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Chemical and Biological Measures against Sclerotinia spp. The Causal of Foliage Blight Disease of Cucumber and Pepper Plants in Egypt- A Review

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Abstract*— Severe outbreaks of Sclerotinia foliage blight disease symptoms with sclerotial formation were observed as first occurrence during a disease survey of growing cucumber and pepper plants during the cool season, 2011 in the Protected Cultivation Station, Ministry of Agriculture located at Tookh province, Qalubiya governorate, Egypt. The isolated causal pathogens for cucumber and pepper foliage blights were identified as Sclerotinia sclerotiorum (Lib.) de Bary and S. minor Jagger, respectively. This is thought to be the first report of these fungi to cause foliage blights on cucumber and pepper in Egypt. As control measures antagonistic agents and fungicides against the growth both pathogenic fungi under in vitro conditions were evaluated. The obtained results showed complete growth inhibition was recorded for both S. sclerotiorum and S. minor at 100ppm of Top-sin-M and Ridomil Gold, while Rizolex-T gave the same effect at 200ppm. The fungicide, Previcure had inhibitor effect on the mycelia growth of both fungi only at the high concentration 800ppm. Moreover, the antagonistic fungi (Trichoderma spp.) showed superior inhibitory effect against the growth of pathogenic fungi compared with bacterial isolates (B. subtilis & P. florescence). No significant differences between the tested antagonistic microorganisms and commercial biocides were observed. Recently, interest has been shown in combining microbial bio-control agents with other chemical components to increase their activity against plant pathogens. To reach the proposed aim, determination of the efficacy of some plant-derived essential oils, plant resistance inducers and plant extracts in combination with bio-control agents against the growth of Sclerotinia foliage blight pathogens S. sclerotiorum and S. minor was carried out under in vitro conditions. The obtained results in the present study has shown the potential of tested materials as effective inhibitors against pathogenic fungi when combined factors with antagonistic bio-agents.

The present work summarizes and reviewed all attempts towards control of Sclerotinia spp. starting from survey of detected infected cucumber and pepper plants with Sclerotina foliage blight in plastic houses under protected cultivation system up to isolation of the causal pathogen and evaluating some control measures under laboratory conditions. This work was carried out during a project supported by the Science and Technology Development Fund (STDF), Egypt.

Index: Antagonistic Antagonism, Bacillus subtilis, Cucumber, Essential Oils, Fungicides, Plant Resistance Inducers, Pepper, Pseudomonas fluorescens, Sclerotinia sclerotiorum, S. minor, Sclerotinia foliage blight, Trichoderma harzianum, T. Viride.

I. INTRODUCTION

Cucumber and pepper crops are cultivated throughout the world as important vegetables or a food source. In Egypt, most vegetable crops except potato are cultivated in the plastic house conditions during the winter season. The plastic house conditions are favorable for occurrence of fungal diseases on the crops due to the high humidity. Especially, Sclerotinia rot is apt to readily occur under cool and moist conditions [1,2]. Severe outbreaks of stem or fruit rot symptoms with sclerotial formation were observed during a disease survey of vegetable crops in the Protected Cultivation Station, Ministry of Agriculture located at Tookh province, Qalubiya governorate, Egypt during the cool season 2011 [3,4]. The disease was recorded as Sclerotinia stem rot, Sclerotinia fruit rot, fruit and stem rot, fruit rot, stem blight and rot, white mold or pink joint depending on the species of the infected crops [5]. It has been reported that *Sclerotinia sclerotiorum* (Lib.) de Bary and *S. minor* Jagger cause Sclerotinia rot in a variety of plants [1,2,5,6,7]. Some mycological and pathological characteristics of *S. sclerotiorum* and *S. minor* causing Sclerotinia rot of vegetable crops were also reported [8]. As control measures, chemical fungicides are successfully reported to control *Sclerotinia* spp. In this regards, it was reported that among the fungicides tested, the most effective fungicides in inhibiting the scle-rotial germination were Ridomil gold, Benlate, Tecto-60 and Topsin-M [9]. Also, *In Vitro* test, Rizolex-T and Topsin-M at 200 ppm was completely inhibited the growth of *R. solani* and *F. oxysporum*, respectively [10], meanwhile among the fungicides tested, Ridomil MZ 72 WP (metalaxyl 8%+ mancozeb 64% WP) was found to give the best protection against Sclerotinia root rot incidence in both common and Tartary buckwheat (by 50 and 56% protection of root rot incidence over the control, respectively) and thereby in-creased the plant stand [11]. Some successes have been recorded using biological methods of control. These have included the application of specific fungal antagonists such as *Coniothyrium minitans*;

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Sporidesmium sclerotivorum and *Trichoderma hamatum* [12,13], as well as inoculation into the rhizosphere of nonspecific fungal antagonists such as Trichoderma and Gliocladium species [14]. Both specific and nonspecific fungal antagonists have been reported to actively parasitize sclerotia, mycelia and ascospores of several Sclerotinia species [15]. The activity of the antagonistic fungi *Coniothyrium minitans* and *Trichoderma hamatum* against *Sclerotinia* spp. in both laboratory and greenhouse assays was reported [16]. Although other studies have been conducted to determine the effects of chitinolytic fungi and bacteria on the growth and development of a number of soilborne fungal pathogens [17,18]. Also, three isolates, *Serratia marcescens*, *Streptomyces viridodiasticus* and *Micromonospora carbonacea*, significantly reduced the growth of *S. minor in vitro*, and produced high levels of chitinase and b-1,3-glucanase [19]. Moreover, it was reported that [20] four bacterial strains, *Pseudomonas chlororaphis*, *Bacillus amyloliquefaciens*, *Pseudomonas* sp. and *B. amyloliquefaciens* had been found to have biocontrol activity in vitro assays against *Sclerotinia sclerotiorum*, the causal agent of stem rot of canola**.** Few studies have been conducted on the detailed characteristics of the disease occurrence and the pathogenicity of the causal Sclerotinia species on different crops.

The management strategy followed by the farmers included an unwise, intensive use of fungicides. This strategy was not a satisfactory solution for controlling Sclerotinia disease. Recently, interest has been shown in combining microbial bio-control agents with other chemical. The increasing interest in pesticide alternatives was due to the toxicity implication of pesticides for humans. Therefore, there will be an increasingly driven demand motivated by different priorities such as health benefits, cost, ecological benefits, ethical issues, food safety and sustainability of supply. Biological control approach depends upon the establishment and maintenance of a threshold population of introduced bio-agent into the soil, and a drop in viability below that level may eliminate the possibility of biological control. Many soil edaphic factors, including temperature, moisture, pH and nutrition influence the survival and establishment of the bio-agent and their interaction with the pathogens. Plant products are characterized as having a wide range of volatile compounds could be used as alternative anti-bacterial and anti-fungal treatments [21]. On the other hand, many investigations reported the use of potassium salts (K_2HPO_4 or KNO_3) as a chemical agent for induction of plant resistance [22,23]. Furthermore, Humic and Fulvic acids have been early recorded to have appositive effect against plant pathogens and their cells biological activities [25,26].

Against this background and the demand for natural products as raw material for new antifungal agents, the objective of the present work was aimed to get a suitable formula could be used for controlling such diseases. To reach the proposed aim the pathological aspects of the causal *Sclerotinia* spp. as well as the efficacy of some biological and chemical measures for controlling *Sclerotinia* spp. under laboratory conditions. Moreover, determination of the efficacy of some plant-derived essential oils, plant resistance inducers and plant extracts in combination with bio-control agents against the growth of Sclerotinia foliage blight pathogens *S. sclerotiorum* and *S. minor* was carried out under *in vitro* conditions.

II. DISEASES SURVEY

The most reported areas for high production of vegetables under protected cultivation system in Egypt were subjected to survey the infected plants with Sclerotina foliage blight during the winter season of 2011 [3]. The surveyed plants were Cucumber and Pepper. Sclerotinia foliage blight was detected in cucumber and pepper plants grown only under protected cultivation system at Tookh location at Kalubia governorate, Egypt [3,4]. The percentage diseases incidence was recorded at different commercial plastic houses distributed in five governorates, *i.e.* Giza, Cairo, Kalubia, Ismaelia and Behiera. The average percentages of infected plants with Sclerotina foliage blight disease were calculated as the number of infected plants in relative to the total number of examined plants grown in plastic house [26]. They recorded the occurrence of the diseases in 18 out of 28 surveyed plastic houses during the growing winter season, 2011. The mean of disease severely occurred as high as 3.4% in pepper and as low as 2.8% in cucumber plants. Their data also showed variation in disease incidence between cultivated varieties. Cucumber cv. Hisham significantly expressed as the highest susceptible cultivar to disease infection followed by D.P.162 and Hykal cvs., respectively. Meanwhile, no significant differences were observed between the two monitored pepper cultivars. A sever Sclerotina foliage blight disease symptoms were observed on cucumber, sweet and hot pepper plants grown in the Protected Cultivation Station, Ministry of Agriculture located at Tookh province, Qalubiya governorate. Disease symptoms on cucumber (Fig. 1) become visible on plant branches, leaves, flowers and fruits. In a cool moist environment, which normally provided under greenhouses conditions, watery soft lesions occur at sites of infection and the tissues are rapidly covered with white fluffy mycelium. The leaves become chlorotic and necrotic, and stems become girdled and die. On the dead plant tissue, as well as within tissue such as stems and fruits, fungal sclerotia are formed. Meanwhile, disease symptoms on pepper appeared as brown lesions formed at the apical part of branches in affected plants. These lesions may expand and girdle the twigs, thereafter, bud wilt and twig death follows. A cottony white mycelium develops around diseased areas with black irregularly shaped sclerotia, also watery soft rot of formed fruits take place (Fig. 2). In this regard, Sclerotinia stem rot

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of pepper has been reported in Florida, Iowa, Texas, Massachusetts and Connecticut and this was considered the first report of the disease on pepper in Ohio [27]. Sclerotinia foliar blights was the first report of bell pepper as a host of *S. minor* in the Salinas Valley, California, USA [28] and also, the first report of *S. sclerotiorum* on cucumbers in Iran was recorded during autumn [29], in greenhouses the disease infection of pepper plants with pathogen *S. sclerotiorum* increases during favorable conditions and could infect pepper flower causing flowers and bud rots [30]. Furthermore, Sclerotinia blights, caused by *Sclerotinia sclerotiorum* is also reported on different host plants, i.e. beans, peanuts and occasionally on chilli plants [31,32].

Fig. 1. Typical symptoms of natural infection with Sclerotinia blight disease of Cucumber plants grown under protected cultivation

III. CAUSAL ORGANISMS AND THEIR PATHOGENIC ABILITY

Sclerotinia foliage blight disease symptoms appeared as different lesions varied in its sizes on the shoot system of diseased plants [26]. Diseased plant samples showing these symptoms were collected from commercial plastic houses and subjected to isolation trails. Lesion pieces (2-3 cm^2) cutted from the diseased plant parts were placed into plates containing sterilized 2% water agar (WA) after surface-sterilizing with 1% sodium hypochlorite solution for 1 minute. The plates were incubated for 1-2 days at 22^{o}C. The fungi grown from the lesion pieces were transferred to potato dextrose agar (PDA) slants and sub-cultured for identification processes. Identification for the isolated fungi carried out according to [33,34]. The isolated fungus of cucumber diseased plants identified as *Sclerotinia sclerotiorum* (Lib.) de Bary. Meanwhile, the isolated fungus of sweet and hot pepper plants identified as *Sclerotinia minor* Jagger. According to the available literature, this is thought to be the first record of Sclerotinia foliage blight disease of cucumber and pepper plants in Egypt. Pathogenic ability of *S. sclerotiorum* and *S. minor* to induce foliage blight infection of cucumber and sweet pepper seedlings was tested under greenhouse conditions [26]. Transplants (5 weeks-old) of two cultivars D.P. 162 & Hisham of Cucumber (*Cucumis sativus* L.) and Khyrratte & Kaha of sweet pepper (*Capsicum annuum* L.) were used for pathogenicity test. The obtained results revealed that the two tested fungi are able to induce foliage blight symptoms of either cucumber and pepper plants. Data also show that there was no significant difference between the two fungi either for host or their cultivars tested. Data also showed that *S. sclerotiorum* was more aggressive for inducing the disease infection than *S. minor* for both host plants and consequently their cultivar varieties. The soil borne plant pathogens *Sclerotinia sclerotiorum* and *S. minor* cause severe economic losses of many vegetable and ornamental crops around the world and they have been reported to be the pathogen of Sclerotinia rot of various crops [1,5,28]. In general, sclerotia of *Sclerotinia* spp. can survive in nature for many years [12] and play the important role as an inoculum source of the disease occurrence [2].

Fig. 2. Typical symptoms of natural infection with Sclerotinia foliage blight disease of pepper plants grown under protected cultivation system

It has not yet been studied on the sclerotial density and viability of the two Sclerotinia spp. in the field of vegetable crops. There have been reports on differences in virulence of S. sclerotiorum isolates to individual plants and in susceptibility of some plants to the isolates [8,35]. Moreover, it has been also reported that there are differences in

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susceptibility of cultivars or lines of some crops to the pathogen [36,37]. However, the present study showed that there was no significant difference in virulence of S. sclerotiorum isolates to cucumber and pepper crops, as reported by previous workers in other crops [38,39]. On the other hand, S. minor was relatively less virulent on the two tested host plants cultivars than on another one, suggesting that there might be some differences in susceptibility of these cultivars to S. minor.

IV. SENSITIVITY AGAINST FUNGICIDES

The major value of any chemical compound as a disease control agent ultimately depends on the mode of action of the molecule at the physiological level on one or more components of the life cycle of the pathogen. Eruptive germination of sclerotia followed by mycelial growth occurs when *S. minor* and *S. sclerotiorum* initiate disease infection to plants. Suppression of these activities should reduce the capacity of these pathogens to cause disease in the field. Laboratory test of the fungicidal effect against *S. sclerotiorum* and *S. minor* is a simple approach for understanding a small sector of chemical system on Sclerotinia foliage blight disease of cucumber and pepper plants caused by these pathogenic fungi. The inhibitory effect of four fungicides against the *S. sclerotiorum* and *S. minor* fungal growth was evaluated under *in vitro* conditions [26]. They recorded that the reduction in the growth of both tested fungi was correlated to the increasing concentrations of Rizolex-T, Topsin-M or Ridomil Gold in medium. They added that data also indicate that the two tested fungi varied in their sensitivity against the fungicides used. Complete growth inhibition was recorded for both *S. sclerotiorum* and *S. minor* at 100ppm of Topsin-M and Ri-domil Gold, while the same effect was recorded at 400ppm and 200ppm of Rizolex-T for both species of Sclerotinia, respectively. Moreover, the growth of *S. minor* showed more positive response to Rizolex-T, Topsin-M and Ridomil Gold concentrations, than the growth of *S. sclerotiorum*. In this concern, significant decrease in mycelial growth and sclerotial production, of the fungus of *S. sclerotiorum*, with higher concentration of fungicide was recorded [9]. Mycelial growth was most sensitive to Benlate, Ridomil gold, Tecto-60 and Topsin-M at both the concentrations of 100 and 200ppm. Similar results concerning the inhibitor effect of Rizolex-T and Topsin-M at different concentrations against soil-borne fungi, *R. solani* and *F. oxysporum* were also [40,41,42]. Furthermore, Rizolex-T could control root rot of cowpea caused by *R. solani* and *F. solani* when used as seed dressing or soil drench in solarized soil [43]. Also, lupine seeds dressing with recommended dose of Rizolex-T resulted in significant reduction in root rot incidence caused by *R. solani*, *S. rolfsii* and *F. solani* [44].

V. SENSITIVITY AGAINST ANTAGONISTIC AGENTS

The inhibitory effect of the abovementioned fungal and bacterial antagonistic agents (*T. harzianum, T. viride, T. hamatum, Bacillus subtilis, Pseudomonas fluorescens*) and commercial biocides products, *e.g*. Plant guard, Biocure-F, Biocure- D, Rhizo-N against the linear growth of *S. sclerotiorum* and *S. minor* pathogenic fungi using the modified dual culture technique [45] was studied by [26] *in vitro*. Their obtained results revealed that all tested agents could drastically reduce the linear growth of root pathogenic fungi. The antagonistic fungi (*Trichoderma* spp.) showed superior inhibitory effect against the growth of pathogenic fungi compared with bacterial isolates (*B. subtilis* & *P. fluorescence*). No significant differences between the tested antagonistic microorganisms and commercial biocides were observed. The antagonistic fungi had a greater significant effect on the retardation of growth compared with the bacterial agents. The antagonistic bacteria also showed significant differences among the tested isolates. The highest inhibitory effect was recorded for *B. subtilis*. The commercial biocides products, *e.g*. Plant guard, Biocure-F, Biocure-D, Rhizo-N showed lesser effect in this regard. In the present study, the fungal and bacterial antagonists evaluated demonstrated an inhibitor effect against *Sclerotinia* spp. under *in vitro* conditions. These results are also confirmed by several researchers [12,14,16,19,46]. Also, it was recorded that an antagonistic bacterium strain BSH-4 (*Bacillus pumilus*) was isolated from rhizosphere soil of cucumber in the greenhouse. Dual culture test indicated that the strain was highly inhibitory to *Sclerotinia sclerotiorum* [47]. In this regard it was reported that microorganisms can play an enormously important role in plant disease control. As naturally occurring resident antagonists, they can be managed or exploited to achieve the desired results. Biological control with introduced microorganisms presents challenges not encountered with naturally occurring parasitic organisms. When used, natural enemies do not depend on the target pest as a host, which is the case with most antagonists of plant pathogens. Recent research on the use of introduced antagonists has to be considered in two ways: (i) antagonists, like the pathogen, should be adapted to the host plant to be protected, in addition to their ability to inhibit or compete with the target pathogen; and (ii) antagonists that can be applied directly and precisely to the infection court need not be able to spread or even persist in the environment. These two considerations for biological control sparked the current and much more successful effort with plant associated microorganisms as agents introduced for biological control of plant pathogens [49].

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VI. EFFECT OF ESSENTIAL OILS ON ANTAGONISTIC ABILITY

Application of essential oil is a very attractive method for controlling plant diseases. Essential oils and their components are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multipurpose functional uses [49]. Recently, interest has been shown in combining microbial bio-control agents with other chemical components to increase their activity against plant pathogens. Essential oils have been used successfully in combination with a variety of treatments, such as antibacterial agents, mild heat and salt compounds [50]. Also among other chemical products, aromatic plants possess essential oils resulting from secondary metabolism. These substances have a great economic potential, especially in the food, pharmaceutical and perfumery sectors. Thus, the number of studies on the chemical composition and biological properties of these oils, as well as the taxonomic, environmental and cultivation factors that lead to variation in their quantity and quality, has been increasing [51]. Three essential oils at concentrations of 0.25, 0.5, and 1.0% for their effect on the antagonistic ability of tested microorganisms against *S. sclerotiorum* and *S. minor* fungal radial growth, were evaluated [52] through *in vitro* tests. Pure-grade of the essential oils, *i.e.* cinnamon (*Cinna-momum verum* L.), clove (*Syzygium aromaticum* L.) and thyme (*Thymus vulgaris* L.) were used. The *in vitro* antagonistic ability of tested microorganisms was preformed [45] as abovementioned procedures. In this work [52], stated that, in general all tested essential oils enhanced the antagonistic ability of fungal bio-agents. In this regards, Cinnamon oil have superior enhancement for increasing the antagonistic efficacy of *T. harzianum*, and *T. viride* followed by Thyme and Clove oils at all used concentrations. Data also showed that the antagonistic efficacy was increased as the concentration increased of tested essential oils. Similar trend was also observed for the antagonistic ability of bacteria bio-agents, that Cinnamon oil have more enhancing effect on the antagonistic ability than that of both Thyme and Clove oils at all tested concentrations. Moreover, it was observed that the pathogenic fungal growth fluctuated at the same used concentration depending on the antagonist. In this regard, it was observed that the pathogenic fungal growth reduced between 77.7-83.3% and 72.2-72.2% [52] when grown in dual culture against *B. subtilis* and *P. fluorescens*, respectively in growth media supplemented with Cinnamon oil at concentration of 1% comparing with control treatment which ranged as 24.4-21.1% and 13.3-13.3%, in respective order to the two bio-agents. In this concern, essential oils are promising alternative compounds which have an inhibitory activity on the growth of pathogens. It is possible that essential oils could be used in plant disease control as the main or as adjuvant antimicrobial compounds. Recently, there has been considerable demand for the discovery of new natural antimicrobials [53]. Plant products with antimicrobial properties have notably obtained attention as possible applicants in order to prevent bacterial and fungal growth. This means that essential oils could be used as alternative anti-bacterial and antifungal treatments [21]. It is evident from reviews [54,55] that some plant extracts and essential oils exhibited antifungal properties. Essential oil of *Juniperus communis* may be applicable against a range of damping-off diseases [56]. Furthermore, studies on the effectiveness of nine essential oils to control the growth of mycotoxins producing moulds revealed that, clove, cinnamon and oregano were able to prevent the growth of *Aspergillus parasiticus* and *Fusarium moniliforme* [57].

VII. EFFECT OF LANT RESISTANCE INDUCERS ON ANTAGONISTIC ABILITY

Recently, the concern of many investigations for the use of abiotic factors for induction of plant resistance against several diseases have been reported. Potassium salts (K_2HPO_4 or KNO_3) as a chemical agent for induction of plant resistance had great attention in many of these reports [22,23]. In this concern, spraying cucumber plants with K_2HPO_4 induced resistance against downy and powdery mildews and increased fruit yield per plant under commercial greenhouse conditions [58,59,60,61,62]. Furthermore, there has been considerable interest in the use of sodium bicarbonate, potassium bicarbonate and potassium phosphate for controlling various fungal diseases in plants [58,59,63,64,65]. Spraying plants with either sodium bicarbonate or potassium bicarbonate solution provided good control of several plant diseases [62,65,66]. Also, calcium chloride had been recorded to have antifungal effect and provided broad-spectrum protection against the postharvest pathogens of *P. expansum* and *B. cinerea* in apple [67]. Moreover, under *in vitro* and *in vivo* conditions, all tested concentrations of hydrogen peroxide, calcium chloride and chitosan were able to reduce the linear growth and spore germination of *B. cinerea; R. stolonifer; P. digitatum* and *P. italicum* and complete inhibition of linear growth and spore germination was obtained with concentrations of 1.5 and 2.0% of all treatments [68]. Humic acid (HA) is a heterogeneous mixture of many compounds with generally similar chemical properties it performs various functions in the soil and on plant growth. Humic substances have been early recorded to have appositive effect against plant pathogens [25,69]. Also, many studies [24,70] showed that Fulvic acid (FA) have a greater effect on cells biological activities than humic acids (HA) compounds. The addition of 500 mgl^{-1} of humic acids on the growth medium completely eliminated the inhibition of *P. ultimum* by *R. radiobacter* [33]. Furthermore, *In vitro*, humic acid at 15.0% (v/v) reduced significantly the radical growth and spore germination of *Fusarium solani* the causal agent of dry root

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rot [71]. Similar records confirm the obtained results in the study carried out by [52]. They evaluated the effect of the chemical plant inducers on the antagonistic ability of different microorganisms against the growth of the *S. sclerotiorum* and *S. minor* fungi *in vitro*. The tested antagonistic microorganisms were *T. harzianum*, *T. viride*, *B. subtilis* and *P. fluorescens* The tested chemical inducers were Sodium bicarbonate and Potassium bicarbonate, Calcium chloride and potassium mono -hydrogen phosphate (at concentrations of 1.0, 2.0 and 4.0%, v/v), humic and folic acids (mixture,1:1) at concentrations of 0.2, 0.4 and 0.6%, (v/v). The plant inducers were added individually to conical flasks containing sterilized PDA before its solidifying to obtain the proposed concentrations. Twenty ml of amended media were poured into 9 cm diameter Petri dishes, and another set of untreated PDA plates was used as control. The antagonistic ability of tested microorganisms was preformed following the method described by [45] as abovementioned procedures. Results obtained by [52] showed the efficacy of some plant inducers on the antagonistic ability of *T. harzianum* and *T. viride* against *S. sclerotiorum* and *S. minor*. Presented data showed that concentrations used of calcium chloride enhance significantly the antagonistic ability of *T. harzianum* and *T. viride*, respectively. Only, *T. harzianum*, exhibit complete reduction (100%) for the two pathogenic fungal growth at concentration of 2 and 4% comparing with the range of 48.8-50% in medium free of calcium chloride. Similar observation was recorded by [52] with *T. viride* at concentration of 4% comparing with the range of 31.1-33.3% in medium free of calcium chloride. Similar trend was also observed with the mixture of Humic and Folic acids whereas concentrations of 0.4 and 0.6% increased the antagonistic ability of *T. harzianum* and *T. viride* causing complete reduction (100%) of pathogenic fungal growth comparing to (48.8-50.0%) and (31.1-33.3%) respectively in medium free of Humic and Folic acids mixture. Also, the obtained data revealed that Potassium bicarbonate have significant enhancement effect on the antagonistic ability of tested fungi followed by Sodium bicarbonate and Potassium mono-hydrogen phosphate, respectively. Data also revealed that Potassium bicarbonate concentrations showed lesser effect on the antagonistic ability of tested fungal bio-agents. In this concern it was observed that the reduction in linear growth of pathogenic fungi against antagonists fluctuated referring to concentration used for each pathogenic fungus. Enhancement of antagonistic ability of tested fungal bio-agents could be arranged in descending order as *T. harzianum* and *T. viride*. Furthermore, [52] stated that the effect of Calcium chloride on the antagonistic ability of bacteria *B. subtilis* and *P. fluorescens* against *S. sclerotiorum* and *S. minor* was also evaluated *in vitro*. They added that the antagonistic efficacy of antagonistic bacteria against pathogenic fungal growth increased as the concentration of calcium chloride increased in growth media. In this regard, all tested pathogenic fungi showed high sensitivity against the antagonist *B. subtilis* where their growth reduced by 81.1, 83.3% in the presence of calcium chloride at 4% in growth media. Another feature at a lesser extent was observed with *P. fluorescence* that they could reduced the fungal growth of *S. sclerotiorum* and *S. minor* to 75.5, 72.2%, respectively at the same concentration of 4%. They also added that concerning Humic and folic acids mixture the obtained results showed that the efficacy of the antagonistic ability of tested bio-agents increased in parallel with increasing the concentrations of Humic and folic acids mixture reaching its maximum at the highest concentration.

In this regards, highest inhibition in pathogenic fungal growth of *S. sclerotiorum* and *S. minor*, was observed when they grown against the antagonistic bacteria *B. subtilis* and *P. fluorescens* in the presence of 0.6% of Humic and folic acids mixture in the growth media. Regarding the effect of different concentrations of Potassium, Sodium bicarbonates and Potassium mono-hydrogen phosphate on the antagonistic ability of bacteria against pathogenic the obtained data revealed that different concentrations of chemicals used have positive effect for enhancing the efficacy of antagonistic ability of tested bio-agents [52]. Potassium mono-hydrogen phosphate showed superior effect in this regard followed by Potassium bicarbonates and Sodium bicarbonate, respectively. Data also showed that the minimum growth records for *S. sclerotiorum* and *S. minor* was 36mm (60.0%) and 39mm (56.6%) against *B. subtilis* on medium supplemented with 4% of Potassium mono-hydrogen phosphate comparing, in respective order, with 58mm (35.5%) and 66mm (26.6%) in medium free of chemical tested.

VIII. EFFECT OF PLANT EXTRACTS ON ANTAGONISTIC ABILITY

The use of plants or plant materials as fungicides is of a great importance and needs more attention [72] and various plant products like gum, oil, resins etc. are used as fungicidal compounds [73,74]. In this regards, several workers studied the effect of various plants extracts against pathogenic microorganisms. Extracts of *Eupartrium cannabinum* completely inhibited the mycelia growth of *Pythium de-baryanum*, *R. solani* and *S. rolfsii* [75]. Some plant extracts and essential oils exhibited antifungal properties [55]. The effect of plant extracts, on the antagonistic ability against pathogenic fungal growth was estimated by several investigators under *in vitro* conditions. The efficacy of some plant extracts on the antagonistic ability of fungal and bacterial bio-agents against pathogenic fungi under laboratory conditions was studied [52]. They evaluated the inhibitory effect of three extracted leaves, *i.e*. Halfa Bar (*Cymbopogon Proximus*); Ginger (*Zingiber officinale*) and Bay laurel (*Laurus nobilis*) *in vitro* test. Their obtained data

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revealed that all plant extract at different concentrates could enhance the antagonistic ability of tested fugal bio-agents. In this concern, the antagonistic efficacy of *T. harzianum* against pathogenic fungi increased in respective order in media free of plant extracts at the highest concentration (4%) of Halfa Bar, Ginger and Bay laurel, respectively. Meanwhile, the antagonistic efficacy of *T. viride* against pathogenic fungi also increased in media free of plant extracts at the highest concentration of tested extracts. Furthermore, the effect of some plant extracts on the antagonistic ability of bacteria and yeast against pathogenic fungi was also evaluated *in vitro.*

On the other hand, the efficacy of plant extracts on the antagonistic ability of bacterial bio-agents was also evaluated [52]. Their data revealed that all tested concentrations of plant extracts could enhance the efficacy of antagonistic ability of bacterial bio-agents. Data also, showed that no announced increase in the antagonistic ability was observed. Significant increase in antagonistic ability was observed only with the highest concentration of tested plant extracts. Moreover, no significant differences were observed between the tested Halfa Bar, Ginger and Bay laurel plant extracts at all used concentrations.

Several reports indicated that plant spices containing carvacrol, eugenol and thymol (phenolic compounds) had the highest antibacterial performances [76]. Alkaloids, flavonoids, isoflavonoids, tanins, cumarins, glycosides, terpens and phenolic compounds were synthesized by plants as secondary metabolites [51]. In agricultural studies, these compounds have broad-spectrum activities against fungi, nematodes, and insects [77, 78, and 79]. Plant spices offer a promising alternative for food safety and plant protection. Inhibitory activity of spices and their derivatives on the growth of bacteria, yeasts, fungi and microbial toxin synthesis was reported [53, 80]. Moreover, antifungal activity of spices and their derivatives were studied by viable cells count, mycelial growth and mycotoxin synthesis. However, there is little information on spices and their derivatives action on/in a fungal cell. In general, inhibitory action of natural products on moulds involves cytoplasm granulation, cytoplasmic membrane rupture and inactivation and/or inhibition of intercellular and extracellular enzymes. These biological events could take place separately or concomitantly culminating with mycelial growth inhibition [81].

IX. CONCLUSION

The wide and indiscriminate use of chemical fungicides has been the cause of the appearance of resistant plant pathogens, leading to the occurrence of serious diseases. Due to this, there is an increasing interest to obtain alternative antimicrobial agents for using in plant disease control systems. One of the main procedures used in the research of biologically active substances is a systematic screening for the interaction between microorganisms and plant products. The influence of some plant products and chemical inducers on the efficacy of the antagonistic ability of the bio-control agents received attention in the present study. For this reason, laboratory tests for the biological antagonistic ability of the bio-agents, *i.e. Trichoderma harzianum, T. viride, Bacillus subtilis* and *Pseudomonas fluorescens* against the soil-borne plant pathogens *Sclerotinia sclerotiorum* and *S. minor* are considered a simple approach for understanding as s small sector of biological systems in disease control under stress of different combined antifungal factors. Hence, the objective of this study was to determine if essential oils, plant resistance inducers and plant extracts could provide enhancing effect to the antagonistic ability of fungal and bacterial against pathogenic fungi *S. sclerotiorum* and *S. minor*. The obtained results in the present study has shown the potential of tested materials as effective inhibitors when combined factors with antagonistic bio-agents. Considering their attribute and broad spectrum activities, successful development of such compounds in combination with bio-agents as antifungal could promise success in multipurpose biorational alternatives to conventional fungicides for the management of plant diseases. The promising obtained results in the present study could suggest that greater reductions in the incidence of *Sclerotinia* spp. foliar blights probably will require an integrated disease management approach, employing useful cultural, chemical and biological control methods as one component.

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