The Efficacy of Some Fungicides Alternatives on the Antagonistic Ability of Some Bio-Control Agents in vitro (Review Article)

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ABSTRACT

The effect of plant resistance inducers, i.e. Potassium mono hydrogen phosphate salt and Calcium chloride, mixture of Humic & Folic acids (AF); some essential oils, Cinnamon, Clove and Thyme as well as plant extracts Halfa Bar, Ginger and Bay laurel on the antagonistic ability of three isolates of Trichoderma spp. as well as one isolate of each of Bacillus subtilis, Pseudomonas fluorescens and Saccaromyces cerevisiae against the linear growth of the root pathogenic fungi was evaluated In vitro. Obtained data revealed that concentrations used of calcium chloride enhance significantly the antagonistic ability of T. harzianum, T. viride and T. hamatum, respectively. Similar trend was also observed with the mixture of Humic and Folic acids. The antagonists T. harzianum and T. viride resulted in reduction of pathogenic fungal growth to 100% at concentration of 0.2% and at 0.4%, respectively. Also, the obtained results revealed that Potassium mono-hydrogen phosphate have significant enhancement effect on the antagonistic ability of tested fungi followed by Sodium bicarbonate and Potassium bicarbonate. As for essential oils, Cinnamon oil have superior enhancement for increasing the antagonistic efficacy of T. harzianum, T. viride and T. hamatum followed by Clove and Thyme oils at all used concentrations. Data also showed that the antagonistic efficacy was increased as the concentration increased of tested essential oils. All plant extract at different concentrates could enhance the antagonistic ability of tested fugal bio-agents in respective order when halfa bar, ginger and bay laurel added to growth media. As for bacterial and yeast antagonists, the obtained results revealed that the antagonistic efficacy of B. subtilis, P. fluorescens and S. cerevisiae against pathogenic fungal growth increased as the concentration of either calcium chloride, or the mixture of Humic and folic acids increased in growth media. Also, different concentrations of Potassium, Sodium bicarbonates and Potassium mono-hydrogen phosphate have positive effect for enhancing the efficacy of antagonistic ability of tested bio-agents. Moreover, in general Thyme oil had more enhancing effect on the antagonistic ability than that of both Cinnamon and Clove oils at all tested concentrations. As for plant extracts, all tested concentrations could enhance the efficacy of antagonistic ability of bacterial and yeast bio-agents. The present review summarizes studies aimed to evaluate different control measures of fungicides alternatives approaches, e.g. some plant resistance inducers, essential oils and plant extracts on the antagonistic ability of some fungal, bacterial and yeast bio-agents in vitro. This work was carried out during a project supported by the Science and Technology Development Fund (STDF), Egypt, grant No. 1059.

Key words: Antagonistic ability, antagonistic bacteria and yeast, Chemical plant resistance inducers, Essential oils, fungal bio-agents, Plant extracts, Root pathogenic fungi, soil borne pathogenic fungi.

Introduction

Plant diseases caused by soil-borne pathogens play an important role in the destruction of natural resources in agriculture. Root rot disease, caused by soil-borne pathogenic fungi including Pythium spp., Rhizoctonia spp., and Fusarium spp. Sclerotinia spp. and other pathogenic fungi cause widespread, serious economic loss both in greenhouse and field production systems under conditions favorable for disease development. Chemical pesticides have been extensively used for control fungal plant disease but their employment favored the selection of fungicides resistant strains as well as negative effect on non-target organisms and environment (Benitez et al., 2004).

Plant pathogenic fungi are ubiquitous in intensive agricultural areas and are extensively controlled by using a large number of inorganic and organic chemical fungicides. No doubt these chemicals are effective in crop protection; however, they exhibit negative environmental and economic aspects, such as pollution of the soil–water system, a specificity and selection of resistant phytopathogenic populations, which lead to soil quality deterioration, rather than high costs of production (Iacomi-Vasilescu et al., 2004). It therefore appears that an urgent investigation of possible alternative, environmentally safe, bioactive natural organic molecules able to
control phytopathogenic fungi in soil is needed. Application of biological control using antagonistic microorganisms has proved to be successful for controlling various plant diseases (Sivan, 1987).

In this respect, the development of alternative methods for plant pathogens is of great interest not only for scientists but also for agriculture. Biological control agents are risk free both for environment and non-target organisms, and could reduce the use of chemical products. Most bio-control agents (BAs) have varied performance in different environmental conditions. Some of this variability has been attributed to differences in physical and chemical properties found in natural environments where bio-control agents are applied (Thomashow and Weller, 1996; Duffy et al., 1997). The growth medium used to produce these agents, has a profound effect on them and their products. The accurate incorporation of nutrients has improved the biomass production of BAs, but unexpectedly did not enhance (Slininger et al., 1996) or even decreased the bio-control efficacy (Moënne-Loccoz et al., 1999). Recognition of the environmental factors that regulate the growth and bio-control efficacy of antagonist bacteria is an essential step towards advancing the level and reliability of their bio-control potential (Duffy and Defago, 1999). Commercial production of disease-suppressive strains of bacteria such as P. fluorescens and B. subtilis as bio-control agents in postharvest diseases requires low cost and high biomass production while maintaining their bio-control efficacy (Costa et al., 2001). A successful disease-control program could involve just a single practice, but the long term reduction of disease losses generally requires the application of several control measures. 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.

The present review focuses on cited reports concerning recorded compounds that are safe to humans and the environment as well as could enhance the antagonistic ability of introduced fungal bio-agents to the soil. Several in vitro studies conducted with the efficacy of some chemical plant resistance inducers, essential oils and plant extracts on the antagonistic ability of some bio-agents, i.e. Trichoderma harzianum, T. viride, T. hamatum, Pseudomonas fluorescens, Bacillus subtilis and the yeast Saccharomyces cerevisiae are included in present review.

(A) Effect of some chemical plant resistance inducers on antagonistic ability:

- **Fungal bio-agents:**

Many investigations concerned with the use of abiotic factors for induction of plant resistance against several diseases. The effect of chemical plant resistance inducers on the antagonistic ability of the fungal antagonistic agents against the linear growth of the root pathogenic fungi was evaluated in vitro (Abdel-Kader et al., 2012a). They tested the antagonistic ability of Trichoderma harzianum, T. Viride and T. hamatum. These antagonistic fungi were isolated from cucumber, cantaloupe, tomato and pepper. The introduction of these agents in the soil through protected cultivation systems and showing root rot disease symptoms (El-Mougy et al., 2011). The results obtained by Abdel-Kader et al., (2012) revealed that concentrations used of calcium chloride enhance significantly the antagonistic ability of T. harzianum, T. viride and T. hamatum, respectively. Only, T. harzianum, exhibit complete reduction (100%) in all pathogenic fungal growth at concentration of 2 and 4% comparing with the range of 47.7-61.1% in medium free of calcium chloride. Similar observation was recorded with T. viride at concentration of 4% comparing with the range of 37.7-46.6% in medium free of calcium chloride. Meanwhile, T. hamatum showed less response to the calcium chloride concentrations as enhancement factor to antagonistic ability. Similarly, calcium chloride at 2% (20 mg/ml) obviously inhibited spore germination and germ tube growth of R. stolonifer PDA medium (Tian et al. 2002). This result further supports the results of Wisniewski et al. (1995), who found that calcium chloride might reduce fungal infection through direct inhibition of spore germination and growth. Maouni et al. (2007) reported that in vitro, calcium chloride significantly reduced pear fruit decay caused by A. alternata and Penicillium expansum when used at 4 and 6%. Furthermore, calcium chloride was reported to suppress growth of the citrus mould pathogen Penicillium digitatum (Droby et al.1997). It is also known that addition of calcium chloride can also improve the activity of biocontrol agents (Droby et al. 1997; McLaughlin et al. 1990).

The efficacy of Potassium, Sodium bicarbonates and Potassium mono-hydrogen phosphate on the antagonistic ability of Trichoderma spp. were also studied (Abdel-Kader et al., 2012a). They reported that Potassium mono-hydrogen phosphate have significant enhancement effect on the antagonistic ability of tested fungi followed by Sodium bicarbonate and Potassium bicarbonate, respectively. Their 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*, *T. viride* and *T. hamatum*.

In this respect, many workers also reported that potassium and sodium bicarbonate showed inhibitory effects against several pathogenic fungi. In addition, bicarbonate salts has been shown to have a considerable inhibitory effect on several fungi and causes the collapse of hyphal walls and shrinkage of conidia (Punj and Grogan, 1982 and Ziv & Zitter, 1992). Hypothesis have been proposed for the inhibitory mechanisms of bicarbonate as hydrogen ion concentration of bicarbonate salts has been shown to have a profound inhibitory effect on sclerotia and conidia germination of *S. rolfsii* and *S. fulginea*, respectively (Punj and Grogan, 1982 and Homma et al., 1981).

As for the mixture of Humic and Folic acids similar trend was also recorded (Abdel-Kader et al., 2012a). Their reported data revealed that concentrations of 0.4 and 0.6% increased the antagonistic ability of tested fungi. They added that antagonists resulted in reduction of pathogenic fungal growth to 100% which was observed at concentration of 0.2% for *T. harzianum* and at 0.4% for *T. viride* comparing to (47.7-61.1%) and (37.7-46.6%) for *T. harzianum* and *T. viride* respectively in medium free of Humic and Folic acids mixture. Also, *T. hamatum* showed lesser response to Humic and Folic acids mixture concentrations that its antagonistic ability was able to reduce pathogenic fungal growth from the range of (44.4-56.6%) in control treatment up to (71.5-82.2) in medium contains 0.6% Humic and Folic acids.

A number of studies have demonstrated that humic acids (HA) and fulvic acids (FA) are able to control plant diseases caused by various soil-borne phytopathogenic fungi (EL-Masry et al., 2002; Loffredo et al., 2007). In contrast, Loffredo et al., (2008) evaluated two concentrations of humic and fulvic (HS) acid on the growth *in vitro* of one plant pathogenic, *Sclerotinia sclerotiorum*, and two antagonistic, *Trichoderma viride* and *T. harzianum*. They found that the presence of HS in the growing medium caused a relevant inhibition of the mycelial growth of *S. sclerotiorum* and a marked stimulation of sclerotial formation that was exhibited as early appearance and numerical increase. On the contrary, the same HS treatments generally did not inhibit the growth of the two *Trichoderma* species.

- **Bacterial and yeast bio-agents:**

The effect of Calcium chloride on the antagonistic ability of bacteria and yeast against some soil-borne pathogenic fungi was evaluated *in vitro* (Abdel-Kader et al., 2012b). They recorded that the antagonistic efficacy of *Bacillus subtilis*, *Pseudomonas fluorescens* and *Saccaromyces cerevisiae* 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 100% in the presence of calcium chloride at 4% in growth media. Another feature at a lesser extent was observed with *P. fluorescens* and *S. cerevisiae* that they could reduced the pathogenic fungal growth to (46.6-77.7%) and (65.5-87.7%), respectively at the same concentration of 4%. Many researchers have shown that calcium plays an important role in the inhibition of postharvest decay of fruits (Conway and Sams, 1985; Conway et al., 1992) and in enhancing the efficacy of postharvest bio-control agents (Conway et al., 1991; Wisniewski et al., 1995).

Postharvest calcium treatment of apples provided broad-spectrum protection against the postharvest pathogens of *Penicillium expansum* and *Botrytis cinerea* (Saftner et al., 1997). The addition of CaCl2 (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 (Wisniewski et al., 1995). The efficacy of controlling grey mould and blue mould rots in apples was enhanced when *Trichosporon* sp., even at a low concentration of 105 CFU mL⁻¹, was applied in the presence of CaCl2 (2% w/v) in an aqueous suspension (Tian et al., 2001). Moreover, Tian et al., (2002) reported that combining CaCl2 with the yeast suspensions significantly enhanced the biocontrol activity of *Colletotrichum guilliermondii* in peaches and *Pichia membranefaciens* in nectarines to Rhizopus rot. The same effects on bio-control activity, achieved by the addition of calcium, were also observed by using the yeasts of *Pichia guilliermondii* (Droby et al., 1993) and *Candida* spp. (Wisniewski et al., 1995) as postharvest bio-control agents. The addition of calcium directly inhibited the number of pathogens and indirectly increased the ability of the yeast to inhibit the growth of pathogens and the resistance of fruit to pathogens (Tian et al., 2001). Pathogen–antagonist interactions inside the wound, such as competition for space and nutrients and the production of lytic enzymes on attachment of the antagonist to the mycelium, are believed to be the main mechanisms of inhibiting diseases by fungal pathogens (Chalutz et al., 1988; Arras, 1996). Competition for nutrients has been frequently cited as a mechanism of bio-control by antagonistic yeasts such as *Pichia, Candida* and *Cryptococcus* spp. (Arras, 1996; Elad, 1996).

Regarding the effect of some plant resistance inducers on the antagonistic ability of bacteria and yeast against pathogenic fungi with different concentrations of Potassium, Sodium bicarbonates and Potassium mono-hydrogen phosphate was evaluated *in vitro*. Abdel-Kader et al., (2012b) reported that Potassium mono-hydrogen phosphate showed superior effect in this regard followed by Potassium bicarbonates and Sodium bicarbonate, respectively. They added that *Pythium* sp. was more sensitive to *B. subtilis*, *P. fluorescens* and *S. cerevisiae*, than
that observed with *S. sclerotiorum* and *M. phaseolina* to the same antagonistic bacteria and yeast tested isolates. Many investigators reported the use of some safely chemicals in combination with bio-agents for enhancing the biological activity. Sodium bicarbonate has been successfully used in combination with bacterial and yeasts bio-control agents to enhance control of postharvest decays on citrus, pome, and stone fruits (Smilanick et al., 1999; Wisniewski et al., 2001). Previous research has indicated that ammonium molybdate and sodium bicarbonate could enhance the efficacy of biological control (Droby et al. 2003; Obagwu and Korsten 2003; Gamage et al. 2004; Yao et al. 2004). Also, Janisiewicz et al., (2005) recorded that the addition of sodium bicarbonate reduced apple decay caused by *Penicillium expansum* when combined with the yeast *Metschnikowia pulcherrima* more than each treatment alone. The inhibitory effect of sodium bicarbonate on microorganisms may be due to a reduction of cell turgor pressure that causes a collapse and shrinkage of hyphae and spores, resulting in fungistasis (Fallik et al., 1997). Droby et al. (2003) observed that bio-control activity by Candida oleophila against *P. expansum* and *B. cinerea* in apples and *Monilinia fructicola* and *Rhizopus stolonifer* in peaches was enhanced by the addition of sodium bicarbonate. Furthermore, 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 (Qin et al., 2006).

The effect of Humic and folic acids as a mixture on the antagonistic ability of bacteria and yeast against some soil-borne pathogenic fungi was evaluated in vitro by several investigators. Abdel-Kader et al., (2012b) reported 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, complete inhibition in pathogenic fungal growth of *A. solani, F. solani*  *F. oxysporum, R. solani, S. rolfisi, M. phaseolina* and *Pythium* sp., when grown against the antagonistic bacteria *B. subtilis* in the presence of 0.6% of Humic and folic acids mixture in the growth media. This observation was also recorded for *S. serevisiae* that its antagonistic ability was increased at concentration of 0.6% of Humic and folic acids mixture to be able to cause complete inhibition in the growth of *F. solani, F. oxysporum, R. solani* and *S. rolfisi*. Also, minimum fungal growth of the pathogen *S. sclerotiorum* and *S. minor* was recorded at the highest concentration 0.6% of Humic and folic acids mixture when grown against *B. subtilis* and *S. serevisiae*. *Pseudomonas fluorescns* showed a lower response to all concentrations of Humic and folic acids mixture for increasing their antagonistic ability. In this regards, Charest et al., (2005) investigated the in vitro influence of humic substances (HS) on the inhibition of *Pythium ultimum* by two compost bacteria, *Rhizobium radiobacter* (*Agrobacterium radiobacter*) and *Pseudomonas aeruginosa*. They found that HS enhanced the microbial antagonism when added to a culture medium. Also, Prakash et al., (2010) reported that bio-solubilization of humic acid enhances plant growth and bio-control efficacy against phyto-pathogenic organism.

**(B) Effect of some essential oils on antagonistic ability:**

- **Fungal bio-agents:**

It is well established that some plants contain compounds able to inhibit the microbial growth (Naqui et al. 1994). These plant compounds can be of different structures and different mode of action when compared with antimicrobials conventionally used to control the microbial growth and survival (Nascimento et al. 2000). Potential antimicrobial properties of plants had been related to their ability to synthesize, by the secondary metabolism, several chemical compounds of relatively complex structures with antimicrobial activity, including alkaloids, flavonoids, isoflavonoids, tannins, cumarins, glycosides, terpenes, phenylpropanes, organic acids (Nychas 1996). The aesthetic, medicinal and antimicrobial properties of plant essential oils have been known since ancient times. Essential oils, i.e. Cinnamon, Clove and Thyme at different concentrations were evaluated for their effect on the antagonistic ability of fungal antagonists against soil-borne pathogenic fungi, in vitro conditions. In this concern (Abdel-Kader et al., 2012a) reported, in general, that all tested essential oils enhanced the antagonistic ability of fungal bio-agents. They added that, Cinnamon oil have superior enhancement for increasing the antagonistic efficacy of *T. harzianum, T. viride* and *T. hamatun* followed by Clove and Thyme oils at all used concentrations. In addition, the antagonistic efficacy was increased as the concentration increased of tested essential oils. Their data also showed that the antagonistic ability of *T. harzianum* against tested pathogenic fungi in control treatment recorded between (48.8-61.1%) and enhanced up to (77.7-88.8%); (68.8-88.8) and (66.6-88.8) at concentration of 1% of Cinnamon, clove and Thyme oils, respectively. Similarly, the antagonistic ability increased from (50.0-61.1%) up to (82.2-88.8%), (75.5-88.8) and (77.7-83.3%) for *T. viride* at 1% of Cinnamon, clove and Thyme oils, respectively. Also, in respective order the antagonistic ability of *T. hamatun* enhanced from (50.0-61.1%) in control up to (54.4-88.8), (73.3-86.6) and (74.4-84.4) at 1% of tested oils.

Numerous studies on the fungicidal and fungistatic activities of essential oils have indicated that many of them have the power to inhibit fungal growth. Thyme oil proved to be extremely effective as a fumigant as well
as a contact fungicide against a range of the economically significant fungi *Alternaria* spp., *Aspergillus* spp., *Botrytis cinerea*, *Erysiphe graminis* (Aleyah and Avice 1997). The information was found in the literature concerning mode of action of essential oils on/in the fungal cell in order to promote fungistatic or fungicide effect. 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 mycelium germination inhibition (Campo et al. 2003).

Also, it is reported that plant lytic enzymes act in the fungal cell wall causing breakage of b-1,3 glycan, b-1,6 glycan and chitin polymers (Brull and Coote 1999). The mode by which microorganisms are inhibited by essential oils and their chemical compounds seem to involve different mechanisms. It has been hypothesized that the inhibition involves phenolic compounds, because these compounds sensitize the phospholipid bilayer of the microbial cytoplasmic membrane causing increased permeability and unavailability of vital intracellular constituents (Juven et al. 1994). Reports indicated that essential oils containing carvacrol, eugenol and thymol (phenolic compounds) had the highest antibacterial performances (Kim et al. 1995).

• **Bacterial and yeast bio-agents:**

  Great interest has been shown in combining microbial bio-control agents with other chemical components to increase their activity against post-harvest pathogens (Droby et al., 1998). Essential oils are considered a promising alternative with many having antimicrobial properties. However, very high concentration is needed when applied to real food systems (Hammer et al., 2003; Ahmet et al., 2005). Also, application of essential oil is a very attractive method for controlling postharvest diseases. Reported data of Abdel-Kader et al., (2012b) showed the effect of some essential oils on the antagonistic ability of bacteria and yeast against pathogenic fungi *in vitro*. Their obtained results revealed that, in general, Thyme oil had more enhancing effect on the antagonistic ability than that of both Cinnamon and Clove oils at all tested concentrations. They added, it was observed that the pathogenic fungal growth fluctuated at the same used concentration depending on the antagonist. In their work, it was observed that the pathogenic fungal growth reduced between 66.6-88.8%, 66.6-83.3% and 61.1-68.8% when grown in dual culture against *B. subtilis*, *P. fluorescens* and *S. cerevisiae*, respectively in growth media supplemented with Thyme oil at concentration of 1%. It was reported that 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 multi-purpose functional use (Ormancey et al., 2001). Essential oils have been used successfully in combination with a variety of treatments, such as antibacterial agents, mild heat and salt compounds (Karatzas et al., 2000). Abd-Alla et al., (2009) reported that the yeast, *Saccharomyces cerevisiae*, *Candida tenuis* and the commercial backing yeast of *Saccharomyces cerevisiae* mixture (CBY) and/or peppermint, melon and rose essential oils were evaluated for their *in vitro* activity against the fungal growth of *Botrytis cinerea*, *Rhizopus stolonifer* and *Alternaria alternate* the causal agents of tomato fruit decay. *Saccharomyces cerevisiae* mixture (CBY) proved itself to have the highest inhibitory effect on the growth of the pathogenic tested fungi followed by the two other yeast isolates *S. cerevisiae* and *C. tenuis*. All the tested concentrations of peppermint oil had not negative effect against the viability of tested yeasts, while significant reduction in the populations of all yeast isolates was observed at melon and rose oils treatments even at the lowest concentration tested. Peppermint oil showed superior inhibitory effect against the growth of tested pathogenic fungi followed by rose and melon oils, respectively. Mixtures of peppermint oil with any of yeast isolate showed high inhibitor effect against the pathogenic fungal growth compared with rose and melon oils mixtures.

  They added that, under storage conditions, application of carnauba wax formula containing either *S. cerevisiae* or *S. cerevisiae* (CBY) combined with peppermint oil (1%) enhanced the efficacy of decay incidence of tomato fruits (gray mould, soft rot and black rot) caused by all the tested pathogenic fungi, *i.e.* *Botrytis cinerea*, *Rhizopus stolonifer* and *Alternaria alternate* during storage reaching up to 100% under artificial inoculation better than each individual component. On the light of their study, it could be concluded that the application of carnauba wax containing 1% peppermint oil combined with *S. cerevisiae* or *S. cerevisiae* (CBY), could control several post-harvest diseases of tomato fruit without affecting tomato fruit quality under storage conditions.

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(C) Effect of some plant extracts on antagonistic ability:

- **Fungal bio-agents:**

  The use of plants or plant materials as fungicides is of a great importance and needs more attention (Bodde 1982) and various plant products like gum, oil, resins etc. are used as fungicidal compounds (Daoud et al. 1990; Dwivedi et al. 1990). Several reports indicated that plant spices containing carvacrol, eugenol and thymol (phenolic compounds) had the highest antibacterial performances (Kim et al. 1995). Alkaloids, flavonoids, isoflavonoids, tanins, cumarins, glycosides, terpenes and phenolic compounds were synthesized by plants as secondary metabolites (Simões et al. 1999). 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. Moreover, the mode by which microorganisms are inhibited by spices and their chemical compounds seem to involve different mechanisms. It was hypothesized that the inhibition involves phenolic compounds, because these compounds sensitize the phospholipid bilayer of the microbial cytoplasmic membrane causing increased permeability and unavailability of vital intracellular constituents (Juen et al. 1994).

  The effect of some plant extracts, *i.e.* Halfa Bar, Ginger and Bay laurel on the antagonistic ability of *Trichoderma* spp. against some soil-borne pathogenic fungi was estimated *in vitro* (Abdel-Kader et al., 2012a). Their reported data revealed that all plant extract at different concentrates could enhance the antagonistic ability of tested fungal bio-agents. In this concern, the antagonistic efficacy of *T. harzianum* against pathogenic fungi increased from the range of (48.8-61.1%) in media free of plant extracts up to (57.7-73.3%), (60.0-73.3%) and (58.8-73.3%) 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 from the range of (50.0-61.1%), in media free of plant extracts up to (61.1-68.8%), (58.8-71.1%) and (57.7-71.1%) at the highest concentration. The recorded increase in antagonistic ability of *T. hamatum* was from (52.2-61.1) up to (61.1-71.1%), (60.0-75.5%) and (62.2-74.4%) in respective order with concentration (4%) of Halfa Bar, Ginger and Bay laurel extracts. Also, Haikal (2007) reported that the efficacy of biological control of cucumber root-rot caused by the pathogenic fungus *F. solani* was improved by using aqueous extract of aerial parts of *A. indica; Z. spina-christi* and *Z. coccineum* in combination with the bio-control agent *T. harzianum*.

- **Bacterial and yeast bio-agents:**

  Many reports conducted with study the effect of some plant extracts on the antagonistic ability of bacteria and yeast against pathogenic fungi was also evaluated *in vitro*. Recorded data of Abdel-Kader et al. (2012b) revealed that all tested concentrations of tested plant extracts could enhance the efficacy of antagonistic ability of bacterial and yeast bio-agents. Their 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 extracts at all used concentrations. In this regard, also many researchers stated that several higher plants and their constituents have been successfully used in plant disease control. The use of antifungal plant extracts as a component of integrated disease management can be prove useful. The present review demonstrated potential efficacy for enhancing the antagonistic ability of tested bacterial and yeast bio-agents against various soil borne pathogenic fungi. Similar reports are cited in literature, Sarovenan and Marimuthu (2003) reported that *A. indica* has improved the biological control of *F. oxysporum* f. sp. *cubense*, the causal agent of wilt disease in banana seedlings, when mixed with the biocontrol agents such as *Pseudomonas fluorescens* or *T. harzianum* and *T. viride*. Also, Radha and Padma (2011) reported that it was clear that the methanol extract of *Majorana hortensis* (majoram) leaves significantly increases the cell viability of the yeast *Saccharomyces cerevisiae*.

**Conclusion:**

Hence, the objective of this review was to determine if plant resistance inducers, essential oils and plant extracts could provide enhancement effect to antagonistic ability against soil-borne pathogenic fungi. Considering their attribute and broad-spectrum activities, successful development of such compounds as antifungal would not only provide a potent tool for control of root rot pathogenic fungi, but also could promise success in multipurpose bio-rational alternatives to conventional fungicides for the management of other plant diseases.

The present results may lead to the conclusion that since application of plant resistance inducers, essential oils and plant extracts is proved to be applicable, safe and cost-effective method for controlling plant diseases. Also, the use of them in agriculture could be a suitable alternative for inclusion in disease control systems and
could act sometimes as main or adjuvant antimicrobial compounds and do not leave a toxic residue in the product. Therefore, this strategy have had favorable results, where the addition of a biological control agent in combination with plant resistance inducers, essential oils and plant extracts could be resulted in increased symptomless plant stand over the biological agent.

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