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Role of Plant Growth Regulators in the Interaction between Phytopathogenicity of *Sclerotinia sclerotiorum* and their Host Plants

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xes

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List of Abbreviations:

ACC	1-aminocyclopropane-1-carboxilic acid
DCNA	2,6-dichloro-4-nitraniline
2,4-D	2.4-Diphenol acetic acid
ABA	Abscisic Acid
A.I	Active Ingredient
AVG	Aminoethoxyvinylglycine
ANOVA	Analysis of Variance
CRD	Complete Randomize Design
DFMO	D,L, -difluoromethylornithine
Et.	Ethephon
GA_3	Gibberellic Acid
GA	Gibberellins
gr	Gram
JA	Jasmonic Acid
L	Liter
Me.JA	Methyl Jasmonate
mM	Millimole
ml	Milliliter
NAA	Naphthalene Acetic Acid
NPK	Nitrogen, Phosphorus, Potassium
ppm	Part Per million
PR	Pathogenesis-related proteins
PME	Pectinmethilesterase
PCNB	Pentachloro-nitrobenzene
PAL	Phenylallanine ammonia lyase
PGRs	Plant Growth Regulators

	Polamines
PG	Polygalacturonase
PDB	Potatoes Dextrose Broth
PDA	Potatoes Dextrose Agar
SS.I	*Sclerotinia sclerotiorum* isolated from Israel
SS.P	*Sclerotinia sclerotiorum* isolated from Palestine
VSP	Vegetative storage proteins

Abstract

The effect of synthetic plant growth regulators (PGRs) on *Sclerotinia sclerotiorum* was investigated *in vitro* and *in vivo*, on bean and cucumber plants. The results showed that naphthalene acetic acid (NAA) has a potential to inhibit fungus *in vitro* and *in vivo*. It reduced white mold disease severity on bean and cucumber plants at concentrations up to 500 μg/ml active ingredient. Ethephon and gibberellic acid (GA3) promoted both mycelium growth rate and white mold disease severity on plants at concentrations up to 400 μg/ml. Methyl jasmonate (MeJA), and absiscic acid (ABA) decreased mycelium growth of the fungus *in vitro*. MeJA decreased bean and cucumber white mold disease at concentrations up to 250 μg/ml. ABA increased disease development on bean and cucumber plants at concentrations up to 300 μg/ml. Aminoethoxvinyleglycine (AVG) used as an ethylene-inhibitor agent significantly inhibited white mold lesions development *in vivo* and decreased white mold disease severity on bean and cucumber plants at concentrations up to 300 μg/ml.

Ethylene was produced by the fungus *S. sclerotiorum* when grown on shaking PDB medium amended with 10 mM methionine at a rate of 400 μl/g/h.

Chapter 1

General Introduction

1.1 The fungus *Sclerotinia sclerotiorum*

1.1.1 History, and host range

The fungus *Sclerotinia spp.* had been described by Libert (1837) who called this species *Peziza sclerotinia*. However, de Bary (1884) renamed it as *Sclerotinia sclerotiorum*. Thus, the proper name for the fungus is *S. sclerotiorum* (Lib.) de Bary. It belongs to class Ascomycetes, and family Sclerotiniaceae which was erected in 1945 by Whetzel (Purdy, 1979).

Sclerotinia sclerotiorum is an important plant-pathogenic fungus causing white mold, which is a serious yield-limiting disease of many hosts. It is non-specific and omnivorous. Adams (1975) reported that *S. sclerotiorum* hosts belonged to 64 plant families, 225 genea, 361 species.

1.1.2 Epidemiology

Species of the genus *Sclerotinia* can function either as soil-borne or air- borne pathogens. Infections of aboveground plant parts result from ascopores inoculum or mycelium, whereas soil-line infection may result either from ascospores or sclerotia. Below-ground infection, however, results from mycelial germination of soil-borne sclerotia. Accordingly, the

e two types of infection incited by *Sclerotinia s*pp. is quite different. Effects of weather factors on their incidence and development differ considerably (Abawi &Grogan, 1979).

White mold epidemics of beans incited by *S. sclerotiorum* are initiated by ascospores produced by sclerotia (Abawi &Grogan, 1975, 1979). Only sclerotia in the top 2-3 cm of the soil are functional because apothecia with stipes longer than 3 cm are rarely produced under field conditions. Sclerotia present in and outside bean fields also can provide ascosporic inoculation for bean white mold epidemics.The mycelium has been also reported to infect bean (Natti, 1971).

Epidemics of white mold of bean occur in the field prior to blossoming because mature bean blossoms usually serve as an energy source for the fungus. Ascospores completely colonize mature blossoms at continuous leaf wetness and constant temperatures of 20-25°C and senescent blossom within 2-3 days (Abawi &Grogan, 1975). Mycelial growth from these colonized blossoms produces infections by contact with leaf, stem and pod tissues. There seems to be a positive correlation between bean plant age and percentage of white mold infection. The infection reached 100% at 40-day old plants (Abawi &Grogan, 1975). Under moist conditions, leaf, stems and pod tissues in contact with infected plants develop water-soaked lesions. These lesions continue to enlarge and in a

red with dense, white mycelial mat. Usually sclerotia are produced on the surface of the mycelium within 7-10 days. Plant-to-plant infection occurs only through direct hyphal growth from previously infected tissues. Asexual spores are not produced by *S. sclerotiorum.*

1.1. 3 Physiology of disease development

1.1.3.1 Colonization of tissue

Exclusive intercellular penetration of infection hyphae through tissue is enhanced by enzymes capable of degrading the middle lamella of host cells (Lumsden, 1976; 1979). Three pectolytic enzymes produced by *Sclerotinia spp.* serve the pathogen.

The endo-polygalacturonase (PG), undoubtedly, is essential for successful advance of the pathogen during the very early stages of pathogenesis. Beside demethylation of pectin, pectinmethylesterase (PME) and PG probably are responsible for hydrolysis of the middle lamellae of cells, thus, enabling the fungus to move rapidly through tissues in an intercellular manner. The ability to produce large quantities of enzyme *in vitro* was associated with isolates of *S. sclerotiorum* that were most virulent on bean plants (Lumsden, 1976; 1979). In addition, the endo-PG was found by Lumsden to readily macerate susceptible bean and cucumber tissues.

probably is essential for rapid action of endo-PG (Lumsden, 1976; 1979). These enzymes work together to degrade highly methylated pectin. The PME demethylates pectin in the middle lamella, forming pectate, which is the preferred substrate for *Sclerotinia spp.* exo- and endo-PG.

PME is also active during the latter stages of pathogenesis at which time the exo-PG is most active. Exo-PG hydrolyzes pectate more readily than pectin, and therefore, exo-PG and PME also would work in concent to degrade middle lamella pectin. The production of exo-PG is correlated with growth of *Sclerotinia* and may play a role in the nutrition and development of the pathogen in plant tissues (Lumsden, 1976; 1979).

1.1.3.2 Nutrition during pathogenesis

The nutrition of *Sclerotinia spp.* during all stages of disease development is probably the most important factor in determining success or failure in the establishment of disease in the host tissues. Even before infection, the availability of a food base is usually a prerequisite for successful infection (Abawi &Grogan, 1975, de Bary, 1976).

During infection the fungus organizes itself into specialized infection hyphae, which require a considerable amount of energy and an abundant, readily available source of nutrients (Abawi &Grogan, 1975). In

e of host plant tissue induce a greater amount of infection hyphae formation than non-host media.

Cellulase, hemicellulase, exo-PG, phosphatetidase, proteolytic enzymes may play a nutritional role in pathogenesis. The action of these enzymes on cell wall and cell content can provide abundant carbon and nitrogen supply that is essential for the extensive metabolic activity of *S. sclerotiorum* (Bauer *et al*, 1977; Hanlock, 1967; Lumsden, 1970; 1976; 1979).

The permeability of infected host cells increased toward infection area due to the greater osmotic pressure of pathogen hyphae and solutes. The fluid surrounding the hyphae could result in an osmotic flow of water from other regions. This fluid allowing the transport of the nutrients from distant area takes part in enzyme reactions (Lumsden,1979).

1.1.4 Biochemistry of disease development

Cell wall-degrading enzymes capable of destroying cellular components and production of oxalic acid are associated with disease development caused by *S. sclerotiorum*. Oxalic acid was first noticed by Maxwell & Lumsden, (1970) and were detected 1.1, 31.4 and 48.3 mg of oxalate per gram dry weight in 0, 2, and 4 day after inoculation of bean tissue with *S. sclerotiorum*, respectively. The most active enzymes are as follows.

Pectolytic enzymes are associated with a quantitative decrease in the pectic substance content and are localized in infected bean tissue in host cell middle lamellae (Lumsden, 1976). Several pectolytic enzymes are produced both in diseased bean tissue and in culture of *S. sclerotiorum*. Lumsden (1976) detected a viscosity–reducing PG as early as 12 hour after inoculation of bean plants. The activity reached a peak in 24 hour after inoculation. At about the time of irreversible establishment (up to 48 h) another peak of viscosity – reducing activity occurred.

1.1.4.2 Pectinmethilesterase (PME)

PME was found to occur early in pathogenesis in diseased bean tissue and to be associated with the advancing margins of lesions through disease development (Lumsden, 1976). Its role in the demethilation of bean host plant pectin was also demonstrated.

1.1.4.3 Cellulases and Hemicellulases

These enzymes often have been associated with *Sclerotinia spp.* and pathogenesis of white mold disease (Barakai–Golan, 1974; Lumsden, 1976). Their role in pathogenesis is the production of insoluble cellulose attributed to C_1 enzyme, and hydrolysis of cellobiose to glucose by β-glusidase. The optimum pH for C_x enzymes is 3.0, and if this series of enzymes is operational in the degradation of native cellulose, *S.*

to have the complete system and can utilize native cellulose as an energy source. Abundant cellulose is produced adaptively in tissues and the content of alpha cellulose declines substantially with the age of an infection and alteration of its structure.

1.1.4.4 Phosphatidase

This enzyme is capable of hydrolysing the phosphatide component of cell membranes. It is produced abundantly in culture and is detectable early in disease development in bean (Lumsden, 1970). The enzyme is inductive, extracellular, activated by calcium, and has activity optimum of pH 4.0.

1.1.4.5 Proteolytic enzyme

Proteolytic enzyme is potentially responsible for the degradation of host protoplasm and, possibly, cell wall constituents. It was detected in *S. sclerotiorum* and *S. minor*, in infected tissue of diseased celery, cucumber, carrot and bean extracts (Khare & Bompeix, 1976). The enzyme was detected two days after inoculation and increased to a maximum after 10 days. The optimum pH for protease activity is 3.0.

1.1.5 Sclerotium formation and composition

When ascospores or mycelium of *S. sclerotiorum* were placed on a solid nutrient medium (e.g., potatoes dextrose agar, PDA) hyphae grew out to form a thin film of growth over the surface. When the colony reaches the edge of the container (plate) or when growth is otherwise restricted, the

and produces white mold of mycelium covered with small liquid droplets. As sclerotium increases in size, the surface begins to darken until it becomes black, (sclerotia appear within ~ 1 week). Sclerotia often are formed at the edge of the plate but sometimes they cover the substrate in a series of concentric circles or some other regular pattern. These patterns appear to be due to endogenous rhythms in some isolates of the fungus (Tourneau, 1979; Humpherson *et al.*, 1977). Sclerotia are dark–colored hardened compact masses of fungal tissues which is formed in three stages; (1) initiation formation; (2) development and formation of full-sized sclerotia; and (3) maturation formation of a compact mass with dark–colored external pigmented cells. Under appropriate conditions, Sclerotia germinate in one or more of three ways: myceliogenic - the formation of hyphae; carpogenic – the formation of sexual fruiting bodies; and sporogenic – the production of sexual spores. Both carpogenic and myceliogenic ways occur in *S. sclerotiorum* (Tourneau, 1979; Chet & Henis, 1975).

The composition of sclerotia grown in culture is different from those collected from commercial bean fields. The histochemical data showed 2% crude fat, 3.5 - 5% ash, 20 - 25% protein, % carbohydrates, (mainly β-glycan, and monosaccharides such as glucose and fructose) are different

in culture contained less than collected from bean field (Le Tourneau, 1979).

1.1.6 Ecology

Sclerotinia spp. spend approximately 90% of their life cycle in soil as sclerotia (Adams & Ayers, 1979). Established sclerotia spread from field to field, and from one geographical area to another by several means. Wind blown ascospores can be the major means of transfer from field to field. Sclerotia or mycelium in infected host tissue can be transferred also by seedlings, farm equipment, animals and man (Adams & Ayers, 1979). The spreading of manure on fields has been shown to be a likely means of introducing the pathogen to uncontaminated fields.

Abawi and Grogan (1975) determined the inoculum density of *S. sclerotiorum* at depths of 0-2.5, 2.5-10, and 10-17.5 cm in a bean field in New York. The numbers of sclerotia at all depths were approximately 7, 2, and 0.5 sclerotia per kg soil before plowing and near zero after planting, respectively. The sclerotial inoculum density of *S. sclerotiorum* can be increased by two ways: (1) the production of secondary sclerotia in soil; (2) the production of sclerotia on host. The sclerotia of *S. sclerotiorum* are capable of forming secondary sclerotia in soil in the absence of a host (Williams *et al.*, 1965).

S. sclerotiorum near the soil surface do not remain viable for more than 1 year (Adams *et al.*, 1979). However, Adams *et al.*, (1979) reported that 3 – 5 year period in the case of bean white mold and 3 years rotation was not an effective control practice.

1.1.7 Factors affecting survival

Temperature and soil pH appeard to be of minimal importance in affecting survival. In Adams & Ayers study (1979) sclerotia introduced to small field plots with different soil textures and pH, survived well during the winter months. *In vivo* studies showed that normal soil temperatures (10-30°C) did not adversely affect survival. A constant soil temperature of 35°C for a week or more, however, reduced survival time of *Sclerotinia spp*. (Adams, 1975).

Moore (1949) reported that nearly 100% of the sclerotia were killed when soil was flooded with water for 26-31 days. This phenomenon was thought to be due to activities of various members of the soil microbiota.

The most significant soil factor affecting survival of sclerotia appears to be biological. More than 30 species of fungi and bacteria have been implicated by various workers as antagonists or mycoparasites of *Sclerotinia spp* (Papavizas *et al.*, 1990; Huang *et al.*, 1976; Turner *et al.*, 1975). Data on parasitic or antagonistic activity of most species of fungi against *S. sclerotiorum* under natural conditions are generally lacking.

fungi belonging to the genera *Acrostalagmus, Fusarium, Gliocladium, Hormodendrum, Mucor, Penicillium, Trichoderma*, and *Verticillium* were described as parasitic on sclerotia of *Sclerotinia triforliorum* based on *in vitro* tests (Papavizas *et al.*, 1990; Huang *et al.*, 1976; Turner *et al.*, 1975).

1.2 Plant growth regulators (PGRs) and plant disease

Plant growth regulators are a group of naturally occurring or synthetically produced, organic substances, which influence physiological processes at low concentration (Davies, 1995). The processes influenced include mainly growth, differentiation and development, though other processes, such as stomatal movement, may also be affected (Davies, 1995).

The role of PGRs in pathogenesies of plant disease is not clearly identified. There is increasing evidence that both the pathogen and the host have the capacity to synthesize various growth regulators, and alterations in their levels and/or sensitivity as a result of plant pathogen interaction are related to disease susceptibility or resistance reaction (Singh *et al.*, 1997). Research work on these subjects has provided new insights into our understanding of pathogenesis and manipulation of disease resistance (Singh *et al.*, 1997).

between pathogens and PGRs in more details will be described in the following items.

1.2.1 The role of auxin naphthalene acetic acid (NAA) in plant disease

NAA is a synthetic plant growth regulator. It was found to act similar to Indole Acetic Acid (IAA) and commonly replaces it in exogenous application. Because IAA is rapidly broken down to inactive products by light and microorganisms, NAA is applied exogenously in chemical thinning. It prevents premature fruit drop, and increases root development in micropropagation (Giafaga, 1995). Some investigations indicated that NAA is a potential antifungal agent (Nakamura *et al.*, 1978; Tomita *et al.*, 1984; Michniewicz *et al.*, 1988).

Auxin has been correlated with plant pathogenic bacteria *Agrobacterium tumefaciens* producing crown gall when infecting dicotyledonous plants. Crown gall formation is due to the enzymes tryptophan monooxigenase, and indoleacetamide hydrase. These two enzymes are responsible for the conversion of tryptophan to indoleacetamide and the hydrolysis of the indoleacetamide to auxin IAA (Klee *et al.*, 1991).

Other gall forming bacteria; *Pseudomonas savastanoi* and *Eriwinia herbicola* were reported to produce auxin in infected host plant tissue (Kosuge *et al.*, 1986; Manulis et. al., 1991)

xins in growth and development of fungi is not yet clearly elucidated. Working with *Neurospora crassa,* Japanese researchers have demonstrated that auxins may act as regulator of conidial germination (Nakamura *et al.*, 1978) and growth of mycelium (Tomita *et al.*, 1984). According to these workers, auxin may control fungal cell elongation and sporulation and that auxin may act as regulator of growth and differentiation in fungi-like organisms and higher plants.

Further studies on the role of auxin in the growth and development of *Fusarium culmorum* were conducted *in vitro*. The results indicated that auxin at 10^{-9} M and 10^{-7} M concentrations, introduced into the medium of pH 5, 7, 9 at 5 days incubation, slightly stimulated mycelial growth, and at 10^{-5} M concentration it strongly inhibited this process. Auxin at 10^{-9} and 10^{-7} M concentration increased spores production and accelerated their germination compared with control at pH 7, while auxin at 10^{-5} M concentration inhibited sporulation and spore germination at pH 5, 7 and 9 (Michniewicz *et al.*, 1987).

NAA, IAA, 2,4-Diphenol acetic acid (2,4,D) and Abscisic acid (ABA) were exogenously applied in North America to control early blight of potato (*Solanum tuberosum* L.), incited by *Alternaria solani* (Melinda *et al.*, 1991). The results indicated that NAA, 2,4-D produced accurate

of early blight when applied at 10.7 mM and 4.0 mM, respectively.

1.2.2 The role of gibberellic acid (GA_3) in plant disease

Giberellins (GA) were isolated from the fungus *Gibberella fujikuroi*, in which they occur in large quantities as secondary metabolites. Of the 89 known giberellins, only 64 have been identified in higher plants, 12 are present only in *Gibberella*, and 13 are present in both kinds of organisms (Sponsel, 1995).

GA_3, which is the end product of GA metabolism in *G. fujikuroi*, has been commercially available for many years. It has high biological activity, and is applied to dwarf or rosette plants, dormant buds, dormant and germinating seeds (Sponsel, 1995).

Shklyar (1965) claims that GA_3 has no significant effect on microorganisms. A similar opinion with reference to fungi has been expressed by Evans (1984). The basis for negating that GA_3 acts as a growth factor in fungi, has been particularly the results of experiments in which growth retardants were used in cultures of *Fusarium moniliforme* (Evans, 1984). A study of the effect of giberellic acid on the smut of wheat caused by *Claviceps purpurea* Tul. was conducted in saprophyte and parasite cultures. The results indicated that GA inhibited sporulation (Ostrovskky *et al.*, 1961). A study on the effect of auxin and gibberelin on

porulation in some yeast strains (Kaminska *et al.*, 1967). Sullia (1968) reported that spraying the leaves of *Cassis tora* and *Crotolaria medicogineae* with GA_3 caused an increase in the number of fungi in the rhizospheres of these plants. In a comprehensive study on the effect of GA_3 on *Neurospora crassa*, Nakamura *et al.*, (1978) reported that GA_3 stimulated spore germination and elongation of young hyphae (Tomita *et al.*, 1984). GA_3 at 10^{-9}, 10^{-7}, 10^{-5} M was found to stimulate slightly mycelium growth, and to stimulate significantly sporulation and spore germination at 10^{-7} and 10^{-5} M under optimal condition in *Fusarium culmorum in vitro* (Michhniewicz *et al.*, 1987; 1988). In addition, it was reported that GA was produced endogenously *in vitro* by *Fusarium culmorum* (Michhniewicz *et al.*, 1983; 1988).

1.2.3 The role of ethephon in plant disease

Ethephon is a synthetic plant growth regulator that became available in the early 1970. Ethephon (2-chloroethyl phosphonic acid) is stable at pH values of 4 or less, but at higher pH values the compound decomposes to produce ethylene, chloride and phosphate ions. Since the cytoplasmic pH >4 when ethephon is absorbed, ethylene is formed inside the cell (Giafaga, 1995). Ethephon, as ethylene releasing agent, is exogenously applied in many agricultural practices such as mechanical harvesting, fruit ripening, flower delaying period, and others.

is a gaseous plant growth regulator produced by almost all plants and plant organs as well as by a large number of microorganisms.

The role of ethylene in disease resistance is difficult to interpret, because ethylene may have a promotive, inhibitory, or no effect on disease development (Archers *et al.*, 1975).

Table 1.1 Relationship between ethylene and plant disease development*

Host	Pathogen
1. Promotion of disease development	
Lemon	*Diplodia* Pole Evans
Strawberry	*Botrytis cinerea* Pers. Ex. Fr.
Barley	*Helminthosporium sativum* Pammel
Various flowering	Unspecified fungi
2. Inhibition of disease development	
Mung bean	*Rhizictonia solani* Kukn
Tangerines	*Colletotrichum gloesporioides* Penz.
Tomato	*Verticillium albo-atrum* Reinke, Bertheir
Tobacco	Tobacco mosaic virus
3. No effect on disease development	
Tomato	*Fusarium oxysporum* Schlecht
Potato	*Phytophtora infestants* Mont., de Bady
Pepper	*Erwinia carotovora*
Celery	*Botrytis cinerea* Pers. ex. Fr.

* Biles *et al.* (1990)

inhibition of disease developments after ethephon treatment of various plants, has been reported (Biles *et al.*, 1990). Also ethephon was reported to increase the resistance of cucumber to *Erysiphe cichoracearum* DC. (Dehlne *et al.*,1982).

Contrastly, ethylene was found to promote the anthracnose of cucumber (*Cucumis sativus*) caused by *Colltotrichum lagenarium* (Biles *et al.*, 1990). In a study on the role of ethylene in the regulation of growth and development of the fungus *Botrytis cinerea* Pers.ex. Fr., ethylene was applied as ethephon (Ethrel) (Kepezynska, 1993). Ethephon was found to slightly stimulate (at $7x10^{-7}$, $7x10^{-6}$ and $7x10^{-5}$ M) or to inhibit (at $7x10^{-4}$M) hyphal growth of *B. cinerea in vitro* and on apples. However, Elad (1992) reported that ethephon increased the severity of grey mold on leaves of *Senecio sp.*

Studies on ethylene effects *in vitro* and *in vivo* on growth of certain post harvest fruit-infecting fungi indicated that ethylene up to 10^3 μg/ml concentration had no significant effect on percent spore germination of *Alternaria alternata* Fr. Keissler, *Colletotrichum gloesporioides* (Penz.) Arx., *Penecillium expansum* LK. Ex. and *Rhizopus stolonifer* (Fr.) Lind (El Kazzaz *et al.*, 1983). The higher concentration of ethylene in El Kazzaz work compared to air control increased the percent of spore germination of *P. digitatum* Sacc., *P. italicum* Wehmerand, and *Trielaviopsis paradoxa*

ene effect on mycelium growth rate of fungi growing on PDA was small and not significant (El Kazzaz *et al.*, 1983). To control the black root rot of soybean caused by *Cylindrocladium crotalaria*. Ethephon was exogenously applied at different concentration (2000 μg/ml, 4000 μg/ml) and was found to increase significantly root rot necrosis (Fortnum, 1983).

1.2.4 The role of methyl jasmonate (MeJA) in plant disease

MeJA is methylester of jasmonic acid (JA) which plays a potential role in plant growth, development and plant defense (Meyer *et al.*, 1984). Both JA and MeJA are widespread in plant kingdom and exhibit biological activity when exogenously applied to plant (Staswich, 1995). It is also produced by *Botryodiplodia* and *Giberella fujikroi* and red algae. The quantities of JA that have been estimated by various methods, ranged from about 10 ng to 3μg per gram fresh weight (Meyer *et al.*, 1984). Jasmonic acid concentration has been estimated in immature bean seeds (*Phaseolus vulgaris*) as 0.7 μg per gram fresh weight (Yamane *et al.*, 1981).

MeJA is usually more active than JA when applied exogenously (Vick *et al.*, 1987). This may result from the volatility of the ester, and the fact that it is lipophilic. MeJA is as well a fragrant oil, which contributes to the distinctive aroma of certain fruits and flowers (e.g., jasmine). These characteristics have led to its synthesis for the perfume industry.

mmercial product, which has been widely used by plant researchers, is not pure (Vick *et al.*, 1987).

Anderson (1989) suggested that volatile MeJA may be effective as a gaseous hormone in plants like ethylene. The concentration of atmospheric MeJA effective in this respect was estimated to be around 80nM, indicating that plants are quite sensitive to jasmonate. Farmer and Ryan (1990) concluded that MeJA, volatilized from one plant, signals a defensive response to another, when both are enclosed together in a small chamber.

Jasmonates, produced by some fungi, may be more directly involved as antifungal agent increasing plant defense (Paul *et al.*, 1995).

During jasmonate pathway, the oxidation products of lipoxygenase also affect fungal growth and some lipoxygenase inhibitors are antifungal agent (Jensen *et al.*, 1992). MeJA inhibited the growth of mycorrhizal fungi at lower concentration (by 10^{-7}) (Gogal, 1991).

Jasmonic acid and MeJA, applied to potato and tomato plants in the glasshouse, induced local protection against *Phytophthora infestans* at 62.5 μg/ml and systemic protection at 1 μg/ml (Cohn *et al.*, 1993). At 1 μg/ml the jasmonate did not inhibit fungal mycelial growth on agar. At high concentration > 1 μg/ml, the jasmonates are phytotoxic, causing slight chlorosis in potato and tomato plants in 2-3 day after spray application. Jasmonates at 100 μg/ml, applied to the adaxial surface of potato leaves 2h

th *Phytophthora infestans*, resulted in 92% and 100% disease control, respectively. Similar level of control was achieved by appling it up to 5 day before inoculation. After 8 days the level of protection decreased to 67 and 77% protection with 100 and 1000 μg/ml, respectively. The nature of the induced resistance of potato and tomato is not known. Also no sesquiterpene phytoalexine were detected in spray level of potatoes or tomatoes, nor was there evidence of enhanced level of chitinases or β,3-gluconases (Cohen *et al.*, 1993).

In another study, jasmonates applied in growth chamber as atopical spray (10 μg/ml) to barley plants gave protection from powdery mildew caused by *Erysiphe graminis* f. sp. *hordei* (Schweizer *et al.*, 1993). The level of protection was short-lived, and declined to 80% protection when plants were inoculated 3 days after treatment. At 1 μg/ml, jasmonic acid caused a weak chlorosis, but at 10 μg/ml it caused necrosis of the leaf tips. Schweizer *et al.* (1993) considered that the protection against barley mildew may have not been exerted through an induced resistance mechanism but through a more direct effect on the fungus. There is not enough published information on the effectiveness of jasmonates in controlling plant disease in the field.

Abscisic acid (ABA) in plant disease

ABA is a true phytohormone and a potential physiological inhibitor, endogenous and exogenous. It occurs in the plant in low concentrations. In most tissues its level range from 10 to 50 ng/g fresh weight ($4x10^{-8}$ M – $2x10^{-8}$M). Only in water-stressed leaves, developing seeds and dormant buds and seeds, its levels are higher than 10^{-6} M (Daniel *et al.*, 1996). The level of ABA increases and decreases dramatically in several kinds of tissues in response to environmental and developmental changes. When leaves of mesophylic plants are water stressed, ABA levels can rise to 50-fold within 4-8 hours, apparently, due to a greatly increased rate of biosynthesis. When plants are rewatered, the ABA levels drop to pre-stressed levels within 4-8 hours (Daniel *et al.*, 1996).

ABA was also detected in the *Cercospora rosicola* (rose pathogen) and was found to be produced in relatively large quantities in its growth medium (Asante *et al.*, 1977). Other investigators reported that ABA was produced by phytopathogenic fungi of the genera *Botrytis, Ceratocystis, Fusarium,* and *Rhizoctonia* (Dorffling *et al.*, 1984). These latter scientists indicated the need to study the role of ABA in pathogenesis.

Studying "Kernal" bunt, a serious disease of wheat caused by the fungus *Neovossia indica* (Mitra) Mundkhur, Singh *et al.*, (1997) revealed that this disease was difficult to control. ABA and D, L–alpha–difluoromethylornithine (DFMO) were used for controlling the disease.

that ABA was a potent inhibitor of growth, sporidiogenesis and teliospore germination of *N. indica* under culture condition. ABA at 100μM concentration and above completely suppressed the pathogen growth *in vitro* (Singh *et al.*, 1997)

1.3 Control of white mold caused by *S. sclerotiorum*

White mold has not been controlled consistently and economically. The pathogenecity of the causal fungus under different conditions and the ability of sclerotia to withstand adverse conditions, allow it to be a successful pathogen on many crops. Methods of control that have been employed with varying degrees of success include: protectant chemicals, materials that inhibit germination or destroy sclerotia, and cultural practices, such as crop rotation, sanitation, and reduced irrigation, sclerotium parasites, resistant cultivars and microclimate modifications (Hawthorne, 1974; Marcum *et al.*, 1977; Hunter *et al.*, 1978; Rodke *et al.*, 1986; Casale *et al.*, 1986; Krishna *et al.*, 1996; and Ferraz *et al.*, 1999)

1.3.1 Chemical control

1.3.1.1 Foliar protectants

As for the prevention of most diseases, chemicals controlling disease caused by *Sclerotinia spp.* must be applied before the infection occurs. Since many sclerotinia diseases are initiated by colonization of senescent plant organs, the fungicide must be applied to prevent

e organs. For crops such as lettuce, myceliogenic germination of sclerotia causes direct infection. Thus soil surface coverage (black plastic mulch) near the plant and timing of fungicide application are the most important factors in obtaining control (Marcum *et al.*, 1977). Benomyl (methyl 1-[butyl carbamoyl]–2-benzimidazole- carbamate), Pentachloro-nitrobenzene PCNB, and (2,6-dichloro-4-nitraniline) (DCNA) were partially effective for control when applied as a single spray immediately on lettuce plants (Hawthome, 1974). Aerial application has been the method of fungicide treatment and more effective than ground application as a root drench.

Hunter *et al.* (1978) found that if the bean whole plant or only bean blossoms were sprayed with benomyl, effective control resulted when plants subsequently were inoculated with a suspension of *S. sclerotiorum* ascospores.

1.3.1.2 Soil treatment

The fumigant methyl bromide was applied to *S. sclerotiorum*–infested fields reduced the disease by preventing carpogenic germination of sclerotia. Herbicides: trifluralin, atrazine, simazine, metribuzin, and pendimethalin were applied to soil-infested with *S. scrlerotiorum* in laboratory study (Rodke *et al.*, 1986). Herbicides stimulated carpogenic germination measured by the percentage of germinated sclerotia and by

apothecia that developed per sclerotium. Linuron and DNBP inhibited germination and apothecium development (Rodke *et al.*, 1986).

Herbicides; metribuzin and diuron (at 50 μg /ml) were found to inhibit the mycelium growth of *S. sclerotiorum* on agar, and to reduce the number of sclerotia produced (at 100μg/g) in soil (Casale *et al.*, 1986). Apothecia that developed in the latter work appeared normal and produced ascospores. Atrazin and simazine were less inhibitory to mycelial growth and had no effect on the number of sclerotia producing stipes, even at 10 μg /g soil. In the presence of atrazine or simazine, however, apothecia either did not develop or were abnormally shaped and produced no ascospores.

1.3.2 Culture modifications

Crop Rotation is a common disease control method that often has been advocated for control of *Sclerotinia* diseases. Sclerotia survive in a soil at least for 3 years, and tillage operations generally assure the presence of sclerotia.

Deep plowing has been recommended to control white mold of bean in the fields of low inoculum density, but plowing to depth of 25 cm did not affect disease severity in Nebraska (Steadman,1983). On the other hand, deep plowing in a field with a history of severe lettuce drop caused

in significant reductions of the mean numbers of sclerotia and lettuce drop incidence occurred immediately after deep plowing. However, disease incidence was significantly greater in the second crop season because of deep plowing distributed the sclerotia from highly aggregated pattern prior to plowing to less aggregated pattern. So, deep plowing is not exepted to function effectively in highly infested fields (Krishna *et al.*, 1996).

Reduction in the number of irrigations, especially those at the end of the season, can lower disease incidence in the absence of rainfall (Steadman, 1983).

In a study to determine the effect of soil moisture and levels of organic matter on the carpogenic germination of *S. sclerotiorum* and subsequent infection of *Phaseolus vulgaris*, it was shown that carpogenic germination increased by high soil organic matter content (Ferraz *et al.*, 1999). Control of soil moisture and grass mulching may reduce the production of apothecia of *S. sclerotiorum* even in soils rich in organic matter (Ferraz *et al.*, 1999).

1.3.3 Biological control

In the past few years, biocontrol research reported that many species of fungi and bacteria as well as insects and other organisms can be considered as parasites or antagonists of *Sclerotinia spp*. However, most

sed on laboratory observations or tests, and little information is available on their activity and effectiveness under natural or field situations.

Papavizas *et al.* (1990) found soil-amended with *Gliocladium virens* to be effective in reducing sclerotia germination of *S. rolfsii*. Huang and Hoes (1976) indicated that *Coniothrium minitans* could effectively control the population of *S. sclerotiorum* in sunflower field. Turner and Tribe (1975) also found that up to 65% of sclerotia of *S. trifoliorum* were destroyed in the soil by application of a pycnidial dust preparared from *C. minitans*.

1.3.4 Disease resistance

Crop and cultivar resistance to *S. sclerotiorum* was not investigated enough because the fungus has an extremely wide host range. Bean genetic resistance to the fungus was first noted by de Bary in 1887, who found that *Phaseolus multiflorus* (*P. coccineus*) was seldom attacked by the fungus whereas *Phaseolus vulgaris* (common bean) cultivars were destroyed by it. Adams *et al.* (1973) confirmed that *P. coccineus* (scarlet runner bean) was resistant. Abawi *et al.* (1978) also reported resistance in *P. coccineus* and *P. coccineus* X *P. vulgaris* hybrids. These workers noted that all *P. vulgaris* accessions were relatively susceptible.

the current research

The present work was amied at:

Investigating the role of plant growth regulators PGRs (naphtalene acetic acid, gibberrellic acid, ethephon, methyl jasmonate, abcissic acid, and aminoethoxyvinylglycine) in the interaction between *S. sclerotiorum* as a plant pathogen and their host plants, namely, bean (*Phaseolus vulgaris*) and cucumber (*Cucumis sativus*), in an attempt to explore their potential in controling white mold disease , and

Studying the potential of *S. sclerotiorum* to produce natural phytohormones such as ethylene which plays an important role in the mechanism of plant disease.

Chapter 2

Materials and Methods

The role of plant growth regulators in host-parasite interaction was evaluated in the system of *Sclerotinia sclerotiorum* against bean (*Phaseolus vulgaris*) and cucumber (*Cucumis sativus*) *in vitro*, *in vivo*, and on host whole plants.

2.1 Plant growth regulators (PGRs)

Six synthetic plant growth regulators (phytohormones) were used in this work (Table 2.1)

Table 2.1 Plant growth regulators used in this work.

Hormone	Active ingredient (%)	Commercial name	Manufacturer
Naphtalene Acetic Acid (NAA)	20	Alphatop	Fine Agrochemical, England
Gibberellic Acid (GA_3)	4	PRO-gibb	CTC, Israel
Ethephon (Et.)	48	Ethrel	AGAN, Chemical Manufactures, LTD., Israel
Methyl Jasmonate (MeJA)	2.5	Methyl Jasmonate	Asia Riezel, Israel
Abscisic Acid (ABA)	99	ABA, A-1049	Sigma, Germany
Aminoethoxyvinylglycine (AVG)	86	AVG	Sigma, Germany

Test isolates of *S. sclerotiorum* were either recovered from infected bean (*P. vulgaris*) and cucumber (*C. sativus*) plants, collected from various greenhouses and open agricultural fields located in different Palestinian agricultural areas, or obtained from The Israeli Agricultural Research Organization (ARO).

At least 25 isolates were cleaned by sub-culturing on potato dextrose agar (PDA) medium amended with 250 mg/L chloramphenicol, and kept lgophilized in skimmed milk in deep freeze at -30°C. Three isolates of *S. sclerotiorum* (SS.P10, SS.18 and SS.P 26) was used in all experiments.

Table 2.2 Isolates of *Sclerotinia sclerotiorum* used in this study

Isolate	Collection site	Plant	Year
SS.P 10	Jericho, Palestine	Bean	1998
SS.I 18	Agriculture Research Center, Israel	Bean	1998
SS.P 26	Jericho, Palestine	Cucumber	1998

2.3 Effect of plant growth regulators *in vitro*

PDA (Oxoid) medium was prepared as follows: 39 g/L of PDA and 250 mg/L chloramphenicol (Sigma C-0378) were suspended in deionized

y stirring on magnetic hot plate. 250 ml of medium was transferred into 300-ml flasks and autoclaved at 125°C for 15 minutes.

Growth regulators were incorporated in PDA medium (set on shaking water bath at 37-39°C) to give final concentrations of active ingredient (μg/ml) of NAA 0, 1, 10, 50, 100, 150, and 200;GA_3 0, 10, 50, 100, 150, and 200; ethephon 0, 10, 50, 100, 150, and 200; MeJA 0, 10, 50, 75, 100, and 150; ABA 0, 25, 50, 75, 100, and 150, and AVG 0, 10, 50, 100, 150, and 200.

250 ml PGR-amended culture medium were dispensed into eighteen, 90-mm diameter petri plates (~14ml per dish) and allowed to solidify. Five mm diameter mycelial plugs of six-day old cultures of *S. sclerotiorum* isolates (SS.P10, SS.I18, and SS.P26) were used to inoculate 6 replicate plates each concentration. The plates were incubated in the dark at 22°C. Colony diameter was measured at 44, 68, and 74 hours. Mycelium growth rate (cm^2/day) in the plates was calculated using the following equation:

$$\mathbf{R = \{[(D/2)^2 - (d/2)^2)] * \pi\}/T}$$

where R, mycelium growth rate; D, average diameter of colony (cm); d, disc dimeter (cm); π = 3.14, and T, time of incubation (day).

lant growth regulators on bean and cucumber detached leaves

2.4.1 The effect of PGRs on bean detached leaves

Two week-old bean seedlings were replanted in 15*30 cm (breadth *height) pots and incubated in a glasshouse. The planting medium comprised a mixture of peatmoss and perlite 2:1 by volume. Plants were irrigated and a 20:20:20 NPK fertilizer was added twice a weak. At flowering phase, 40 days after seeding, healthy young leaves were collected and placed on upper surface in the bottom of plastic boxes (40x25x15cm), on a plastic mesh platform. The plastic mesh was placed on sterilized wet towel paper to preserve enough humidity in the box. Each box contained six leaves each with three leaflets.

The experimental design used was completely randomized design (CRD), where each six leaflets were considered as replicates for each treatment in each box. The leaves were sprayed with the PGRs solution using a microsprayer. The concentrations of PGRs solution used were as follows:

NAA	0	200	300	400	500	600	μg/ml A.I.
GA3	0	50	100	150	200	300	μg/ml A.I.
Ethephon	0	50	150	250	300	400	μg/ml A.I.
MeJA	0	50	75	100	150		μg/ml A.I.

		100	150	200	250	μg/ml A.I.
AVG	0	10	50	100	200	μg/ml A.I.

After having absorbed the PGRs solution, the detached leaves were inoculated by placing a 5-mm diameter agar block, taken from 6-day-old culture of three isolates of *S. sclerotiorum* (SSP.10, SSP.26, and SSI.18) growing on PDA, on the lower surface of the leaflet. Six leaflets (replicates) were used for each isolate per box. The boxes were moistened, covered by transparent plastic film and incubated at 22°C with 12 hours photoperiod.

Evaluation of disease development on bean detached leaves was carried out by careful examination of rotting leaf area around the inoculum disk at 93 hours by measuring the lesion diameter. Disease growth rate was calculated using the equation mentioned earlier in section 2.3.

2.4.2 The effect of plant growth regulators on cucumber detached leaves

Three week-old cucumber seedlings (cv. dalellia) were replanted in 15*30cm (B*H) plastic pots. The planting mixture comprised of peatmoss and perlite (2:1 v/v). The plants were grown in glasshouse. Plants were irrigated and fertilized with commercial fertilizers (20:20:20 NPK + trace elements) twice a week. At flowering phase, about 30 days after seeding, healthy young leaves were collected and placed on upper surface in the bottom of plastic boxes (40x25x12cm), on a plastic mesh platform. The

on sterilized wet towel papers to preserve humidity in the box. Six leaves were used in each box.

The CRD was used in this experiment, where six replicate leaves were used for each isolate in the same concentration of PGRs. The leaves were sprayed with the PGRs solution using a microsprayer. PGRs concentrations used were as follows:

NAA	0	200	300	400	500	600	µg/ml A.I.
GA3	0	50	100	150	200	300	µg/ml A.I.
Ethephon	0	50	150	250	300	400	µg/ml A.I.
MeJA	0	50	75	100	150		µg/ml A.I.
ABA	0	50	100	150	200	250	µg/ml A.I.
AVG	0	10	50	100	200		µg/ml A.I.

After having absorbed the PGRs solution, detached leaves were inoculated using 5-mm disk of inoculum from a 6-day old culture of three isolates of *S. sclerotiorum* (SSP.10, SSP.26, and SSI.18) growing on PDA. and placed (mycelium-side down), on the lower leaf surface. Six replicate were used for each isolate in the same box. The boxes were moistened, covered by transparent plastic film and incubated at 22°C and 12 hours photoperiod.

Evaluation of the disease development on cucumber detached leaves was carried out by careful examination of rotting leaf area around the

ours. Disease development rate was calculated using the equation mentioned earlier in section 2.3.

2.5 The effect of plant growth regulators on white mold disease severity on bean and cucumber plants

2.5.1 The effect of PGRs on white mold disease severity on bean whole plant

Two-week-old bean seedlings were replanted in 15*30 cm (B*H) pots in a mixture of peatmoss and perlite (2:1 v/v). Plants were irrigated and fertilized with commercial fertilizer (20:20:20 NPK plus trace elements) twice a week. Twelve, 40-day old plants, for each concentration, were sprayed until saturation by the following PGRs: NAA: 0, 200, 400, and 600; GA_3: 0, 50, 150, and 250; Et.: 0, 200, 400, and 600; MeJA.: 0, 75, 150, and 250; AVG: 0, 100, 200, and 300, and ABA: 0, 100, 200, and 300μg/ml active ingredient. After two hours, when the PGRs solution had been adsorbed, each 4 plants were presprayed with each concentration of PGRs were inoculated with homogenized mycelium suspended in deionized sterile water containing (g/L) 2 glucose, and 1 potassium dihydrogen phosphate (KH_2PO_4) of one of three isolates. Each plant was sprayed with 20 ml of mycelium suspension of *S. sclerotiorum* isolates (SS.P10, SS.I 18, and SS.P26). Plants were covered with transparent plastic

idity, and incubated in a growth chamber at 22°C, with photoperiod of 12 hours.

The development of white mold disease was evaluated by estimation of disease severity (%) at 11 days. Disease severity was rated on the basis of a 0-100% scale, where 0= no visible symptoms; 1-10% = small, circular and irregular spots on lower leaves; 11-20% = leaf symptoms enlarged as lesions on lower leaves; 21-30% = small, circular and irregular spots begin to appear on upper leaves; 31-40% = leaf symptoms enlarged as lesions on upper leaves; 41-50% = rot symptoms appeared on leaves, petiols and stem ; 51-60% = wilt brownish lesions clearly appeared on leaves , petiols and stem; 61-70%= white mold mass begin to appear on 50-60% of whole plant; 71-80% = wilt leaves defoliated; and 81-100% = plants completely wilted and died.

Experiment design is complete randomize, which consider each 4 plants were inoculated with mycelium suspension of one of three isolates as replicates for each treatment.

2.5.2 The effect of PGRs on white mold disease severity on cucumber whole plant

Seventeen-day-old cucumber seedlings were replanted in 15*30 cm (B*H) pots in a mixture of peatmoss and perlite (2:1 v/v). Plants were irrigated and fertilized with commercial fertilizer (20:20:20, NPK plus trace elements) twice a weak. Each twelve, 30-day old plants, were sprayed until

ving PGRs: NAA: 0, 200, 400, and 600; GA_3: 0, 50, 150, and 250; Et.: 0, 200, 400, and 600; MeJA.: 0, 75, 150, and 250; AVG: 0, 100, 200, and 300, and ABA: 0, 100, 200, and 300μg/ml active ingredient. After two hours, when the PGRs solution had been adsorbed, each 4 plants were presprayed with each concentration of PGRs were inoculated with the homogenized mycelium suspension (of one of three isolates) in deionized sterile water containing (g/L) 2 glucose and 1 potassium dihydrogen phosphate (KH_2PO_4). Each plant was sprayed with 20ml of mycelium suspension of *S. sclerotiorum* isolates (SS.P10, SS.I 18, and SS.P26). Plants were covered with transparent plastic sacks to preserve humidity, and incubated in a growth chamber at 22°C, with photoperiod of 12 hours.

The development of white mold disease was evaluated by estimation of whole plant disease severity at 11 days as mentioned in section 2.5.1.

Experiment design is complete randomize, which consider each 4 plants were inoculated with mycelium suspension of one of three isolates as replicates for each treatment.

2.6 Ethylene production by *S. sclerotiorum*

To determinate *in vitro* ethylene production by *S.sclerotiorum*, the isolate SS.I18 was first grown on PDA at 22°C. After 6 days, the surface mycelium was removed by scraping with sterile scalpel from three plates

tube with 5 ml of sterile deionized water and the suspension was asptically homogenized (Homogenizer Polytron PT-3100). From mycelium suspension 0.5 ml, was used to inoculate the autoclaved, aluminum foil covered neck flasks containing 10 ml of potato dextrose broth (PDB) amended with 10 mM/l methionine (Sigma M-6039). Eighteen flasks were inoculated and three flasks were used as control. Flasks were setted on a rotovator shaker at 200 rpm and incubated at 22°C. After 2, 4, 6, 8, 10, and 12 day periods, three flasks were used to estimate ethylene production as follows.

Flask covers were removed and flasks were sealed with a rubber (Suba) seal and set on rotovator shaker at 200 rpm for two hour. Then a 5-ml sample of gas was removed from the flask by disposable plastic syringe and injected into a gas chromatograph (Varian 3400) fitted with a pre-column for ethylene and calibrated by standard gas (1*ppm*).

Dry weight of mycelium was measured after filtration and exposed to two-hour drying at 80°C on filter paper. Flask volumes were measured by graduate cylinder (= 47ml). Ethylene production after 2, 4, 6, 8, 10, and 12 days was calculated using the following equation:

Ethylene production (μl/g/hr) = (R*V)/(W*D)

Where, R, gas-chromotography reading (*ppm*); V, volume of air in the flask (ml); W, mycelium dry weight (gr); and D, duration (hours)

experiment, 11 isolates of *S. sclerotiorum* (SS.P13, SS.I24, SS.P26, SS.I18, SS.P10, SS.I21, SS.P22, SS.I16, SS.I17, SS.P11 and SS.P7) were employed and the above outlined procedure repeated.

11 isolates of *S. sclerotiorum* were studied in the experiment in three replicates and the experiment was repeated twice.

2.7 Statistical analysis

The results of the effect of PGRs on (mycelium growth rate of *S.sclerotiorum*; white mold lesions growth on bean and cucumber detached leaves after incubation 93 hours , and on white mold disease severity (%) on bean and cucmber plants at 11 day after infestation were analyzed statistically using one-way analysis of variance (ANOVA) to test for significance, and the Tukey test was used for means separations by Sigma Stat. Software Program (1999)

Data of ethylene production by 11 isolates of *S. sclerotiorum* were analyzed statistically using one-way repeated measurment analysis of variance (ANOVA) to test for significance, and Tukey test was used for means separations (Sigma Stat. Program).

Chapter 3

Results

3.1 Effect of PGRs on mycelial growth rate of *Sclerotinia sclerotiorum* in vitro

Data on the effect of PGRs on mycelium growth rate (cm^2/day) of three isolates of *S. sclerotiorum* (SS.P10, SS.I18 and SS.P26) grown on PDA-amended with different concentration of PGRs, are presented in Table 3.1 and Fig 3.1.

Napthalene acetic acid (NAA) significantly ($P<0.05$) decreased the mycelium growth rate of three isolates of *S. sclerotiorum* (SS.P10, SS.I18, and SS.P26) growing on PDA at 1, 10, 50, 100, 150, and 200 μg/ml concentrations compared with the control. At 200 μg/ml, the mean of mycelium growth rate of three isolate was reduced to 0.4 cm^2/day (=2.9% of mycelium growth rate of control). The inhibitory effect of NAA increased significantly with increasing PGR concentration (r^2 =0.72) (Fig.3.1 and Table 3.1).

Gibberellic acid (GA_3) and ethephon (Et.) did not significantly ($P>0.05$) affect mycelium growth rates of *S. sclerotiorum* isolates at the concentrations used (<100μg/ml of GA_3, <150μg/ml of Et.) (Fig.3.1, Table 3.1) though there was a declining trend. The mean mycelium growth

ificantly at 200 μg/ml of GA_3 (51%), $r^2 = 0.91$ and Et. (72%), $r^2 = 0.82$, compared to the control.

Methyl jasmonate (MeJA) significantly ($P<0.05$) reduced mycelium growth rate of the three isolates of *S. sclerotiorum* at 10, 50, 75, 100, and 150 μg/ ml. Mycelium growth was highly inhibited at 150 μg/ml (0.34 cm^2/day; 2%) compared with the control. A negative correlation ($Y = -0.0899\ X + 10.922$; Y- mycelium growth rate (cm^2/day), X- concentration (μg/ml), $r^2=0.79$) between concentration of MeJA and mycelium growth rate was observed (Fig. 3.1 and Table 3.1).

Abscisic acid (ABA) and aminoethoxyvinylglycine (AVG) significantly ($P<0.05$) reduced mycelium growth rate of three isolates of *S. sclerotiorum* growing on PDA-amended at 25, 50, 75, 100, and 150 μg/ml of ABA, and at 10, 50, 100, 150 and 200 μg/ml of AVG. The effect of ABA and AVG on mycelium growth rate indepented on their concentrations, so the effect of 50, 75, 100, and 150 μg/ml of ABA and the effect of 100, 150, and 200μg/ml of AVG on mycelium growth was similar (Fig 3.1 and Table 3.1).

growth rate (cm^2/day) of three isolates of *S. sclerotiorum* grown on PDA-amended with different concentration of PGRs incubated at 22°C.

PGRs (μg /ml)	Isolate SS.P10	Isolate SS.I18	Isolate SS.P26
NAA 0	13.62*±1.5 a**	13.54±1.2 a	12.39±1.1a
NAA 1	8.36 ± 0.9 b	7.85± 0.7 b	10.16± 0.6 b
NAA 10	5.93 ±1.4 c	6.6± 1.0cg	7.65± 0.5cg
NAA 50	2.58± 0.9d	3.12± 0.2d	3.96±1.3 c
NAA 100	0.75± 0.2ef	1.00± 0.18 ef	1.06± 0.16 ef
NAA 150	0.59± 0.2ef	0.37± 0.11 ef	0.37± 0.2ef
NAA 200	0.40±0.08 ef	0.33± 0.12 ef	0.08 ± 0.17ef
GA_3 0	13.62±1.5 a	13.55± 1.2a	12.39±1.06 a
GA_3 10	11.76± 2.2 a	10.03± 1.7a	12.03 ± 0.75 a
GA_3 50	11.62± 1.6a	11.13± 1.6a	11.27± 0.83.a
GA_3 100	9.32 ±1.3 b	7.99 ±1.23 b	8.75 ± 0.41 b
GA_3 150	8.61± 0.62 b	8.62± 1.9 b	8.00 ± 0.4 b
GA_3 200	7.55± 2.5 b	6.9± 1.25 b	6.91± 0.4 b
Et. 0	15.52± 1.1a	15.78± 0.8 a	11.16± 0.7 b
Et. 10	14.86± 0.8 a	14.82±1.6 a	11.23± 1.3b
Et. 50	12.01± 0.7 a	10.66± 2.3 b	11.73± 0.8 b
Et. 100	12.66± 2.8 a	11.96 ± 3.2 b	11.62±1.2 b
Et. 150	11.56 ±1.1 b	10.16±1.7 b	11.12±1.1 b
Et.200	11.04 ±0.5 b	9.53±2.2 b	10.4 ± 1.1b
MeJA 0	15.52 ± 1.1a	15.78± 0.8a	11.06 ± 0.7 b
MeJA 10	10.69±1.3 b	11.38± 0.9 b	9.19±1.3 b
MeJA 50	3.80± 1.3 ch	3.1± 0.5ch	3.08± 0.6ch
MeJA 75	1.67± 0.5 dh	2.01± 0.3 dh	1.82± 0.3 dh
MeJA 100	0.63± 0.2 de	0.91± 0.2 de	1.04± 0.1 de
MeJA 150	0.3 ± 0.07 de	0.34 ±0.06de	0.38 ±0.05 de
ABA 0	13.55± 0.5 b	17.78 ± 2.4 a	14.27±1.9 b
ABA 25	6.56±1.5 c	9.56±1.2 c	9.81± 0.8 c
ABA 50	6.68 ±1.5 d	6.86± 0.9 d	6.88± 1.2 d
ABA 75	4.62± 0.8 e	5.45±1.2 e	5.91±1.0 e
ABA 100	4.74 ± 1.1 e	5.13±1.1 e	5.32± 0.9 e
ABA 150	4.31± 0.5 e	4.97±1.0 e	4.57± 0.9 e
AVG 0	12.05± 1.3 a	13.80± 0.7 a	11.41± 0.3 a
AVG 10	10.59± 0.6 b	8.05±1.35 b	8.80±1.0 b
AVG 50	7.08± 0.7 c	8.72± 0.8 b	10.84± 1.2 a
AVG 100	5.42± 0.8 d	5.27± 0.9 d	5.89± 0.7 d
AVG 150	3.98± 0.3 de	2.71± 0.4 de	3.98± 0.4 de
AVG 200	3.68± 0.5 de	2.81± 0.3 de	2.28± 0.4 de

** Means followed by the same letter within a column or row for each PGR treatment are not statistically significant according to Tukey multiple comparison test (P<0.05).
*Mean of six replicates ± standard deviation.
Plant growth regulators (PGRs); Naphthlene acetic acid (NAA); Gibberellic acid (GA3); Ethephon (Et); Methyl jasmonate (MeJA); Abscisic acid (ABA); and Aminoethoxy vinylglycine (AVG);

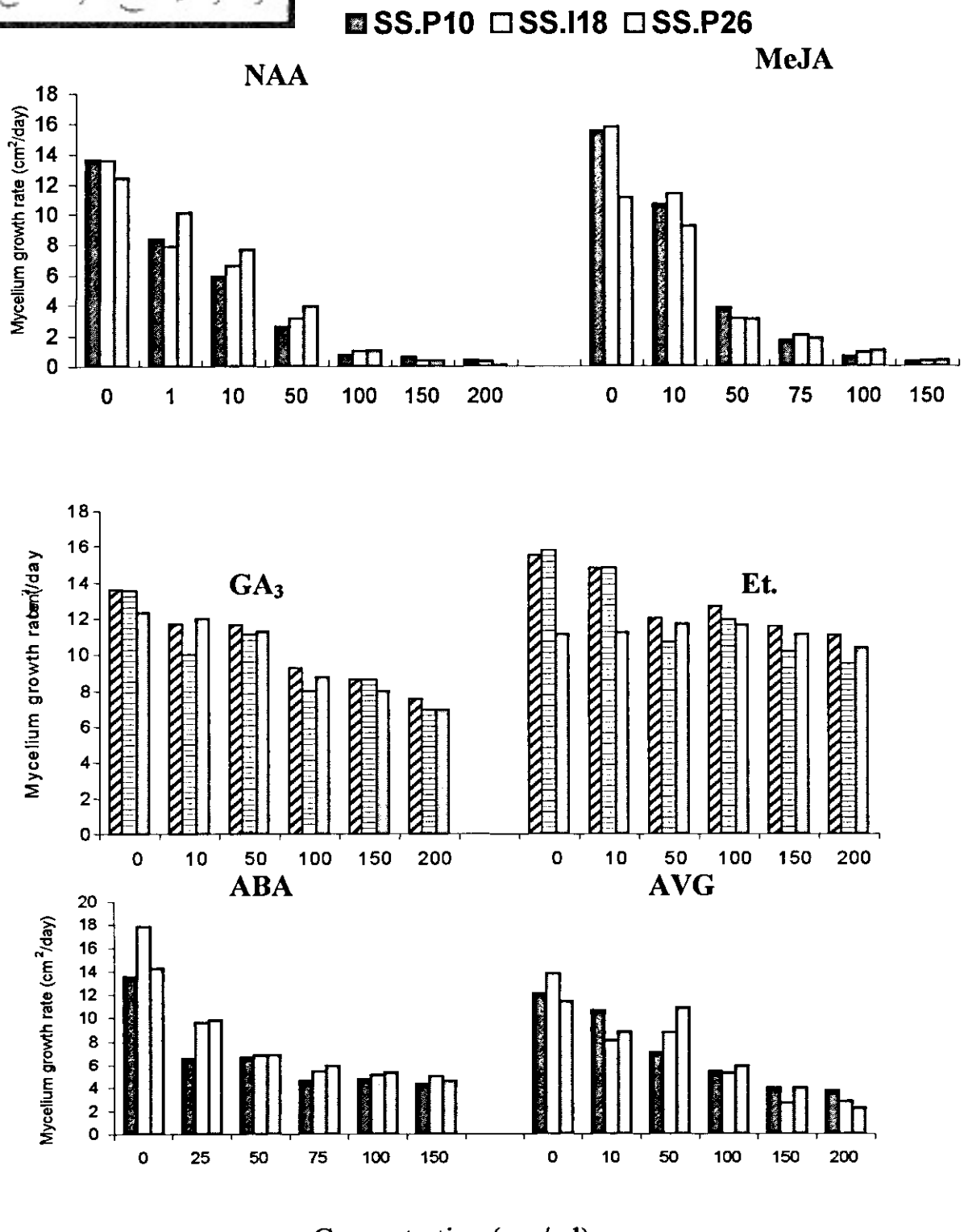

Figure 3.1 Effect of naphthalene acetic acid (NAA); methyl jasmonate (MeJA); gibberellic acid (GA_3); ethephon (Et.); abscisic acid (ABA); and aminoethoxyvinylglycine (AVG) on mycelium growth rate (cm^2/day) of three isolates of *S. sclerotiorum* grown on PDA-amended with different concentration of PGRs incubated at 22°C.

3.2 The effect of PGRs on white mold disease development on leaves

The development of white mold disease on cucumber and bean detached leaves pre-treated with different concentration of PGRs was evaluated by measuring disease lesions growth rate (cm^2/day) 93 hours after inoculation. Lesion area is dark and soft rot (Table 3.2, and Table 3.3)

NAA significantly ($P<0.05$) reduced development of white mold lesions on bean and cucumber detached leaves pretreated with 200, 300, 400, 500, and 600 μg/ml of NAA (Fig 3.2, Table 3.2, and Table 3.3). No significant differences in lesion areas developed on bean detached leaves caused by the three isolates at concentrations of 200, 300 and 400μg/ml, were detected. The increase in concentration did not affect significantly the lesion area, either. At concentrations of 500 and 600 μg/ml significant variations were observed between the isolates. White mold lesions caused by isolate SSP.26 was significantly larger than those caused by isolates SSP.10 and SSI.18. In addition, chlorosis was observed on bean leaves treated with 600 μg/ml of NAA (Fig 3.2, Table 3.2, and Table 3.3).

GA_3 did not significantly ($P>0.05$) affect white mold lesions development on bean detached leaves pretreated with <150 μg/ml GA_3; or

leaves pretreated with <300 μg/ml GA_3. Lesion development on bean detached leaves, on the other hand, increased at concentrations of 200, and 250μg/ml.

Ethephon slightly increased lesion development on bean detached leaves at lower concentrations (<150μg/ml). Higher concentrations (>250 μg/ml), considerably increased lesion development with increasing concentrations (Fig.3.2). Lesion development on cucumber detached leaves pretreated with 50, 150, and 250 μg/ml Et. was not affected by the concentration, while it was significantly increased at 300 and 400 μg/ml (Fig. 3.2, Table 3.2, and Table 3.3).

MeJA significantly (P<0.05) decreased white mold lesions development on bean and cucumber detached leaves pretreated with 50, 75, 100 and 150 μg/ml (Fig.3.3, Table 3.2 & 3.3). Isolates effect differed significantly at 50 and 100μg/ml of MeJA on bean detached leaves, and at 50 and 75μg/ml on cucumber leaves. A negative correlation was observed between the MeJA concentration and the rate of disease lesion development. At 150 μg/ml the mean lesion growth rate was 14% ($Y = -0.0209\ X + 3.0381$; Y- lesion growth rate (cm^2/day), X- concentration (μg/ml) , r^2= 0.84) and 9% ($Y = -0.0212\ X + 3.1088$; Y- lesion growth rate (cm^2/day), X- concentration (μg/ml) , r^2= 0.94) on bean and cucumber

ctively, compared to mean growth rate of control (Fig 3.3, Table 3.2, and Table 3.3).

AVG significantly reduced white mold disease development on both bean and cucumber detached leaves pretreated with 10, 50, 100, and 200 μg/ml (Fig. 3.3, Table 3.2, and Table 3.3). The mean disease lesion growth rate was 2 % (r^2=0.45) and 4.6 % (r^2 = 0.98) on bean and cucumber pretreated detached leaves with 200 μg/ml of AVG, respectively.

Abscisic acid did not significantly (P>0.05) affect white mold lesions development on cucumber leaves pretreated with 50, 100, 150, 200 and 250 μg/ml. While it slightly reduced lesions development on bean leaves at 50, 100, 150, and 200 μg/ml, and slightly stimulated lesions development on bean at 250 μg/ml.

l lesions growth rate (cm^2/day) on bean detached leaves treated with different concentrations of PGRs after 4-days incubation at 22° C.

PGRs (μg/ml)	Isolate SS.P10	Isolate SS.I18	Isolate SS.P26
NAA 0	6.2*±1.0 a**	5.9±1.6 a	5.7±3.0 a
NAA 200	1.6± 0.6 bd	1.3± 0.3 bd	1.6± 0.3 bd
NAA 300	1.1± 0.5 bd	1.1± 0.2 bd	1.0± 0.4 bd
NAA 400	1.0± 0.3 bd	0.8± 0.2 bde	1.0 ± 0.5 bd
NAA 500	0.1± 0.1 ce	0.5± 0.2 ce	1.0± 0.3 cd
NAA 600	0.1± 0.1 ce	0.4± 0.1ce	0.7± 0.2 ce
GA_3 0	5.1±1.5 cf	4.6±1.0 cf	4.9±1.3 cf
GA_3 50	6.1±1.7 c	6.3± 0.8 c	7.4± 1.7 ce
GA_3 100	7.1±2.3 ce	7.1± 2.6 ce	8.2±1.2 cd
GA_3 150	7.6±1.6 ce	7.3± 0.6 ce	7.9 ± 1.5 cd
GA_3 200	8.6± 0.5 bd	8.9± 1.0 b	9.0± 0.8 b
GA_3 300	10.3± 2.2 a	11.6± 1.8 a	10.4±1.6 a
Et. 0	2.0± 0.8 bc	3.9± 1.6 bc	3.3± 0.7 bc
Et. 50	3.2± 0.9 bc	4.2 ± 1.3 bc	3.8± 0.9 bc
Et. 150	3.8± 0.9 bc	4.3 ± 0.6 bc	3.5 ± 1.0 bc
Et. 250	5.2± 1.7 b	5.6 ± 1.4 b	5.8 ± 1.3 b
Et. 300	6.8 ±1.0 a	7.2± 1.6 a	7.5± 0.8 a
Et.400	8.4 ± 1.7 a	8.1± 2.3 a	9.1± 1.0 a
MeJA 0	5.4 ± 1.3 a	5.1± 1.6 a	4.3± 0.6 a
MeJA 50	3.9± 2.0 b	2.8± 0.9 b	3.3± 0.7 b
MeJA 75	1.5± 0.7 cd	1.2± 0.7 cd	1.3 ± 0.6 cd
MeJA 100	0.9± 0.5 cde	0.4± 0.2 ce	0.7± 0.2 ce
MeJA 150	0.7± 0.3 ce	0.7± 0.2 ce	0.6± 0.2 ce
AVG 0	5.4± 1.3 a	5.1± 1.6 a	4.3± 0.6 a
AVG 10	1.6± 0.9 b	1.2± 0.4 bd	1.8± 0.4 b
AVG 50	0.9± 0.2 bd	0.9± 0.2 bd	0.7± 0.3 bd
AVG 100	0.4± 0.1 ce	0.4 ± 0.2 ce	0.38± 0.1 ce
AVG 200	0.1± 0.1 ce	0.1± 0.1 ce	0.1± 0.08 ce
ABA 0	6.7 ± 0.9 a	7.1 ± 0.6 a	6.6 ± 0.8 a
ABA 50	5.1 ± 0.9 ce	5.1 ± 0.7 ce	4.9 ± 1.5 ce
ABA 100	5.5 ± 0.6 be	5.6 ± 1.5 be	5.3 ± 1.1 be
ABA 150	5.4 ± 0.5 be	5.4 ± 0.5 be	5.1 ± 1.9 be
ABA 200	6.1 ± 1.0 ad	6.6 ± 1.1 ad	7.1 ± 1.5 ad
ABA 250	8.1 ± 1.1 ad	7.1 ± 0.8 ad	7.6 ± 1.8 ad

*Mean lesions growth rate (cm^2/day) of six replicates± SD

** Means followed by the same letter within a column or row for each PGR treatment are not statistically significant according to Tukey multiple comparison test (P<0.05).

Plant growth regulators (PGRs); Naphthlene acetic acid (NAA); Gibberellic acid (GA3); Ethephon (Et); Methyl jasmonate (MeJA); Abscisic acid (ABA); and Aminoethoxy vinylglycine (AVG);

Table 3.3 White mold lesions growth rate (cm^2/day) on cucumber detached leaves treated with different concentration of PGRs after 4-days incubation at 22 °C.

PGRs (μg/ml)	Isolate SS.P10	Isolate SS.I18	Isolate SS.P26
NAA 0	2.9*± 0.5 a**	3.3± 0.3 a	3.7± 0.3 a
NAA 200	1.8± 0.2 bc	1.6± 0.4 bc	1.6± 0.2 bc
NAA 300	1.3± 0.4 bc	1.1± 0.2 bc	1.1± 0.5 bc
NAA 400	0.9± 0.2 bcd	0.8± 0.2 bcd	0.7± 0.07 bcd
NAA 500	0.3± 0.2 bcd	0.3± 0.1 bcd	0.4± 0.2 bcd
GA_3 0	5.1± 0.4 a	4.6 ± 0.3 a	4.9± 0.3 a
GA_3 50	6.3± 0.2 a	6.3± 0.5 a	7.3± 0.3 a
GA_3 100	7.1± 0.3 a	7.1± 0.6 a	8.2 ± 0.4 a
GA_3 150	6.9± 0.5 a	6.0 ± 0.2 a	6.0 ± 0.2 a
GA_3 200	6.5± 0.5 a	7.4± 0.2 a	5.6± 0.2 a
GA_3 300	6.4± 0.2 a	6.2± 0.2 a	6.3± 0.2 a
Et. 0	2.9± 0.5 b	3.3± 0.3 b	3.7± 0.3 ab
Et. 50	2.6± 0.5 b	2.1± 0.1 b	2.1± 0.3 b
Et. 150	3.2± 0.3 b	3.3± 0.4 b	3.3± 0.2 b
Et. 250	3.4± 0.6 b	3.7± 0.4 ab	3.6± 0.4 ab
Et. 300	4.5± 1.0 a	4.3± 0.7 a	4.0± 0.4 a
Et.400	5.2± 0.7 a	4.8± 0.7 a	4.7± 0.5 a
MeJA 0	5.4± 1.3 a	5.1± 1.6 a	4.3± 0.6 a
MeJA 50	3.6± 1.2 b	2.7± 1.2 be	3.3± 0.7 b
MeJA 75	2.1± 0.6 ce	1.6± 0.7 c	1.6± 0.6 c
MeJA 100	1.0± 0.5 cf	0.9± 0.3 cf	1.0± 0.3 cf
MeJA 150	0.4± 0.3 df	0.4± 0.2 df	0.4± 0.2 df
AVG 0	5.4± 1.3 a	5.1± 1.6 a	4.3± 0.6 be
AVG 10	4.0± 1.2 be	4.3± 0.9 be	4.8± 0.7 be
AVG 50	3.6± 0.6 be	3.6± 0.7 be	4.2± 0.6 be
AVG 100	1.9± 0.5 cf	2.1± 0.3 cf	2.4± 0.3 cf
AVG 200	0.2± 0.3 df	0.3± 0.2 df	0.2± 0.2 df
ABA 0	4.6±1.2 a	3.9±0.9 a	4.1±1.4 a
ABA 50	3.5±0.3 a	3.2±0.5 a	3.9±0.8 a
ABA 100	3.7±0.3 a	3.3±1.3 a	3.9±1.1 a
ABA 150	3.2±0.3 a	3.5±0.5 a	3.6±0.2 a
ABA 200	3.2±0.9 a	4.0±1.1 a	3.4±0.5 a
ABA 250	4.9±1.6 a	3.8±0.3 a	3.8±0.8 a

*Mean lesions growth rate (cm^2/day) of six replicates± SD

** Means followed by the same letter within a column or row for each PGR treatment are not statistically significant according to Tukey multiple comparison test (P<0.05).

Plant growth regulators (PGRs); Naphthlene acetic acid (NAA); Gibberellic acid (GA3); Ethephon (Et); Methyl jasmonate (MeJA); Abscisic acid (ABA); and Aminoethoxy vinylglycine (AVG);

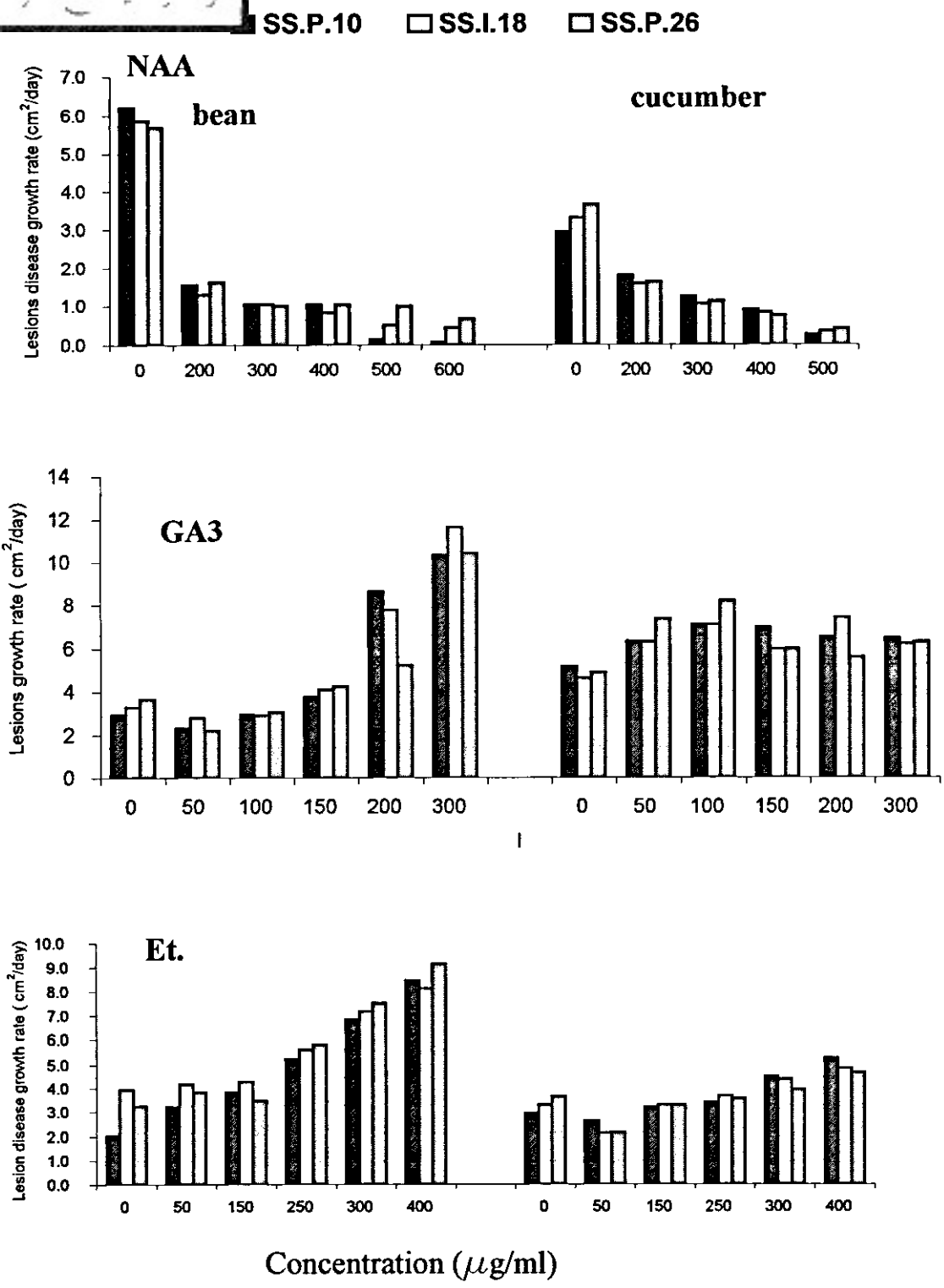

Figure 3.2 Effect of naphthalene acetic acid (NAA); gibberellic acid (GA_3); and ethephon (Et.); on white mold lesion growth rate (cm^2 / day) caused by three isolates of *S. sclerotiorum* on bean and cucumber detached leaves after 4-day incubation period at 22°C.

PGRs on white mold severity on bean and cucumber plants

Development of white mold disease on bean and cucumber plants pretreated with different concentrations of PGRs was estimated visually as whole plant disease severity (%) after 11 days of incubation (Table 3.4, Table 3.5, Fig. 3.4, and Fig. 3.5)

Table 3.4 White mold disease severity (%) caused by three isolates of *S.sclortiorum* on bean whole plant, pretreated with different concentrations of plant growth regulators after 11 days incubation at 22°C

PGRs(μg/ml)	Isolate SS.P10	Isolate SS.I18	Isolate SS.P26
NAA O	72.5*±5.6 a**	60 ±3.5 a	60 ±7.9 a
NAA 200	36.7 ±2.3 bc	46.2 ±6.2 b	43.7 ±4.7 b
NAA 400	37.5 ±2.9 bc	33.7 ±9.4 bc	32.5 ±6.9 bc
NAA 600	63.8 ±4.8 a	60 ±4 a	62.5 ±2.9 a
GA_3 0	46.2 ±6.3 c	62.5 ±5 bd	46.2 ±2.5 c
GA_3 50	63 ±2.4 bd	61.8 ±7 bd	61.2 ±4.8 bd
GA_3 150	68.2 ±3.5 a	77.5 ±6.4 a	70 ±4.1 a
GA_3 250	72±2.9 a	82.5 ±2.9 a	73.7 ±2.5 a
Et. 0	33.7 ±2.5 d	36.2 ±4.8 dg	30 ±4.8 d
Et. 200	43.7 ± 2.5 cg	48.7 ±2.5 cf	48.7 ±2.5 cf
Et. 400	55.2 ±4.1 bf	62.5 ±2.9 be	63.7 ±2.5 be
Et. 600	66.3 ±2.5 ae	75 ±4.1 a	68.8 ±2.5 a
MeJA 0	33.8 ±2.5 a	36.3 ±4.8 a	30 ± 4.1 a
MeJA 75	25 ±4.1 bd	27.5 ±2.9 bd	28.8 ±2.5 ad
MeJA 150	18.8 ±4.8 ce	25 ±4.1 cd	22.5±2.5 cd
MeJA 250	13.8 ±2.5 c	17.5 ±2.9 ce	17.5 ±2.9 ce
AVG 0	72.5 ±9.6 a	75 ±10.8 a	76.3 ±4.8 a
AVG 100	43.8 ±4.8 b	18.8 ±2.5 cf	42.5 ±2.9 b
AVG 200	36.3 ±2.5 de	22.5 ±2.9 df	31.3 ±4.8 de
AVG 300	32.5 ±2.9 de	27.5 ±2.9 df	22.5 ±2.9 df
ABA 0	31.3±2.5 cd	16.3±2.5 c	31.3±6.3 cd
ABA 100	35.0±4.1 cd	33.8±4.8 cd	35.0±4.1 cd
ABA 200	37.5±2.9 bd	35.0±4.1 bd	40.0±7.1 b
ABA 300	41.3±4.8 b	40.0±4.1 b	55.0±7.2 a

*Mean of white mold disease severity (%) of four replicate ± SD

** Means followed by the same letter within a column or row for each PGR treatment are not statistically significant according to Tukey multiple comparison test (P<0.05).
Plant growth regulators (PGRs); Naphthlene acetic acid (NAA); Gibberellic acid (GA3); Ethephon (Et); Methyl jasmonate (MeJA); Abscisic acid (ABA); and Aminoethoxy vinylglycine (AVG);

d disease severity (%) caused by three isolates of *S. sclortiorum* on cucumber whole plant, pretreated with different concentrations of plant growth regulators after 11 day incubation at 22 °C.

PGRs (μg/ml)	Isolate SS.P10	Isolate SS.I18	Isolate SS.P26
NAAO	37* ±8.3 a**	33.7 ±9.6 a	30 ±6.1 a
NAA 200	30 ±11.5 a	27.5 ±8.6 a	30 ±9.1 a
NAA 400	27.5 ±8.7 a	22.5 ±2.8 a	22.5 ±2.8 a
NAA 600	23.7 ±4.8 a	21.2 ±2.5 a	18.7 ±2.5 b
GA_3 0	37.5 ±9.5 ac	33.7 ±11 ab	30 ±7 ab
GA_3 50	38.7 ±4.8 ac	31.2 ±8.5 ab	27.5 ±2.9 ab
GA_3 150	46.2 ±4.8 a	37.5 ±2.9 ac	33.7 ±2.5 ac
GA_3 250	47.5 ±2.9 a	50 ±7.1 a	35 ±4.1 ac
Et. 0	30 ±4.1 c	30 ±4.1 c	27.5 ±2.9 c
Et. 200	38.7 ±2.9 b	45 ±4.1 bd	40 ±4.1 bd
Et. 400	60 ±4.1 a	50 ±4.1 bd	51.2 ±2.5 bd
Et. 600	65 ±4.1 a	65±4.1 a	62.5 ±2.9 a
Me.JA 0	45 ±4.1 a	52.5 ±2.9 a	53.7 ±2.5 a
MeJA 75	27.5 ±2.9 b	21.2 ±2.5 b	17.5 ±2.9 be
MeJA 150	18.7 ±4.7 ce	17.5 ±2.9 ce	17.5 ±2.9 ce
MeJA 250	15 ±2.8 ce	13.7 ±2.5 de	13.7 ±2.5 de
AVG 0	45 ±4.1) a	52.5 ±2.9 a	53.7 ±2.5 a
AVG 100	17.5 ±2.9 b	15 ±4.1 b	12.5 ±2.9 bd
AVG 200	12.5 ±2.9 bd	8.7 ±4.8 ce	7.5 ±2.9 ce
AVG 300	11.2 ±2.5 bd	8.7 ±2.5 ce	6. ±2.5 ce
ABA 0	13.8 ±2.5 d	22.5 ±2.9 d	25 ±4.1 d
ABA 100	31.3 ± 2.5 c	28.8 ±2.5 c	41.3 ±4.8 be
ABA 200	48.8 ±8.5 be	48.8 ±8.5 be	45±4.1 be
ABA 300	65.0 ±4.1 a	70.0 ±4.5 a	48.3 ±2.4 be

*Mean of white mold disease severity (%) of four replicate ±SD

** Means followed by the same letter within a column or row for each PGR treatment are not statistically significant according to Tukey multiple comparison test (P<0.05).

Plant growth regulators (PGRs); Naphthlene acetic acid (NAA); Gibberellic acid (GA3); Ethephon (Et); Methyl jasmonate (MeJA); Abscisic acid (ABA); and Aminoethoxy vinylglycine (AVG);

NAA significantly ($P<0.05$) reduced mean white mold disease severity (%) at 200, and 400 μg/ml of bean plants. Differences between the virulence of different isolates at the same concentrations were not significant. At 600 μg/ml NAA, white mold severity on bean and cucumber for three isolates except SSP.26 on cucumber plants was not affected. The latter concentration also caused leaf chlorosis. Mean disease severity in bean plants was significantly higher at 600 μg/ml compared with those at 200 and 400 μg/ml.Cucumber white mold disease severity was not significantly ($P>0.05$) affected at 200, 400 and 600 μg/ml NAA compared with the control (Fig 3.4, Table 3.4, and Table 3.5).

GA_3 significantly ($P<0.05$) increased white mold disease severity on beans at 50, 150 and 250 μg/ml (Fig 3.4, Table 3.4, and Table 3.5). Disease severity increased as concentration of GA_3 increased. However, disease severity was not affected on cucumber plants at 50, 150, and 250 μg/ml of GA_3. Variation in disease severity between isolates was not significant at these same concentrations.

Et. significantly ($P<0.05$) increased bean and cucumber white mold disease severity at 200, 400, and 600 μg/ml. A positive correlation ($Y = 0.0617\ X+34.217$; Y- disease severity (%), X- concentration (μg/ml), $r^2=0.99$) was found between concentration and white mold disease severity (Fig.3.4, Table 3.4, and Table 3.5). Isolates differed significantly ($P<0.05$)

0 μg/ml on bean plants, and at 400μg/ml on cucumber plants. Chlorosis and necrosis were observed on bean and cucumber plants treated with 600 μg/ml of ethephon.

MeJA significantly (P<0.05) reduced bean and cucumber white mold disease severity at 75, 150, and 250 μg/ml (Fig.3.5, Table 3.4, and Table 3.5). A negative correlation ($Y= -0.0678\ X+32.74$, r^2=0.99 on bean, $Y= -0.1357\ X+42.0051$, r^2=0.75 on cucumber, Y- disease severity (%) and X-concentration (μg/ml) was found between concentration of MeJA and white mold disease severity of bean and cucumber plants. This concentration, however, induced leaf chlorosis on plants.

AVG significantly (P<0.05) reduced the development of white mold disease on bean and cucumber plants treated with 100, 200, and 300 μg/ml of AVG (Fig.3.5, Table 3.4, and Table 3.5). The variability between the three isolates in relation to disease development was observed in 100 μg/ml on bean plants, and in 200 and 300 μg/ml on cucumber plants.

ABA significantly (P<0.05) increased white mold disease severity on cucumber plants pretreated with 100, 200, and 300 μg/ml of ABA, and on bean plants at 200 and 300 μg/ml (Fig.3.5, Table 3.4, and Table 3.5). The variability between the isolates in relation to disease development was observed at 300μg/ml on bean and cucumber plants.

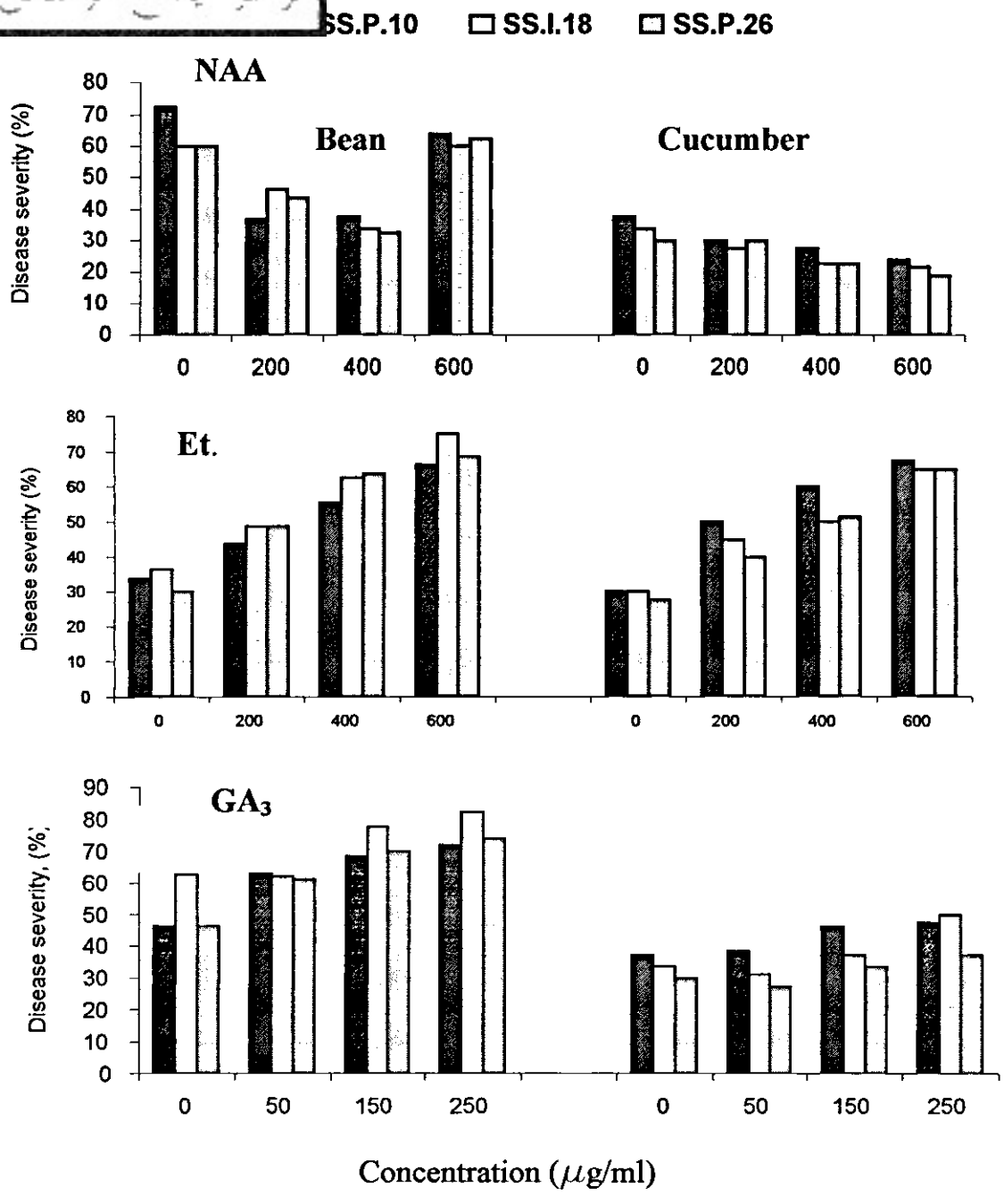

Figure 3.4 Effect of naphthalene acetic acid (NAA); gibberellic acid (GA_3); and ethephon (Et.) on white mold disease severity (%) causing by three isolates of *S. sclerotiorum* on bean and cucumber plants pretreated with different concentration of PGRs after 11 days incubation at 22°C.

duction by *S. sclerotiorum*

Experiment 1.

Ethylene was produced by the *S. sclerotiorum* isolate SS.I18 in shake PDB medium amended with 10 mM methionine. Ethylene production was detected after 3, 4, 5, 6, 8, and 10 days of incubation. Production reached a peak of 400.3 μl/g /hr at day 6 followed by a decline to low levels at day 10. Data on ethylene production are presented in Table 3.6, and Fig.3.6

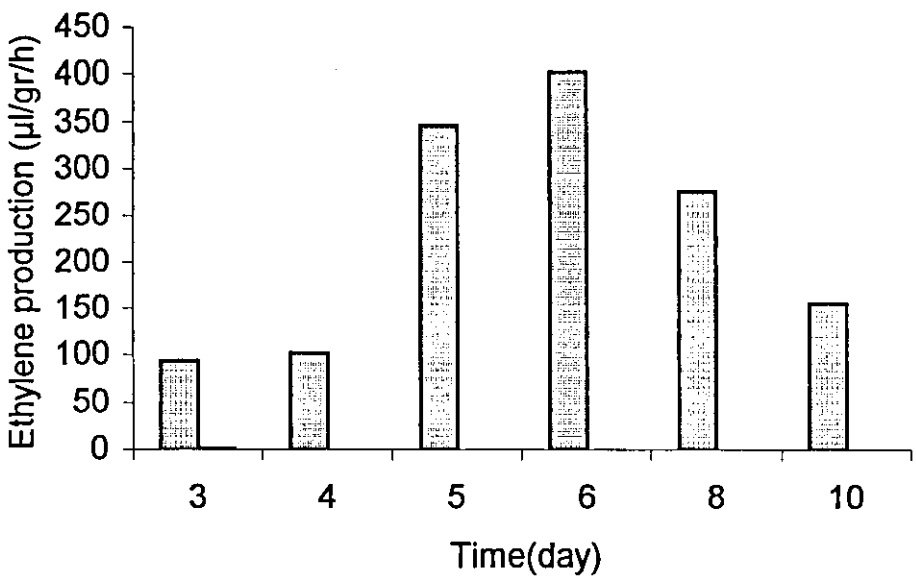

Figure 3.6 Ethylene production (μl/g/hr) by isolate SS.I18 of *S. sclerotiorum* growing on PDB amended with 10mM methionine after 3, 4, 5, 6, 8, and 10 days of incubation at 22 °C.

oduction (μl/g/hr) by isolate SS.I18 of *Sclerotinia sclerotiorum* growing on PDB-amended with 10mM methionine after 3, 4, 5, 6, 8, and 10 days of incubation at 22 °C.

Incubation time (day)	3	4	5	6	8	10
Ethylene production* (μl/g/hr)	94.6 ±41a	101.9 ±63 a	345.6 ±56b	400.3 ±75c	276.3 ±93b	155.4 ±24a

* Mean of three replicates ±SD

Experiment 2

Eleven isolates of *S. sclerotiorum* were grown on shaking culture PDB amended with 10mM methionine. Ethylene production was detected after 6 days of incubation at 22°C, and the amount of ethylene produced by the fungus, reached a peak on day 6 as well. The experiment was repeated twice and the results are presented in Table 3.7.

Mean of ethylene production by 11 isolates was 373 μl/g/hr with a range of 267.2-588, in the 1st experiment, and it was 367 μl/g/hr with a range 265-576.9 in the 2nd experiment.

production (μl/g/hr) by different isolates of *S. sclerotiorum* growing on PDB amended with 10mM methionine after 6 days of incubation at 22°C

Isolate	1st Experiment	2nd Experiment
Control	0±0	0±0
SS.P13	267.2* ±42** c	265 ±123 c
SS.I24	292.4 ±55 b	359.6±55 b
SS.P26	352.2 ±51b	396.8±39 b
SS.I 18	402.3 ±142 b	384.2±66 b
SS.P10	320.3 ±90 b	284.1±48 b
SS.I 22	304.5 ±165 b	340.5±125 b
SS.I 17	588.7 ±91 a	576.9±101 a
SS.I 16	474.7 ±95 a	459.9±112 a
SS.P11	401.9 ±72 b	279.1±38 b
SS.P7	354.7 ±105 b	297.8±152 b
SS.I 21	341.8 ±57 b	391.5±165 b

* Mean of three replicates ±SD

** Means followed by the same letter within a column or row are not statistically significant according to Tukey multiple comparison test (P<0.05).

Chapter 4

Discussion

4.1 Role of NAA in the development of white mold disease on bean and cucumber plants

The results of this study revealed that NAA reduced mycelium growth rate of *S. sclerotiorum in vivo* at concentrations < 200 μg/ml due to its toxicity to the fungus. It also reduced fungus lesion development *in vivo* and white mold disease severity on bean and cucumber plants at concentration up to 600 μg/ml. Similar results were observed by Michniewicz *et al.* (1987) who reported that NAA at 10^{-5}M strongly inhibited mycelium growth, sporulation, and spore germination of *Fusarium culmorum in vitro*, whereas it increased spore production and germination at low concentrations (10^{-7} and 10^{-9} M). NAA, exogenously applied at 10.7 mM, and 4.0 mM, was found by Melinda *et al.* (1991) to increase the resistance of potato plants to early blight incited by *Alternaria solani*. The present results are also in agreement with those of Gruen (1959) who indicated that the auxin was not a growth and development regulation factor in fungi, but it stimulated fungal growth only under unfavorable conditions. Many reports e.g., Michniewicz *et al.* (1987) and Melinda *et al.* (1991) have pointed out that auxin acts as a growth and

g factor, while its role in growth and development processes may vary in different species.

The velocity and flux of auxin transport varies depending on many factors; including nature of auxin, and nature and maturity of tissues. NAA moves slightly slower than other auxins, and the flux transport rate of NAA in bean tissue is slow (Hertel & Flory, 1968). So, when NAA is exogenously applied on bean and cucumber plant tissues, it accumulates in the upper cell layer where it initiates a short- lived partial resistance against the fungus. Also the fungus *S. sclerotiorum* penetration through tissue is enhanced by enzymes capable of degrading the middle lamella of the host cell. Optimum pH-value range of enzymes pectinase, pectinmethilesterase (Lumsden, 1976), cellulases, hemicellulases (Baraki-Golan, 1974; Lumsden, 1976), phosphatidase (Lumsden, 1970), and proteolytic enzymes (Khare *et al.*, 1976) are 4.3 - 5.5. While pH-value of NAA spray solutions used in this study were 6.63 - 7.69 depending on concentrations (Appendix A). The latter pH values may reduce the enzyme activity and thus the penetration of *S. sclerotiorum* of host cells.

NAA increased white mold disease severity on bean plants at 600 μg/ml compared to lower concentrations when exogenously applied, propably because it stimulates endogenuos ethylene production in plant tissue at high concentrations (Saniewski, 1990). In turn, the exogenous

600μg/ml, and endogenous ethylene produced, propably enhanced the protein synthesis (Abeles, 1969), caused phytotoxic, chlorosis of bean plant tissues, and increased the penetration of fungal mycelium.

4.2 Role of GA_3 on bean and cucumber white mold disease

The present results revealed that GA_3 significantly reduced mycelium growth rate of *S. sclerotiorum in vitro* at 150 and 200 μg/ml propably because GA_3 slightly reduced pH value of medium (Appendix A), whereas GA_3 increased lesions devlopment on bean leaves at 200, and 300 μg/ml. However, GA_3 stimulated white mold disease development on both bean and cucumber plants at higher concentrations (>150μg/ml). Similar results on the role of GA_3 in fungi development were observed by Nakamura *et al.* (1978) who found GA_3 to stimulate spore germination of *Neurospora crassa*. They therefore, concluded that GA_3 acts as a growth regulator of conidial germination of *N. crassa* similar to its action in higher plants. Michniewiez *et al.* (1983, 1988) found that GA_3 slightly stimulated mycelium growth of *Fusarium culmorum in vitro* at 10^{-9},10^{-7}, and 10^{-5} M and essentially stimulated sporulation and spore germination at 10^{-7} and 10^{-5}M. In addition, these authors concluded that GA_3 acts as a regulator of the metabolism of nitrogen compounds and carbohydrates. Tomita *et al.*

the same conclusion that GA_3 is a growth and differentiation regulator in fungi just as it is in higher plants.

Gibberellins perform a variety of activities in infected plants. These hormones promote plant cell elongation which is the most common effect they induced on plants. They stimulate α- and β-amylase action, which affects carbohydrate degradation and the hydrolysis of starch, providing respiratory substrate for the pathogen. Where a constitutive α- amylase was present, this role for GA_3 would be superfluous, and stimulation of cellulase and stimulation or inhibition of ethylene production depended on the plant species (Pegg, 1981).

The complex interaction between exogenous application of GA_3 against white mold disease on bean and cucumber plants has not been studied enough. The stimulating effect of GA_3 on white mold disease is probably due to the sensivity of plant tissue. In this process, elongation of plant cells and enhancment of α-amylase, β-amylase and cellulase activities stimulates the hydrolysis of starch and cellulose providing the fungus causing white mold with a simple food like glucose to live and thrive on (Lumsden 1976 ,1979).

The pH-value of GA_3-spray solutions in the present work were 3.46 - 4.92 (Appendix A) which is equivilant to the range for *S. sclerotiorum*

3 - 5.5) found by (Lumsden 1970,1976; Baraki-Golan, 1974; Khare *et al.*, 1976). Hence, these enzymes acted under optimum conditions and enhanced the degradation of host cells enabling the fungus to penetrate rapidly intercellulary .

4.3 Role of ethephon in bean and cucumber white mold disease development

The exogenous application of ethephon slightly decreased mycelium growth rate of *S. sclerotiorum* at concentrations of 100, 150 and 200 μg/ml, propably because it did not release ethylene at pH <4 (Giafaga, 1995), medium pH range was 3.18-3.21 (Appendix 1). ethephon increased white mold lesions development on bean and cucumber leaves at 250, 300 and 400 μg/ml. Also, it significantly increased bean and cucumber white mold severity (%) at 200, 400 and 600 μg/ml. The present results clearly demonstrate that Et. undoubtedly increase white mold disease on bean and cucumber plants. This finding is in agreement with that of Biles *et al.* (1990) who found Et. to promote anthracnose of cucubmer caused by *Colletotrichum lagenarium*; with that of Kepezynska (1993) who found Ethephone to slightly stimulate the *Botrytis cinerea in vitro* and on apple plants; with that of Elad (1992)who found Et. to increase gray mold severity on leaves of *Senecio sp.*; and also with that of Fortnum (1983)

increase black root rot of soybean caused by *Cylindrocladium crotalaria.*

Ethephon as an ethylene releasing agent, is stable at pH-values of 4 or less, but at higher pH-values it decomposes to produce ethylene. Since the cytoplasmic pH is greater than 4, ethylene is released inside the cell after ethephon has been adsorbed by plant tissue (Giafaga, 1995). Ethylene increases the glucosamine and chitin contents which are major constituents of fungal cell walls, and hence, ethylene may have stimulated a *S. sclerotiorum* mycelium growth *in vivo* by supplying the fungus with glucosamine and chitin. Similar results were obtained by El-Kazzaz (1983). The increase of glucosamine increased the growth of *B. cineria* and P*enicillium italicum in vitro* and *in vivo*. On the other hand, endogenous ethylene, produced by the *S. sclerotiorum*, and ethylene produced as a result of exogenously applied ethephon were known to hasten several biochemical and physiological alteration, including proteolysis and other hydrolytic activities, stimulation of oxidative enzymes, loss of chlorophyll, and decline in photosynthetic rate (Abeles, 1973). Exogenous application of ethephon, also changes protein composition in treated tissue, increases the pathognesis-related proteins content, and decreases rubisco (a major plant soluble protein) and other enzymes which accelerate protein degradation enhancing the disease severity (Vera *et al.*, 1990).

l jasmonate (MeJA) on bean and cucumber white mold

MeJA significantly decreased mycelium growth rate of *S. sclerotiorum in vitro* and reduced white mold disease severity on both detached leaves and whole plants of bean and cucumber, in this study. A positive correllation between MeJA concentration and its inhibitory effects was found. This indicates that MeJA has a potential of being used to control white mold disease on bean and cucumber plants. At 250μg/ ml, it gave 73% and 82% white mold disease control on bean and cucumber plants, respectively. On the other hand, it induced chlorosis on bean and cucumber leaves. Similar results were observed by Cohn (1993) who applied MeJA exogenously to control late blight caused by *Phytophthora infestants* on potato and tomato plants in greenhouse. He reported that MeJA gave 92% and 100% control of late blight disease on potato and tomato leaves, respectively. At concentration of 100 μg/ml, also, MeJA induced chlorosis.

When applied as a topical spray against barley powdery mildew caused by *Erysiphe graminis f.sp. hordei*, jasmonates gave 80 % protection to plants inoculated 3-days after treatment (Schweizer *et al.*, 1993). The level of protection was however, short-lived, and necrosis occur on leaf tips at 10 μg/ml. Several investigators had attempted to explain low dose function of

luces partial resistance in diseased plants. Bohlmann (1991) indicated that partial resistance is because of the accumulation of the pathogenesis-related proteins (PR) that are located in plant cell walls and inside vacuoles. Leaf thionins are toxic for fungal and bacterial cells and are assumed to play a role in the defense against pathogens (Bohlmann, 1991). Staswick (1990) reported that vegetative storage proteins (VSPs) are accumulated in vegetative organs in the epidermis and paraveinal mesophyll cells of leaves as well as in bundle sheath tissues. The interaction between MeJA and systemin, ethylene, ABA, and electric current in plant tissue initiates defence to pathogen (Bressan *et al.*, 1994; Shigemi *et al.*, 1997). MeJA induced partial resistance against white mold on bean and cucumber plants propably due to accumulation of vegetative storage proteins (VSPs) and thionins in plant tissues.

Chlorosis caused by exogenous application of MeJA at high concentrations (150 and 250 μg/ml) observed on bean and cucumber plants, may be due to MeJA promotion of degradation of cell wall polysaccharides and the chlorophyll (Parthier 1990) and inhibition of phytosynthetic activities (Popova *et al.*, 1988). Similar observations were made by Miyamoto (1997) and Vedo (1996) who reported that MeJA promoted the degradation of cell wall polysaccharides in petioles of *Phaseolus vulgaris* plants.

scisic acid on white mold disease development on bean and cucumber

ABA slightly decreased mycelium growth rate of *S. sclerotiorum* growing on PDA-amended with different concentrations up to 150 μg/ml due to ABA nature, that reduced water content in ABA- amended culture. Similar result observed by Singh (1997), who found that the water content of ABA-treated cultures fell to 13% from the control value of 70% in untreated cultures. On the other hand, it stimulated the white mold disease on bean and cucumber plants at concentrations up to 300 μg/ml because ABA effects on plant cell membrane; stomata closure (Ya'acov *et al.*, 1990); and turgidity of tissue due to their effect on unique species of phosphatidylcholine (PC), and dipalmitoylphosphatidylcholine (DPPC) (Demandre *et al.*,1987). This interaction sutitation in plant tissue may reduce the sensevitiy of plant tissue and enhance the fungus to pentrate tissue and increase white mold disease. This result was not substantiated by Singh *et al.* (1997) who found in his investigation on controlling the causative agent of (Karnal) bunt, a serious disease of wheat caused by fungus *Neovossia indica*. ABA at 10, 50, and 100 μM to be a potent inhibitor of growth, sporidiogenesis, and teliospore germination of *Neovossia indica* under culture conditions. The latter workers also reported that ABA application at 10 μM gave a worth result to reduce endogenous levels of different polyaminase, i.e., putrescine, sprmidine and

As) are simple aliphatic compounds that are present in all living cells and required for optimal growth and development in many biological systems. Additional factor which may also be involved in growth inhibition is the loss of cellular turgidity by mycelial hyphae of *Neovossia indica* cultured in the presence of 10 μM ABA.

4.6 Role of aminoethoxyvinylglycine (AVG) on bean and cucumber white mold

In the present work, AVG reduced mycelium growth rate of *S. sclerotiorum* grown on PDA-amended with concentrations up to 150 μg/ml. It also inhibited the development of white mold on tissues and plants of bean and cucumber. At 300μg/ml, AVG gave 63% and 86% white mold disease severity reduction on bean and cucumber plants, respectively.

This result may be attributed to the inhibitory effect of AVG on ethylene production by fungi and/or plant tissue (Bae Gy *et al.* 1996; Shellie 1999). AVG is used as ethylene inhibitor agent. Endogenous ethylene produced both by fungi and/or plant tissue is reduced by AVG application. AVG inhibits 1-aminocyclopropane-1-carboxylic acid (ACC) synthase activity of ethylene production pathway in plants (Bae Gy *et al.* 1996).

G on disease development in plant tisuues infected by *B. cinerea* had been investigated by Elad (1990), who found AVG to inhibit grey mold disease development in bean and tomato leaves at $5*10^{-4}$ M, and to prevent completely the disease at $5*10^{-3}$ M. He also found that ethylene production was drastically reduced in both tomato and bean leaves pretreated with $5*10^{-3}$ M AVG, while the treatment with $5*10^{-4}$ M was effective in reducing ethylene production only up to 7 days of incubation.

The influence of AVG on white mold disease development on both bean and cucumber tissues and plants, may therefore be due to inhibiton ethylene production by both the fungus *S. sclerotiorum* and infected tissues due to inhibition of ACC synthase (known enzyme involved in ethylene production and it also inhibits transaminases such as phenylallanine ammonia lyase, PAL). Furthermore it may be due to inhibition of protein synthesis. This situation may be responsible for the reduction of disease development on bean and cucumber plant tissues pretreated with diffferent concentration of AVG.

uction by *Sclerotinia sclerotiorum*

Ethylene is produced by a range of plant pathogenic bacteria and fungi. Hag and Curtis (1968) studied various groups of fungi and reported that 58 of 228 species examined produce ethylene. The ratio of ethylene producing to non-producing species in different groups of fungi was 1:31 in Phycomycetes, 1:10 in Ascomycetes, 1:6 in Basidomycetes, and 1:4 in Fungi Imperfecti. Altaf (1997) reported that *B. cinerea* produces ethylene. There have been, to the best of my knowledge, no reports however on ethylene production by *S. sclerotiorum*.

In the present investigation, ethylene was produced by *S. sclerotiorum* isolates grown on PDB amended with 10mM methionine reaching a peak at day 6 (400 μl/g/h), followed by a decline to a low level at day 10 (155 μl/g/h). Ethylene production by 11 isolates ranged from 267.2 to 588.7μl/g/h.

Sclerotinia sclerotiorum, similar to a number of other phytopathogenic fungi (*Verticillium*, *Fusarium*, *Colletorichum*, and *Penicillium digitatum* seems to use methionine as a precursor of ethylene (Tzeng *et al.*, 1984, Chalutz *et al.*, 1977).

Ethylene production in *S. sclerotiorum* reached a peak at day 6 followed by a decline at day 10, probably, because fungus biomass

ion and available methionine was reduced due to consumption by fungus. Similar results were obtained in several investigations that studied relations between ethylene production and mycelium weight. In shake culture, ethylene production by P. *digitatum* peaked at about 280 nl/g/h after 4day incubation and then declined (Chalutz *et al.*1977). In *V. dahilae* ethylene rate was 3.11 nl/g/h (Tzeng *et al.* 1984), and in *B. cinerea* the rate was very high (780 μl/g/h after 2 day incubation) and declined (74 μl/g/h) after 3 days (Altaf, 1997)

All *S. sclerotiorum* isolates tested in this work produced ethylene, reaching a maximum productivity (267.2-588.7 μl/g/h) at day 6. The variability between isolates of *S. sclerotiorum* may be due to genetic variation of the isolates. Similar variability of ethylene production between isolates of *Endothia gyrosa* and *Cytospora eucalypticola* has been observed by Wilkes *et al.* (1989).

Conclusions

Naphthalene acetic acid (NAA) reduced white mold disease severity on bean and cucumber plants due to inhibition effect on fungus and induced short-lived partial resistance in plant tissues.

Ethephone and giberellic acid (GA3) promoted white mold disease severity on plants because of reduction of plant tissues sensivity to the fungus.

Methyl jasmonate (MeJA) decreased bean and cucumber white mold disease because it induces partial resistance due to accumulation of the pathogenesis-related proteins and /or vegtative storage proteins, that are located in plant cell walls, and inhibition effect on the fungus.

Absiscic acid (ABA) increased white mold disease development on bean and cucumber plants because ABA affected on unique species of phosphatidycholine (PC) and dipalmitoylphosphatidylcholine (DPPC) that reduce the sensevity of plant tissue and enhance the fungus to pentrate tissue.

Aminoethoxvinyleglycine (AVG) decreased white mold disease severity on bean and cucumber plants because AVG reduce endogenous

h by fungi and/or plant tissue due to inhibition by 1-aminocyclopropane-1-carboxylic acid (ACC).

Ethylene was produced by the fungus *S. sclerotiorum* growing on shaking PDB medium amended with 10 mM methioniane and reach a peak at day 6 at rate of 400 μl/g/h.

Results releaved high correlation between PGRs and white mold disease development. NAA, MeJA and AVG may be potentially used for controlling white mold disease on bean and cucumber, while GA_3, ABA and ethephone stimulate disease development.

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pH-values of different concentrations of plant growth regulators in spray solutions and in PDA medium amendment suspensions (1 medium: 1 distilled water) at 25°C.

Plant growth regulators	Concentration (μg/ml)	pH of spray solutions	pH of PDA medium
NAA	0	7.2- 7.3	4.1
	10	*	3.16
	50	6.63	3.19
	100	6.65	3.26
	150	6.73	3.28
	200	7.01	3.42
	250	7.15	*
	300	7.27	*
	400	7.32	*
	500	7.39	*
	600	7.69	*
GA_3	10	*	3.78
	50	4.92	3.77
	100	4.7	3.75
	150	3.83	3.74
	200	3.58	3.22
	300	3.46	*
ET.	10	*	3.36
	50	3.26	3.28
	100	*	3.26
	150	2.88	3.18
	200	*	3.21
	250	2.71	*
	300	2.66	*
	400	2.44	*
MeJA	10	*	3.20
	50	7.31	3.20
	75	*	3.30
	100	7.88	3.40
	150	8.06	3.45
	200	8.14	*
	250	8.34	*
	1000	8.92	*
AVG	10	6.27	4.06
	25	6.29	*
	50	6.34	4.05
	75	6.36	*
	100	6.46	4.05
	150	6.48	4.08
	200	6.61	4.12
	300	6.74	*
ABA	25	*	3.94
	50	4.40	3.94
	75	*	3.97
	100	4.59	3.97
	150	4.66	3.99
	200	4.79	*
	300	4.90	*

• **Not measured**

ANOVA tables

Experiment	D.F treat.	D.F residual	D.F total	SS treat.	SS residual	SS total	MS treat.	MS residual	F
NAA on PDA	20	102	122	2673	62.55	2736	133.7	0.613	218.0
GA_3 on PDA	17	90	107	505.7	193.7	699.4	29.75	2.152	13.83
Et.on PDA	17	90	107	362.4	225.5	588	21.32	2.5	8.50
Me.JA on PDA	17	90	107	3064.4	46.66	3111	180.26	0.51	347.62
ABA on PDA	17	90	107	1548	163.7	1711	91.04	1.82	5.005
AVG on PDA	17	90	107	1322	67.25	1390	77.79	0.747	104.1
GA_3 on bean leaves	17	90	107	382.6	269.6	652.2	22.5	2.99	7.51
Et. on bean leaves	17	89	106	446.3	145.4	591.8	26.25	1.63	16.07
Me.JA on bean leaves	14	75	89	282.7	61.39	344.1	20.2	0.818	24.67
ABA on bean leaves	17	90	107	390.3	172.6	562.9	22.9	1.9	11.97
AVG on bean leaves	14	75	89	283.1	33.21	316.3	20.22	0.44	45.66
NAA on cucu. leaves	14	75	89	95.78	8.12	103.9	6.84	0.108	63.16
GA_3 on cucumber leaves	17	90	107	86.82	2.90	377	5.1	3.2	1.58
Et. on cucumber leaves	17	90	107	77.98	24	102	4.58	0.266	17.2
Me.JA on cucu. leaves	14	75	89	247.9	48.93	296.8	17.7	0.65	27.14
ABA on cucu. leaves	18	95	113	48.9	121.72	170.7	2.7	1.3	2.1
AVG on cucu. leaves	14	75	89	247.9	48.93	296.8	17.71	0.65	27.14
NAA on bean plants	11	36	47	8398.72	1191.75	9590.47	763.52	33.1	23.06
GA_3 on bean plants	11	36	47	5438.16	719.5	6157.66	494.37	19.98	24.73
Et. on bean plants	11	36	47	9677.72	375.75	10053.47	879.79	10.43	84.29
Me.JA on bean plants	11	36	47	2126.56	443.75	2570.31	193.32	12.32	15.68
ABA on bean plants	11	36	47	3464.1	818.7	4282.8	314.9	22.7	13.84
AVG on bean plants	11	36	47	19780.72	993.75	20774.47	1798.24	27.6	65.14
NAA on cucumber plants	11	36	47	1341.66	2050	3391.66	121.97	56.94	2.14
GA_3 on cucumber plants	11	36	47	2230.72	1418.75	3649.47	202.79	39.41	5.14
Et. on cucumber plants	11	36	47	9039.06	518.75	9557.81	821.73	14.41	57.02
Me.JA on cucumber plant	11	36	47	10439.06	343.75	10782.8	949	9.549	99.38
ABA on cucumber plants	11	36	47	12762.1	804.2	13566.3	1160.2	22.34	51.93
AVG on cucumber plants	11	36	47	19780	993.8	20770	1798	27.6	65.14

دور منظمات النمو النباتية في العلاقة بين القدرة المرضيه لفطر *Sclerotinia sclerotiorum* وعوائله

الخلاصة

تمت دراسة أثر منظمات النمو النباتية كطريقة حديثة لمكافحة فطـــر *Sclerotinia sclerotiroum* المسبب لمرض العفن الابيض في محصولي الفاصوليا والخيار علي ثلاث مستويات: البيئة المغذية، الأنسجة النباتية والنباتات الكاملة. وأظهرت النتائج ما يلي.

أدى استخدام الأوكسين نفثا لين أستيك اسيد (NAA) بتركيزات لغاية 500μg/ml على تقليل معدل نمو الفطر على الوسط المغذي والأنسجة النباتية، وأظهر الاوكسين قدرة عالية علـــى مكافحـــة مرض العفن الابيض في محصولي الفاصوليا والخيار؛ وعزي ذلك الى مقدرة الأوكسين على إحـــداث مناعة ذاتية مؤقتة في الأنسجة النباتية.

عمل حامض الجبريللين(GA_3) أو مادة Ethephon، التي تعمل على انبعاث غاز هرمون الأيثيلـين، عند استخدامهما بتركيزات تصل إلى 400 μg /ml، عملتا على زيادة معدل نمو الفطر على الوســط المغذي والأنسجة النباتية ،وعلى زيادة المرض على نباتات الفاصوليا والخيار. وقد عـــزى ذلـــك إلى مقدرة حامض الجبريللين على تشجيع أنزيم الاميليز والسيليز اللذين يعملان على زيادة إنتاج الجلوكـــوز

وتحفيز نمو الفطر. أما مادة Ethephon فتعمل عند ادمصاصها في الأ

هرمون الأيثلين الذي يعمل بدوره على تحطيم جدران الخلايا وزيادة احداث العدوى.

وادى استخدام استر حامض الجسمونيت (MeJA) بتراكيز 250 µg /ml إلى تقليل معدل نمـــو الفطر على الوسط المغذي والأنسجة النباتية، واظهر مقدرة عالية علي مكافحة المرض بنسـبة , 73% 82% على محصولي الخيار والفاصوليا بالترتيب؛ وعزى هذا إلى مقدرة الهرمون على إحداث مناعـــة ذاتية في الأنسجة النباتية. وادى استخدام حامض الأبسيسيك بمعدل 250 µg /ml ، إلى خفـــض معدل نمو الفطر علي الوسط المغذي ، بينما ادى الى تشجيع المرض على نباتات الفاصوليا و لم يوثر على نباتات الخيار. وادى استخدام مركب Aminoethoxvinyleglycine (AVG) كمثبط لغـــاز الأيثيلين المنتج من الفطر أو من الأنسجة النباتية بتركيزات 300 µg /ml، الى مكافحة المرض بنسبة 86%, 63% على نباتات الخيار والفاصوليا بالترتيب.

وجرى فى هذه الدراسة فحص قدرة الفطر على إنتاج غاز الأيثيلين بتنميتة علـــى الوســـط المغـــذي (PDB) المزوج مع10 mM من الحمض الأميني الميثونين حيث ظهر أنه ينتج غاز الايثيلين بحـــد أقصى 4 0 0 µl/g/h في يوم التحضين السادس، حيث يبـــدأ أنتاجـــه في الانخفـــاض ليصـــل إلى 155 µl/g/h في اليوم العاشر.