field-grown wheat (Glandorf et al. 2001,

Bakker et al. 2002).

Diverse microorganisms secrete and excrete other metabolites that can interfere with pathogen growth and/or activities. Many microorganisms produce and release lytic enzymes that can hydrolyze a wide variety of polymeric compounds, including chitin, proteins, cellulose, hemicellulose, and DNA (K.K.Pal, 2009). Biocontrol based on competition for rare but essential micronutrients, such as iron, has also been examined. Iron is extremely limited in the rhizosphere, depending on soil pH. In highly oxidized and aerated soil, iron is present in ferric form (Lindsay 1979), which is insoluble in water (pH 7.4) and the concentration may be as low as 10^{-18} M. This concentration is too low to support the growth of microorganisms, which generally need concentrations approaching 10^{-6} M. To survive in such an environment, organisms were found to secrete iron-binding ligands called siderophores having high affinity to sequester iron from the micro-environment. Almost all microorganisms produce siderophores, of either the catechol type or hydroxamate type (Neilands 1981). Kloepper et al. (1980) were the first to demonstrate the importance of siderophore production as a mechanism of biological control of *Erwinia carotovora* by several plant-growth-promoting *Pseudomonas fluorescens* strains A1, BK1, TL3B1 and B10. And, a direct correlation was established in vitro between siderophore synthesis in fluorescent pseudomonads and their capacity to inhibit germination of chlamydospores of*F. oxysporum* (Elad and Baker 1985, Sneh et al. 1984). As with the antibiotics, mutants incapable of producing some siderophores, such as pyoverdine, were reduced in their capacity to suppress different plant pathogens (Keel et al. 1989, Loper and Buyer 1991). The increased efficiency in iron uptake of the commensal microorganisms is thought to be a contributing factor to their ability to aggressively colonize plant roots and an aid to the displacement of the deleterious organisms from potential sites of infection.

A number of strains of root-colonizing microbes have been identified as potential elicitors of plant host defenses. Some biocontrol strains of *Pseudomonas* sp. and *Trichoderma* sp. are known to strongly induce plant host defenses (Haas and Defago 2005, Harman 2004). In several instances, inoculations with plant-growth-promoting rhizobacteria (PGPR) were effective in controlling multiple diseases caused by different pathogens, including anthracnose (*Colletotrichum lagenarium*), angular leaf spot (*Pseudomonas syringae* pv. *lachrymans* and bacterial wilt (*Erwinia tracheiphila*). A number of chemical elicitors of SAR and ISR may be produced by the PGPR strains upon inoculation, including salicylic acid, siderophore, lipopolysaccharides, and 2,3-butanediol, and other volatile

substances (Van Loon et al. 1998, Ongena et al. 2004, Ryu et al. 2004).

Currently, fundamental advances in computing, molecular biology, analytical chemistry, and statistics have led to new research aimed at characterizing the structure and functions of biocontrol agents, pathogens, and host plants at the molecular, cellular, organismal, and ecological levels.

Induced Systemic Resistance (ISR)

Studies on mechanisms of ISR are suggested to be valuable in extension of PGPR-elicited ISR to practical agriculture. It has been suggested that mixtures of PGPR strains with different mechanisms of interactions might more reliably benefit plants than would individual PGPR strains (Raupach and Kloepper, 1998). Choudhary et al. (2007) elaborately described induced resistance and its mechanism of action in plants. Plants have the ability to acquire enhanced level of resistance to pathogens after exposure to biotic stimuli provided by many different PGPRs. These in association with plant roots elicit a steady state of defense or ISR in plants. This is often referred to as rhizobacteria- mediated ISR. PGPR-elicited ISR was initially observed in carnation, common bean and in cucumber with reduced susceptibility to Fusarium wilt and halo blight. Several bacteria that colonize root systems by seed applications Include P. putida and B. pumilus (Thomma et al., 2001).

Induced resistance is a physiological ‘‘state of enhanced defensive capacity’’ elicited by specific environmental stimuli, whereby the plant’s innate defenses are potentiated against subsequent biotic challenges. This enhanced state of resistance effective against a broad range of pathogens and parasites (van Loon, 2000). Besides ISR, there is another defined form of induced resistance so- called systemic acquired resistance (SAR), which can be differentiated on the basis of the nature of the elicitor and the regulatory pathways involved. SAR can be triggered by exposing the plant to virulent, avirulent, and non-pathogenic microbes. Depending on the plant and elicitors, a set period of time is required for the establishment of SAR wherein accumulation of pathogenesis-related pro- teins (chitinase and glucanase), and salicylic acid (SA) takes place. Unlike SAR, ISR does not involve the accumulation of pathogenesis-related proteins or salicylic acid, but instead, relies on pathways regulated by jasmonate and ethylene (Yan et

al., 2002). A network of interconnected signaling pathways regulates induced defenses of plants against pathogens. The primary components of the network are plant signal molecules – SA, jasmonic acid (JA), ethylene (ET), and, probably nitric oxide (NO) (Devendra K. Chaudhary, 2009).

Use of bacterium biocontrol against plant viral diseases

Nowadays, plant virus diseases can cause epidemics with great economical losses in agriculture worldwide and are mainly controlled either by conventional breeding introducing resistance genes or with the use of genetically engineered plants. Unfortunately, due to their genetic plasticity, viruses can overcome genetic resistance of the plants. Thus, the investigation of alternative, more flexible strategies for the control of virus diseases is needed.

Bacillus subtilis and its related species have long been used as biological control agents, mainly in the plant rhizosphere. There, they have been adopted to grow in diverse environmental niches, under nutrient deprivation and other abiotic and biotic stresses, due to their ability to form highly resistant dormant endospores (reviewed in Earl et al., 2008). Rhizosphere adaptation allows them to outgrowth soil borne fungal pathogens and pests (reviewed by Borris et al., 2011). Moreover, such species grow in close association with plant root surfaces where they form biofilms (Bais et al., 2004) and act as plant-growth-promoting rhizobacteria (PGPRs). PGPRs can produce components such as hormones, nutrients and growth factors that are helpful in plant growth and yield (Babalola, 2010). Besides their beneficial effects into plant growth, PGPRs can trigger plant resistance to several phyto-pathogens either by producing siderophores, bacteriosins and antibiotics as antagonistic activities (Beneduzi et al., 2012) or by inducing resistance. As described formerly by Van Loon et al. (1998), induced resistance is a state of enhanced defensive capacity of the plant caused either by biotic or abiotic elicitors. There are two main forms of induced resistance, Systemic Acquired Resistance (SAR) and Induced Systemic Resistance (ISR)(Pieterse et al., 1996). Generally, pathogenic organisms and chemicals can activate SAR in contrast to PGPR-mediated resistance that is referred as ISR. SAR depends on the accumulation of the endogenous salicylic acid that finally triggers the transcription of the Pathogenesis Related genes (*PRs*). PGPR-mediated resistance utilizes a complex array of defence responses to yield systemically induced immunity to

subsequent infection utilizing a network of small-molecule hormones mainly jasmonic acid and ethylene (reviewed in Pieterse et al. 2009). PGPRs are also thought to cause a constitutive level of systemic priming, a sensitized state in which plants display either faster and/or stronger, activation of the various cellular defence responses that are induced following attack by pathogens or insects or in response to abiotic stress (Conrath et al., 2006). Thus, the advantages from two distinct defence mechanisms, direct suppression of the disease and defence priming, can be combined. Furthermore, the expression of the one defence mechanism can activate another, for example localized expression of Pathogen Associated Molecular Pattern (PAMP) triggered immunity (PTI) and Effector Triggered Immunity (ETI) has the ability to elicit SAR, which is mostly based on priming (Ahmad et al., 2010).

In field and greenhouse conditions chemicals such as acinobenzolar-S-methyl known as BTH can achieve SAR. BTH is a functional analogue of salicylate and it is shown to induce an artificial systemic resistance to viruses. For example, BTH can protect tobacco plants from *Tobacco mosaic virus* (TMV) (Friedrich et al., 1996) and *Tomato spotted wilt virus* (TSWV) (Mandal et al., 2007) and tomato plants from *Cucumber mosaic virus* (CMV) (Anfoka, 2000). However, the application of BTH results in reduced growth and stunted plants, a fact that can lead to an economical cost.

Although ISR and SAR trigger different regulatory pathways, the induced resistance is similar, as they can protect plants from a broad range of pathogens, including viruses (Ryu et al., 2004, Faoro and Gozzo, 2015). To date, studies reporting an antiviral action of PGPR and especially of *Bacillus* spp. are scarce. In tomato, *Bacillus* strains mediated protection against *Tomato mottle virus* (ToMoV) (Murphy et al., 2000) and CMV (Murphy et al. 2003; Zehnder et al., 2000). Moreover, *B. pumilus* SE34 significantly reduced symptom development and CMV accumulation in *Arabidopsis thaliana*. Wang and co-workers (2009) showed that *Bacillus* spp. elicited ISR to TMV in tobacco plants with the participation of the *PR*, *NPR1* and *Coi1* genes, resulting in reduced TMV disease severity and virus accumulation. So far, only three *P. fluorescens* strains, CoP-1, CoT-1 and CHAO, are shown to protect tomato plants from TSWV through the induction of *PAL* that activates the secondary metabolism and especially the

phenylpropanoid pathway (Kandan et al., 2002, Kandan et al., 2005).

Zeolite: its implementation in agricultural systems

The disposal of mine and metallurgical waste without treatment presents a very difficult problem, which if ignored leads to a legacy of pollution that covers an infinite time scale. The wastes from mining and smelting practices are devoid of the major plant nutrients (Bloomfield et al., 1982) and soil contaminated by them commonly remain barren or colonized only by metal tolerant plants (Brooks et al., 1998). Such barren sites have no defence against wind erosion, leaching by rain and surface run-off. As a consequence toxic metal elements are free to migrate and invariably terminate in the food chain. It has long been appreciated that phytoremediation provides an answer to this problem, but it has always been difficult to sustain plant growth on such inhospitable soils (Walker et al, 1996).

The symbiosis between the agricutural and geological sciences is not a recent phenomenon. Crop production strategies and patterns depends on the existence and maintenance of fertile soil and agronomists rely on knowledge of mineralogy and geochemistry of clays and other soil constituents. In the animal sciences, the addition of crushed limestone to chicken feed to strengthen egg shells is well known, as is the use of bentonite as a binding agent in pelletized anima1 feed stuffs (Fredrick Mupton). Aqueous leachate analysis has demonstrated a correlation between shoot growth and the mobilization of cations in the soil pore water (Peter J. Leggo, Be´atrice Lede´sert , Graham Christie, 2006).

The demand for fresh water is increasing worldwide due to fast population growth and improvement in living standards. Conflicts between water use for irrigation and other uses have created interest in exploring the use of sea and other recyclable sources such as wastewate. However, use of poor quality water for irrigation may lead to soil salinity and its associated problems. Accumulation of salts in the root zone affects plant performance through creation of water deficit and disruption of ion homeostasis (Munns, 2002) which in turn cause metabolic dysfunctions. These stresses change hormonal status and impair basic metabolic

processes (Munns, 2002; Loreto et al., 2003) resulting in growth inhibition and reduction in yield (Mass, 1993). Irrigation management practices aim for the efficient use of saline water by maintaining salt accumulation in the root zone at lower levels and cultural practices may dramatically improve the performance of crops in saline environments. Soil permeability problems may be prevented or corrected by using soil or water amendments. One of the root biophysical cause of falling per capita food grain production is reported to be soil resource degradation. In order to resolve this trend of soil base degradation, it is necessary to either expand the land base under cultivation or to intensify crop production per unit of land. Soils are either low fertile or made less fertile due to the removal of nutrients without adequate replenishment by intensive farming (Kulasekaran Ramesh, Ashis Kumar Biswas, Jayaraman Somasundaram, Annangi Suba Rao). Improved nutrient use efficiency in production agriculture is a research priority for both agronomic and environmental reasons. To minimize groundwater contamination caused by nitrate and phosphate during irrigation after application of fertilizers and maximize the efficiency of input fertilizers, slow release fertilizers (SRFs) have drawn great attention recently. Current additives to SRFs include natural occurring minerals that have high cation exchange capacity as well as synthetic cation and anion exchange resins (Zhaohui Li, Yingpeng Zhang, and Yan Li3, 2013).

Recently, one group of minerals has emerged as having considerable potential in a wide variety of agricultural processes. This group of minerals is the zeolite group. The unique ionexchange, dehydration-rehydration, and adsorption properties of zeolite materials promise to contribute significantly to many years of agricultural and aquacultural technology (Walker et al., 2003).

Most of the initial research on the use of zeolites in agriculture took place in the 1960s in Japan, Japanese farmers have used zeolite rock for years to control the moisture content and malodor of animal wastes and to increase the pH of acidic volcanic soils. The addition of small amounts of the zeolites clinoptilolite and mordenite to the normal protein diet of pigs, chickens, and ruminants gave noticeable in-creases in the body weight and general “health” of the animals (Mimato Hideo, 1968). The use of zeolites in rations also appeared to reduce odor and associated pollution problems and to provide a means of regulating the

viscosity and nitrogen retentivity of animal manure. These same zeolites were also found to increase the ammonium content of rice paddy soils when added with normal fertilizers. Zeolites are crystalline, hydrated aluminosilicates of alkali and earth metals that possess infinite, three-dimensional crystal structures. They are further characterized by an ability to lose and gain water reversibly and to exchange some of their constituent elements without major change of structure. They are among the most common elements in the sedimentary rocks and among the most common in the turfaceous rocks.

Zeolites have many important tasks such as ion exchange, filtering, odour removal, chemical sieve, water softener and gas absorption. Therefore, apart of agriculture, numerous examples of their application are cement and brick production, stabilization of soil, building materials, paint components with anticorrosive property, defluorination of industrial wastes, desulphurization of flue gas, methylene blue and mercury removal, copper recovery from wastes, fixation of phosphates, chlorinated phenol removal and neutralization of acid wastes, cleanup of sewerage, and both heavy metal, and ammonium ion removal (Kocakusak et al., 2001). Identification of zeolite as a mineral goes back to 1756, when a Swedish mineralogist, Fredrich Cronstet, began collecting some well formed crystals from a copper mine in Sweden. They were named "Zeolite" from the Greek words meaning "boiling stones", that is, because of ability to froth when heated to about 200°C. After their discovery, zeolites were considered as (E. Polat et al. J. Fruit Ornam. Plant Res. 184 Special ed. vol. 12, 2004: 183189) minerals found in volcanic rocks for a period of two hundred years. Fortunately in 1950s, they were rediscovered and reported to exist on all the continents (Anonymous, 2004a). In the world, their commercial production and use started in 1960s, but in Turkey they were first discovered in 1971. To date, more than forty types of zeolites have been reported by different research groups. Among these minerals, analcime (sometimes known as analcite), clinoptilolite, erionit, chabazite, mordenite, and philipsite are well known (Doğan, 2003). Also, more than 150 zeolites have been synthesized. Some of the common synthetics are zeolites A, X, Y, and ZMS5. Those naturally occurring and synthetic minerals are used commercially because of their unique adsorption, ion exchange, molecular sieve, and catalytic properties. Extensive research is very important to assure that the source of natural zeolites can provide their sufficient quantity with uniform

characters and unique properties (cation exchange capacity, pH and B content) for application and commercial processing. Research is under progress to enhance the use of zeolites (Kütük et al., 1996). There are many minerals that show similar cage like framework structures or have similar properties and/or are associated with zeolites, but actually are not them. Therefore, zeolites without well defined chemical characteristics may cause severe problems in their application (Kocakuşak et al., 2001).

The commercial use of natural zeolites is still in its infancy, but more than 300,000 tons of zeolite-rich tuff is mined each year in the United States, Japan, Bulgaria, Hungary, Italy, Yugoslavia, Korea, Mexico, Germany, and the Soviet Union. Natural zeolites have found applications as fillers in the paper industry, as lightweight aggregate in construction, in pozzolanic cements and concrete, as ion-exchangers in the purification of water and municipal sewage effluent, as traps for radioactive species in low-level wastewaters from nuclear facilities, in the production of high purity oxygen from air, as reforming petroleum catalysts, as acid-resistant absorbents in the drying and purification of natural gas, and in the removal of nitrogen compounds from the blood of kidney patients (F.A. Mumpton, 1978).

Zeolites are composed of pores and cornersharing aluminosilicate (AlO4 and SiO4) tetrahedrons, joined into 3dimensional frameworks. The pore structure is characterized by cages approximately 12Å in diameter, which are interlinked through channels about 8Å in diameter, composed of rings of 12 linked tetrahedrons (Kaduk and Faber, 1995). The pores are interconnected and form long wide channels of varying sizes depending on the mineral. These channels allow the easy movement of the resident ions and molecules into and out of the structure. Zeolites have large vacant spaces or cages within and resemble honeycomb or cage like structures. The presence of aluminium results in a negative charge, which is balanced by positively charged cations (Ersin Polat, Mehmet Karaca, Halil Demir, and, Naci Onus, 2004).

Although, there are no certain figures on the total amount of zeolites in the world, it is well known that they are present on all the continents with varying mineral contents and kinds. According to reports of 2001, the total consumption of zeolites was 3.5 million tons of which 18% came from natural resources and the rests from

synthetics such as A, X, Y, and ZMS5 (Öz et al., 2003). Clinoptilolite and chabazite are most commonly consumed. The use of natural zeolites has been continuously increasing over last years. Such countries as Cuba, USA., Russia, Japan, Italy, South Africa, Hungary and Bulgaria have important resources of these minerals and production potentials (Anonymous, 2004a). Also, Turkey has substantial zeolite resources, estimated to be approximately 50 billion tons, mainly consisted of clinoptilite ores. In this country, zeolite production in 2002 was 25 000 tons of which 80% was used internally and the rest exported to the USA, France, Italy, Israel, and United Kingdom. According to the General Directorate of Mineral Research and Exploration, Turkey, resources of clinoptilolite in the ManisaGördes region are estimated at 2 billion tones (Anonymous, 2004a).

Clinoptilolite originally received its Greek name, meaning "*oblique feather stone*" because it was thought to be the monoclinic phase of the mineral ptilolite (as in "*oblique ptilolite*") but it was later found the earlier named mineral was mordenite; consequently the name ptilolite, is no longer in use. Clinoptilolite, one of the most useful naturally occurring zeolites, is applied as a chemical sieve, feed and food additive, as well as gas and odour absorber. Suitability for such applications is due to its large amount of pore spaces, a high resistance to extreme temperatures and chemically neutral basic structure. Clinoptilolite can easily absorb ammonia and other toxic gases from air and water and thus can be used in filters, both for health reasons and odour removal. The properties such as high absorption level, ion exchange capacity, catalysis, dehydration activity and easily shapeable features make clinoptilolite important in plant production. Pure or composite clinoptilolite added to soil improves its physical and chemical characteristics (Anonymous, 2004a). Zeolites are able to lose and gain water reversibly and to exchange extra framework cations, both without change of crystal structure. The large structural cavities and the entry channels leading into them contain water molecules, which formhydration spheres around exchangeable cations. On removal of water by heating at 350–400°C, small molecules can pass through entry channels, but larger molecules are excluded—the so called ‘‘molecular sieve’’ property of crystalline zeolites. The uniform size and shape of the rings of oxygen in zeolites contrasts with the relatively wide range of pore sizes in silica gel, activated alumina, and activated carbon, and the Langmuir shape of their adsorption isotherms allows zeolites to remove the last trace of a particular gas from a system (e.g.,H2Ofrom

refrigerator Freon lines). Furthermore, zeolites adsorb polar molecules with high selectivity. Thus, polar CO2 is adsorbed preferentially by certain zeolites, allowing impure methane or natural gas streams to be upgraded. The quadrupole moment of N2 contributes to its selective adsorption by zeolites from air, thereby producingO2-enriched products. The adsorption selectivity for H2O, however, is greater than for any other molecule, leading to uses in drying and solar heating and cooling. The weakly bonded extraframework cations can be removed or exchanged readily by washing with a strong solution of another cation. The CEC of a zeolite is basically a function of the amount of Al that substitutes for Si in the framework tetrahedra; the greater the Al content, the more extraframework cations needed to balance the charge. Natural zeolites have CECs from 2 to 4 milliequivalents/g (meq/g), about twice the CEC of bentonite clay. Unlike most non crystalline ion exchangers, e.g., organic resins and inorganic aluminosilicate gels (mislabeled in the trade as ‘‘zeolites’’), the framework of a crystalline zeolite dictates its selectivity toward competing ions. The hydration spheres of high field-strength cations prevent their close approach to the seat of charge in the framework; hence, cations of low field strength are generally more tightly held and selectively exchanged by the zeolite than other ions. Clinoptilolite has a relatively small CEC ('2.25 meq/g), but its cation selectivity is Cs > Rb > K > NH4 > Ba > Sr > Na > Ca > Fe > Al > Mg > Li. This preference for larger cations, including NH4 1, was exploited for removing NH4-N from municipal sewage effluent and has been extended to agricultural and aquacultural applications (1, 2). Clinoptilolite and natural chabazite have also been used to extract Cs and Sr from nuclear wastes and fallout. Most zeolites in volcanogenic sedimentary rocks were formed by the dissolution of volcanic glass (ash) and later precipitation of micrometer-size crystals, which mimic the shape and morphology of their basalt counterparts. Sedimentary zeolitic tuffs are generally soft, friable, and lightweight and commonly contain 50–95% of a single zeolite; however, several zeolites may coexist, along with unreacted volcanic glass, quartz, K-feldspar, montmorillonite, calcite, gypsum, and cristobaliteytridymite. Applications of natural zeolites make use of one or more of the following properties: (*i*) cation exchange, (*ii*) adsorption and related molecularsieving, (*iii*) catalytic, (*iv*) dehydration and rehydration, and (*v*) biological reactivity. Extrinsic properties of the rock (e.g., siliceous composition, color, porosity, attrition resistance, and bulk density) are also

important in many applications. Thus, the ideal zeolitic tuff for both cation-exchange and adsorption applications should be mechanically strong to resist abrasion and disintegration, highly porous to allow solutions and gases to diffuse readily in and out of the rock, and soft enough to be easily crushed. Obviously, the greater the content of a desired zeolite, the better a certain tuff will perform, *ceteris paribus*.

Applications of zeolite:

Clinoptilolite in agriculture improves the efficiency of used fertilizers, thus promotes better plant growth and consequently enhances the yield. For instance, Torii (1978) reported that the application of zeolite at the rate of 48 tons/acre increased apple yield by 1338%. This mineral used in the amount of 2 to 8 kg/tree, can contribute to a better new orchard establishment. Zeolites are used successfully in the cultivation of a wide variety of crops including cereals, vegetables, grapes and other fruits (Burriesci et al., 1984; Anonymous, 2004ab). Zeolites added to fertilizers help to retain nutrients and, therefore, improving the long term soil quality by enhancing its absorption ability. It concerns the most important plant nutrients such as nitrogen (N) and potassium (K), and also calcium, magnesium and microelements. Zeolite can retain these nutrients in the root zone to be used by plants when required. Consequently this leads to the more efficient use of N and K fertilizers by reducing their rates for the same yield, by prolonging their activity or finally by producing higher yields. Large losses of fertilizers which move out of the root zone (leaching) often happen in sandy soils, which lose their capability to retain high nutrient levels (Flanigen and Mumpton, 1981; Mumpton, 1981). Therefore an application of zeolites will enhance the plant growth and development by reducing the loss of nutrients (Anonymous, 2004a).

Natural zeolites can absorb CO, CO2, SO2, H2S, NH3, HCHO, Ar, O2, N2, H2O, He, H2, Kr, Xe, CH3OH and many other gases and can thus be used to collect them or control odours. Therefore, those minerals are being used in intensive animal husbandry sheds, significantly reducing the content of ammonia and H2S, which cause undesirable odours. NH4+ absorbed zeolite becomes a natural enriched slow release fertilizer. High ammonia absorption capacity makes it a very effective natural way to control high levels of this gas generated in fish farms. It can be used in the filtration systems or simply be broadcast over the water surface

as it is totally harmless to water life (Anonymous, 2004a). Additionally, food crops growing in soil containing high amounts of Pb, Cd, and Cu can be protected by the absorption ability of zeolites. Also, research showed that S90 uptake by plants was significantly reduced.

Zeolites may hold water up to 60% of their weight due to a high porosity of the crystalline structure. Water molecules in the pores could be easily evaporated or reabsorbed without damage to such structures (Kocakuşak et al., 2001). Zeolites assure a permanent water reservoir, providing prolonged moisture during dry periods; they also promote a rapid rewetting and improve the lateral spread of water into the root zone during irrigation. This results in a saving in the quantity of water needed for irrigation. Furthermore, high absorption capacity makes zeolites a carrier of agricultural pesticides.

Zeolite with a negative charge provides an ideal trap for positive cations such as sodium, potassium; barium and calcium, and positively charged groups such as water and ammonia. Both carbonate and nitrate ions are attracted by the negative charge within zeolites. Therefore, alkali and soil alkali metallic cations are attracted in the same way and water can be absorbed by zeolites (Mumpton, 1999). Absorbed cations are relatively mobile due to their weak attraction, and can be replaced using the standard ion exchange techniques, making zeolites good ion exchangers.

Unlike other soil amendments (e.g. lime) zeolite does not break down over time but remains in the soil to improve nutrient retention. Therefore, its addition to soil will significantly reduce water and fertilizer costs by retaining beneficial nutrients in the root zone. The porous structure of natural zeolite helps to keep the soil aerated and moist as well as active for a long time. Zeolite is not acidic but marginally alkaline and its use with fertilizers can help buffer soil pH levels, thus reducing the need for lime application. This mineral is therefore very beneficial in the construction of golf courses and sport fields where the resulting irrigation and maintenance costs can be very substantial.

Similar to their synthetic counterparts, the high adsorption capacities in the dehydrated state and the high ion-exchange capacities of many natural zeolites make them effective carriers of herbicides, fungicides, and pesticides.

Clinoptilolite can be an excellent substrate for benzyl phosphorothioate to control stem blasting in rice (88). Using natural zeolites as a base, Hayashizaki and Tsuneji (26) found that clinoptilolite is more than twice as effective as a carrier of the herbicide benthiocarb in eliminating weeds in paddy fields as other commercial products. Torii (82) reported that more than 100 tons of zeolite were used in Japan in 1973 as carriers in agriculture. A Russian patent was issued to Aleshin, et a], (2), for grouting compound containing 3 to 5 percent clinoptilolite to control herbicide percolation from irrigation canals to ground waters.

Tomato (*Solanum lycopersicum* L.)

The commercial tomato belongs to a species most frequently referred to as *Lycopersicon esculentum* (Miller). The correct latin name of this species has been the subject of much discussion which has not been fully resolved. The alternative names *Solanum lycopersicum L.*, *Lycopersicon lycopersicum L* (Karsten) have appeared in the literature. *Lycopersicon esculentum* was first proposed for the tomato by Miller in 1768, replacing the earlier Linnean name *Solanum lycopersicum*. However, the authors conclude that because of its long and popular usage, the name *Lycopersicon esculentum* (Mill) should be preserved (J. Rudich).

Lycopersicon is a relatively small genus within the extremely large and diverse family *Solanaceae*. The family is currently considered to consist of around 90 genera (D' Arcy, 1979). These are mainly divided between two sub families, *Solanoideae*, and *Cestroideae*.

The division between the major subfamilies is based on different patterns of embryo development. Genera assigned to the *Solanoideae* have a coiled embryo of more or less uniform diameter. Whilst in the latter the embryo is typically straight or only slightly curved. A large number of morphological, chemical and cytogenetic differences accompany this basic division (J. Rudich).

All species in the genus *Lycopersicon* are typical of the *Solanoideae* subfamily, each having an identical genome formula (2n=2x=24). Sub-family *Solanoideae* is further divided into tribes. *Lycopersicon* belongs to the largest tribe in the family, Tribe *Solaneae*. This tribe consists of around 18 genera, ranging from

Lycopersicon, which is one of the smallest, to the closely related genus *Solanum*, which is the largest in the family. *Solanum* includes around 1500 species which is one of the largest and most diverse genera of vascular plants (Humziker, 1979). The two genera are separated on the basis of unique anther morphology.

The small genus of *Lycopersicon* is currently thought to consist of the cultivated tomato, *L. esculentum* and seven closely related wild *Lycopersicon* species (Rick, 1976). Earlier taxonomic treatments have become inadequate as the number of species and races collected from South America have increased (Muller, 1940). Muller subdivided the genus into two groups; *Eulycopersicon* consisting of colored fruited species, and, *Eriopersicon* consisting green fruited species. This split based on fruit color is arbitrary and does not correspond to more fundamental differences (J. Rudich).

Lycopersicon esculentum has become widely disseminated all over the world due to its value as a crop. The original site of domestication is uncertain, although the balance of evidence suggests Mexico. The cherry tomato (*L. esculentum var. cerasiforme*) is almost certainly the direct ancestor of the modern cultivated forms. Cherry tomatoes are the only wild tomatoes found outside South America. Collections of this taxon has been made from the centre of evolution of the genus in Peru; from most of the Central American states; and from widely separated regions such as Zambia, Borneo and Hawaii (J.Rudich).

All representatives of *L. esculentum* are self compatible and inbreeding. Domestication has involved the selection of the progressive withdrawal of the stigma within the anther cone ensuring automatic self pollination (Rick, 1976). Domestication has clearly involved continued selection for larger-fruited forms. Nevertheless, modern tomato varieties are extremely closely related to the wild species *L. esculentum var. cerasiforme* and the two groups can be freely intercrossed (J. Atherton).

The tomato has been bred for fruit production under a wide range of environmental conditions, from the short season cold climates to the warm humid tropics and to the hot arid deserts. Wherever man has attempted to grow tomatoes he has had to contend with numerous diseases. Before the introduction of resistant varieties, *Fusarium* wilt was perhaps the most destructive disease to tomatoes. In

some areas of the western US the processing tomato industry was virtually destroyed by beet curly top virus which developed because of the extensive sugar beet production. In other temperate regions, diseases such as late blight, *Septoria* leaf spot, bacterial canker and bacterial speck have built up to epidemic proportions, completely ruining crops (J. Atherton; J. Rudich, 1986).

While bacterial wilt and bacterial spot have devastated plantings in the warm humid tropics, protected crops have equally been plagued by damaging diseases. *Fusarium* crown rot, corky root, *Didymella* stem rot, black dot, leaf mould and tomato mosaic have all caused serious problems for glasshouse tomato growers. Today over 200 diseases are reported to affect the tomato plant. Generally covered production of the crop is typified by such environmental conditions, such as high humidity, poor air circulation and low light intensity. Diseases which are common in such conditions are grey mould, late blight, powdery mildew and pith necrosis. Soil borne organisms are favored by poor soil sterilization and sanitation practices as well as by cool soil or root zone temperatures (J.Rudich).

Tomato spotted wilt virus (TSWV) is one of the most important diseases affecting tomatoes, occasionally leading to losses of up to 100%. Because of the importance of this virus disease, considerable research has been carried out in recent years on the viral particle, disease vectors, transmission and control methods. Genetic resistance appears as the best solution to control this disease. It may be complemented with other strategies of control aimed to reduce inoculum amount or to avoid transmission, since these strategies have been shown to be ineffective to stop the disease in themselves. Efforts should be made to study virus variability, since this would help the development of TSWV resistant tomato varieties as well as the establishment of more precise diagnostic methods for TSWV (Salvador Roselló, María José Díez, Fernando Nuez).

Biocontrol of soilborne diseases of tomato caused by *Rhizoctonia solani* and *Pythium ultimum* alone or in combination with *Sclerotium rolfsii* and *Fusarium oxysporum* f. sp. *lycopersici* were recently studied in the greenhouse and field. Soilborne diseases of pepper caused by the first three pathogens were also studied alone or in combination with *Phytophthora capsici*. Tomato and pepper seeds were treated with biomass of *Gliocladium virens* (Gl-3) and *Burkholderia cepacia* (Bc-F), individually and in combination, and planted in

pathogen-infested soilless mix. Seedling stands for tomato from treated seeds were comparable to that in non-infested soilless mix. Although seed treatments with individual biocontrol agents reduced damping-off in peppers, only the Gl-3 + Bc-F treatment resulted in stands similar to the non-infested control. When healthy seedlings of both crops were transplanted into pathogen-infested soil/soilless mix in the greenhouse, and supplementary root drenches of suspensions of Gl-3, Bc-F, and Gl-3 + Bc-F were applied, the plant fresh weight was significantly greater and the disease severity (DSI) significantly less than for infested controls. When transplants were set out into infested field plots, the combined Gl-3 + Bc-F application resulted in greater fresh weight and lower DSI for pepper, and greater fruit yield for tomato than those obtained with either Gl-3 or Bc-F alone (W. Mao; J.A. Lewis).

Arabidopsis thaliana

Arabidopsis thaliana L. (Kingdom, Plantae; Order, Brassicales; Family,Brassicaceae; Genus, *Arabidopsis*; Species, *A.thaliana*) is an annual species with a present worldwide distribution after expansion from its native geographical range in Eurasia and North Africa (Hoffmann 2002). Europe/North Africa has been proposed as a main centre of origin of A. thaliana (Hoffmann 2002). Within this region, the Iberian Peninsula is a centre of genetic diversity, and the genetic structure of A. thaliana in Iberia suggests that it harboured multiple glacial refugia during the Pleistocene, which contributed to the species; re-colonization of Europe in post-glacial times (Pico´ et al. 2008). In the Iberian Peninsula, A. thaliana can be found in a variety of anthropic and wild habitats (Pico´ et al. 2008) where plants flower mainly in spring. Demographical analyses show that populations are built of two or one cohorts of plants that either germinate in the autumn and overwinter as rosettes, or germinate in the spring. The occurrence and demographic relevance of each cohort depends on the site and the climatic conditions (Montesinos et al. 2009). For over 20 years, A. thaliana has been developed as the model organism for molecular plant genetics, including the analysis of the mechanisms of resistance to parasites (Sommerville & Koornneef 2002). More recently, A. thaliana has been increasingly developed as a model for plant ecology and evolutionary genetics (Mitchell-Olds & Schmitt 2006), including the study of the consequences of herbivory and parasitism on plant fitness, and plant–parasite coevolution (Kover & Schaal 2002; Salvaudon et al. 2005; Goss & Bergelson 2006). These analyses have not considered viral parasites, and there is currently no information on what viruses infect A. thaliana in nature or on the possible consequences of viral infection on the population

dynamics and genetics of A. thaliana.

The plant was first described in 1577 in the Harz Mountains by Johannes Thal (1542–1583), a physician from Nordhausen, Thüringen, Germany, who called it *Pilosella siliquosa*. In 1753, Carl Linnaeus renamed the plant *Arabis thaliana* in honor of Thal. In 1842, the German botanist Gustav Heynhold erected the new genus *Arabidopsis* and placed the plant in that genus. The genus name, *Arabidopsis*, comes from Greek, meaning "resembling *Arabis* " (the genus in which Linnaeus had initially placed it).

There are over 750 natural varieties of *A. thaliana* found around the world. All varieties have various differences dependent on where in the world they are found. This variation among different environments suggests that all strains of *A. thaliana* have a common ancestor and have adapted evolutionarily over time.

A. thaliana is a predominantly self-pollinating plant with an outcrossing rate estimated at less than 0.3% (Abbott, RJ; Gomes, MF (1989). An analysis of the genome-wide pattern of linkage disequilibrium suggested that self-pollination evolved roughly a million years ago or more (Tang C, Toomajian C, Sherman-Broyles S, Plagnol V, Guo YL, Hu TT, Clark RM, Nasrallah JB, Weigel D, Nordborg M, August 2007). Meioses that lead to self-pollination are unlikely to produce significant beneficial genetic variability. However, these meioses can provide the adaptive benefit of recombinational repair of DNA damages during formation of germ cells at each generation (Harris Bernstein, Carol Bernstein and Richard E. Michod,2011). Such a benefit may have been sufficient to allow the long-term persistence of meioses even when followed by self-fertilization. A physical mechanism for self-pollination in Arabidopsis is through pre-anthesis autogamy, such that fertilisation takes place largely before flower opening.

Botanists and biologists began to research *A. thaliana* in the early 1900s, and the first systematic collection of its mutations was performed around 1945.It is now widely used for studying plant sciences, including genetics, evolution, population genetics, and plant development. Although *A. thaliana* has little direct significance for agriculture, it has several traits that make it a useful model for understanding the genetic, cellular, and molecular biology of flowering plants.

Arabidopsis thaliana has been successfully implemented in the study of the subdicipline of plant pathology, that is, the interaction between plants and disease-causing pathogens. The use of arabidopsis has led to many breakthroughs in the advancement of knowledge of how plants manifest plant disease resistance. The reason most plants are resistant to most pathogens is through nonhost resistance.

This is, not all pathogens will infect all plants. An example where arabidopsis was used to determine the genes responsible for nonhost resistance is *Blumeria graminis*, the causal agent of powdery mildew of grasses. Arabidopsis mutants were developed using the mutagen ethyl methanesulfonate and screened to determine which mutants had increased infection by *B. graminis* (Stein, M; Dittgen J; Sánchez-Rodríguez C; Hou BH; Molina A; Schulze-Lefert P; Lipka V; Somerville S., 18 March 2006). The mutants with higher infection rates are referred to as PEN mutants due to the ability of *B. graminis* to penetrate arabidopsis to begin the disease process. The PEN genes were later mapped to identify the genes responsible for nonhost resistance to *B. graminis*.
In general, when a plant is exposed to a pathogen, or non pathogenic microbe, there is an initial response, known as PAMP-triggered immunity (PTI), because the plant detects conserved motifs known as pathogen-associated molecular patterns (PAMPs) (Knepper, Caleb; Day, Brad, March 2010). These PAMPs are detected by specialized receptors in the host known as pattern recognition receptors(PRRs) on the plant cell surface. The best-characterized PRR in *A. thaliana* is FLS2 (Flagellin-Sensing2), which recognizes bacterial flagellin, (Gomez-Gomez, L; Boller T, 5 June 2000) a specialized organelle used by microorganisms for the purpose of motility, as well as the ligand flg22, which comprises the 22 amino acids recognized by FLS2. Discovery of FLS2 was facilitated by the identification of an *A. thaliana* ecotype, Ws-0, that was unable to detect flg22, leading to the identification of the gene encoding FLS2. FLS2 shows striking similarity to rice XA21, the first PRR isolated in 1995.

A second PRR, EF-Tu receptor (EFR), identified in *A. thaliana*, recognizes the bacterial EF-Tu protein, the prokaryotic elongation factor used in protein synthesis, as well as the laboratory-used ligand elf18 (Zipfel, C; Kunze G; Chinchilla D; Caniard A; Jones JD; Boller T; Felix G,19 May 2006). Using *Agrobacterium* mediated transformation, a technique that takes advantage of the natural process by which *Agrobacterium* transfers genes into host plants, the EFR gene was transformed into *Nicotiana benthamiana*, tobacco plant that does not recognize EF-Tu, thereby permitting recognition of bacterial EF-Tu (Lacombe S, Rougon-Cardoso A, Sherwood E, Peeters N, Dahlbeck D, van Esse HP, Smoker M, Rallapalli G, Thomma BP, Staskawicz B, Jones JD, Zipfel C, 28 April 2010) thereby confirming EFR as the receptor of EF-Tu.

Both FLS2 and EFR use similar signal transduction pathways to initiate PTI.*A. thaliana* has been instrumental in dissecting these pathways to better understand the regulation of immune responses, the most notable one being the mitogen-activated protein kinase (MAP kinase) cascade. Downstream responses of PTI

include callose deposition, the oxidative burst, and transcription of defense-related genes (Zhang, J; Zhou JM, 2010).

PTI is able to combat pathogens in a nonspecific manner. A stronger and more specific response in plants is that of effector-triggered immunity (ETI). ETI is dependent upon the recognition of pathogen effectors, proteins secreted by the pathogen that alter functions in the host, by plant resistance genes (R-genes), often described as a gene-for-gene relationship. This recognition may occur directly or indirectly via a guardee protein in a hypothesis known as the guard hypothesis. The first R-gene cloned in *A. thaliana* was RPS2 (resistance to *Pseudomonas syringe* 2), which is responsible for recognition of the effector avrRpt2 (Kunkel, BN; Bent AF; Dahlbeck D; Innes RW; Staskawicz BJ., 1993) The bacterial effector avrRpt2 is delivered into *A. thaliana* via the Type III secretion system of *P. syringae pv tomato* strain DC3000. Recognition of avrRpt2 by RPS2 occurs via the guardee protein RIN4, which is cleaved. Recognition of a pathogen effector leads to a dramatic immune response known as the hypersensitive response, in which the infected plant cells undergo cell death to prevent the spread of the pathogen (Axtell, MJ,; Staskawicz BJ, 2003).

Arabidopsis Thaliana is a model organism used to determine specific defense mechanisms of plant-pathogen resistance. Plants have special receptors on their cell surfaces that allow for detection of pathogens and initiate mechanisms to inhibit pathogen growth (Zipfel, C.; Robatzek, S.; Navarro, L. (2004).

Lettuce (*Lactuca sativa L.*)

Lettuce is an annual herb of the daisy family Asteraceae, grown prominently as a as a leaf vegetable but sometimes also for its stems and seeds. First cultivated by ancient Eygptians, it was turned into the food plant grown for its succulent leaves, from a weed whose seeds were used to produce oil. Greeks gave the name lactuca to the plant. It is generally grown as a hardy annual, and is easily cultivated in low temperatures to prevent it from flowering quickly. It belongs to kingdom Plantae, under the order Asterales belonging to family Asteraceae, genus Lactuca and species L.sativa.

Lettuce leaves contain small amounts of opiate-like substance, *lactucarium* ("lettuce opium"), which is a mild sedative. Lettuce was used to treat anxiety, insomnia, and neurosis in the ancient times. Lettuce is rich source of antioxidants such as quercetin, caffeic acid, vitamins A and C. It was shown that ethanol

extract of lettuce injected subcutaneously, significantly decreased accumulation of lipofuscin pigment granules ("age granules") in brain of mice under accelerated ageing regimen (the mice were administered D-galactose). Lettuce originates from the wild *Lactuca serriola* found in the Mediterranean and Near East and has been transformed from an erect plant with bitter leaves to various cultivars including ones with distinctive heads of chlorophyll deficient leaves. Other wild relatives of genus *Lactuca* (*L. aculeata*, *L. scarioloides*, *L. azerbaijanica*, *L. saligna* and others) also most likely contributed to the cultivated lettuce gene pool. Usually, the wild species landraces are only partially cross-fertile with *L. sativa*. Occasional inter-species hybrids are used to introduce disease resistance genes into garden lettuce's stock.

Biocontrol Agent K 165 (*Paenibacillus alvei*)

Paenibacillus alvei (formerly known as Bacillus alvei) belongs to the domain of bacteria, Phylum Fimicutes; Class Bacilli; Order Bacillales; Family Paenibacillaceae; and Genus Paenibacillus. The strains of this species grow in vortex-like or branched patterns. Its strain, specifically acts as biocontrol against a considerable amount of plant diseases. It has the ability to protect Arabidopsis thaliana against Verticilium dahliae . A direct antagonistic action of strain K165 against *V. dahliae* was ruled out, making it likely that K165-mediated protection results from induced systemic resistance (ISR) in the host. K165-mediated protection was tested in various *Arabidopsis* mutants and transgenic plants impaired in defense signaling pathways, including NahG (transgenic line degrading salicylic acid [SA]), *etr1-1* (insensitive to ethylene), *jar1-1* (insensitive to jasmonate), *npr1-1* (nonexpressing NPR1 protein), *pad3-1* (phytoalexin deficient), *pad4-1* (phytoalexin deficient), *eds5/sid1* (enhanced disease susceptibility), and *sid2* (SA-induction deficient). ISR was blocked in *Arabidopsis* mutants *npr1-1*, *eds5/sid1*, and *sid2*, indicating that components of the pathway from isochorismate and a functional NPR1 play a crucial role in the K165-mediated ISR. Furthermore, the concomitant activation and increased transient accumulation of the *PR-1*, *PR- 2*, and *PR-5* genes were observed in the treatment in which both the inducing bacterial strain and the challenging pathogen were present in the rhizosphere of the *A. thaliana* plants (S.E.Tjamos, E.J. Paplomatas, 2005). In the present study, the action of K165 was exploited against the different isolates of Cucumber mosaic virus, Potato virus Y and Tomato spotted wilt virus.

EXPERIMENTAL PROCEDURES

The present study having the rubric "Evaluation of biocontrol strategies against a range of plant pathogens" was undertaken in the summer (April) of 2015 and stretched for one year. The experiments were scheduled (from April-December,2015) as in three replications for biocontrol agent (*Paenibacillus alvei*) K165 and virus(*CMV,PVY,TSWV*) studies in tomato plants (*Solanum lycopersicum* var.Belladona); and, plant virus co-evolution studies in *Arabidopsis thaliana* mutant plants namely *bamh1, mpk6, mpk3* and *wild type col-o* against *TRV, CMV* and *PVY*. The above activities were carried out at the greenhouse of Laboratory of Plant Virology, Benaki Phytopathological Institute, Athens (Greece).

The other part of the research of using natural zeolite from Polyegos and Kimolos islands was carried out from January2016-May 2016. Zeolite was tested as soil remediation strategy against soil-borne plant pathogens namely *Rhizoctonia solani* (strain AG2 1), *Pythium ultimum* (strain 1313) and *Sclerotinia sclerotiorum.* There was one replication per pathogen and the experiments were carried out in the greenhouse of Laboratory of Plant Pathology, Agricultural University of Athens, Athens (Greece).

The details of materials used and methods employed in the present investigation are described under the following headings:

1. Materials and layout of the design
2. Observations recorded
3. Statistical analysis
4. Molecular analysis

Induction of resistance to viruses in *Solanum lycopersicum* var. Belladona by the biocontrol agent *K165*: The experimental material for this study consisted of tomato plants of variety Belladona used to observe the induced systemic induction, if triggered, by the bacterium biocontrol *Paenibacillus alvei* K165 against three different viruses, namely, *TSWV* (*Bunyaviridae*), *CMV* (*Bromoviridae*), *PVY* (*Potyviridae*). The protective activity of K165 has been attributed to the triggering of plant defence mechanisms via a SA-dependent pathway and the reduction of *Verticillium dahlae* microsclerotia germination (Tjamos et al., 2003; Antonopoulos

et al., 2008). K165 is also known to mediate protection in *Arabidopsis* mutants npr1-1, eds5/sid1, and, sid2, which were blocked in ISR pathway (Tjamos et al., 2005).

Plant materials: Tomato (*Solanum lycopersicum* var. Belladona) seeds (from Syngenta) were sown in the seed pans in a fecund soil mixture (Pot Grond P). Twelve days after seedlings (DAS), seedlings were transplanted in separate pots, under controlled greenhouse conditions i.e. 22 degree Celsius temperature and 60-70% humidity. The plants were watered timely. The plants used for maintaining the virus were: *Nicotiana benthamiana* for *Tobacco rattle virus* (*TRV*), whose leaves were harvested at 3 days post inoculation (dpi) to prepare the viral inoculum; *Nicotiana tabaccum* for *CMV* and *PVY* (leaves harvested at 7 dpi to prepare the viral inoculums); and, *Nicotiana rustica* for *TSWV* (leaves harvested at 14 dpi to prepare the viral inoculum). The check plants used were: *Chenopodium quinoa* for *Cucumber mosaic virus* (*CMV*) and *Tomato spotted wilt virus* (*TSWV*); *Nicotiana benthamiana* for *Potato virus Y* (*PVY*); and, *Chenopodium quinoa* for *TRV*.

Bacterial Culture Preparation: A rifampicin-resistant mutant of the biocontrol strain K165 with biocontrol activity against *V. dahliae* in glasshouse and field experiments was used in this study (Tjamos et al. 2004). Drenching of K165 was done in the soil as an induced resistance bioassay. The antagonist was grown in liquid culture of nutrient broth and glycerol (Leliot and Stead 1987) in an orbital incubator at 180 rpm at 30°C for 18 h. Bacterial suspension was centrifuged at 8,000 rpm for 10 min, was resuspended in 50 mM phosphate buffer, pH 7.02. The concentration of the biocontrol agent in the liquid formulation was 108 CFU per ml.

Source of Viral Inoculum: The isolates of *CMV*, *PVY*, and, *TSWV* (from sample collection of Laboratory of Plant virology, Benaki Phtyopathological Institute) were used for this experiment. The isolates were maintained on different plants (mentioned above) in the greenhouse. Fresh leaves with systemic symptoms of *CMV, PVY*, and, *TSWV* were harvested at 7 & 14 days post inoculation (DPI) respectively, and were used as the source of inoculum. The *TSWV* isolate, source leaf, was stored at – 80 degree Celsius in the refrigerator which was used to infect the plants for the use of source inoculum.

Challenge inoculation: K165 treated plants and the control stock were challenged by the mechanical inoculation with *CMV, PVY* & *TSWV*. Inoculum was prepared by grinding systemically infected leaves in 0.1 M phosphate buffer, pH 7.0, containing 0.57 grams of trinatrium-phosphate-12-hydrate, 0.1 gram of DIECA in 50 milliliters of water at the rate of 1:10 tissue and buffer ratio (wt/vol).For PVY inoculums, activated carbon is also added in the paste in order to facilitate the infection of the virus in plants. Debris, in the extract, was crushed thoroughly to make the inoculum slack and properly mixed. Carborundum powder is sprinkled on the third leaf (for CMV&PVY infection), and, on the second leaf (for TSWV infection) which are broadly expanded and then kept for 2 minutes. Inoculum was maintained on ice and was rubbed gently on the above leaves by the hands (index finger dipped in the inoculum), with the hand gloves worn. Inoculations were done in the greenhouse, under controlled conditions, at 25 to 30°C. After inoculation, plants were lightly misted with water. Plants were inoculated at six days post treatment (DPT) of biocontrol bacterium *K165* for *CMV* and *PVY*, and, 5 DPT for *TSWV*, at the rate of 20ml/plant for all the viruses. K165 was drenched again in the soil of the infected potted plants at 7 days post inoculation (@20ml/plant) for *CMV* & *PVY*, and, 2 days post inoculation for *TSWV* (@10ml/plant).

Assessment of viral infection, phytotoxicity, and statistical analysis: After inoculation, plants were inspected daily for symptom development. Local infection was confirmed, for *CMV*, by testing the half portion of inoculated leaves (200 mg from randomly selected 5 plants) both from control and K165 treated plants by enzyme-linked immunosorbent assay (ELISA) following the lab protocol (Laboratory of plant virology, Benaki Phytopathological Institute). LOEWE protocol was followed for the ELISA for *TSWV*.

Table 1. Layout for the *CMV*- K165 bioassay experiments:

Trial	No. of K165 treated plants	Control plants	ELISA (dpi)
1	15	15	7 & 12
2	24*	25	7 & 32

*One plant in the K165 stock in the 2nd trial was destroyed.

Table 2. Layout for the *PVY*- K165 bioassay experiments:

Trial	No. of K165 treated plants	Control plants	ELISA (dpi)
1	15	15	7,9 & 13
2*	25	25	7, 11 & 32**

* PVY son 41 isolate of *PVY* which is genetically modified for GFP gene was used for this trial.
** Only positive samples of 7 & 11 dpi were sampled for 32 dpi.

Table 3. Layout of *TSWV*-K165 bioassays experiments:

Trial	No. of K165 treated plants	Control plants	No. of K165 drenching	ELISA (dpi)
1	30	30	2	14,16*,21** & 27***
2	50	50	2	9, 14, & 29
3	72	72	2	14 and 16

*Systemic leaves of the negative samples of 14 dpi
**Inoculated leaves of negative samples of 14 dpi
*** Systemic leaves of positive samples of all the experiments

Study of virus resistance in different *Arabidopsis thaliana* genotypes:

Plant material: The plant material used was ecotype of *Arabidopsis thaliana*, namely *Columbia (Col-o)* wild type; and three different mutant lines, namely *Mitogen- Activated protein kinase* loss of function mutants *mpk3* and *mpk6*, and *bam3* mutants. The seeds of the above lines were obtained from Laboratory of Plant pathology, Agricultural University of Athens. The seeds were also produced at the greenhouse of the Laboratory of Plant Virology at Benaki Phytopathological Institute by bagging the *Arabidopsis* plants of different lines and collecting their seeds after desiccation of the plants. All seeds were stored at 4 degree Celsius

Seed treatment: The seeds of different *Arabidopsis* mutants were kept in separate small tubes soaked in water and kept in the refrigerator at 4 degree Celsius. After two days the seeds were taken out and washed with 75% ethanol for two minutes, thereafter, in 25% bleach for four minutes in small tubes, and then finally washed with water. The same day they are sprinkled in different pots, carrying seeds of different mutant lines, in a fecund soil mixture (Pot Grond P) and covered with a polythene sheet holed at some points to maintain the microenvironment for the tender seedlings. After 16 days they are transplanted in different pots which are small in size.

Viral Inoculum: The different mutants were infected by *Tobacco rattle virus* (strain P0049) genetically modified for the green fluorescent protein (*GFP*) gene was maintained on the leaves of *Nicotiana benthamiana* and was harvested at 3 days post inoculation to prepare the viral inoculum for the infection of *Arabidopsis* plants. The plants were infected with the virus by preparing the viral paste in 0.1 M phosphate buffer mixing it with carborundum powder and applying it gently on three rosette leaves of the plants with the help of ear buds. In the 3rd trial the inoculated leaves were also marked with the help of a marker after the inoculation process.

Table 4. Layout of the *Arabidopsis*- virus bioassays

Trial	Virus	No. of plants infected				ELISA (dpi)
		Col-o	*mpk3*	*mpk6*	*bam3*	
1	*CMV*	20	20	20	20	21 dpi
	PVY	20	20	20	20	17 dpi
2	*CMV*	22	22	22	22	16 dpi
	TRV	18	17	13	0	(2,5,9)* dpi
3	*CMV*	40	40	40	40	10 dpi

*The *TRV* infected plants were observed under UV lamp at different days post inoculation to see the systemic foci of the virus through the *GFP* gene signal.

Assessment of viral infection: The different mutant lines of *Arabidopsis thaliana* were tested for the presence of the virus through the process of enzyme linked immune sorbent assay (ELISA) following the lab protocol of Laboratory of Plant Virology (Benaki Phytopathological Institute). Whole plants were sampled for the test leaving the roots. For the first trial there were no estimations made as the plants died before the examination.

Use of zeolite to control various soil-borne plant pathogens:

Zeolite mineral: The zeolite mineral used was natural and was from the Greek islands of Polyagos and Kimolos. The mineral was mixed in a fecund soil mixture either when the seeds were sown, or at the time of transplanting of the plants.

Plant material: The plant material used to study the fungal and oomycete pathogenesis was *Lactuca sativa* L., var. Tom thumb, which is considerably susceptible to plant pathogens *Rhizoctonia solani, Pythium ultimum and Sclerotinia sclerotiorum*. The seeds were sown in the seed pans and then were infected with the fungus, after two weeks, at the time of transplanting.

Cultivation of the fungal inoculum: *Rhizoctonia solani, Pythium ultimum*, and, *Sclerotinia sclerotiorum* were used to assess the plant protection efficiency of zeolite and for inoculum in bioassays. *Rhizoctonia solani* strain AG2-1, *Pythium ultimum* strain 1313 and *Sclerotinia sclerotiorum* strain from the Laboratory of Plant Pathology, Agricultural University of Athens were used. The fungal strains were routinely grown on Potato Dextrose Agar (PDA) prior to use in sporangium germination or biocontrol experiments. For the production of fungal sporangia of each fungus, a mycelia disk from 4-5 days old PDA culture (kept at 24 degree Celsius) was transferred to a fresh sterile PDA plate and kept in the incubation chamber under controlled conditions. After 5-day incubation at 27°C, 5-mm-diameter mycelial disks were excised, placed in sterile petri dishes containing PDA (prepared in the laboratory) and kept in cold and dark conditions. The fungus was harvested for inoculation after one week in *R. solani* and *P.ultimum* and after two weeks in *Sclerotinia sclerotiorum.*

Assays to assess the biocontrol efficiency of zeolite against the fungal and oomycete strains: The fungus was harvested after two weeks, broadly for all the three pathogens. There were two treatments for each pathogen, namely, the control stock of lettuce plants (out of which 20 plants were grown on normal soil, another 20 were the grown plants of the seeds sown in 20% zeolite i.e., 16 parts of soil and 4 parts of zeolite mixed, which were transplanted in normal soil and were labeled as 20% zeolite seed. The rest 20 plants in the control stock were lettuce plants transplanted in zeolite mixed soil at the rate of 20% (i.e., 16 parts of soils and 4 parts of zeolite). For each pathogen, the treatments were the same, as, 40 plants infected with the pathogen in normal soil (positive control), pathogen infected 40 plants in zeolite seed 20% and 40 plants transplanted in 20% zeolite. The process of the harvesting of fungus was carried out in the greenhouse. The fungal and

oomycete colony (20 petri plates having the full grown colony of the fungus) was blended with 500 ml of water to prepare the inoculums. 40 petri plates were required to prepare the inoculum for 20 plants. The inoculum was then mixed in the soil, with the other required material (eg., zeolite), as per the treatment. The plants were transplanted using this inoculum infected soil.

Analysis of root rot (*Rhizoctonia solani*), damping off (*Pyhtium ultimum*) and white mold (*Sclerotinia sclerotiorum*): All the three pathogen symptoms were recorded after every two days after the onset of symptom development for 6 days in *R. solani*, 17 days for *P. ultimum* and 15 days for *S. sclerotiorum.* Plants were evaluated for disease symptoms on a 1 to 5 scale, with 1= healthy seedling, 2= infected seedling; primary root firm but infected, 3=infected seedling with primary root infected, 4= dead seedling, 5= dead seed. A score of 1 or 2 was considered healthy and free of disease. The experiment was done only once with 40 plants in each treatment. Disease ratings were plotted over time to generate disease progress curves. The area under the disease progress (AUDPC) curve was calculated by the trapezoidal integration method (Campbell and Madden, 1990).

Table 5. Layout of the zeolite- fungi bioassays

	Control plants			Infected plants		
	Healthy lettuce (Mock)	Lettuce 20% zeolite	Lettuce 20% zeolite seed	Positive control	Pathogen infected 20% zeolite plants	Pathogen infected 20% zeolite seed plants
Pythium ultimum	20	20	20	40	40	40
Rhizoctonia solani	20	20	20	40	40	40
Sclerotinia sclerotiorum	20	20	20	40	40	40

Molecular Analysis:

RNA isolation and qPCR determination of PR1and LOX transcript level:
Five plants from each treatment were harvested at 3 and 7 days post inoculation (dpi) for quantification of *Pythium ultimum* by q PCR. For each sampled plant, the aerial parts were cut at soil level (stored at -80 degree Celsius), rinsed with sterile distilled water and ground to a fine powder using an autoclaved mortar and pestle

in the presence of liquid nitrogen (D. Gkizi, S.E. Tjamos, 2015). For each sample total RNA was extracted from 100 mg of ground tissue using TRIzol (Invitrogen) according to the manufacturer's instructions. The RNA samples were treated with DNAse 1 (Fermentas) to eliminate traces of contaminating genomic DNA. The RNA concentration was measured in spectrophotometer (ND-1000; Nanodrop). First strand c DNA was synthesized using Superscript 2 (Invitrogen) following the manufacturer's procedure. PCR efficiency for each amplicon was calculated by employing the linear regression method on log (flourescence) per cycle number data.

Statistical Analysis

Data of various treatments (for relative AUDPC) was subjected to mean separation by multiple range test when a significant $p \leq 0.05$ F test was obtained. A student T – test was done to analyze the data for viral infection.

RESULTS

Bioassays of fungal and Oomycete resistance in lettuce plants

Zeolites are minutely porous minerals which are composed of aluminium, silicon and oxygen counteractions and are commonly used as commercial adsorbents and catalysts. It was discovered by the virtue of its adsorbing property when the Swedish mineralogist Axel Fredrick Cronstedt, in 1975, heated the mineral *stilbite* and found that it rapidly produced large amounts of steam from water that had been adsorbed by the mineral, upon its heating. In the agricultural sector the use of zeolite has gained momentum mainly in the areas of soil treatment or water management. Clinoptilolite (a natural form of zeolite) has been efficiently monitored for its function for as a slow releaser of potassium and nitrogen in the soil if it is deficient. The mineral is also claimed to be a water moderator i.e., absorbing the water up to 55% of its weight and then slowly releasing it in the root zone when the plants demand. This action is noteworthy for both nursery growers and large scale farmers as it prevents water logging in the root zone and thus making the conditions unfavorable for stem and root rot pathogens such as *Rhizoctonia, Sclerotinia* and *Pythium*. When we follow the movement of water in the root zone, there arises the concern of cation exchange capacity of the used soil for the plantation. Cation exchange capacity of soil can be defined by the ability of the soil particles to able to hold the exchangeable ions within itself by the virtue of its dry weight and to be later exchanged by the soil water solution. It is in a way a measure of the soil fertility, nutrient retention capacity and groundwater contamination with the cation accumulation. Zeolite deposits are fairly distributed across the Greek islands. The majoirity of Greek zeolite rock samples contain HEU type (hellandite-clinoptilote) zeolite. The cation exchange capacity of the zeolite rock samples from the islands of Polyegos and Kimolos was observed to be 153 meq and 96 meq/100 grams of the zeolite rocks respectively (E. Tzamos, and A. Filipiddis; 2007). The CEC value of the zeolite from the above two islands denoted that it can adsorb the water and nutrients from the soil at a qualitative rate, and hence can be used to combat various soil borne diseases (fungal) in the plants by providing the required amount of water and nutrients to the plants when in need.

Influence of different application procedures of natural zeolite in protection and triggering of host defence mechanisms in lettuce plants against *Rhizoctonia solani, Pythium ultimum* and *Sclerotinia sclerotiorum*

In the present study entitled "Evaluation of biocontrol strategies against a range of plant pathogens" the second segment was rapt for determining the efficacy of natural zeolite against three soil borne plant pathogens. The soil borne pathogens chosen were *Rhizoctonia solani* (strain AG2 1*), Pythium ultimum* (strain 1313), and *Sclerotinia sclerotiorum*. The stock of natural zeolite was obtained from the Greek islands of Polyegos and Kimolos. The plant chosen for the experiment was *Lactuca sativa* (var. Tom thumb), seeing its susceptibility to the above three pathogens. The study was conducted from the January 2016 to March 2016 in the green house of Laboratory of Plant Pathology, Agricultural University of Athens, under controlled conditions. The experiment schedule was forked into two halves; in which the first half investigated the efficacy of zeolite against fungi *Pythium ultimum* and *Rhizoctonia solani* in a trial constituting 40 plants in each treatment [i.e., positive control, 20%zeolite added after transplanting ('zeolite 20%) and 20% zeolite added at the time of the sowing of the seeds (zeolite seed)]. The same procedure was (with the same number of plants) was done in the second half with the *Sclerotinia sclerotiorum* investigation.

Zeolite confers partial resistance to lettuce against *Sclerotinia sclerotiorum*

The efficacy of zeolite as soil amendment against *Scelrotinia sclerotiorum* was examined by employing two different application procedures: growing the lettuce seeds with 20% zeolite (abv. as zeolite seed) and amendment of the transplant soil plug with 20% zeolite at the time of transplanting of the seedlings (abv. as 20% zeolite). Zeolite applications significantly reduced the white mold symptom advancement in lettuce compared to the control treatment (Fig. 1). However, growing lettuce seed in 20% zeolite (zeolite seed) as a soil amendment was more effective against the white mold fungus than the addition of 20% zeolite in the transplant soil plug.

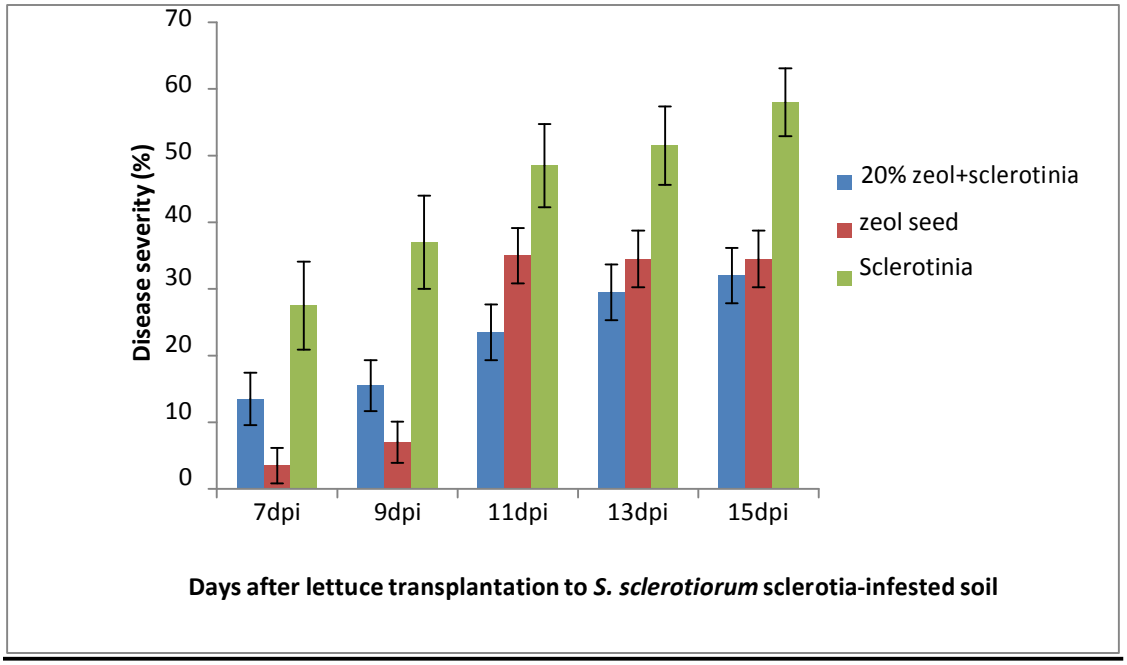
In the case of lettuce seed grown with 20% zeolite ("zeolite seed") the fungal advancement was slow as compared to the other treatment. The white mold

symptoms started 7 dpi, when the fungal symptoms were observed in the control treatment very clearly and in 20% zeolite transplant soil plug a bit mildly, progressing rapidly until a plateau was reached at 13 dpi. The symptoms were delayed in the zeolite seed treatment but the disease rapidly progressed from 9dpi to 11dpi bouncing from 7% to 35% and then it remained stable all through. Whilst, in the 20% zeolite transplant soil plug the disease gained a stable initiation from the starting point, where at 7dpi the disease progress was 13.5% and grew to 32% at 15 dpi compared to the 58% of the control treatment (Fig.1a). Statistical analysis of the relative AUDPC values unveiled that both zeolite seed and 20%zeolite transplant were able to confer the same level of resistance to the lettuce plant against *S.sclerotiorum.* The resistance conferred by both the application procedures had a statistically significant difference than the control treatment (Fig. 1b).

Lettuce seeds grown in 20% zeolite amended soil (zeolite seed) are susceptible to *Pythium ultimum*

Pythium ultimum is a very important root rot causing plant pathogen, attacking most of the beneficial crops. The rare ability of its swimming spores make the disease incidence even more faster. The pathogen likes moist and humid soils to flourish and evade the host plant's physiology. The addition of zeolite, in any manner, further braced the growth of the pathogen thereby attacking the plant with more vigor than the control treatment (Fig. 2). The plants grown with 20% zeolite in the nursery seemed to be more vulnerable to the disease having been attacked by the pathogen at an early stage of inoculation i.e. 6dpi causing maximum infection of 18% as compared to the other treatments and reaching to 34.6% at 17dpi compared to the 31% of the control treatment. There were similar results for the 20% zeolite transplant soil plug treatment that too caused the infection up to 34% at 17 dpi (Fig. 2a). As per the statistical analysis of relative AUDPC curve, there was a statistically significant difference noticed between the 20% zeolite seed treatment and the positive control (Fig. 2b). The disease progress results very categorically pointed out that the addition of zeolite would not be good for the cultivation of the crops that are prone to the root rot pathogen. It even specified that incorporation of the zeolite mineral in any way in the soil would make the crop more prone to the infection caused by *Pythium ultimum.*

(a)



(b)

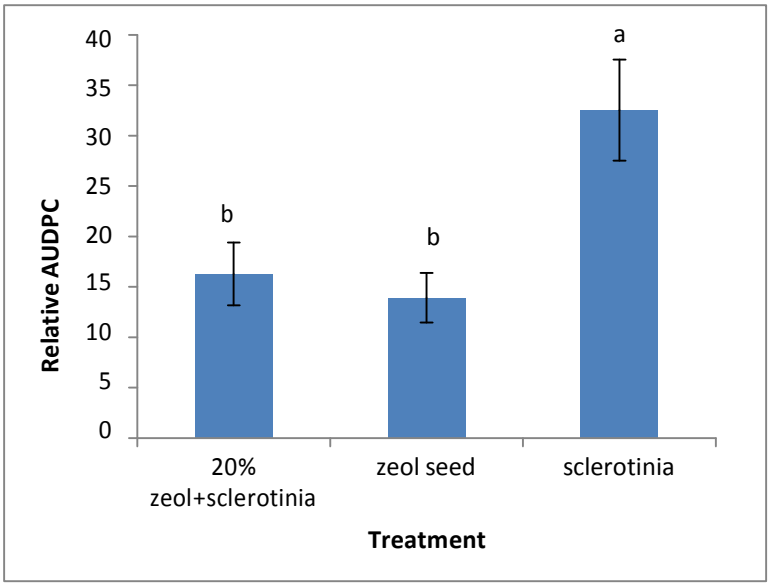
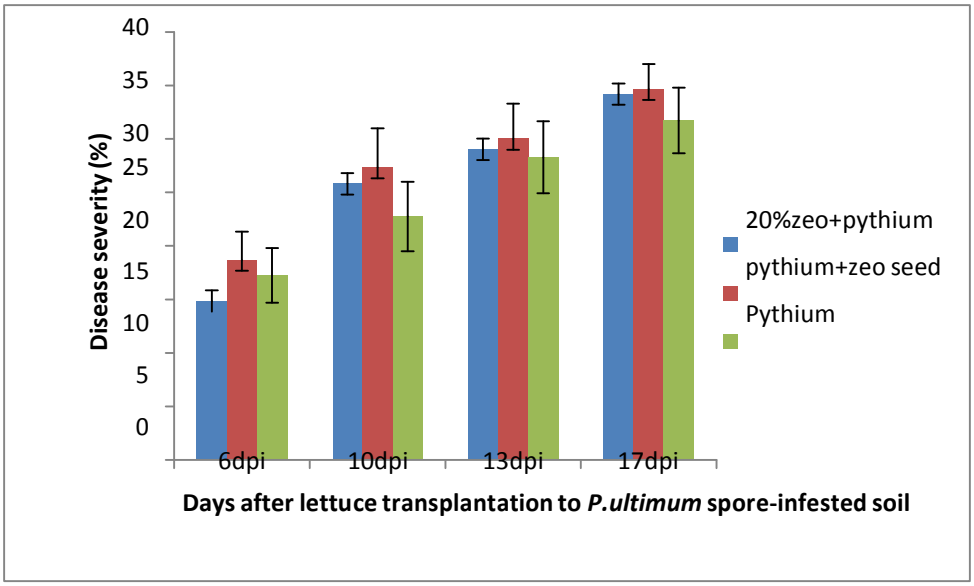


Figure 1 White mold (*S.sclerotiorum*) disease severity on lettuce plants either grown in soil amended with 20% zeolite and then transplanted in *S.sclerotiorum* infested soil, or grown in normal soil and transplanted to soil amended with 20% zeolite and infested with sclerotia of the fungus (a). Disease ratings were plotted over time to generate disease progression curve and the area under the disease progression curve was calculated by the trapezoidal integration method (Campbell and Madden, 1990). (b) Results expressed as relative AUDPC i.e. the disease level

as a percentage of the maximum possible area for the whole period of the experiment (D.J. Angelopoulou, E.J. Naska, S.E. Tjamos; 2014). Columns with different letters are significantly different according to the Fisher's least significant difference test at $p \leq 0.05$.

Figure 1.1 Pictorial depiction of the lettuce plants infected with *S. sclerotiorum* at 18 days post inoculation. The treatments zeolite seed and zeolite 20% seems to be in tolerant mode with little effect in its appearance as compared to control plants. Though the plants are short in height as compared to the mock plants.

(a)



(b)

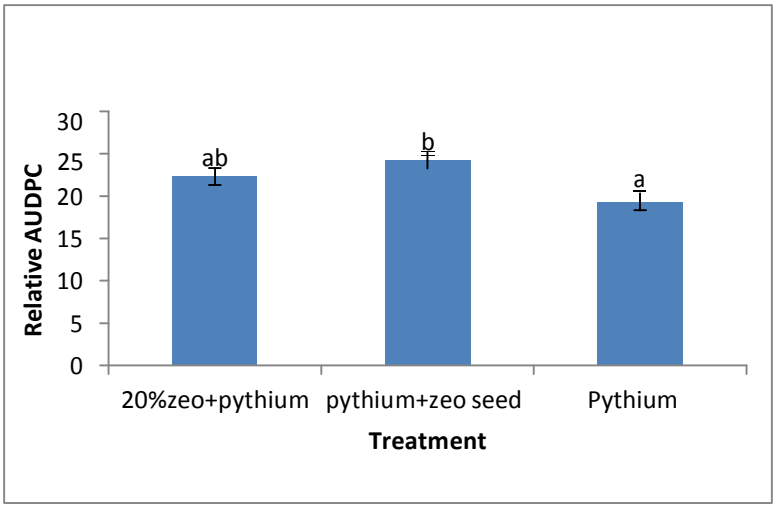


Figure 2 Stem rot (*P. ultimum*) disease severity on lettuce plants either grown in soil amended with 20% zeolite and then transplanted in *P. ultimum* infested soil, or grown in normal soil and transplanted to soil amended with 20% zeolite and infested with sclerotia of the oomycete (a). Disease ratings were plotted over time to generate disease progression curve and the area under the disease progression

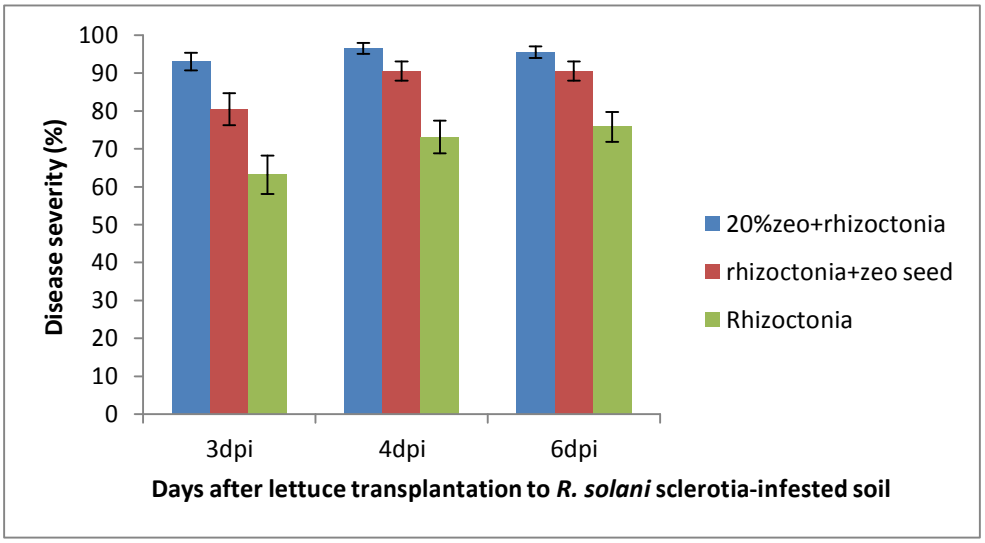
curve was calculated by the trapezoidal integration method (Campbell and Madden, 1990). (b) Results expressed as relative AUDPC i.e. the disease level as a percentage of the maximum possible area for the whole period of the experiment (D.J. Angelopoulou, E.J. Naska, S.E. Tjamos; 2014). Columns with different letters are significantly different according to the Fisher's least significant difference test at $p \leq 0.05$.

Figure 2.1 *Pythium ultimum* infected lettuce plants with the mock plants at 18 days post inoculation. Zeolite seed treatment seems to be the most infected. Control plants are comparatively taller than the zeolite treatments, be it, 20% zeolite or zeolite seed.

***Rhizoctonia solani* (strain AG2 1) stresses the lettuce plants in the presence of zeolite and causes them to die rapidly**

The application of zeolite resulted in sudden death of the lettuce plants infected with the sclerotia of *R. solani* .The onset of symptom development was expeditious in both the treatments and the plants drooped down within three days of fungal inoculation. The progress of the disease was always higher in the zeolite 20% treatment and the zeolite seed and it remained same all through. Whereas, in control plants the disease progressed from 60% at 3 dpi to 75% at 6 dpi. The zeolite treatments showed 90% disease severity in the plants (Fig 3a). In this case, statistical analysis of relative AUDPC values showed that there was a significant difference between the zeolite treatments and the control plants (Fig. 3b). Furthermore, the use of zeolite in any way was unsuitable for the plants to combat against *R. solani* infection. The plants transplanted in 20% zeolite and *R. solani* infested soils encountered much aggressive evasion of *R. solani*.

(a)



(b)

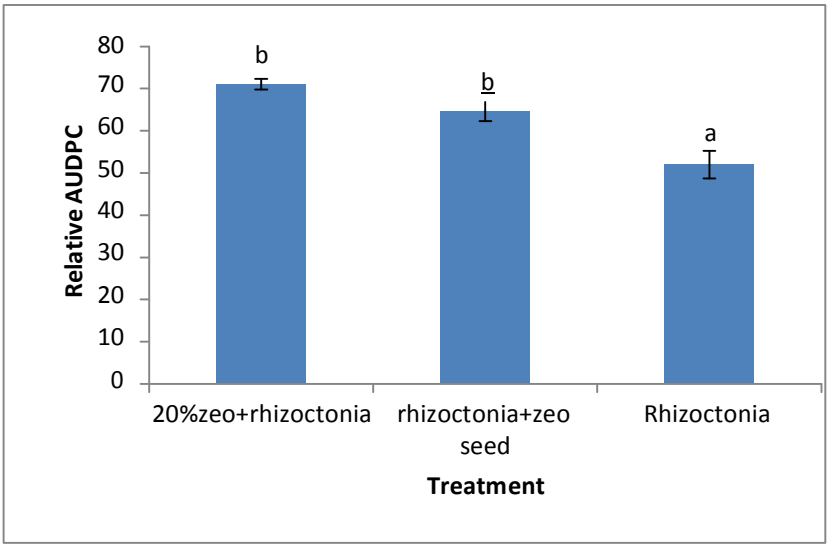


Figure 3 Root rot (*Rhizoctonia solani*) disease severity on lettuce plants either grown in soil amended with 20% zeolite and then transplanted in *R. solani* infested soil, or grown in normal soil and transplanted to soil amended with 20% zeolite and infested with sclerotia of the fungus (a). Disease ratings were plotted over time to generate disease progression curve and the area under the disease progression curve was calculated by the trapezoidal integration method (Campbell and Madden, 1990). (b) Results expressed as relative AUDPC i.e. the disease level as a percentage of the maximum possible area for the whole period of the experiment (D.J. Angelopoulou, E.J. Naska, S.E. Tjamos; 2014). Columns with different letters are significantly different according to the Fisher's least significant difference test at $p \leq 0.05$.

(a) **(b)**

(c)

Figure 3.1 Pictorial depiction of the rapid fungal (R. solani) advance and the plant death caused by it in (a) zeolite 20% (b) zeolite seed and (c) control treatments. The photographs were taken one day post inoculation.

qPCR determination of *PR1* and *LoX* transcript levels in *Pythium ultimum* infested plants

The transcript levels of two plant defence genes were examined by qPCR analysis to see if they could be induced by the addition of zeolite and also to observe if there could be a differential induction between the zeolite 20% and zeolite seed treatments. The expression of L-gulono lactone oxidase gene is well studied in lettuce plants. The study of expression of the genes in two different treatments could be beneficial for the analysis of a better technique. The qPCR analysis revealed that *PR1* was fairly expressed in all treatments. It was overexpressed in plants grown in Zeolite 20% normal soil and zeolite 20% *Pyhtium ultimum* infested soil. There was also a notable expression of the gene in the zeolite seed and *pythium*-zeolite seed treatment but was less than the zeolite 20%. There was a comparatively less expression of the gene in the control treatment (Fig. 4a). However in the case of *Lox* gene, plants responded to the presence of zeolite by expressing it moderately in zeolite 20% and *pythium*-zeolite 20% treatments. Whilst in zeolite seed treatments the gene was under-expressed and was very less expressed in the control treatment (Fig. 4b). The analysis was done on the samples collected at one day post inoculation. Plants with zeolite 20% responded well with a fair expression of the genes.

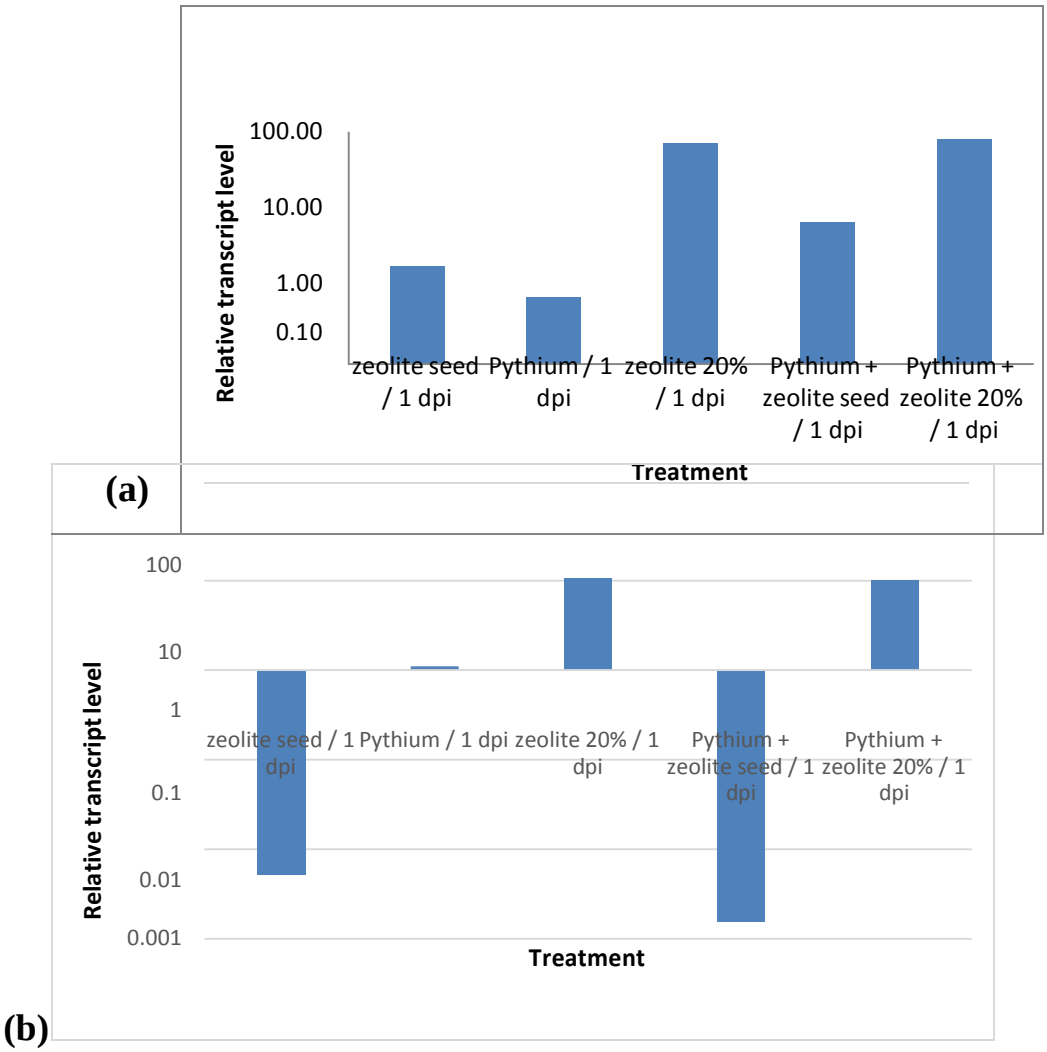


Figure 4 Changes in relative transcript abundance of PR1 (a) and LOX (b) in lettuce plants grown in soil amended with zeolite; lettuce seeds grown in 20% zeolite and later transplanted to *Pythium* infested soil (zeolite seed) and lettuce seeds grown in normal soil and later transplanted to zeolite 20%-*pythium* infested soil (zeolite 20%). Total RNA was isolated from above ground parts of the plant at a day post inoculation, converted to cDNA, and used as template in qPCR assays. Transcript levels of the genes were normalized to the expression of *actin* measured in the same samples and expressed relative to the normalized transcript levels in the mock plants.

Bioassays for virus resistance in *Arabidopsis* mutants

Challenge infection of *Cucumber mosaic virus (CMV)* and *Tobacco rattle virus (TRV)* on *Arabidopsis* mutants:

Cucumber mosaic virus (isolate from Benaki Phytopathological Institute) and *Tobacco rattle virus* (isolate P0049 GFP) were used to observe the resistance potential of *Arabidopsis thaliana* mutants, namely, *Col* wt plants, *bam, Mpk3* and *Mpk 6*. Only *mpk6, mpk3* and *col* wt plants were challenged by the *TRV* infection. The experiments were conducted in the Laboratory of Plant Virology at Benaki Phytopathological Institute. According to prominent evidences β-amylase is over-expressed in *Arabidopsis thaliana* plants upon infection by the obligate biotrophic protest *Plasmodiophora brassicae* (Jubault et al. 2013). Also, Engelsdorf et al. (2013) showed that starch-free mutants were more resistant towards the fungal biotroph *Erysiphae cruciferrarum* and more susceptible towards the hemibiotroph *Colletotrichum higginsiannum*. It is possible that reduced carbohydrate availability influences susceptibility differentially in interactions with biotrophic and hemibiotrophic fungal pathogens. Moreover, it was studied that BAM mutants are partially resistant to and negative regulators of Verticilium wilt (D. Gkizi, S.E. Tjamos; 2015). Therefore, in the present study an effort has been made to study the resistance potential and tolerance level of several *Arabidopsis* mutants against the viruses. In the case of *TRV* there was only one trial conducted for the experiment having 18 Col (wild type), 17 mpk3 and 13 mpk6 plants infected with the virus and kept under controlled conditions in the greenhouse. The plants were examined for the systemic foci of the virus by observing virus particles laden with fluorescent protein under an ultraviolet lamp, at 2, 5 and 9 days post inoculation. Broadly, there was no viral advance observed in any part of the plant (be it stem or leaves). Injuries occurred to the plant tissues at the time of inoculation could only be seen.

BAM mutants are partially resistant to *Cucumber mosaic virus*

In the bioassays conducted for virus resistance in wild type and three different *Arabidopsis* mutants it was found that the *BAM* mutants offered a moderate resistance to *CMV* as compared to the others. There were two replications carried out to observe the growth of *Arabidopsis* (mutants) against the virus. In the first trial there were 21 plants in each genotype that were challenged by the viral

infection through artificial inoculation (on the rosette leaves). The plants were sampled at 18 dpi. Whereas in the second trial, there were 40 plants infected with the virus in each genotype (which were separated in two blocks kept in same situation). The plants were sampled at 15 dpi in this case. Upon sampling of the whole plant an enzyme assay (ELISA) was conducted using the lab (BPI) protocol. The results obtained through 'Microplate manager 6" plate reader were in the form of absorbance [of optical density (O.D.)] values of the viral titres that were absorbed through the machine on the basis of the color. When inferenced, the results depicted that *bam* mutants had less positive samples as compared to the other genotypes. On an average count there was a plateau of 90% infection in *mpk6* and *mpk3* mutants, 81% in wild type (*Col*) plants and 51% infection in the *Bam* mutants (Fig.5a). The concentration of the virus was depicted by the absorbance values which showed less presence of virus in the bam mutants and very high percentage in the mpk6 mutants (Fig. 5b).

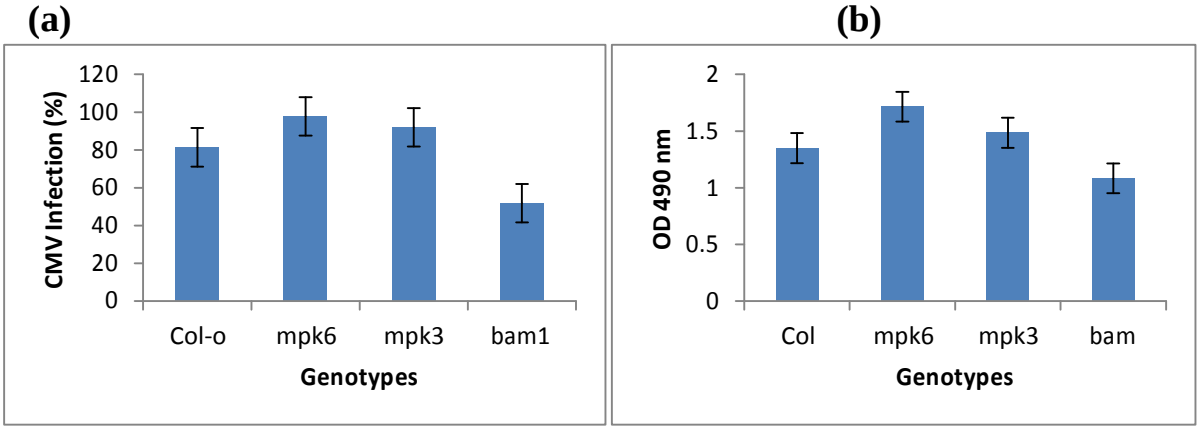


Figure 5 Graphical presentation of the *Cucumber mosaic virus* infection in the different *Arabidopsis thaliana* genotypes. (a) Disease severity (%) and (b) Concentration of the virus in the plant samples obtained by calculating the mean of the absorbance values of the samples through ELISA.

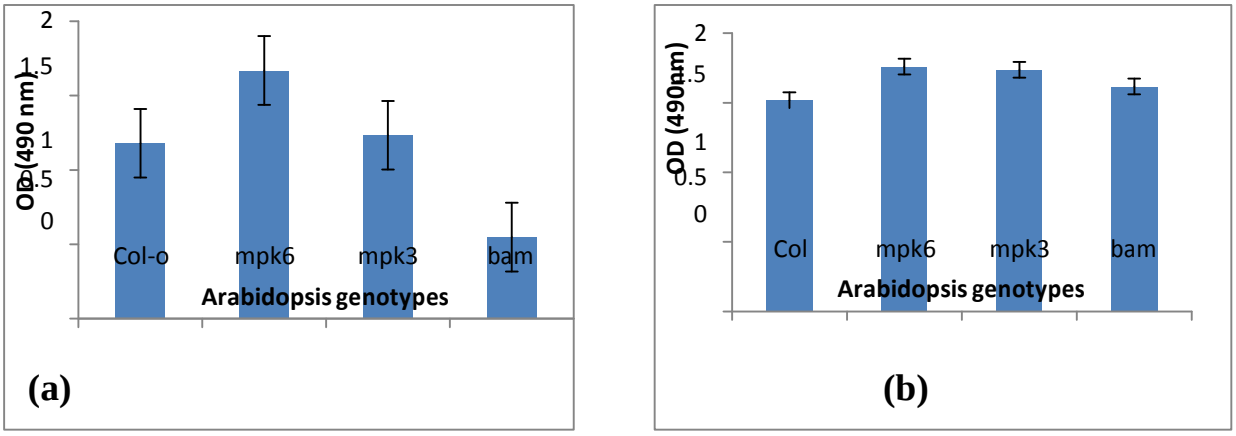


Figure 5.1 Concentration of Cucumber mosaic virus in different Arabidopsis thaliana genotypes in two different trials of the same experiment. The graph depicts the mean of the absorbance values obtained through ELISA. (a) Trial 1st had 21 plants for each genotype that were challenged by the viral inoculation and tested at 15 days post inoculation (b) Trial 2nd had 40 plants in each line that were tested at 10 days post inoculation (dpi)

Bioassays of virus resistance in Tomato

ISR induction has proved to be a promising strategy to control virus disease, particularly by seed bacterization with a mixture of plant growth-promoting rhizobacteria. However, the use of any of these treatments should be integrated with cultivation practices that reduce vector pressure by the use of insecticides, or by Bt crops (Franco Faoro, Franco Gozzo, 2013). The most efficient defense response relies on *R* genes that encode suitable proteins capable of recognizing viral elicitors. Many of these R-proteins conserve a domain characterized by leucine-rich repeat motif sequences. Other domains cooperate with leucine-rich repeat sequence proteins conferring successful resistance activation. Lectins, which generally bind to glucan molecular matrices, are also involved in viral resistance. A peculiar *Arabidopsis* Jacalin-type lectin, RTM1 (RESTRICTED TEV MOVEMENT 1) mediates resistance to *tobacco etch virus* (TEV). A similar protein, encoded by the lectin gene JAX1, promotes resistance to poty viruses. This type of lectin-mediated resistance apparently does not depend on HR or SAR signaling (Y.Yamaji et al., 2012). One of the most striking instances is the control of tomato spotted wilt virus (TSWV) in *N. tabacum* (A.s. Csinos, H.R. Pappu,

2001). In this pathosystem the most effective treatment was performed before transplanting (C. Nischwitz et al., 2008). After transplanting, a combination of *BTH* and the insecticide imidacloprid proved to be more suitable to contain the disease (K.R. Cherry, A.L. Mila, 2001).

The present study entitled "Evaluation of Biological Control Strategies against a range of plant pathogens" was undertaken using three replications for Tomato spotted wilt virus with two blocks (in trial 2nd and 3rd), and two replications in Cucumber mosaic virus and Potato virus Y with one block in both. The K165 mediated resistance against the above viruses was tested in the greenhouse and Laboratory of Plant Virology at Benaki Phytopathological Institute. The results obtained were categorized as here under:

Effect of *Paenibacillus alvei* (strain K165) on *Tomato spotted wilt virus* infection:

The leaves of tomato plants (var. Belladona) did not show necrotic or wilt symptoms after infection with the *TSWV*. Eventually there were very few tall plants 29 days after inoculation with the virus (16 out of 50 plants in the K165 treated block in the 2nd trial). Drenching with the bacillus was done twice, once five days before the inoculation of the plants with the virus and the other two days post inoculation (dpi) at the rate of 20ml per plant. Drenching of soil was done with the liquid formulation of the bacillus. The physiology of the plant remained same throughout the experiment, as such there was no visible

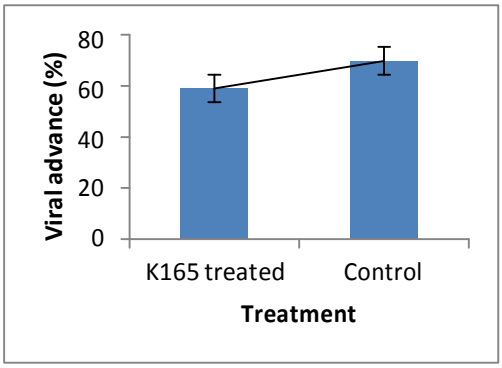


Figure 6. Systemic infection of *TSWV* in tomato plants var. Belladona, showing the percentage of the plants infected which were treated with the bacillus Paenibacillus alvei (strain K165), and, the control stock. Disease estimations were taken 2 and 3 weeks after inoculation.

difference between the K165 treated plants and the control stock. Likewise there was no significant difference noticed in the viral titres of K165 treated (1.854±0.498) and the control stock (1.60±0.734). Probably the root colonization by the bacillus did not elicit a a significant induced systemic response (ISR), neither it lowered the titre of TSWV in tomato.As per the figure (Fig.6), there was 59.03% infection in the K165 treated stock and 69.83% infection in the control stock. There was an enzyme assay conducted of infected plants (positive depicted by ELISA) at 28 dpi in the second trial to observe the K165 recovery in the plants. But the resistance offered and the viral titre remained the same depicting the inability of the bacillus against the viral antagonist. Colonization with the bacillus seemed to have increased the no effect on the viral systemic foci as the systemic leaves when tested showed the presence of the virus. Though the surface area of the leaf remained the same.

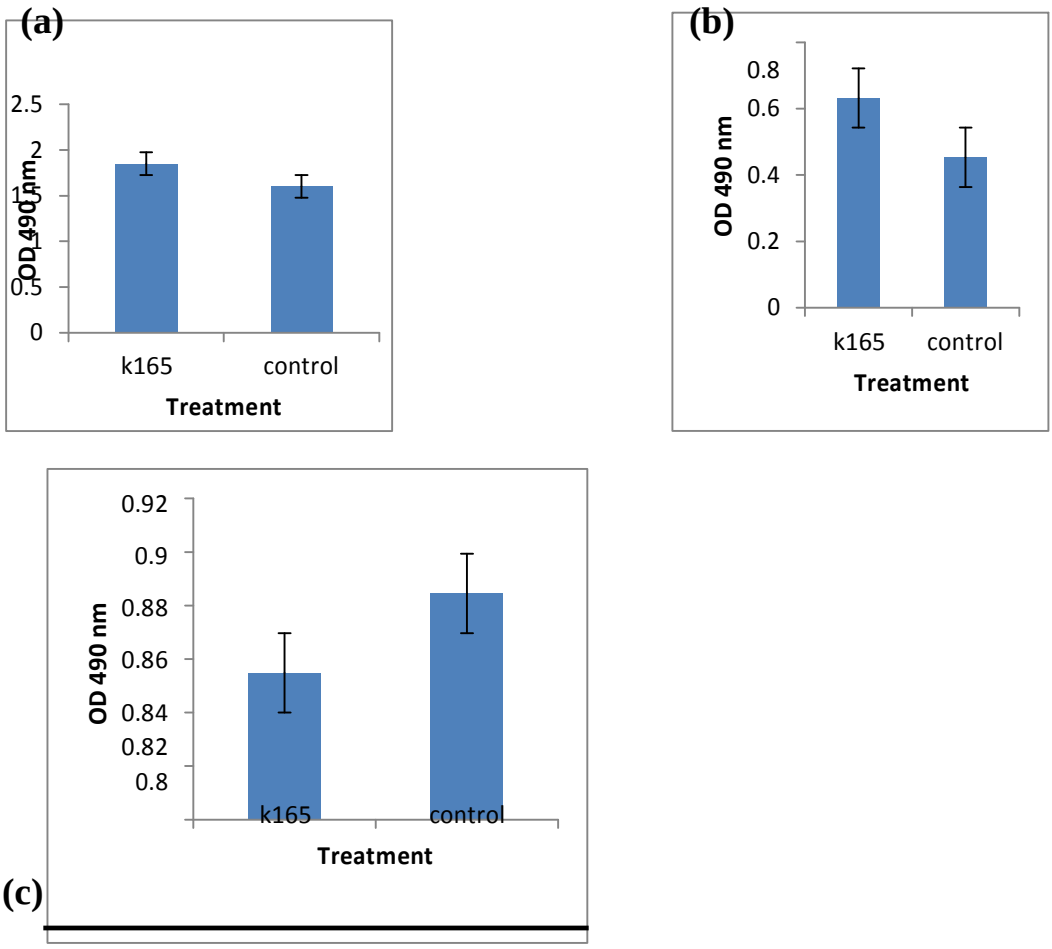


Figure 6.1 Concentration of *TSWV* in the systemic leaves of tomato plants for different trials of the experiemnt. Estimations are based on mean of the absorbance

value obtained through ELISA done at (a) 27dpi for trial 1st (b) 29 dpi for trial 2nd and (c) 16 dpi for trial 3rd.

***Potato virus Y* and K165 bioassays**

There were two replications done to observe the K165 mediated resistance in the tomato plants against the seriuosly damaging potyvirus. Inoculation of the virus was done five days post treatment (DPT) of the bacillus K165 and the first sampling was done at 7dpi. Drenching of soil with the liquid formulation was repeated 5 days post inoculation of the virus.

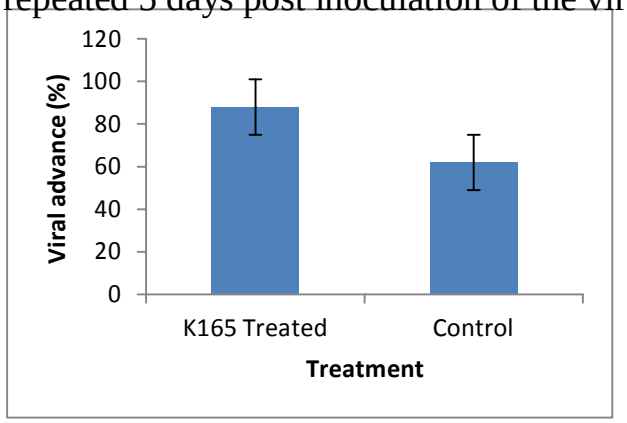
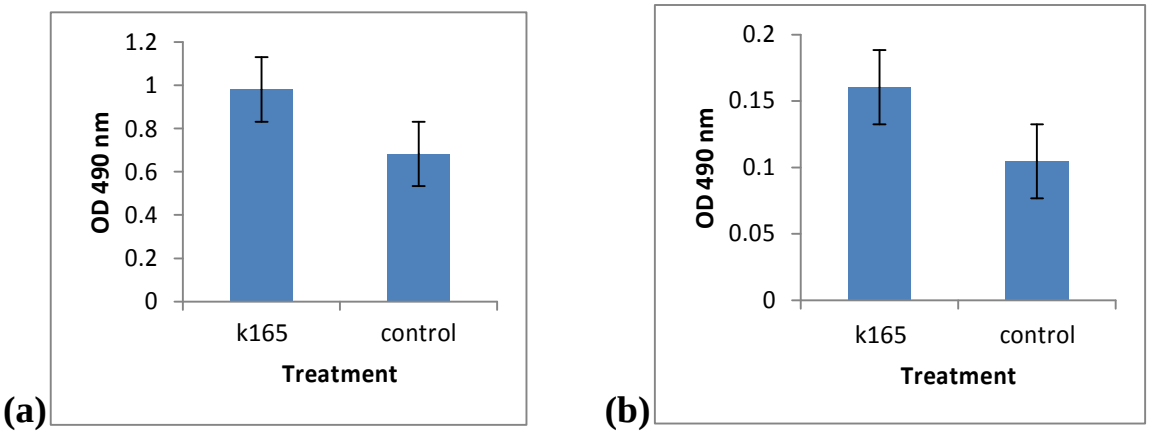


Figure 7. Disease estimations of *PVY* infection in tomato (Var. Belladona) plants. The infection % is greater in the K165 treated plants. Observations were done at 7, 11 and 32 dpi.

The bacillus seemed to influence the infection systemically in the plants and did'nt elicited the resistance pathway. The infection percentage in the bacillus treated plants was 88%, in contrast there was only 62% infection in the control (untreated) stock. The viral titers remained same in both the stocks without any significant difference.



Figyre 7.1 Concentration of *PVY* in the systemic leaves of tomato plants for different trials of the experiemnt. Estimations are based on mean of the absorbance value obtained through ELISA done at (a) 11 dpi for trial 2nd (b)7 dpi for trial 1st.

***Cucumber mosaic virus* and K165 bioassays**

Solubilization of phosphates by rhizobacteria can improve the uptake of phosphate (Glick 1995; Kim et al. 2002), thereby leading to increased plant growth. Certain volatile metabolites, including 2,3-butanediol and acetoin, produced by root-colonizing microbes also enhance plant growth (Han et al. 2006; Ryu et al. 2003). The criterion used to define ISR is to observe separation on the plant between the site of pathogen attack and the colonization site for the inducing bacteria. ISR occurs in many plants including carnation, cucumber, tobacco, tomato, bean, radish, and Arabidopsis (Van Loon et al. 1998). ISR does not require a salicylic acid-dependent pathway (Pieterse et al. 1996, 2002; Ryu et al. 2004b; Van Loon et al. 1998). Several microbial determinants have been associated with elicitation of ISR; 2,4-diacetylphloroglucinol (Iavicoli et al. 2003), the O-antigen from lipopolysaccharide (Van Peer and Schippers 1992), and the volatile organic compound, 2,3-butanediol (Han et al. 2006; Ryu et al. 2004a). Induced resistance is a valuable strategy for control of plant pathogens, due to its long-lasting effects against a broad range of pathogens. Chemical elicitors including salicylic acid, *b*-aminobutyric acid (BABA), and benzo(1,2,3) thiadiazole-7-carbothioic acid S-methyl ester (BTH, ActigardTM) also exist (Vallad and Goodman; 2004). However, through *Paenibacillus alvei* the approach is more eco friendly and it also induces the high level of production of defence related proteins. In the present study K165 was used against Cucumber mosaic virus in tomato plants (var. Belladona). However, against the viral pathogen the bacillus did not elicited the ISR mechanism and infact caused a bit more infection than the untreated plants.

The infection was 90% in the bacillus drenched plants and 86.5% in the un-drenched plants.

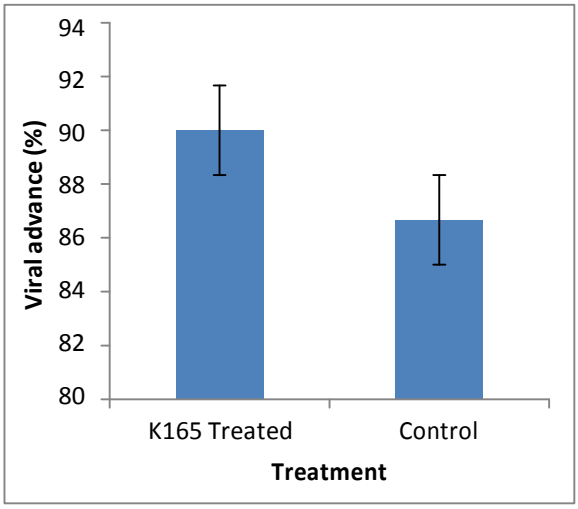


Figure 8. Systemic spread of *CMV* in tomato (var. Belladona) plants. The estimations were observed at 7, 11 and 32 dpi.

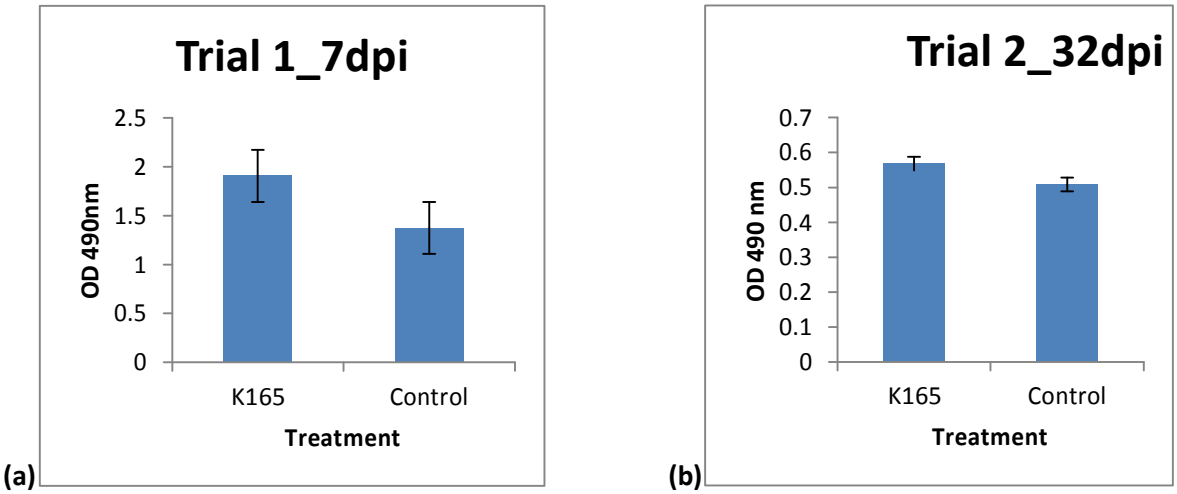


Figure 8.1 Concentration of *CMV* in the systemic leaves of tomato plants for different trials of the experiemnt. Estimations are based on mean of the absorbance value obtained through ELISA done at (a) 7dpi for trial 1st (b)32 dpi for trial 2nd.

Conclusion

Sustainable agriculture has always been a desire for the farmers and agricultural scientists. The term encapsulates a huge understanding of ecosystem services. Satisfying human needs for food and fiber through agricultural practices and maintaining the quality of environment and natural resources is the major idea of sustainable agriculture. Agriculture is an age old phenomenon, probably since the existence of mankind on earth who cultivated the land and utilized it for food. With the passage of time it became an occupation to feed a large scale of population and thus started the never ending use of chemicals in it. Plant diseases that occurred on main crops encouraged the use of more chemical control.

Study of symptoms, causes and mechanisms of development of plant diseases has an extremely practical purpose as it allows for development of methods to control it. Methods of control vary from one disease to another depending on the kind of pathogen, host, and interaction of two and many other different variables. In controlling diseases plants are treated as populations rather than individuals. Most serious diseases of crop plants appear on a few plants in area year after year, spread rapidly, and are difficult to cure once they have begun to develop. Therefore almost all control methods are aimed at protecting plants from becoming diseased rather than curing them after they have become diseased. Most biological and some cultural control methods are targeted at improving the resistance of the host or favoring microorganism antagonistic to the pathogen. Eventually, the present study entitled 'Evaluation of biological control strategies against a range of plant pathogens' is also an initiative to test the biocontrol potential of natural zeolite as a soil amendment against rot and mold causing soil borne pathogens *Rhizoctonia solani*, *Pyhtium ultimum* and *Sclerotinia sclerotiorum* in lettuce plants and, the use of biocontrol bacterium *Paenibacillus alvei* to help the tomato plants to resist evasion of *Cucumber mosaic virus*, *Tomato spotted wilt virus* and *Potato virus Y*. The study also focused on testing the resistance of different mutants of *Arabidopsis thaliana* against different viruses.

The first line of active defense of plants against the pathogens relies on the recognition of common features of microbial pathogens, such as flagellin (the major protein of bacterial flagellum), lipopolysaccharides, glycoproteins and

chitin. These microbial determinants are referred to as Pathogen associated molecular patterns (PAMPs) and are sensed by host encoded pathogen recognition receptors (PRRs) which encode transmembrane receptor-like kinases. Upon PAMP detection, PRRs trigger a series of immune responses, for instance, reactive oxygen species (ROS) production, differential expression of genes which ultimately leads to basal immunity or PAMP triggered immunity (Martine Boccara, Alexis Sarazin,Oliver Voimet; 2014). The encounter of divergent virulent determinants and disease resistance (R) genes is also an important event in providing the first hand resistance.

Zeolites are known to be useful in combating the plant diseases as they can be used as carriers of herbicides, fungicides and pesticides. Clinoptilolite can be an excellent substrate for benzyl phosphorothioate to control stem blast in rice (Fredrick Mupton, 2008). Using natural zeolites as a base, Hayashizaki and Tsuneji (2013) found that clinoptilolite is more than twice as effective as a carrier of the herbicide benthiocarb in eliminating weeds in paddy fields as other commercial products. Whereas, the biocontrol bacterium *Paenibacillus alvei* has a remarkable background of imparting resistance through triggering of host defence systems in aubergene plants against *Verticillium dahliae* in different formulations and application methods (E.J. Naska and S.E. Tjamos; 2014). In field and greenhouse conditions chemicals such as acinobenzolar-S-methyl known as BTH can achieve SAR. BTH is a functional analogue of salicylate and it is shown to induce an artificial systemic resistance to viruses. For example, BTH can protect tobacco plants from *Tobacco mosaic virus* (TMV) (Friedrich et al., 1996) and *Tomato spotted wilt virus* (TSWV) (Mandal et al., 2007) and tomato plants from *Cucumber mosaic virus* (CMV) (Anfoka, 2000). However, the application of BTH results in reduced growth and stunted plants, a fact that can lead to an economical cost.

Although ISR and SAR trigger different regulatory pathways, the induced resistance is similar, as they can protect plants from a broad range of pathogens, including viruses (Ryu et al., 2004, Faoro and Gozzo, 2015). To date, studies reporting an antiviral action of PGPR and especially of *Bacillus* spp. are scarce. In tomato, *Bacillus* strains mediated protection against *Tomato mottle virus* (ToMoV) (Murphy et al., 2000) and CMV (Murphy et al. 2003; Zehnder et al., 2000). Moreover, *B. pumilus* SE34 significantly reduced symptom development and CMV accumulation in *Arabidopsis thaliana*. Wang and co-workers (2009) showed that

Bacillus spp. elicited ISR to TMV in tobacco plants with the participation of the *PR*, *NPR1* and *Coi1* genes, resulting in reduced TMV disease severity and virus accumulation. So far, only three *P. fluorescens* strains, CoP-1, CoT-1 and CHAO, are shown to protect tomato plants from TSWV through the induction of *PAL* that activates the secondary metabolism and especially the phenylpropanoid pathway (Kandan et al., 2002, Kandan et al., 2005).

The present study revealed that the use of zeolite as a soil amendment in the case of white mold fungus *Sclerotinia sclerotiorum* helped the plant to tolerate the pathogen to a level that the disease severity in the zeolite seed treatment was almost half of the control treatment. In the zeolite seed treatment the advance of the disease was slow from the initial days of the inoculation and it continued to be the same full spread of the fungus (Fig. 1). The disease severity in the zeolite 20% (added at the time of transplanting) was also less compared to the control. In the other cases of *R. solani* and *Pythium ultimum* there was no protection observed. In R. solani the fungal attack was so expeditious that it caused the death of the plants just a day after the inoculation. In *P. ultimum* treatments there was a greater presence of oomycete in the zeolite treatments than the control treatment, though there was an expression of *PR 1* gene (in moderate quantities) in all the treatments and *Loxidase* gene in the zeolite 20% treatment (Fig.4). Zeolite, thus can be used an effective soil amendment strategy if the field is infested with *Sclerotinia sclerotiorum*. Soil amendment with 20% zeolite would rather be more effective. However this study needs a further support of molecular research to support it entirely. Observing the differential disease pattern in the lettuce plants in response to different fungi it is quite clear that zeolite has a key role to play in the disease advancement or check if it is present in the soil.

In the case of K165 and virus bioassays there was no efficient control by the biocontrol bacterium against the viruses. There was no significant difference between the *bacillus* drenched stock and the control treatment when infected with *Tomato spotted wilt virus*. Whereas, the tomato plants that were drenched with the bacterium showed higher presence of virus in them as compared to the control treatment in the case of *Potato virus Y* and *Cucumber mosaic virus* (as per the mean absorbance values).The delivery of fungal and bacterial BCAs capable of colonizing the rhizosphere and achieving their biocontrol potential is a key issue in the use of biocontrol inoculants for protection of crops against plant pathogens

(Gowen, 2006). Probably the mode of application didn't suited the biocontrol bacterium K165 against the use of viruses. Maybe the BCA is not efficient against highly infectious plant viruses such as *TSWV, PVY* and *CMV*. The fungal BCA in liquid formulation was possibly not fast in colonizing the rhizosphere than the system spread of the virus.

Plants have developed an elaborate immune system by the virtue of which they can encounter the attacking pathogen and resist to it. Similarly pathogens keep adapting themselves by the newest of the resistant strategies adopted by the host plant. Likely the *BAM* mutants by the virtue of *β-amylase* gene could stay partially resistant to highly infectious *Cucumber mosaic virus* strain (Fig.5)

It is clear that effective crop protection requires integration of a number of different approaches, including biologically based methods. Integrated Crop Management (ICM) can be a tool for sustainable agriculture. ICM includes cost-effective production of high quality crops, with priority given to ecologically safe methods of cultivation and minimizing the use crop protection chemicals and biological control strategies are an integral part of it.

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