CHAPTER 2

REVIEW OF LITERATURE

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2.1. Tomato

Tomato (*Lycopersicon esculentum* Mill.) is a herbaceous fruiting plant. Itis grown both commercially and non-commercially in over 140 countries for its diverse edible fruit. China mainland, India, United States of America, Turkey and Egypt are the top five tomato fruit-producing countries**(FAOSTAT, 2014)**(Table 1) and, for more than 20 years, annual production has been rapidly increased. Most recent statistics estimate world production at approximately 161 million tons of fresh and processing tomatoes per year, with such levels having more than doubled since 1990.

Table 1: World production of tomato, 2012

Location	Production (ton)
World	161793834
China mainland	50000000
India	17500000
United States of America	13206950
Turkey	11350000
Egypt	**8625219**

The high level of tomato consumption and the concentration and availability of several vitamins, minerals and antioxidants makes the fruit important to human nutrition. The nutritional quality of tomato is mainly determined by its carotenoid, potassium, vitamin C and vitamin A contents. Ripe tomatoes have high levels of carotenoids, of which carotenes make up between 90 and 95% **(Guil-Guerrero and Rebolloso-Fuentes, 2009)**. In particular, the pigment lycopene, which is the most abundant carotene in red tomatoes, has gained much attention due to its antioxidant properties and is known to reduce the risks of many forms of cancer and heart attacks **(Clinton, 2005)**.

Other antioxidant compounds in tomatofruit include flavonoids and phenolic acids. Flavonoids and phenols are regarded as potentially healthbenefiting compounds since they are implicated in the prevention of human inflammatory andcardiovascular diseases as well as cancer **(Mutanen *et al.*, 2011)**.

Recently, there has been more emphasis on tomato production not only as source of vitamins, but also as a source of income and food security.

Arie *et al.* (2007) reported that tomato has been a good model plant to analyze plant-pathogen interactions and its prospects for the future are promising. An international consortium named International Solanaceae Genomics Project (SOL) is proceeding with whole genome sequencing of tomato. In order to be relevant in the post-genomic era, accumulation of information on tomato pathogen interactions is important. Tomato is one of the most popular vegetables worldwide; however, its cultivation has been limited by an abundant attack by pathogens. In order to establish effective control methods to control them, analysis of tomato-pathogen interactions is also important.

2.2. Tomato diseases

A simple definition of plant disease is any disturbance that interferes with a plant's normal structure, function, or economic value **(Persley, 1993)**.

The most costly and common diseases of tomato are caused by a number of different organisms namely bacteria, fungi, oomycetes, viruses and nematodes**(Jones *et al*., 1991)** (Table 2).

A crop cultivar can be defined as resistant, tolerant or susceptible to these biotic stresses, dependent upon its ability to prevent infection and the severity of the symptoms caused. In resistant crops, the plant avoids infection by the initiation of plant defence responses and as such, symptoms (if any) are low. On the contrary, if a crop is susceptible then the infection is successful, the disease causing agent colonizes plant tissues and the crop develops disease symptoms. Tolerant crops are unusual in that they are successfully infected and colonized by pathogenic organisms but the plant will exhibit reduced symptoms and similar yields to resistant cultivars.

Tomato diseases can be spread by air, soil (media), water, seed or vector and can infect aerial parts of the plant as well as the roots, the latter often being more expensive and difficult to control due to location and microbial complexities in the root environment **(Jones, 2008)**.

Table 2: Commonly occurring and economically important tomato diseases (the causal agent)

Disease	Microorganisms
Fungi	
Late blight	*Phytophthora infestans*
Verticillium wilt	*Verticilliumalbo-atrum*
Verticillium dahlia	
Anthracnose	*Colletotrichum coccodes*
	Colletotrichum dematium
	Colletotrichum gloeosporioides
Early blight	*Alternaria solani*
Cercospora leaf mold	*Pseudocercospora fuligena*
Fusarium wilt	*Fusarium oxysporum f.sp. Lycopersici*
Fusarium crown and root rot	*Fusarium oxysporum f.sp. radicis-lycopersici*
Powdery mildew	*Oidiopsis sicula*
Pythiumdamping-off and fruit rot	*Pythium aphanidermatum*
	Pythium arrhenomanes
	Pythium debaryanum
	Pythium myriotylum
	Pythium ultimum
Rhizoctoniadamping-off and fruit rot	*Rhizoctonia solani*
Gray leaf spot	*Stemphylium lycopersici*
Septoria leaf spot	*Septoria lycopersici*
Leaf mold	*Cladosporium fulvum*
Bacteria	
Bacterial spot	*Xanthomonas campestris*
Bacterial wilt	*Ralstonia solanacearum*
Bacterial speck	*Pseudomonas syringae pv. Tomato*
Bacterial canker	*Clavibacter michiganensis*
Root Mat	*Agrobacterium rhizogenes*
Viruses	
Tomato mosaic	*Tomato mosaic virus*
Tomato fern leaf	*Cucumber mosaic virus*
Curly top	*Curly top virus*
Tomato bushy stunt	*Tomato bushy stunt virus*
Tomato etch	*Tobacco etch virus*
Potato virus Y	*Potato virus Y*
Tomato necrosis	*Alfalfa mosaic virus*
Tomato spotted wilt	*Tomato spotted wilt virus*
Tomato mosaic	*Pepino Mosaic Virus*
Nematodes	
Root-knot	*Meloidogyne spp.*
Sting	*Belonolaimus longicaudatus*
Reniform	*Rotylenchus reniformis*
root lesion	*Pratylenchus spp.*

false root-knot x	*Pratylenchus spp.*
potato cyst nematodes	*Globodera spp.*
Stunt	*Tylenchorhynchus spp.*

2.2.1. Fungal diseases

Fungi are the most important common cause of plant disease **(Persley, 1993)**, since they are the most widespread and destructive parasites of plants **(Ingold and Hudson, 1993)**.

The effect of fungi on plants can be devastating. Cellular structure can be destroyed, physiological functions of the plant impaired, and rates of metabolism and metabolic pathways can be altered. These biological processes have widespread consequences for infected plants because the impairment of any one function or organ has effects on others **(Moore, 1996)**.

Prior to fungal penetration of a plant, many factors influence the sequence of events from germ tube emergence to attachment, adhesion, appresorium development, and growth on the plant surface. These may relate directly to endogenous factors such as influences from the environment, competition from other microbes, or factors relating to the host plant such as leaf age, cultivar type, and physiological condition **(Isaac, 1992)**.

Fungi can be extremely destructive for several reasons **(Strange, 1993)**: 1) they sporulate prolifically; 2) the infection cycle (i.e., the time between infection and the production of further infectious propagules - usually spores) may be only a few days; 3) the fungi can rapidly mutate to develop resistance to fungicides; 4) the spores themselves may be carried long distances by the wind; 5) the fungus may produce compounds that are highly phytotoxic and/or a battery of enzymes that destroy the plant's structures; and 6) small lesions caused by some specialized parasites, such as rusts, act as powerful sinks, drawing nutrients away from the economically valuable part of the plant such as the grain, and thus depress yields.

Concerning fungal diseases, tomato producers are confronted with great losses in the production caused by plant pathogens especially such as the soil-borne fungi *Fusarium oxysporum,Alternaria solani* which causes early blightand the Oomycete *Phytophthora infestans* **(Shattock, 2002; Fradin and Thomma, 2006).**

Fusarium solani and *Rizoctonia solani* are the most important soil-borne fungalpathogens, which develop in both cultured andnon-cultured soils, causing the symptoms of dampingoff and root rot diseases to wide range of vegetableand crop plants including tomato **(Abu-Taleb *et al*.,2011)**.

The main focus of this section will be on root rot and early blight.

2.2.1.1. Root Rot

Fusarium solani is one of the most important pathogen for rot disease of plants **(Agrios, 2005)**. Root rot disease caused by *F. solani* was reported by various researchers **(Gupta *et al.*, 2004 and Gaetan *et al.*, 2007)**.

The genus *Fusarium* is imperfecti fungi (Deuteromycotina), and belongs to the Kingdom fungi, order Hypocreales, Family Hypocreaceae **(Fry, 2004)**.

Fusarium species are widely distributed in soil, roots and aerial plant tissues, plant debris, and other organic substrates. They are common in tropical and temperate regions and have also been found in deserts, alpine and arctic areas **(Nelson *et al.,* 1983 and Aoki*et al.,* 2003)**.

The fungus is spreaded by infested plants and soil on farm machinery, drainage water and boots **(Jones, 1997)**. **Koseki and Isobe (2005)** also reported that vegetables could be contaminated during growth from many sources such as soil, water, wild animals, birds and insects.

Plant infection by *Fusarium* can occur at all developmental stages, from germinating seeds to mature vegetative tissues, depending of the host and *Fusarium* species involved **(Mueller *et al.*, 2010)**.

Fusarium species play an important role as plant pathogens, causing a very different symptoms on different plants and under various environmental conditions, such as rot **(Wang and Jeffers, 2000)**, cankers, blights **(Schmale and Gordon, 2003)**and wilts **(Chaimovitsh *et al.,* 2006)**. Certain species are capable of producing dangerous mycotoxins **(Bush *et al.,* 2004)**. Others may be saprophytes that live on dead plant tissues **(Dill-Macky and Jones, 2000)**.

Fusarium solani is a common pathogen of tomato where it causes a wilt, pre- and post-emergence damping off **(Chandra *et al.,* 1983)** and a fruit rot **(Pradeep and Gupta, 1979)**. It has also been recorded causing a foot rot of tomato **(Romberg and Davis, 2007)**.

F. solani diseases are characterised by distinct foliar symptoms that develop after infection at any growth stage.The most conspicuous symptoms begin with chlorotic mottling followed by interveinal chlorosis and necrosis **(Shuxian *et al*., 2000)**. Besides these foliar symptoms, roots are rotten and the vascular system of lower stems is discoloured and reduced **(Rupe *et al.,* 1997)**.

The first symptom in the field is wilting of the leaves, within several days after infection, followed by plant death. On tomato, yellowing occurs along the margins of the oldest leaves, followed by necrosis and collapse of the leaf petiole. Soon after yellowing appears on the seedling, the water conducting tissue (xylem) becomes reddish brown. Plants such as tomato may wilt slowly and still be alive at the end of the harvest **(Jones, 1997)**.

Diseases caused by *Fusarium solani* are a limiting factor in plant production, and they are one of the main causes of the reduction of yields. *F. solani* causes the death of young and adult plants, with consequent economic losses **(Rojo *et al*., 2007)**. The incidence of root rots caused of 10 to 80% losses in different vegetables **(Hadwan and Khara, 1992)**.In recent years, *F. solani* cause the severe damage to tomato cultivars in Egypt **(Haggag, 2008)**.

2.2.1.2. Early Blight

Diseases caused by members of the genus *Alternaria* are among the most common diseases affecting a wide variety of plants including annual plants, vegetables, ornamentals

and trees.The two major features of *Alternaria* species are the production of melanin, especially in the spores, and the production of non-specific as well as host-specific toxins in the case of pathogenic species **(Thomma, 2003)**. Due to the number of plants affected by the species in this genus, it is considered one of the most economically important in term of losses **(Agrios, 2005)**.

Alternaria solani Sorauer, the causal agent of early blight disease in tomato and potato plants, is one of the best-known and most economically important members of the genus *Alternaria***(Chaerani and Voorrips, 2006)**. This species is the causal agent of early blight of potato (*Solanum tuberosum* L.), tomato (*Lycopersicon esculentum* Mill.), and other members of the family *Solanaceae* **(Foolad and Lin, 2001 and Foolad *et al*., 2003)**.The pathogen may also cause collar rot symptoms **(Poysa and Tu, 1996)**. Early blight is considered the more serious of the two diseases **(Maiero *et al.,* 1991)**.

As many species of *Alternaria*, *A. solani* produces toxins. In culture the fungus synthesises two phytotoxins, alternaric acid and zinniol. Alternaric acid is considered to be host specific and causes leaf lesions that are surrounded by a yellow zone. Zinniol affect a wide range of hosts and causes stem wilting and leaf necrosis. The phytotoxins are transported in the vascular tissue of the diseased plant **(Maiero *et al.,* 1991)**.

The pathogen can affect all aerial parts of the plant at all stages of development; however it is frequently first observed affecting older or senescing tissue **(Jones, *et al.*, 1991)**.

Disease symptoms are characteristic dark brown to black lesions with concentric rings, which produce a "target spot" effect **(van der Waals *et al.*, 2004)**. Early blight may affect foliage, stems and in more severe cases, fruits **(Haggag and Farghaly, 2007)**.

Leaves on infected plants develop dark brown spots with concentric rings. A yellow area surrounds the spots and leaves with many spots become completely yellow. The infected tissue can occur anywhere on the leaf. The spots enlarge rapidly and finally the whole leaf dies. Usually the infection starts at the lower leaves, which turn yellow or brown and then droop or fall off. If the climate is favourable, the disease develops rapidly, the spots spread over the leaves and the plant becomes defoliated **(Jones *et al.,* 1997)**.

Disease becomes serious when the season begins with abundant moisture or frequent rains followed by warm and dry weather which are unfavorable for the host and help rapid disease development. Very high temperatures and periods of continued drought check the spread of disease **(Thirthamallappa and Lohithaswa, 2000 and Chaerani *et al*. 2007)**.

Crop losses from early blight results from loss of blighted foliage, along with decreased photosynthesis and increased respiration in apparently healthy tissue. Because physiological changes are difficult to evaluate, crop loss is based on severity of disease.

Alternaria solani is considered as an agent of reduction of yields in tomato crop **(Stone *et al.,* 2000)**. Infected fruit fall to the ground and this causes losses of 30-50 % of the harvest **(Jones *et al.,* 1997)**.

2.3. Management of fungal diseases using bio-and chemical fungicides or systemic acquired resistance agents

Increasing loss of conventional fungicides due to pathogen resistance and general unacceptability in terms of public and environmental risk has favored the introduction of integrated pest management (IPM) programmes. Induction of natural disease resistance (NDR) in harvested horticultural crops using physical, biological and/or chemical elicitors has received increasing attention over recent years, it being considered a preferred strategy for disease management **Terry and Joyce (2004)**.

Spletzer and Enyedi (1999)investigated that root feeding 200 μM SA to tomato plants can (i) significantly elevate foliar SA levels, (ii) induce PR-1B gene expression, and (iii) activate SAR that is effective against *A. solani*.

Heil *et al.* (2000) mentioned that **1)**Although most theories on plant defence assume that costs will result from the production and maintenance of defensive traits, studies on the costs of induced defence against pathogens are comparatively rare. **2)** Shedding focus on fitness costs resulting from the chemical induction of systemic acquired resistance (SAR), a rather unspecific form of defence, which can be induced by and is effective against a broad spectrum of bacteria, fungi and viruses. **3)** Using a model system in which the wheat plants were protected against fungi by `traditional' fungicides with BION®. Treated plants were therefore compelled to invest in defence without gaining any profit from the induction. **4)** Treated plants achieved lower biomass than untreated controls, and developed fewer shoots and ears and therefore produced fewer seeds. The effects were most pronounced in plants that suffered from a shortage of nitrogen, and were observed only when pathogen resistance was induced during lateral shoot production. Later treatment revealed no significant effects. **5)** The differences between treated and control plants can be interpreted as a consequence of allocation costs. Such costs could result from metabolic competition between processes involved in plant growth and the synthesis of defence-related compounds.

Zehnder *et al.* (2000) suggested that specific strains of plant growth promoting rhizobacteria (PGPR)-mediated induced resistance against cucumber mosaic virus(CMV) infection following mechanical inoculation onto tomato can be maintained under field conditions.

Benhamoua *et al.* (2001)reported that oligandrin has the ability to induce systemic resistance in tomato. Exogenous, foliar applications of the fungal protein sensitize susceptible tomato plants to react more rapidly and more efficiently to *F. o.* f. sp. *radicis-lycopersici* attack, mainly through the massive accumulation of fungitoxic compounds at sites of attempted pathogen penetration. Although cell wall modifications do not represent the central core of the oligandrin-mediated host response in tomato, they are part of the multicomponent defense system elaborated to fend off *Fusarium* invasion.

Özgönen *et al.* (2001) tested in vitro the effects of different salicylic acid (SA) concentrations on mycelial development of *Fusarium oxysporum* f.sp *lycopersici* (Fol). Also, two concentrations of SA and *Glomus etunicatum* (GE) were tested on plant development of tomatoes. SA completely inhibited the mycelial development of Fol in vitro at concentrations from 0.6 mM to 1.0 mM and ED_{50} value was found as 0.51 mM. GE could increase dry weight of plant, length of shoot and root growth irrespective whether Fol infected the tomato plants. The root colonization by GE was determined as 62.3% when the Fol was absent and as 53.2% when the plants were infected. However, in different combinations of GE and SA, the root colonization was determined between 19.1

and 34.2%. In pot experiments, the combination of GE and 1 mM SA had the highest effect on infection of Fusarium wilt and disease severity was reduced by 70%.

Cotxarrera *et al.* (2002) reported that*T. asperellum* isolates isolated from the suppressive compost-peat mix appear to have the potential to be a new alternative of biocontrol of Fusarium wilt.

El-Mougy (2002) reported that salicylic acid proved antimicrobial effect on some fungal and bacterial plant pathogens and completely inhibited their growth at certain concentrations under *in vitro* conditions.

He *et al.* (2002) examined the ability of nonpathogenic isolates of *Fusarium oxysporum* (np *Fo*) to induce systemic resistance and defence responses against subsequent challenge with a pathogenic strain of *F. oxysporum* f. sp. *Asparagi* (*Foa*) in *Asparagusofficinalis*. And it was found that isolates of np *Fo* may function as inducers of systemic acquired resistance (SAR) and defence responses against *Foa* invasion in *A. officinalis*.

Qin *et al.* (2003) investigated salicylic acid (SA) and two yeast antagonists, *Rhodotorula glutinis* and *Cryptococcus laurentii*, separately and together for controlling *Penicillium expansum* and *Alternaria alternata* in sweet cherry fruit. Results of in vitro studies showed that SA at low concentrations had little effect on the growth of the yeasts or the pathogens. SA treatment induced a significant increase in polyphenoloxidase, phenylalanine ammonia-lyase, and β-1,3-glucanase activity in cherry fruit, but did not alter the levels of peroxidase. The mechanism by which SA enhanced the biocontrol efficacy of the antagonistic yeast may be related to its ability to induce biochemical defense responses in sweet cherry fruit rather than its fungitoxicity effects on the pathogens.

Achuo *et al.* (2004) investigated the role of salicylic acid (SA) in basal defence and induced resistance to powdery mildew (*Oidiumneolycopersici*) and grey mould (*Botrytis cinerea*) in tomato (*Lycopersicon esculentum*) and tobacco (*Nicotiana tabacum*). It is concluded that the SA-dependent defence pathway is effective against different pathogens in tomato and tobacco.

Dietrich *et al.*(2004) treated *Arabidopsisthaliana* plants that had been cultivated under different nitrogen regimes with BION®, a chemical resistance elicitor. The activities of three enzyme classes functionally involved in resistance (chitinase, chitosanase and peroxidase) were quantified.

Panina *et al.* (2004) found that, in tuber tissues of potato (*Solanum tuberosum* L.) infected with an incompatible race of *Phytophthora infestans* (Mont.) de Bary, the activity of phenylalanine ammonia-lyase and the contents of free and bound salicylic acid (SA) considerably exceeded the corresponding indices in the tissues infected with a compatible race of the oomycete. The accumulation of the free form of SA apparently resulted from both enhanced SA biosynthesis and the liberation from the bound SA forms. SA accumulation in the incompatible host-pathogen combination presumes that SA participated in the local potato resistance to late blight.

Awadalla and Mahmoud (2005) studied the mechanism of resistance to *Fusarium oxysporum* f.sp.*vasinfectum* induced by some chitosan derivatives in susceptible cotton plants. Pretreating cotton seeds with chitosan markedly increased cotton resistance to vascular wilt caused by *Fusarium oxysporum* f.sp.*vasinfectum*.

Faheed *et al.* (2005) reported that treatment of infected tomato plants with 10-6 M salicylic acid enabled plants to tolerate stress due to fungus by increasing the contents of pigments, soluble protein and proline. Therefore, salicylic acid is involved in regulation of resistance against *A. solani*.

Kavroulakisa *et al.* (2005) used a transgenic GUS-expressing strain of the root-infecting fungal pathogen *Fusarium oxysporum* f.sp. *radicis-lycopersici* to inoculate the roots of tomato plants (*Lycopersicon esculentum* Mill.). The data strongly indicate that induction of plant defense response is the main mechanism of biological control mediated by the GM-EPC compost.

Liu *et al.* (2005) studied Benzothiadiazoles (BTH, BION®), as candidate of SAR inducer in the cucumber-*Sphaerotheca fuliginea* system. Disease incidence and index were significantly lower when compared with the water-treated control. Treatments with BION®led to an increase in activities of chitinase and beta-1,3-glucanase in cucumber leaves compared with control plants.

Sarwar *et al.* (2005) immersed seeds of chickpea variety AUG424, susceptible to *Fusarium oxysporum ciceri* (FOC), in two concentrations of salicylic acid (1.0 &1.5 mM) and Bion (0.3& 0.4mM). Wilt disease was significantly reduced with all the treatments in both experiments. On the basis of disease rating done after root cutting, wilt incidence was significantly less in chemically treated plants as compared to control ones. Fresh and dry weights of shoot and root were higher in treated plants as compared to control ones especially in plants grown from Bion treated seeds.

Abo-Elyousr (2006) tested the effect of Bion and BioZell-2000 B against common blight (caused by *Xanthomonas campestris* pv. *phaseoli*) of bean plant. *In vitro* studies, both tested compounds exhibited no inhibitory effect against the pathogen. Under greenhouse conditions, bean variety “Red Kidney" treated by Bion and BioZell-2000 B resulted in marked disease suppression. A high decrease of the disease rate of 68% and 50% was correlated with a reduction in the bacterial population up to 50 % and 45%, respectively.

Faheed and Mahmoud (2006) explained the role of exogenous application of salicylic acid and kinetin in increasing *Phaseolus vulgaris* resistance against tobacco necrosis virus (TNV). Scince it showed partial inhibition of the accumulation of local lesion virus in infectedprimary bean leaves. This inhibition was accompanied with an increase in the peroxidases activity, especially at the lowconcentration used of early treatment salicylic acid and kinetin compared with the un-treated control plants. In addition, theaccumulation of total soluble protein contents were increased in comparison with the untreated control plants.

Oanh *et al.* (2006) tested biotic inducer as *Trichoderma harzianum* and chemical inducers as salicylic acid and Bion. In field experiment, the result showed that the foliar sprays with any of plant inducer reduced the infection percentage of anthracnose on chilli.

Segarra*et al.* (2006) found that in plants treated with the biological control agent *Trichoderma asperellum* strain T-34, the levels of SA and JA did not differ from control plants. *Rhizoctonia solani* diseased cucumber plants showed higher levels of SA and JA compared to non infested controls.

Tosun *et al.* (2006) applied plant disease resistance elicitors, salicyclic acid and Harpin Ea alone and in combination with systemic fungicide phosphorus acid (Agrifos

400) to tomato plants, which were inoculated with *Phytophthora infestans* after treatments. The combinations of SA and Harpin Ea with fungicide were found much more effective than that of single treatments. While SA induced the accumulation of pathogenesis related proteins (PR proteins), chitinase, β-1, 3-glucanase and peroxidase. Surprisingly, fungicide application induced the disease resistance components like the elicitors used in our experiment. The results confirmed that Agrifos 400 is not only effective fungicide against late blight but also a plant activator.

Aktas and Guven (2007) showed that salicylic acid (SA) treatment and challenge by *Uncinula necator* (Schwein.) Burr., significantly induced the systemic acquired resistance components in grapevine(*Vitis vinifera* L. cv. Sultana). Chitinase (CHV, EC 3.2.1.14) and β-1, 3-glucanase (Laminarinase,EC 3.2.1.39) activities increased in SA-treated (100, 200 and 400 ppm) as well as in *U. necator* infected plants and the highest enzymatic activity was observed in leaves treated with 100 ppm ofSA.

Al-Hakimi and Alghalibi (2007) studied the interactive effects of fungi (*Fusarium solani and Rhizoctonia solani*) infection and thiamin or salicylic acid on growth rate of broad bean plants. Fungal infection induced a reduction in growth rate. Application of thiamin or salicylic acid increased growth rate.

El-Khallal (2007) inoculated tomato plants infected by *Fusarium oxysporum* with *Arbuscular mycorrhizal* (AM) fungi and/ or sprayed 3 times with hormonal inducers (JA& SA). Results showed that % of disease incidence in infected plants gradually increased with increasing time of infection (86% at 42 days). Treatments with AM fungi, JA and SA significantly reduced % of disease incidence. AM fungi plus JA had the highest effective (92% efficiency). Results revealed that induction in the uptake of nutrients could be responsible for increasing susceptibility of tomato plants to *Fusarium* wilt disease. The results suggest that reduction in disease incidence, promotion in growth and metabolic activities in tomato plants inoculated with bioagent(AM fungi) and sprayed with elicitors(JA& SA) could be related to the synergistic and cooperative effect between them; which lead to the induction and regulation of disease resistance. Thus, two signal hormones could enhance the biological activity of AM fungi in tomato, potentially through interaction signaling pathways. AM fungi plus JA was more effective than AM fungi plus SA.

Hajhamed *et al.* (2007) used salicylic acid and BION as resistance inducing factorsunder artificial inoculation condition to manage thebacterial soft rot disease of potato caused by *Erwinia carotovora* subsp. *carotovora*. Thetested agents decreased the disease compared with the control. Disease severity was completely reduced when salicylic acid was applied at 0.9 mM, before or at the same time or after inoculation with the pathogen. Efficiency of the inducer agents were increased against the disease by increasing their rates. Salicylic acid was the most effective against the disease compared with BION.

Hibar *et al.* (2007) evaluated the effect of *Trichoderma* spp. on resistance induction in tomato plants against *Fusarium* crown and root rot of tomato (*Lycopersicon esculentum*) caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) *in vivo*, in growth chamber by bringing separately the pathogen and the antagonist to the same plant root level. Results showed that in spite of the physical separation from FORL, *Trichoderma* spp. have significantly reduced disease incidence especially when they were applied one week before inoculation with the pathogen. Light photomicrograph of samples from tomato roots treated with *Trichoderma* spp. showed elaboration of structural barriers

in regions situated within striking distance of the pathogen penetration, formation of cell wall thickenings and occlusion of intercellular spaces by a densely stained material, preventing thus pathogen evolution and penetration.

Mostafa and Gado (2007) found thatthe treatment of ethyl salicylic acid (0.125 ml/liter) led to a great reduction in number of blight spots caused by *Phytophthora infestans* (Mont.)/ potato plants in comparison to non treated plants. Ethyl salicylic acid reduced spot size. Disease severity was reduced in treated plants.

Yu *et al.* (2007) evaluated the efficacy of salicylic acid (SA) in suppressing the blue and gray mould rots in pear fruit and to explore possible mode of action involved. Their results showed that the combined treatment of pear fruit with SA at 100 μg ml^{-1} resulted in a remarkably improved control of *Penicillium expansum* and *Botrytis cinerea* infections. SA at 100 μg ml^{-1}inhibited the blue mold when the inoculation concentrations of *P. expansum* were above 5×10^2 spore per ml *in vivo*, it induced the fruit resistance to the blue and gray mold rots when the time interval between SA treatment and pathogens inoculation was more than 48 h, being associated with a rapid and strong activation of the peroxidase activity in pear fruit.

Cavalcanti *et al.* (2008) sprayed susceptible cacao plants with acibenzolar-S-methyl (ASM; Bion® 50 WG (0.2 g/l) and a heterogeneous chitosan suspension (MCp) from *Crinipellis perniciosa* mycelium. Plants were challenged five days later with a virulent isolate of *Verticilliumdahliae*, under greenhouse conditions. A reduction of Verticillium wilt severity was observed in plants treated by the substances tested, with MCp reaching 80% of ASM protection performance. To evaluate local and systemic activation of defences, cacao leaves exposed to ASM and MCp were assayed for peroxidase, polyphenol oxidase. Local induction of resistance was confirmed by the increase of chitinase and β-1, 3-glucanase activities in the leaves, 4-18 days after spraying.

Mwangi *et al.* (2008) selected the rhizobacteria isolates *Pseudomonas fluorescens* T58, *Pseudomonas putida* 53 and *Bacillus sphaericus* B43, after greenhouse tests for biological control of Fusarium wilt in tomato, were all able to induce systemic resistance (ISR) against *Fusarium oxysporum* f.sp. *lycopersici*. Peroxidase activity in the stem was stimulated by all three bacteria. The increase due to *B. sphaericus* B43 occurred transiently three days after bacteria treatment, while peroxidase activities in plants treated with *P. fluorescens* T58 or *P. putida* 53 increased six days after bacteria treatment.

Shabana *et al.* (2008) investigated the antifungal activity of the phenolic antioxidants (salicylic acid and benzoic acid) against *Bipolaris oryzae* (the causal agent of rice brown spot disease) at different concentrations *in vitro,* as well as the efficacy of their exogenous application in controlling rice brown spot disease under field conditions. *In vitro*, benzoic acid or salicylic acid at 9 mM completely inhibited the growth of *B. oryzae.* Under field conditions, spraying of benzoic acid at 20 mM led to a significant reduction in disease severity (DS) and disease incidence (DI) on the plant leaves.

Abd El-Hai *et al.* (2009) tested seed soaking method or foliar spray of antioxidants (citric acid and salicylic acid at 10 mM) to control of the damping-off and charcoal rot diseases of sunflower. Laboratory results showed that salicylic acid alone or in combination with citric acid completely inhibited the linear growth of both pathogens i.e., *M. phasoliana* and *R. solani in vitro*. The application of Rizolex-T 50 followed by citric acid showed a highest percentage of healthy plants followed by the combination of citric acid and salicylic acid. All treatment of antioxidants significantly reduced the incidence of

charcoal rot disease. On the other hand, no significant differences between Rizolex-T 50 and salicylic acid treatments was shown. Total phenols content was highest due to Rizolex-T 50 application followed by salicylic acid.

Ashour (2009a) stated that applicationofTridex 8%, Bion and salicylic acid caused significant reduction in the linear growth of *Alternaria solani,* the causal of tomato early blight, compared with check treatment. This reduction was gradually increased by increasing the incorporated concentration.

Ashour (2009b) evaluated the efficiency of some systemic fungicides and resistance inducing chemicals (RICs) on management of cantaloupe powdery mildew, caused by *Erysiphe cichoracearum* DC, under greenhouse and field conditions. Greenhouse experiments revealed that application of the tested systemic fungicides as well as RICs (Bion and salicylic acid) significantly reduced the disease severity. However, systemic fungicides were more efficient in this concern than the tested RICs.

Egusa *et al.* (2009) concluded that the pathogenicity of *Alternaria alternata* (Aa), causes Alternaria stem canker on tomato, depends on the production of host-specific AAL-toxin. Pre-inoculation with nonpathogenic Aa or pretreatment an elicitor prepared from Aa reduced disease symptoms by the pathogen. Salicylic acid (SA)- and jasmonic acid (JA)-dependent defense responses in tomato are not involved in the resistance to the pathogen induced by nonpathogenic Aa. The results showed that an alternative and unknown signaling pathway independent of SA- and JA-signaling might modulate the induced resistance by activating the expression of the multiple defense genes.

Mandal *et al.* (2009) demonstrated that exogenous application of 200 mM salicylic acid through root feeding and foliar spray could induce resistance against *Fusarium oxysporum* f. sp. *Lycopersici* (Fol) in tomato. The activity of peroxidase were 4.7 times higher than the control plants. The increase in POD activities was 3.3 times higher than control plants. The salicylic acid-treated tomato plants challenged with Fol exhibited significantly reduced vascular browning and leaf yellowing wilting. The mycelial growth of Fol was not significantly affected by salicylic acid. The results indicated that the induced resistance observed in tomato against Fol might be a case of salicylic acid-dependent systemic acquired resistance.

Ahmed (2010) reported that the combination treatments of the inducers with fungicides increased the levels of defense-related polyphenol oxidase and phenolic substances.

Asgharia and Aghdam (2010) found that salicylic acid (SA), an endogenous plant growth regulator can generate a wide range of metabolic and physiological responses in plants thereby affecting their growth and development. SA as a natural and safe phenolic compound exhibits a high potential in controlling post-harvest losses of horticultural crops.

El-hendawy *et al.* (2010) applied the inducerssalicylic acidand ascorbic acid by seed soaking to compare their effectiveness in inducing resistance against chocolate spot disease (*Botrytis fabae* Sard.) in faba bean under greenhouse and field conditions. It was found that a decrease in disease severity. These results led to the conclusion that, for field application of chemical inducers to develop host resistance, it is important to study their effects on growth in addition to their ability to control diseases.

Haggag *et al.* (2010) found that an increasing in the activity of soluble ornithine decarboxylase (ODC) and polyamine oxidize (PAO), in leaves following treatment with

MJ. Analysis of soluble protein, salicylic acid (SA), peroxidase, chitinase, polyphenoloxidase and phenols in protected plants revealed conspicuous accumulation of such substances. All results showed significant changes in metabolism affected by either viral infection or MJ treatments and also indicated that exogenous MJ plays an important role in the induction of defense mechanism against BtMV infection.

Percival (2010) evaluated several systemic inducing resistance agentsand a conventional triazole fungicide (myclobutanil) on apple scab (*Venturia inaequalis*) development under laboratory conditions. Salicylic acid and salicylic acid derivative inhibited germination of apple scab conidia, subsequent formation of appressoria and reduced leaf scab severity. The synthetic fungicide myclobutanil resulted in the greatest levels of germination inhibition, reduced appressorium development and leaf scab severity.

Sarwar *et al.* (2010) studied induced systemic resistance in chickpea against wilt disease caused by *Fusarium oxysorum* f. sp. *ciceri* (FOC) by treating the seeds with benzo (1,2,3)-thiadizole-7-carbothioic acid - s- methyl ester (Bion), salicylic acid (SA). Reduction in disease was observed in both type of applications but seed dressing was found more effective than soaking method.

Hashem and Hamada (2012) tested salicylic acid (SA) against root rot disease of wheat under field condition. The tested compound significantly reduced the root rot of wheat severity during seedling, flowering and ripening stages. Fresh and dry weights were also affected by application of this compound.

Houssien *et al.* (2010) used *Trichoderma harzianum,* salicylic acid and low dose of thiophanate methyl as recommended fungicide as a new strategy to enhance tomato defense response against wilt disease caused by *Fusarium oxysporum f. sp. Lycopersici* under greenhouse conditions. Changes in various physiological defensesincluding enzymes like polyphenol oxidase (PPO), peroxidase (POD) and acid invertase (AI); total solublephenols; protein were investigated.All applied treatments completelyprotected tomato seedlings against *Fusarium* wilt. Level of all the determined physiological parameters greatly changed in tomatoplants in response to *Fusarium oxysporum (FO),* TH fungi and hormonal elicitor reflected manycomponents of defense signals which leading to the activation of power defense system in tomato againstpathogen attack.

Montaser *et al.* (2012) used the chemical inducer salicylic acid (SA) at three different concentrations (50,100, 200 ppm) to treat tomato seedling by soaking into these to minimize root rot (caused by *Rhizoctonia solani*) and wilt (caused by *Fusarium solani* and *F. oxysporum*) diseases incidence. Moreover, the influence of these chemicals on growth, quantity and quality parameters of tomato plants (cv. Super Strain B) under greenhouse and field conditions were studied. SA inducer significantly reduced root rot and wilt diseases severity either under both greenhouse and field conditions and the efficiency of this compound increased with increasing concentrations. SA inducer significantly reduced mycelial linear growth of all tomato root rot and wilt tested fungi compared with control.

2.4. The pesticides residue

2.4.1. Risks of the intensified use of pesticides

The use of pesticides benefits in increasing agricultural production but the repeated and indiscriminated use of certain pesticides have led to their accumulation in plants, animals, solid and sediments this effecting widespread contamination of the environment(**Dalvi and Salunkhe, 1975**).

Arendse *et al.* (1989) reported that the improper handling and the consumption of contaminated vegetables over long periods could result into chronic poisoning for which long term effect could lead to increased sensitivity to pesticides, and damage to the internal organs such as the liver.

The extensive use of pesticideshas the drawback ofpesticide residues which remain in fruits and vegetables, constituting a potential risk toconsumers. Fruits and vegetables are the foods that receive the highest doses of pesticides (Torres *et al.,* 1996).

Price (2008) stated that pesticides are present in all compartments of agro-ecosystem, but perhaps the real risk to human is through the consumption of pesticide residues in food vegetables.

The World Health Organization (WHO) estimates there are 20,000 unintentional death and three million poisonings caused by pesticide misuse in the third world each year **(Lowell, 2008)**.

Moawad *et al.* (2014) reported that fungicides which are not easily degradable have the greatest adverse effects on soil microbes. These pesticides negatively affect the growth and multiplication of fungi and bacteria and consequently cause the disturbance of the natural soil microbial balance.

2.4.2. Determination of pesticides residue

Sisken and Newel (1971)determined the residues of Vitavax (5, 6-dihydro-2-methyl-l &oxathiin-3-carboxanilide), a systemic fungicide and its sulfoxide, in wheat, barley, oats,peanuts, sorghum, flax, and cotton seeds and extractedseed oils. They found that no residues have been detectedin seed harvested from plants grown from treatedseed.

Khalfallah *et al.* (1998) studied the fate of tetraconazole residues on greenhouse cucumbers. Tetraconazole residues dissipated relatively rapidly, with a half-life of 7 days.

Navarro*et al.* (2002) determined eight fungicides in fruits and vegetables. Recoveries from spiked orange, apple, tomato, artichoke, carrot and courgette samples ranged from 62 to 102% and relative standard deviations were less than 15% in the concentration range 0.05–10 mg/kg. Detection and quantitation limits ranged 3–30 µg/kg and 10–100 µg/kg, respectively, with linear calibration curves up to 10 mg/kg.

Su *et al.* (2003)determined the residue of metalaxyl in apple core, flesh and peel at the initial time, 3 and 6 months after storage under controlled atmosphere conditions. The concentration of metalaxyl residues in apple flesh was $\leq$ 0.22 mg/kg; the mean concentrations in apple core and peel were 0.41±0.18 and 0.79±0.94 mg/kg, respectively.

Amer *et al.* (2007) determined residues and the rate of disappearance of tetraconazole and diniconazole from tomatoes and green beans. The fungicides incorporated into the plants decreased rapidly with a half-life around 3 days for

diniconazole and from 4.5 to 6.5 days for tetraconazole. No residues could be detected in the plants during the period of study of 21 days after field application.

Badaway*et al.* (2009)conducted a field trial to follow the dissipation of the residues of oxamyl and metalaxyl alone or in combination in tomato leaves. The results showed that metalaxyl was affected by tank mixing with oxamyl before spraying time, while oxamyl was not affected when mixed with it.

Sahoo *et al.* (2011) estimated the residue of propamocarb in tomato and soil. Propamocarb presented a distinct peak at retention time of 8.962 min. Consistent recoveries of propamocarb ranging from 87 to 92 percent were observed when samples were spiked at 0.10, 0.50 and 1.00 mg/kg levels. The limit of quantification (LOQ) of this method was determined to be 0.10 mg/kg.

Abd-Alrahman and Ahmed (2012) estimated the dissipation of penconazole in tomatoes fruits cultivated in field using QuEChERS method. Following one application of normal dose 25 mL/100 L water, the average initial deposits of penconazole were observed to be 0.74 and 1.21 mg/kg for tomatoes fruits and soil, respectively. The residues dissipated below the maximum residues limit of 0.2 mg/kg after 15 days. The preharvest interval of penconazole were 15 days.

Abd Al-Rahman *et al.* (2012) evaluated the dissipation rate of three widely used pesticides in growing tomatoes, namely, chlorothalonil, metalaxyl-m and metalaxyl. Tomato samples were collected within 2 weeks after pesticides application. Pre-harvest intervals (PHI) for these pesticides ranged from 3 to 9 days, with the longest being for metalaxyl (9 days), followed by chlorothalonil (6 days). Metalaxyl-m had PHIs of 4 days.

Kundu *et al.* (2012)evaluated the dissipation pattern of fungicide mixture (Benalaxyl-M 4 % + Mancozeb 65 %) WP in grapes at two application rates (2,750 g and 5,500 g/ha). Following the first order kinetics the fungicide Benalaxyl-M dissipates in grapes with half-life (T1/2) value ranges between 2.59 and 2.79 days irrespective of seasons and doses. The dissipation pattern of Mancozeb also follows first order kinetics with half-life (T 1/2) value ranges between 3.86 and 4.93 days irrespective of seasons and doses.

Abd-Alrahman and Ahmed (2013) estimated Dissipation of penconazole in peach, plum, apricot, and mango fruits cultivated in different farms. Following one application of normal dose 25 mL/100 Lwater, the average initial deposits of penconazole were observed to be 0.44, 0.35, 0.66 and 1.12 mg/kg for peach, plum, apricot, and mango, respectively. The residues dissipated below the maximum residues limit of 0.1 mg/kgafter 15, 7, 10 and 21 days for peach, plum, apricot, and mango, respectively. The pre-harvest interval of penconazole were 12, 12, 7 and 21 days for peach, plum, apricot, and mango, respectively. Thus, a waiting period of 21 days was suggested for the safe consumption of penconazole treated mango.

Rani *et al.* (2013)estimated the dissipation of mancozeb and metalaxyl intomato following four applications of acombination formulation Ridomil MZ (mancozeb 64% + metalaxyl 8%) at 0.25 and 0.50% at 10 days interval.Residues of mancozeb dissipated below limitof quantification (LOQ) of 0.25 mg/kgafter 10 and15 days at single and double the application dosage,respectively. Similarly, residues of metalaxyl took 3 and5 days to reach LOQ of 0.02 mg/kg at single and doubledosages, respectively.

Bagi *et al.* (2014) tested the efficacy of azoxystrobin (Quadris, 250 g a.i. L^{-1}) in two doses (187.5 g a.i. ha^{-1} and 250 g a.i. ha^{-1}) and chlorothalonil (Bravo 720-SC, 720 g a.i. L^{-1}) at a rate of 1.44 kg a.i. ha^{-1} for the control of cucumber downy mildew (CDM). While monitoring the degradation of azoxystrobin residues, a decrease in residue levels to 1.0 mg/kg below the maximum residue level (MRL) was observed at the end of the pre-harvest interval (PHI).

Abdellseid and Abd El Rahman (2014) determined the residue of Tetraconazole in tomato samples. At fortification levels of 0.1, 0.5, and 1.0 mg/kg in tomato, it was shown that recoveries ranged from 89.4 % to 95.6 %. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.02 and 0.01 mg/kg, respectively. The dissipation half-life time of tetraconazole residues in tomato was 5.02 days. According to maximum residue limit (MRL) 0.5 mg /kg, the pre harvest interval (PHI) of tetraconazole on tomato was 4 days after the treatment.

Moawad *et al.* (2014)assessed two fungicide tolerant isolates of rhizobia; clover isolate (TA1) and peanut isolate (8) in their capacity to degrade Vitavax and Rizolex. The performance of these isolates in fungicides degradation was tested using the colorimetric assay for Rizolex and the HPLC analysis for Vitavax to detect the degradation products. The Vitavax fungicide did not degrade completely after 240 hours of incubation with rhizobial isolate. The used Rizolex contained blend of Thiram (active ingredient of Rizolex) and Tolcofs methyl fungicides in 1:1 ratio. The biodegradation of Rizolex in the liquid media showed the formation of two new intermediates which were released into the medium indicating the degradation of the tested fungicide by peanut rhizobial isolate No. 8 in 48 hrs of incubation 45% of this compound was degraded.