Journal of
Plant Pathology & Microbiology

Abdel-Kader et al., J Plant Pathol Microb 2013, 4:9
DOI: 10.4172/2157-7471.1000200

Review Article Open Access

Biological and Chemical Resistance Inducers Approaches for Controlling Foliar Diseases of Some Vegetables under Protected Cultivation System

Abdel-Kader MM*, El-Mougy NS and Lashin SM

Department of Plant Pathology, National Research Centre, Giza, 12622, Egypt

Abstract

Different alternative approaches of antagonistic bio-agents, *Trichoderma harzianum, T. Viride, Bacillus subtilis, Pseudomonas flourescens* and *Saccharomyces cerevisiae* and plant resistance inducers, Calcium chloride, Potassium monohydrogen phosphate, Potassium bicarbonate, Saccharin, Ascorbic acid, Chitosan and Humic & Folic acid (mixture) in addition to thyme oil were applied as plant spray to evaluate their efficacy against vegetables foliar diseases incidence was carried under open greenhouse conditions.

The recorded foliar diseases, i.e. Powdery, Downy mildews of Cucumber, Cantaloupe and Pepper as well as Early, Late blights of Tomato were significantly reduced at all treatments either alone or in combinations comparing with untreated plants. Application with either *T. harzianum* or *B. subtilis* showed significant reduction in diseases incidence comparing with the other applied bio-agents. The other bio-agent treatments, *T. viride, P. fluorescens* and *S. cerevisiae* recorded moderate reduction in this concern. Under artificial infestation the most significant reduction in foliar diseases incidence and severity of tested vegetables were recorded in combined treatments of combined chemical inducers and *S. cerevisiae*, i.e. (Chitosan+Thyme oil); (Chitosan+Saccharin); (Chitosan+Calcium chloride+*S. cerevisiae*); (Chitosan+Potassium monohydrogen phosphate); (Saccharin+Potassium monohydrogen phosphate); (Humic & folic+Thyme oil) and (Chitosan+*S. cerevisiae*) comparing with other applied treatments as well as untreated control.

The present review summarizes studies aimed to evaluate different control measures of fungicides alternatives approachs, e.g. some plant resistance inducers, essential oils and bio-control agents on the foliar diseases incidence of some vegetables under greenhouse and plastic house conditions. This work was carried out during a project supported by the Science and Technology Development Fund (STDF), Egypt.

Keywords: Vegetables; Bio-agents; Chemical inducers; Foliar diseases

Introduction

Greenhouse operations have been viewed by many as a means to diversify and improve farm income. These new growers are trying to take advantage of vegetable crop production which, in general, has higher return per unit area than agronomic crops. In addition, greenhouse vegetable growers have recently been able to benefit from the increased demand for specialty horticultural crops, a category which encompasses greenhouse vegetables [1,2]. Vegetables, i.e. Sweet pepper, Tomato, Cucumber, Cantaloupe which grown under protected cultivation system are represent Egypt's largest national crops export. Plant diseases represent an important role of growing vegetables under protected cultivation system and considered a serious problem and limiting factor for their production. Vegetables production in Egypt, is threatened by a large number of fungal, bacterial and viral diseases as well as by pests and insects vectors viral diseases which cause considerable loss in Egyptian quantitative and qualitative vegetables yield. Overall, most vegetable crops under protected cultivation system suffered losses due to the favorable conditions for most diseases as high humidity and temperature. The most prevalent fungal and bacterial diseases on vegetables continue to plague growers because they are difficult to prevent, and remedial disease management tools are generally suppressive at best. Moreover, plant diseases continue to cause severe damage to most agricultural crops during different stages of plant growth resulting in heavy losses of both yield and quality. Considering the regularity with which most serious diseases of crop plants appear in an area year after year, the rapid spread of most plant disease, and the difficulty of curing a disease after it has begun to develop.

So, it is easy to understand why almost all control methods are aimed at protection plant from becoming diseased rather than at curing them after they have become diseased. As a matter of fact, few infectious plant diseases can be satisfactory controlled in the field by therapeutic means, although certain diseases can be cured under managed environmental conditions. Furthermore, some of environmental factors i.e. direct sun, ultraviolet light, and the constant changes in temperature also play an important role in overall natural pest control often obtained under open conditions. The greenhouse literally could protects its plants and consequently their respective pests from these environmental conditions [1,3,4].

The regional problem being addressed is the development of a scientifically and environmentally sound scheme for the sustainable production of pathogen-free vegetables to meet domestic requirements and export market opportunities for Egypt. From an historical perspective, to date, production of pathogen-free vegetable seedlings and commercial vegetable crops have been heavily dependent upon the use (often overuse) of pesticides and other inputs which cause

***Corresponding author:** Mokhtar M. Abdel-Kader, Department of Plant Pathology, National Research Centre, Egypt, E-mail: mokh_nrc@yahoo.com

Received August 12, 2013; **Accepted** September 26, 2013; **Published** September 30, 2013

Citation: Abdel-Kader MM, El-Mougy NS, Lashin SM (2013) Biological and Chemical Resistance Inducers Approaches for Controlling Foliar Diseases of Some Vegetables under Protected Cultivation System. J Plant Pathol Microb 4: 200. doi:10.4172/2157-7471.1000200

important adverse effects on the environment. Experts agree that this is especially true in the case of vegetables. On the other hand, there are serious legal and liability situations that a greenhouse grower could be faced with when using toxic substances in enclosed areas. Under greenhouse conditions the use of pesticides could cause highly volatile toxic vapors, as well as respond differently in numerous other chemical and physical fashions, and thus are considered a potential hazard to the grower [5-7].

Vegetables growing under protected cultivation system occupied about 24.000 Hectares in Egypt [8]. The most recorded serious foliar diseases attacked vegetables growing in plastic houses are Powdery and Downy mildews as well as Early and Late blights causing large yield losses in a number of crops [9-11].

The exceptionally long survival of vegetables disease pathogens along with the susceptibility of different vegetables cultivars rendered the issue of control of a paramount importance. 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. Therefore, there is an increasing interest to obtain alternative antimicrobial agents for using in plant disease control systems.

As control measures, some cultural practices, *i.e*. crop rotation, sowing date, fertilizers and irrigation were tried by many investigators but they failed to provide satisfactory control against plant pathogens. Regarding chemical control, the phytopathogens are not sensitive to selective pesticides as most pesticides have its own mode of action. While many plant diseases have been controlled, in part, by use of chemical pesticides, therefore alternatives to the use of chemicals would be of value. Plant associated microbes used as bio-control agents can play a role in reducing losses to such diseases. Biological control should play a major role in reducing the population of plant pathogens with management systems. Biological control of plant pathogens with antagonists has been the subject of numerous reviews and several books. Promising antagonists that may be useful for controlling soil-borne plant pathogens were also reported. Researchers from all over the world paid more attention towards the development of alternative methods which are, by definition, safe in the environment, non-toxic to humans and animals and are rapidly biodegradable, one such strategy is the use of bio-control agents to control fungal plant diseases. There are several antagonistic microorganisms recorded to be promising candidate and effective for controlling wide range of plant pathogens. Among these bio-agents, species of the genus *Trichoderma* [12], strains *Pseudomonas fluorescens* PCL1751 and *P. putida* PCL1760 [13], and *Bacillus* spp. [14].

A successful disease-control program could involve generally requires the application of several control measures [15]. Generally, IPM is regarded as the use of environmentally safe practices to reduce the disease incidence and development or use of multiple control tactics integrated into a single pest control strategy [16]. The use of different approach in plant disease control, i.e., bio-control agents, plant extracts and natural compounds were used as an IPM program to control powdery mildew of greenhouse crops [15,17].

Among other control measures the use of compound that induce a systemic plant resistance which were successfully used against several plant diseases incidence affected either plant root or shoot systems. There are several fungicides alternatives commercially used for induction of plant resistance against viruses, bacteria, and fungal infections. In this concern, salts have been previously studied as foliar applied control agents for powdery mildews on various horticultural crops, e.g. cucumber, grape, nectarine, mango, and rose can be reduced through foliar applications of phosphate and potassium salts [18-21]. Also, reducing the severity of powdery mildew on *E. japonica* and pumpkin is achieved by application with potassium bicarbonate [22,23]. Sodium bicarbonate or calcium chloride significantly reduced the early blight incidence and severity of tomato plants in pot experiment under artificial infestation with pathogenic fungus [24]. They added that Calcium chloride proved higher efficacy for reducing both disease incidence and severity than that of sodium bicarbonate when applied either alone or combined with *Saccharomyces cerevisiae*. Induction of local and systemic resistance to powdery mildew in cucumber using phosphate foliar applications [21,25]. Moreover, using calcium administered as plant nutrient has been reported to be important for resistance to bacterial wilt [26] resistance in tomato.

The use of pesticides alternatives such as induces resistance compounds and bio-agent microbes to control plant disease and enhance crop production are desirable for the following reasons: 1) chemical pesticides are being severely restricted; 2) the public is demanding reduced pesticide use; and 3) pesticides alternatives are effectively and more safely used.

Biological Control Approaches

A greenhouse study [27] was conducted to evaluate the efficacy of plant spraying with different bio-control agents against incidence of some vegetables foliar diseases. Powdery and Downy mildews of Cucumber, Cantaloupe, Pepper and Early, Late blights of Tomato are subject for this study. Foliar spraying with tested bio-agents, i.e. *T. harzianum, T. viride, B. subtilis, P. fluorescens* and *S. cerevisiae* were applied twice with two weeks intervals starting one week from transplanting. Foliar artificial infestations with *Sphaerotheca fuliginea* and *Peronoplasmopara cubensis* the causal fungi of Cucumber, Cantaloupe and Pepper Powdery and Downy mildews, respectively as well as *Alternaria solani* the causal fungi of Tomato Late blight was carried out as spraying of pathogen suspension, one week after the second antagonistic foliar application. *Phytophthora infestanse* the causal of Tomato Early blight was applied as soil drench through soil irrigation with fungal suspension.

As a result of this experiment [27] all applied bio-agents significantly reduced the recorded foliar diseases comparing with untreated control. Moreover, application with either *T. harzianum* or *B. subtilis* had superior reduction effect on diseases incidence comparing with the other applied bio-agents. Moderate reduction in Powdery mildew incidence was achieved by applied other bio-agent treatments, *T. viride*, *P. fluorescens* and *S. cerevisiae*. Similar trend was also observed for Downy mildew, Early and Late blights.

In this concern several works which conducted with bio-control applications against plant diseases control reported the efficacy of biological control of vegetable foliar diseases by different microorganisms. Biological control using natural products or antagonistic microorganisms in order to reduce the pesticides use and decline of their residues on agriculture products proved to be successful for controlling various plant pathogens in many countries [28]. It is still not expensive and is easy in application however it can serve as the best control measure under restricted conditions.

In addition, its application is safe, un-hazardous for human and avoids environmental pollution [29]. In this regard, Paulitz and Bélanger [30] reviewed that during the past ten years, over 80 bio-control products have been marketed worldwide. A large percentage of these have been developed for greenhouse crops. Products containing *Trichoderma, Ampelomyces quisqualis, Bacillus, Ulocladium* and *Pseudomonas* are being developed to control the primary foliar diseases, Botrytis and powdery mildew in greenhouses could predominate over chemical pesticides.

In the study of Abdel-Kader et al. [27] spraying vegetables, Cucumber, Cantaloupe, tomato and Pepper with the bio-agents, *T. harzianum, T. viride, B. subtilis, P. fluorescens* and *S. cerevisiae* was effectively able to reduce the foliar diseases comparing with untreated control. These results were in agreement with several previous reports. A field experiment [31] was conducted at Rahuri, Maharashtra, India to investigate the efficacy of the culture filtrates of *T. viride*, *T. harzianum*, *T. hamatum, T. longiflorum* and *T. koningii* and the fungicide carbendazim against the powdery mildew (*Leveillula taurica*) of guar. They found that all treatments recorded beneficial effects on growth parameters and disease control. Also, Elad [32] recorded that *Trichoderma harzianum* which can be regarded as a model to demonstrate bio-control under commercial conditions and the mechanisms involved. He added that this bio-control agent controls the foliar pathogens, *Botrytis cinerea* (gray mold), *Pseudoperonospora cubensis* (downy mildew), *Sclerotinia sclerotiorum* (foliar blight) and *Sphaerotheca fusca* (powdery mildew) in cucumber under commercial greenhouse conditions.

Similarly, Deore et al. [33] evaluated culture filtrates of *T. viride*, *T. harzianum*, *T. hamatum, T. Iongiforum* and *T. lconlngll* for the management of powdery mildew of Cluster bean plants caused by *LevelIlula taurlca.* They found that culture filtrates of *Trichoderma* spp. either alone or in combination were found effective against powdery mildew. Also, *Trichoderma harzianum* T39 (TRICHODEX) spray reduced powdery mildew severity caused by *Sphaerotheca fusca* on greenhouse cucumber by up to 97% [34,35]. Furthermore, Deore et al. [36] stated that foliar application of *Pseudomonas fluorescens* combined with a half of the recommended dose of azoxystrobin is of practical significance, since an application of fungicide alone requires three to four further following sprays for an effective control of downy and powdery mildews of cucumber. Also, Yazici et al. [37] suggest that *Paenibacillus macerans, Serratia plymuthica, Bacillus coagulans, Serratia marcescens-GC, Bacillus pumilis* and *Pantoea agglomerans* the bacterial isolates as foliar spray reduced the disease severity of early blight significantly when compared with control and have a good potential to be used as biocontrol agents of *A. solani* in tomato.

The mode of action of biocontrol mechanisms was explained by many investigators. The potential of *Bacillus* sp. to synthesize a wide variety of metabolites with antifungal activity is known and in recent years it has been a subject of experiments [38,39]. Most of these substances belong to lipopeptides, especially from surfactin, iturin and fengicin classes. Not so much is known about the mechanism of antifungal activity of these substances produced by *Bacillus* sp. Some of them (iturin and surfactin) are able to modify bacterial surface hydrophobicity and, consequently, microbial adhesion to surfaces to mycelium [38]. Also, antibiotics of the iturin group were found to act upon the sterol present in the cytoplasmic membrane of the fungi [39,40]. A local isolate of *S. cerevisiae* was reported to have a reduction potential against radial growth of pathogenic fungi *Macrophomina phaseolina* and *Fusarium solani*, the cause of root rot diseases in tomatoes and eggplants [41].

Integrated Fungicidal Alternatives and Yeast (*S. cerevisiae*) Approaches

The use of plant resistance inducers in combination with bio-agents was subjected to evaluation in many reports. In this regards, an interesting alternative to fungicide application for plant disease control involves the use of some organic and inorganic salts with antimicrobial properties generally used in food processing and preservation. Selected organic and inorganic salts are active antimicrobial agents and have been widely used in the food industry.

Many of these salts are effective against a range of micro-organisms; most of them have low mammalian toxicity and therefore have potential for postharvest disease control. Salt treatments can inhibit plant pathogens or suppress mycotoxin production [42,43]. Also, Ashour [44] found that from field experiments, that spraying cantaloupe plants three times with fungicides in alternation with another three sprays with any of calcium chloride or salicylic acid resulted in significant reduction in the disease severity with significant increase in the fruit yield when compared with unsprayed (check) plants. Furthermore, Sodium and ammonium bicarbonate were shown to inhibit fungal pathogens of fruits, field crops, vegetables, and ornamentals [22]. Also, sodium bicar-bonate applied at room temperature at 2 to 4% reduced blue mold caused by *Penicillium italicum* in Citrus fruits [45].

Moreover, beneficial effect of two food additives, ammonium molybdate and sodium bicarbonate on antagonistic yeasts for control of brown rot in sweet cherry was evaluated [46]. They found that application of additives improved bio-control of brown rot on sweet cherry fruit under various storage conditions. It is postulated that the enhancement of disease control is directly because of the inhibitory effects of additives on pathogen growth, and indirectly because of the relatively little influence of additives on the growth of antagonistic yeasts.

The efficacy of foliar spray with some plant resistance inducers and/or bio-agent (*S. cerevisiae*) treatments under open greenhouse conditions was studied [47]. They reported that all applied spray treatments of tested plant resistance inducers individually or in combination with *S. cerevisiae* were able to reduce foliar diseases incidence of artificially infested with the diseases causal agents grown Cucumber, Cantaloupe, Tomato and Pepper grown in pots under open greenhouse conditions comparing with untreated check control. Also, data revealed that the most significant reduction in diseases incidence of Cucumber, Cantaloupe and Pepper Powdery, Downy mildews and Tomato Early and Late blights in combined treatments of chemical inducers and *S. cerevisiae* were (Chitosan+Thyme oil); (Chitosan+Saccharin); (Chitosan+Calcium chloride+*S. cerevisiae*); (Chitosan+Potassium monohydrogen phosphate); (Saccharin+Potassium monohydrogen phosphate); (Humic & folic+Thyme oil) and (Chitosan+*S. cerevisiae*) comparing with other applied treatments as well as untreated control.

Under plastic houses conditions, in another study [48] announced highly significant effect was observed at combined treatments, [Potassium bicarbonate+Thyme oil] and [Chitosan+Thyme oil] followed by treatments, [Calcium chloride+*S. cerevisiae*] and individual treatment [Potassium monohydrogen phosphate], respectively. Moreover, these applied treatments, could suppress both downy and powdery mildews incidence up to 60 days of growing cucumber plants under natural infestation comparing with untreated control which recorded diseases incidence.

In another study [49] under plastic houses conditions, they recorded that the applied treatments caused significant effect on foliar diseases resulted in high reduction in both Early and Late blights diseases incidence comparing with check control. They added that the highest reduction in disease incidence calculated as 100, 100, 85.7% and 100, 100, 76.7% for Early and Late blights at the combined treatment, [Potassium bicarbonate+Thyme oil] followed by treatment, [Chitosan+ Thyme oil] at tomato growth periods of 60, 90 and 120 day, respectively. The other applied treatments, [Calcium chloride+*S. cerevisiae*] followed by individual treatment [Potassium monohydrogen phosphate] showed similar effect on the diseases incidence throughout the growth period in a lesser effect that at 120 days of plant growth they recorded the lowest reduction in diseases incidence calculated as (68.9, 50.8%); (70.9, 49.6%) for Early and Late blights, in respective order.

In the reviewed studies [27,47-50] it was reported that application of different formula of biological and/or chemical plant resistance inducers as foliar spray resulted in reduction of Powdery, Downy mildews of Cucumber, Cantaloupe and Pepper as well as tomato Early blight and Late blight diseases incidence and severity which reflected positively in plant stand and its healthy growth as well as its produced yield. The obtained results showed high efficacy of application plant resistance inducers Calcium chloride, Potassium bicarbonate, Potassium monohydrogen phosphate, Thyme oil, Chitosan, *S. cerevisiae* as foliar spray treatment during the growing growth season against foliar diseases of tomato plants. In this regards, many investigators studied the influence of various salts on microorganisms. These studies focuses on finding compounds that are safe to human and environment. An alternative to pesticide application is that, it may be possible to utilize a scheme of inducible plant defense which may provide protection against a broad spectrum of disease-causing pathogenic microorganisms. Biological control using microbial antagonists has shown potential as an alternative for natural control of plant pathogens instead of synthetic chemical fungicides [51]. Another alternative control method is given by enhancing natural resistance of plants towards the pathogen. Compounds which are triggering plant's own defense mechanisms are termed elicitors.

Similar reports conducted the efficacy of chemical inducers application individual or combined with bio-agents against plant pathogens were cited in literature.

It was reported [52] that *in vitro* activity of sodium carbonate salt against germinated or un-germinated spores of *P. italicum* and *P. digitatum* was higher than that of bicarbonate salt, although their efficacy was similar *in vivo*. They suggested that the original toxicity of the treating sodium bicarbonate solution to the spores is altered by inter-actions in wounds with constituents of the rind and the relatively high pH of these solutions has been proposed to be the mode of action. The use of sodium bicarbonate alone to control postharvest decays of fruit has its limitations [45], but it can be combined with other alternative treatments to synthetic fungicides, resulting in the control that is superior to individual treatments alone. For example, sodium bicarbonate was successfully used in combination with bacterial and yeasts bio-control agents to enhance control of postharvest decays on citrus, pome, and stone fruits [52-54]. These reports are clearly demonstrated in the present study and show that the application of *S. cerevisiae* enhanced the control of foliar vegetables diseases when combined with either sodium bicarbonate or calcium chloride spray. In this concern inoculums used in the present work was more effective where it reached (2×10^4 cfu/mL). Many researchers have shown that calcium plays an important role in the inhibition of postharvest decay of fruits [55,56], and in enhancing the efficacy of postharvest bio-control agents [57,58]. Postharvest calcium treatment of apples provided broad-spectrum protection against the postharvest pathogens of *Penicillium expansum* and *Botrytis cinerea* [59]. The addition of $CaCl_2$ (2% w/v) to the formulation of the yeast bio-control agent, *Candida oleophila,* enhanced the ability of this yeast to protect apples against postharvest decay [58]. The efficacy of controlling grey mould and blue mould rots in apples was enhanced when *Trichosporon* sp. was applied in the presence of $CaCl_2$ (2% w/v) in an aqueous suspension [60]. In a study of Abdel-Kader et al. [48] it was found that calcium chloride application either alone or combined with other plant resistance inducers and/or *S. cerevisiae* was able to reduce foliar disease incidence. Applications of Ca contributed to reducing the intensity of white mold on dry beans, but did not affect bean yield in a soil with high amounts of this nutrient. In the USA, found that foliar-applied Ca was found to enhance both disease control and dry bean yield [61]. Gulya and Miller [62] suggested that Ca may be a nutritional supplement that increases plant resistance to white mold. Nutritional effect is particularly noticeable in the case of Ca compounds with high water solubility, like $CaCl_2$. It has been suggested that plants resistant to *S. sclerotiorum* have higher Ca levels than susceptible ones. Also, Paula Júnior et al. [63] recorded that incidence and severity of white mold on dry bean were significantly reduced with application of calcium chloride and calcium silicate.

Saccharin and Chitosan [48] had shown effective influence for reducing foliar diseases when sprayed either alone or in combined treatment. These records were confirmed with previous reports. Saccharin is a metabolite of probenazole and has also been found to induce resistance in a number of host–pathogen systems, including tobacco mosaic virus [64], barley *Blumeria graminis* f. sp. *hordei* [65] and broad bean *Uromyces fabae* [66]. In barley, saccharin did not induce defenses directly, but rather primed plants against infection by the powdery mildew fungus [65]. Also, Saccharin treatment provided systemic protection of barley against the leaf scald pathogen *R. secalis.* This agrees with previous work which showed protection of barley against powdery mildew [65], broad bean against rust [66] and tobacco against TMV [64]. These data also confirm earlier studies which showed that saccharin treatment primed CAD activity in barley against subsequent challenge with powdery mildew [65]. As for Chiosan, in recent years, the importance of chitosaccha-rides as plant growth promoting and disease control agents has been emphasized [67,68]. CHN (β-1-4 linked *N*-gluco-samine) has been shown to induce defense responses in different plants [69,70]. Chitosan oligomers was found to induce defense responses in grapevine leaves, as evidenced by an accumulation of stilbene phytoalexins, *trans*- and *cis*-resveratrol, ε-viniferins, and piceids, and a stimulation of chitinase and *β*-1,3-glucanase activities [68]. They added that the combination of Chitosan and $CuSO_4$ increased phytoalexin production. This elicitor capacity of Chitosan and/or $CuSO_4$ appeared to be associated with an induced protection of grapevine leaves against gray mold and downy mildew diseases. Furthermore, Hadwiger and McBride [71] recorded that chitosan/copper complex retained a presence on the potato leaf surface late and early blights where infection by either *A. solani* or *P. infestans* occurs. Also, Chitosan enhanced the accumulation of pathogenesis related-proteins such as ss-1,3-glucanase, chninase and PR14 in treated and upper untreated tomato leaves [72]. Their studies with chitosan against tomato late blight suggested thatchitosan displays dual effects: (a) direct interference in developmental stages of *P. infestans* and (b) by lesion formation, leading to disease resistance mechanisms. Moreover, several workers suggested two different mechanisms of chitosan molecule and target microorganism interaction: the first is the adsorption of chitosans to cell walls leading to the cell wall covering, membrane disruption and cell leakage; the second is the penetration of chitosans into living cells leading to the inhibition of various enzymes and interference with the synthesis of mRNA and proteins [73-75].

On the other hand, Humic acid is a suspension, based on potassium humates, which can be applied successfully in many areas of plant production as a plant growth stimulant or soil conditioner for enhancing natural resistance against plant diseases and pests, stimulation plant growth through increased cell division, as well as optimized uptake of nutrients and water [76]. Furthermore, Abd-El-Kareem [77] reported that bean plants treated with humic acid induced resistance against root rot and Alternaria leaf spot. The role of Humic acid in overcoming the harmful effects of chocolate spot and rust diseases in faba bean plant may be due to the increase in chitinase activity [77] and stimulation plant growth through increased cell division, as well as optimized uptake of nutrients and water [78,79] also, regulate hormone level, improve plant growth and enhance stress tolerance [80].

Humic acid is a suspension, based on potassium humates, which can be applied successfully in many areas of plant production as a plant growth stimulant or soil conditioner for enhancing natural resistance against plant diseases and pests [76] which consequently increase yield of plant. Foliar application of Humic acid consistently enhanced antioxidants such as á-tocopherol, â-carotene, superoxide dis-mutases, and ascorbic acid concentrations in turf grass species [59]. These antioxidant may play a role in the regulation of plant development, flowering and chilling of disease resistance [8,82,83]. The effect of spray application with Ascorbic acid as antioxidant against foliar diseases was also observed in the study of Abdel-Kader et al. [48]. They reported that under artificial infestation with Powdery and Downy mildews pathogens, Cucumber; Cantaloupe and Pepper plants expressed disease incidence as 54.4 and 46.6% in medium, meanwhile when sprayed with Ascorbic acid only or combined with *S. cerevisiae* revealed diseases infection estimated as 28.8, 25.5%, and 23.3 and 20.0%, in respective order. Similar effect was also recorded concerning Tomato Early and Late blight which recorded as 18.3 and 14.9% in Ascorbic acid only or combined with *S. cerevisiae* treatments comparing with 33.3% in control treatment, respectively.

Essential oils as natural alternatives that are user friendly and demonstrate low toxicity to humans are desirable to be tested either alone or in combination in the present work. Thyme oil applied alone or in combination showed effective reduction in foliar diseases incidence more than 50%, moreover complete reduction in Tomato Early and Late blights was recorded in Thyme oil combined with either *S. cerevisiae* or Humic & Folic acid (a mixture). In this regards, several investigators reported the antifungal effect of essential oils. Thyme and Egyptian geranium oils are considered antimycotic natural compounds may be useful for inhibition of mold fungi on wood in service or during storage of building materials [84]. Moreover, Momol et al. [85] had the first report on the use of Thymol for controlling a plant disease under field conditions, which indicated that this compound pro-vided effective control of bacterial wilt on susceptible tomato cultivars. Also, Thymol has been reported to have fungicidal activities and fumigation with thymol has been used for control of postharvest fungal diseases [86,87]. Modes of action of the antibacterial property of thymol appeared to include disruption of bacterial cell membrane integrity by altering protein reactions [88,89].

In general, biological control using microbial antagonists has shown potential as an alternative for natural control of plant pathogens instead of synthetic chemical fungicides [51]. The mode of action of antagonistic yeasts may be competition for space and nutrients [51,90], production of cell-wall lytic enzymes [91], and induction of host resistance [92,93]. In large-scale tests, the use of biological control often needed to be combined with low doses of synthetic fungicides to obtain a level of disease control equivalent to synthetic fungicides [94]. In order to completely eliminate the use of synthetic fungicides, more environmentally friendly and harmless compound (s) should be explored to improve the activity of the antagonist. Selected chemicals such as calcium chloride [58,60], chitosan [95], in combination with biological control agents have been demonstrated to give beneficial effects on control of fruit decay. The control is not total, but in combination with bioactive additives it is possible to obtain the efficacy of the chemical standard [96,97]. Another alternative control method is given by enhancing natural resistance of plants towards the pathogen. Compounds which are triggering plant's own defense mechanisms are termed elicitors. Moreover, the bio-control activity of *S. cerevisiae* against pathogenic fungi might have possibly resulted from mycoparasitism [98], secretion of lytic enzymes such as β-1,3 glucanase [99] and production of antibiotics [100].

Conclusion

The preliminarily recorded results in the present review showed that foliar spray with bio-agents can have a considerable activity against Powdery and Downy mildews of Cucumber, Cantaloupe, Pepper and Early, Late blights of Tomato under open greenhouse conditions. Their non chemical properties suggest potentials for commercial formulation and application which could suggested as a broad spectrum use against foliar pathogens under plastic greenhouses conditions.

The present findings demonstrate that plant resistance inducers in combination with bio-agents may have important implications for the future use of antagonistic microorganisms on a commercial scale for controlling such diseases especially under protected cultivation regime.

Hence, the objective of this study was to determine if plant resistance inducers and essential oils could provide enhancement effect against downy and powdery mildews as well as early and late blights. Considering their attribute and broad-spectrum activities, successful development of such compounds as antifungal would not only provide a potent tool for control of vegetables foliar diseases, but also could promise success in multipurpose biorational alternatives to conventional fungicides for the management of other plant diseases.

Acknowledgment

This work was supported financially by the Science and Technology Development Fund (STDF), Egypt, Grant No. 1059.

References

1. Bartok JW Jr (1984) Greenhouse startup and expansion. Greenhouse Manager 3: 57-78.
2. Hochmuth GJ, Homas WD, Sweat MS, Hochmuth RC (2001) Financial Considerations - Florida Greenhouse Vegetable Production Handbook, In the Florida Greenhouse Vegetable Production Handbook. Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.
3. Anonymous (1991) "Engineering practices-commercial greenhouse design and layout". In Engineering Standards. Amer Soc Agric Engineers, St. Joseph, MI.
4. http://www.umass.edu/umext/floriculture
5. Chase AR (1995) Compendium of Ornamental Foliage Plant Diseases. APS Press, St. Paul, MN.
6. Daughtrey ML, Wick R, Peterson J (1995) Compendium of Flowering Potted Plant Diseases. The American Phytopathological Society, St. Paul. Minnesota.
7. http://edis.ifas.ufl.edu
8. Anonymous (2009) 'Yearbook of statistics of Ministry of Agriculture.' Agricultural Economical and Statistical Department, Arab Republic of Egypt: Cairo.
9. Kiss L (2003) A review of fungal antagonists of powdery mildews and their potential as biocontrol agents. Pest Manag Sci 59: 475-483.

10. Yan Z, Reddy MS, Ryu CM, McInroy JA, Wilson M, et al. (2002) Induced systemic protection against tomato late blight elicited by plant growth-promoting rhizobacteria. Phytopathology 92: 1329-1333.

11. Jagadeesh KS, Jagadeesh DR (2009) Biological control of early blight of tomato caused by *Alternaria solani* as influenced by different delivery methods of Pseudomonas gladioli B25. Acta Horticulture 808: 327-332.

12. Chet I, Inbar J, Hadar I (1997) Fungal antagonists and mycoparasites. In: The Mycota IV: Environmental and Microbial Relationships. Wicklow DT and Soderstorm B (eds), 165-184.

13. Kamilova F, Validov S, Lugtenberg B (2009) Biological Control of Tomato foot and root rot caused by *Fusarium oxysporum* f.sp. radicis-lycopersici by Pseudomonas bacteria. Acta Hort (ISHS) 808: 317-320.

14. Sharifi Tehrani A, Ramezani M (2003) Biological control of *Fusarium oxysporum*, the causal agent of onion wilt by antagonistic bacteria. Commun Agric Appl Biol Sci 68: 543-547.

15. Dik A, Wubben J, Elad Y, Kohi J, Shtienberg D (2002) Combination of control methods against powdery mildew diseases in glasshouse-grown vegetables and ornamentals. IOBC WPRS bulletin 25: 5-8.

16. Zinkernagel V, Tischner H, Hausladen H, Habermeyer H, Taborsky V, et al. (2002) Practical application of integrated disease management. Plant Prot Sci 38: 212–222.

17. Napier D, Oosthuyse SA (2000) Use of monopotassium phosphate in the integrated control of powdery mildew. Rivista di Frutticoltura e di Ortofloricoltura 62: 57-58.

18. Belanger RR, Dik AJ, Menzies JG (1997) Powdery mildew: Recent advances towards integrated control. In: Boland GS, Kuykendall LD (eds) Plant-microbe interactions and biological control. Marcel Dekker, New York, USA.

19. Reuveni M, Agapov V, Reuveni M, Raviv M (1994) Effects of foliar sprays of phosphates on powdery mildew (*Sphaerotheca pannosa*) of roses. J Phytopathol 142: 331-337.

20. Reuveni M, Agapov V, Reuveni R (1995) Suppression of cucumber powdery mildew (*Sphaerotheca fuliginea*) by foliar sprays of phosphate and potassium salts. Plant Pathol 44: 31-39.

21. Reuveni M, Agapov V, Reuveni M (1996) Controlling powdery mildew caused by *Sphaerotheca fuliginea* in cucumber by foliar sprays of phosphate and potassium salts. Crop Prot 15: 49-53.

22. Ziv O, Zitter TA (1992) Effects of bicarbonates and film forming polymers on cucurbit foliar diseases. Plant Dis 76: 513-517.

23. Ziv O, Hagiladi A (1993) Controlling powdery mildew in Euonymus with polymer coatings and bicarbonate solutions. Hort Sci 28: 124-126.

24. El-Mougy NS, Abdel-Kader MM (2009) Salts application for suppressing potato early blight. Journal of Plant Protection Research 49: 353-361.

25. Mucharromah E, Kuc J (1991) Oxalates and phosphates induce systemic resistance against disease caused by fungi, bacteria and viruses in cucumber. Crop Prot 10: 265-270.

26. Yamazaki H, Hoshima T (1995) Calcium nutrition affects resistance of tomato seedlings to bacterial wilt. Hort Science 30: 91-93.

27. Abdel-Kader MM, El-Mougy NS, Aly MDI, Lashin SM, Abdel-Kareem F (2012a) Greenhouse biological approach for controlling foliar diseases of some vegetables. Advances in Life Sciences 2: 98-103.

28. Papavizas GC, Lumsden RD (1980) Biological control of soilborne fungal propagules. Ann Rev Phytopathol 18: 389-413.

29. Sivan A, Chet I (1989) Degradation of fungal cell walls by lytic enzymes of *Trichoderma harzianum*. J Gen Microbiol 135: 675-682.

30. Paulitz TC, Bélanger RR (2001) Biological control in greenhouse systems. Annu Rev Phytopathol 39: 103-133.

31. Deore PB, Sawant DM (2001) Management of guar powdery mildew by *Trichoderma* spp. culture filtrates. Journal of Maharashtra Agricultural Universities 25: 253-254.

32. Elad Y (2000) Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. Crop Protection 19: 709-714.

33. Deore PB, Sawant DM, Ilhe BM (2004) Comparative efficacy of *Trichoderma* spp. For the control of powdery mildew of cluster bean. Indian J Agric Res 38: 212-216.

34. Elad Y, Rav David D, Levi T, Kapat A, Kirshner B, et al. (1998) *Trichoderma harzianum* T39 mechanisms of biocontrol of foliar pathogens. In: Lyr H, Russell PE, Dehne HW, Sisler HD (Eds.), Modern Fungicides and Antifungal CompoundsII. Intercept Ltd, Andover, Hampshire, UK.

35. Elad Y, Kirshner B, Yehuda N, Sztejnberg A (1998) Management of powdery mildew and gray mold of cucumber by *Trichoderma harzianum* T39 and *Ampelomyces quisqualis* AQ10. Bio Control 43: 241-251.

36. Anand T, Chandrasekaran A, Kuttalam S, Raguchander T, Samiyappan R (2009) Management of Cucumber (*Cucumis sativus* L.) Mildews through Azoxystrobin-Tolerant *Pseudomonas fluorescens*. J Agric Sci Technol 11: 211-226.

37. Yazici S, Yanar Y, Karaman I (2011) Evaluation of bacteria for biological control of early blight disease of tomato. African Journal of Biotechnology 10: 1573-1577.

38. Ahimou F, Jacques P, Deleu M (2000) Surfactin and iturin A effects on *Bacillus subtilis* surface hydrophobicity. Enzyme Microb Technol 27: 749-754.

39. Moyne AL, Shelby R, Cleveland TE, Tuzun S (2001) Bacillomycin D: an iturin with antifungal activity against *Aspergillus flavus*. J Appl Microbiol 90: 622-629.

40. Worthington PA (1988) Antibiotics with antifungal and antibacterial activity against plant diseases. Nat Prod Rep 5: 47-50.

41. Attyia SH, Youssry AA (2001) Application of *Saccharomyces cerevisiae* as a biocontrol agent against some diseases of Solanaceae caused by *Macrophomina phaseolina* and *Fusarium solani*. Egypt J Biol 3: 79-87.

42. Roinestad KS, Montville TJ, Rosen JD (1993) Inhibition of trichothecene biosynthesis in *Fusarium tricinctum* by sodium bicarbonate. J Agric Food Chem 41: 2344-2346.

43. Singh SN, Chand L (1993) Inhibition of aflatoxin production by garlic extract and sodium bicarbonate. Crop Res 6: 149-154.

44. Ashour AMA (2009) Effect of Application of some Systemic Fungicides and Resistance Inducing Chemicals on Management of Cantaloupe Powdery Mildew Disease. Egypt J Phytopathol 37: 1-8.

45. Palou L, Smilanick JL, Usall J, Vinas I (2001) Control of postharvest blue and green molds of oranges by hot water, sodium carbonate and sodium bicarbonate. Plant Dis 85: 371-376.

46. Qin GZ, Tian SP, Xu Y, Chan ZL, Li BQ (2006) Combination of antagonistic yeasts with two food additives for control of brown rot caused by *Monilinia fructicola* on sweet cherry fruit. J Appl Microbiol 100: 508-515.

47. Abdel-Kader MM, El-Mougy NS, Lashin SM (2012) Efficacy of Different Plant Resistance Inducers against Downy and Powdery mildew Diseases of Pepper under Plastic Houses Conditions. Journal of Applied Sciences Research 8: 3415-3423.

48. Abdel-Kader MM, El-Mougy NS, Aly MDE, Lashin SM (2012) Integration of Biological and Fungicidal Alternatives for Controlling Foliar Diseases of Vegetables Under Greenhouse Conditions. International Journal of Agriculture and Forestry 2: 38-48.

49. El-Mougy NS, Abdel-Kader MM, Aly MDE (2012) Application of plant Resistance Inducers for Controlling Early and Late Blights of Tomato under plastic houses conditions. Journal of Applied Sciences Research 8: 3406-3414.

50. Abdel-Kader MM, El-Mougy NS, Embaby EI (2012) Resistance Inducers Treatments against Downy and Powdery mildews of Cucumber under Commercial Plastic Houses Conditions. Australian Journal of Basic and Applied Sciences 6: 249-259.

51. Droby S, Chalutz E, Wilson CL, Wisniewski M (1989) Characterization of the biocontrol activity of *Debaryomyces hansenii* in the control of *Penicillium digitatum* of grapefruit. Can J Microbiol 35: 794-800.

52. Smilanick JL, Margosan DA, Mlikota F, Usall J, Michael IF (1999) Control of citrus green mold by carbonate and bicarbonate salts and the influence of commercial postharvest practices on their efficacy. Plant Dis 83: 139-145.

53. Wisniewski M, Wilson C, El Ghaouth A, Droby S (2001) Increasing the ability of the biocontrol product, Aspire, to control postharvest diseases of apple and peach with the use of additives. Biological control of fungal and bacterial plant pathogens. IOBC/WPRS Bull 24: 157-160.

54. Janisiewicz WJ, Peterson DL (2005) Experimental Bin drenching system for testing biocontrol agents to control postharvest decay of apples. Plant Dis 89: 487-490.

55. Conway WS, Sams CE (1985) Influence of fruit maturity on the effect of postharvest calcium treatment on decay of Golden Delicious apples. Plant Disease 69: 42-44.

56. Conway WS, Sams CE, McGuire RG, Kelman A (1992) Calcium treatment of apples and potatoes to reduce postharvest decay. Plant Disease 76: 329-333.

57. Conway WS, Abbott JA, Bruton BD (1991) Postharvest calcium treatment of apple fruit to provide broad-spectrum protection against postharvest pathogens. Plant Disease 75: 620-622.

58. Wisniewski M, Droby S, Chalutz E, Eilam Y (1995) Effects of Ca2+ and Mg2+ on *Botryris cinerea* and *Penicillium expansum in vitro* and on the biocontrol activity of Candida oleophila. Plant Pathology 44: 1016-1024.

59. Saftner RA, Conway WS, Sams CE (1997) Effects of some polyamine biosynthesis inhibitors and calcium chloride on *in vitro* growth and decay development in apples caused by *Botrytis cinerea* and *Penicillium expansum*. J Am Soci Hort Sci 122: 380-385.

60. Tian SP, Fan Q, Xu Y, Jiang AL (2002) Effects of calcium chloride on biocontrol activity of yeast antagonists against the postharvest fungal pathogen *Rizopus stolonifer*. Plant Pathol 51: 352-358.

61. Venette JR (1998) Calcium micronutrient may reduce white mold of dry beans. Proceedings of the Sclerotinia Workshop, Minnesota ND, In-Service Extension Worksho, Fargo.

62. Gulya TJ, Miller JF (2007) Advances in the development of sunflower germplasm with resistance to both Sclerotinia stalk rot and head rot, and the possible role of calcium in Sclerotinia resistance. Proceedings of the 2007 National Sclerotinia Initiative Annual Meeting. Minneapolis.

63. Paula Júnior TJ, Vieira RF, Teixeira H, Carneiro JS (2009) Foliar application of calcium chloride and calcium silicate decreases white mold intensity on dry beans. Tropical Plant Pathology 34: 171-174.

64. Siegrist J, Muhlenbeck S, Buchenauer H (1988) B-aminobutyric acid-mediated enhancement of resistance in tobacco to tobacco mosaic virus depends on the accumulation of salicylic acid. Physiol Mol Plant Pathol 53: 223-228.

65. Boyle C, Walters DR (2006) Saccharin-induced resistance to powdery mildew in barley: effects on growth and phenylpropanoid metabolism. Plant Pathol 55: 82-91.

66. Boyle C, Walters D (2005) Induction of systemic protection against rust infection in broad bean by saccharin: effects on plant growth and development. New Phytol 167: 607-612.

67. Trotel-Aziz P, Couderchet M, Vernet G, Aziz A (2006) Chitosan stimulates defense reactions in grapevine leaves and inhibits development of Botrytis cinerea. Eur J Plant Pathol 114: 405-413.

68. Aziz A, Trotel-Aziz P, Dhuicq L, Jeandet P, Couderchet M, et al. (2006) Chitosan oligomers and copper sulfate induce grapevine defense reactions and resistance to gray mold and downy mildew. Phytopathology 96: 1188-1194.

69. Chang MM, Hadwiger LA, Horovitz D (1992) Molecular characterization of a pea beta-1,3-glucanase induced by *Fusarium solani* and chitosan challenge. Plant Mol Biol 20: 609-618.

70. Hadwiger LA, Ogawa T, Kuyama H (1994) Chitosan polymer sizes effective in inducing phytoalexin accumulation and fungal suppression are verified with synthesized oligomers. Mol Plant Microbe Interact 7: 531-533.

71. Hadwiger LA, McBride PO (2006) Low-level copper plus chitosan applications provide protection against late blight of potato. Online. Plant Health Progress.

72. Atia MMM, Buchenauer H, Aly AZ, Abou-Ziad MI (2005) Antifungal activity of chitosan against *Phytophthora infestans* and activation of defense mechanisms in tomato to late blight. Biological agriculture & horticulture 23: 175-197.

73. Chircov SN (2002) The antiviral activity of chitosan (review). Applied Biochemistry and Microbiology 38: 1-8.

74. Rabea EI, Badawy ME, Stevens CV, Smagghe G, Steurbaut W (2003) Chitosan as antimicrobial agent: applications and mode of action. Biomacromolecules 4: 1457-1465.

75. Zheng L, Zhu J (2003) Study on antimicrobial activity of chitosan with different molecular weights. Carbohydrate Polymer 54: 527-530.

76. Scheuerell SJ, Mahaffee WH (2006) Variability Associated with Suppression of Gray Mold (*Botrytis cinerea*) on Geranium by Foliar Applications of Non-aerated and Aerated Compost Teas. Plant Dis 90: 1201-1208.

77. Abd-El-Kareem F (2007) Induced resistance in bean plants against root rot and Alternaria leaf spot diseases using biotic and abiotic inducers under field conditions. Research J of Agricultural and Biological Science 3: 767-774.

78. Atiyeh RM, Lee S, Edwards CA, Arancon NQ, Metzger JD (2002) The influence of humic acids derived from earthworm-processed organic wastes on plant growth. Bioresour Technol 84: 7-14.

79. Chen Y, De Nobili M, Aviad T (2004) Stimulatory effect of humic substances on plant growth. In "Soil organic matter in sustainable agriculture". Boca Raton, FL.

80. Piccolo A, Nardi S, Concheri G (1992) Structural characteristics of humic substances as regulated to nitrate uptake and growth regulation in plant systems. Soil Biochem 24: 373-380.

81. Ziadi S, Barbedette S, Godard JF, Monoti C, Corre LED, et al. (2001) Production of pathogenesis related protein in the cauliflower (Brassica oleacea var. botrytis) downy mildew (*Peronospora parasistica*) pathosystem treated with acidbenzolar-5-methyl. Plant Pathol 50: 579-586.

82. Dmitriev A, Tena M, Jorrin J (2003) Systemic acquired resistance in sunflower (*Helianthus annuus* L.). Tsitol Genet 37: 9-15.

83. Achuo EA, Audenaert K, Meziana H, Hofte M (2004) The salicylic acid dependent defence pathway is effective against different fungi in tomato and tobacco. Plant Pathol 53: 65-72.

84. Yang VW, Clausen CA (2007) Antifungal effect of essential oils on southern yellow pine. International Biodeterioration & Biodegradation 59: 302-306.

85. Momol MT, Olson SM, Pradhanang PM, Jones JB (2005) Evaluation of thymol as biofumigant for control of bacterial wilt of tomato under field conditions. Plant Dis 89: 497-500.

86. Liu WT, Chu CL, Zhou T (2002) Thymol and acetic acid vapors reduce postharvest brown rot of apricots and plums. Hort Science 37: 151-156.

87. Paster N, Menasherov M, Ravid U, Juven B (1995) Antifungal activity of oregano and thyme essential oils applied as fumigants against fungi attacking stored grain. J Food Prot 58: 81-85.

88. Juven BJ, Kanner J, Schved F, Weisslowicz H (1994) Factors that interact with the antibacterial action of thyme essential oil and its active constituents. J Appl Bacteriol 76: 626-631.

89. Walsh SE, Maillard JY, Russell AD, Catrenich CE, Charbonneau DL, et al. (2003) Activity and mechanisms of action of selected biocidal agents on Gram-positive and -negative bacteria. J Appl Microbiol 94: 240-247.

90. Janisiewicz WJ, Tworkoski TJ, Sharer C (2000) Characterizing the mechanism of biological control of postharvest diseases on fruits with a simple method to study competition for nutrients. Phytopathology 90: 1196-1200.

91. El-Ghaouth A, Wilson CL, Wisniewski M (1998) Ultrastructural and Cytochemical Aspects of the Biological Control of *Botrytis cinerea* by *Candida saitoana* in Apple Fruit. Phytopathology 88: 282-291.

92. Arras G (1996) Mode of action of an isolate of Candida famata in biological control of *Penicillium digitatum* in orange fruits. Postharvest Biol Technol 8: 191-198.

93. Droby S, Vinokur V, Weiss B, Cohen L, Daus A, et al. (2002) Induction of Resistance to *Penicillium digitatum* in Grapefruit by the Yeast Biocontrol Agent *Candida oleophila*. Phytopathology 92: 393-399.

94. Droby S, Wisniewski M, El-Ghaouth A, Wilson C (2003) Influence of food additives on the control of postharvest rots of apple and peach and efficacy of the yeast based biocontrol product Aspire. Postharvest Biol Technol 27: 127-135.

95. El-Ghaouth A, Smilanick JL, Brown GE, Ippolito A, Wisniewski M, et al. (2000) Applications of *Candida saitoana* and glycolchitosan for the control of postharvest diseases of apple and citrus fruit under semi-commercial conditions. Plant Dis 84: 243-248.

96. Lima G, Castoria R, Spina AM, De Curtis F (2005) Improvement of biocontrol yeast activity against postharvest pathogens: recent experiences. Acta Hortic 682: 2035-2040.

97. Conway WS, Leverentz B, Janisiewicz WJ, Saftner RA, Camp MJ (2005)

Improving biocontrol using antagonist mixtures with heat and/or sodium bicarbonate to control postharvest decay of apple fruit. Postharvest Biol. Technol. 36: 3: 235-244.

98. Hajlaoui MR, Belanger RR (1993) Antagonism of the yeast like phylloplane fungus *Sporothrix flocculosa* against *Erysiphe graminis* var tritici. Biocontrol Sci Technol 11: 427-434.

99. Punja ZK (1997) Comparative efficacy of bacteria, fungi and yeasts as biological control agents for disease of vegetable crops. Can. J Plant Pathol 19: 35-323.

100. Benyagoub M, Rhlid RB, Belanger RR (1996) Purification and characterization of new fatty acids with antibiotic activity produced by *Sporothrix flocculosa*. J Chem Ecol 22: 405-413.