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Effect of Some Plant Resistance Inducers, Essential Oils and Plant Extracts on Antagonistic Ability of Bacteria and Yeast Bio-agents *In Vitro*

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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 viability of bio-agents, *Bacillus subtilis*, *Pseudomonas fluorescens* and *Saccharomyces cerevisiae* was evaluated *in vitro.* 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 have 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. Further research in this area has the potential to extend the usefulness of natural plant products and other biopesticides in crop production systems.

Key words: soil borne pathogenic fungi, viability of antagonistic bacteria and yeast, plant resistance inducers, some essential oils and plant extracts

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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 soilborne 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). 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 biocontrol 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 biocontrol 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 biocontrol efficacy (Moënne-Loccoz *et al*., 1999). Recognition of the environmental factors that regulate the growth and biocontrol efficacy of antagonist bacteria is an essential step towards advancing the level and reliability of their biocontrol potential (Duffy and Defago, 1999). Commercial production of disease-suppressive strains of bacteria such as *P. fluorescens* and *B. subtilis* as biocontol agents in postharvest diseases requires low cost and high biomass production while maintaining their biocontrol 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.

The best way to ensure success of a disease-management program is to use integrated disease-control measures (Dik *et al*., 2002). 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 (Zinkernagel *et al*., 2002). Also, in order to enhance biocontrol activity of antagonists against fungal pathogens, certain strategies, such as adding calcium salts, carbohydrates, amino acids and other nitrogen compounds to biocontrol treatments, are proposed (Conway, 1982; Conway *et al*., 1987a,b; Janisiewicz *et al*., 1992).

The objectives of the present study were to evaluate the potential effect of some plant resistance inducers, essential oils and aqueous extract of some medicinal plants on the viability of bacterial and yeast bio-control

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agents in order to improve the efficacy of biological control when combined with such fungicides alternatives approach.

Materials and Methods

The effect of chemical plant resistance inducers, some essential oils and plant extracts on the antagonistic ability of the fungal, bacterial and yeast antagonistic agents against the linear growth of the root pathogenic fungi was evaluated *In vitro.* Antagonistic studies of bio-control microorganisms against pathogenic fungi were performed on PDA medium in 9-cm-diameter Petri dishes.

Tested Microorganisms:

The tested soilborne pathogenic fungi were *Alternaria solani, Fusarium solani, F. oxysporum, Rhizoctonia solani, Sclerotium rolfsii, Sclerotinia sclerotiorum, S. minor, Macrophomina phaseolina* and *Pythium* sp.

The tested antagonistic microorganisms (Fungi-Bacteria–Yeast) were *Trichoderma harzianum*, *T. Viride*, *T. hamatum*, *Bacillus subtilis*, *Pseudomonas fluorescens* and *Saccharomyces cerevisiae.*

In vitro laboratory tests:

Abundant bacterial and yeast growth were prepared. For bacterial and yeast inoculua, ten mL of each individual bacterial and yeast isolate were grown for 48 h on nutrient or NYPD broth media and poured into flasks containing sterilized PDA medium. Before solidifying, each flask was rotated gently to ensure equal distribution of bacterial or yeast growth, and then poured into 9-cm-diameter Petri dishes. Inoculated plates were incubated for 48 h at 28±2^{o}C.

For pathogenic fungal growth, 5-mm disk of each tested fungi was transferred to the centre of a PDA dish then incubated at 28±1^{o}C for 7 days.

In vitro, antagonistic studies of bio-control Bacteria and Yeast and pathogenic fungi were performed on PDA medium in 9-cm-diameter Petri dishes supplemented with different concentrations of the tested materials (stated below). A 5-mm disk of each antagonistic bacterial and yeast growth culture was placed onto the PDA, 10mm from the edge of the Petri dish. Another disk of the same diameter of each pathogenic fungal growth culture was placed on the opposite side of the dish at the same distance. The control treatment was inoculated with a culture disk of either pathogenic or antagonistic culture alone at the same conditions. Both experimental and control dishes were assigned to a completely randomized design, with five replicates per treatment. All inoculated Petri dishes were incubated at 28±2^{o}C and the fungal growth diameter away from and towards the antagonist agent was measured after the pathogenic fungal growth in the control treatment had reached the edge of the Petri dish (Ferreira *et al*. 1991). This test was repeated three times and the inhibition was calculated as the percentage reduction in colony diameter growth compared with the control for each particular tested bio-agent.

Tested materials:

- *Chemical plant resistance inducers:*

Different concentrations of Potassium mono hydrogen phosphate salt (K_2HPO_4), Calcium chloride and Humic & Folic acids mixture were tested to study their inhibitory effect on linear growth of pathogenic and antagonistic fungi *in vitro*. Three concentrations of each K_2HPO_4 and Calcium chloride, *i.e.* 1; 2 and 4% (w:v) or 0.2, 0.4, and 0.6 % for Humic & Folic acids mixture were added individually to conical flasks containing sterilized PDA medium to obtain the proposed concentrations, then mixed gently and dispensed in sterilized Petri dishes (10-cm-diameter). Petri dishes were individually inoculated at the centre with equal disks (6-mm) of tested fungal cultures. The average linear growth of fungus was measured after 7 days of incubation at 25 ±2^{o}C.

- *Essential oils:*

Commercial essential oils of Cinnamon (*a.i.* cinnamic, aldehyde, 70-85%)**,** Clove (*a.i.* eugenol, 90–95%) and Thyme (*a.i.* Thymol, 60%) were used in the present work. Essential oils used in the study were obtained from Chemical Industrial Development Company (CID), Egypt. The inhibitory effect of the essential oils was evaluated against the linear growth of the pathogenic and antagonistic fungi *in vitro*. For each of the essential oil, three concentrations, *i.e* 0.0, 0.25, 0.5 and 1% were prepared and tested. Fungal inoculation, incubation conditions and growth measurements and calculations were followed as stated before.

- *Plant extracts:*

Extracts of three plant leaves, *i.e*. Halfa Bar (*Cymbopogon Proximus*); Ginger (*Zingiber officinale*) and Bay laurel (*Laurus nobilis*) were evaluated for their inhibitory effect on fungal linear growth using *in vitro* test. The plant materials kindly obtained from Medicinal and Aromatic Plants Research Department, NRC, Egypt. The materials were washed with distilled water and dried in shade.

The dried plant materials were then finely grinded to powder. Fifty grams of each plant material in powder form was homogenized by laboratory blender in 200 ml of ethanol (96%) and distilled water (20:80, v:v) for 10 min, then left in dark glass bottles for 72 h for tissue maceration. The extracts were filtered through thin cheesecloth sheets. The final extracts were collected separately in other dark glass bottles and exposed to 60°C in water bath for 30 min for ethanol evaporation. The collected extracts were then stored in a refrigerator at 5°C until needed. The extracts were added to sterilized PDA flasks before solidifying to obtain the proposed concentrations of 1, 2 and 4% (v/v). and Bay laurel at concentrations of 1.0, 2.0 and 4.0% (v:v)

All *in vitro* testes concerning adjustment of supplemented media with different concentration of tested chemicals as well as fungal inoculation, incubation conditions and growth measurements and calculations were followed as stated before.

Statistical analysis:

All experiments were set up in a complete randomized design. One-way ANOVA was used to analyze differences between antagonistic inhibitor effect and linear growth of pathogenic fungi *in vitro.* A general linear model option of the analysis system SAS (SAS Institute Inc. 1996) was used to perform the ANOVA. Duncan's multiple range test at $P \leq 0.05$ level was used for means separation (Winer 1971).

Results and Discussion

The effect of Calcium chloride on the antagonistic ability of bacteria and yeast against some soilborne pathogenic fungi was evaluated *in vitro.* Data in Table (1) and Fig (1) showed that the antagonistic efficacy of *Bacillus subtilis*, *Pseudomonas fluorescens* and *Saccharomyces 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 choride at 4% in growth media. Another feature at a lesser extent was observed with *P. flourescencse* 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 biocontrol 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 $CaCl_2$ (2% w/v) to the formulation of the yeast biocontrol 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 $CaCl_2$ (2% w/v) in an aqueous suspension (Tian *et al*., 2001). Moreover, Tian *et al*., (2002) reported that combining $CaCl_2$ with the yeast suspensions significantly enhanced the biocontrol activity of *Colletotricum guilliermondii* in peaches and *Pichia membranefaciens* in nectarines to Rhizopus rot. The same effects on biocontrol 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 biocontrol 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 biocontrol by antagonistic yeasts such as *Pichia*, *Candida* and *Cryptococcus* spp. (Arras, 1996; Elad, 1996).

Table 1: Effect of Calcium chloride on the antagonistic ability of bacteria and yeast against some soil-borne pathogenic fungi *in vitro*

Pathogenic fungi	Antagonistic bacteria and yeast											
	B. subtilis				*P. fluorescens*				*S. cerevisiae*			
	Calcium chloride concentration (%)											
	0	1	2	4	0	1	2	4	0	1	2	4
A. solani	73 [*] a	59 c	33 e	0 h	76 a	61 b	50 c	48 d	72 a	42 d	36 e	20 f
F. solani	64 b	35 e	25 f	0 h	74 a	44 d	36 e	28 f	70 a	39 e	25 f	15 g
F. oxysporum	68 b	45 d	30 e	0 h	73 a	47 d	45 d	25 f	74 a	46 d	37 e	24 f
R. solani	69 b	55 c	38 e	0 h	69 b	57 c	48 d	34 e	69 b	50 c	45 d	31 e
S. rolfsii	68 b	47 d	37 e	0 h	68 b	48 d	40 d	27 f	76 a	48 d	30 e	15 g
S. sclerotiorum	70 a	30 e	25 f	16 g	71 a	44 d	35 e	22 f	71 a	25 f	15 g	11 g
S. minor	66 b	35 e	26 f	17 g	72 a	46 d	40 d	25 f	70 a	27 f	18 g	14 g
M. phaseolina	63 b	57 c	30 e	0 h	70 a	43 d	32 e	24 f	72 a	33 e	27 f	18 g
Pythium sp.	65 b	48 d	27 f	0 h	68 b	41 d	30 e	20 f	73 a	31 e	21 f	16 g

Mean values within columns followed by the same letter are not significantly different ($P \leq 0.05$). [*] Linear fungal growth (mm)

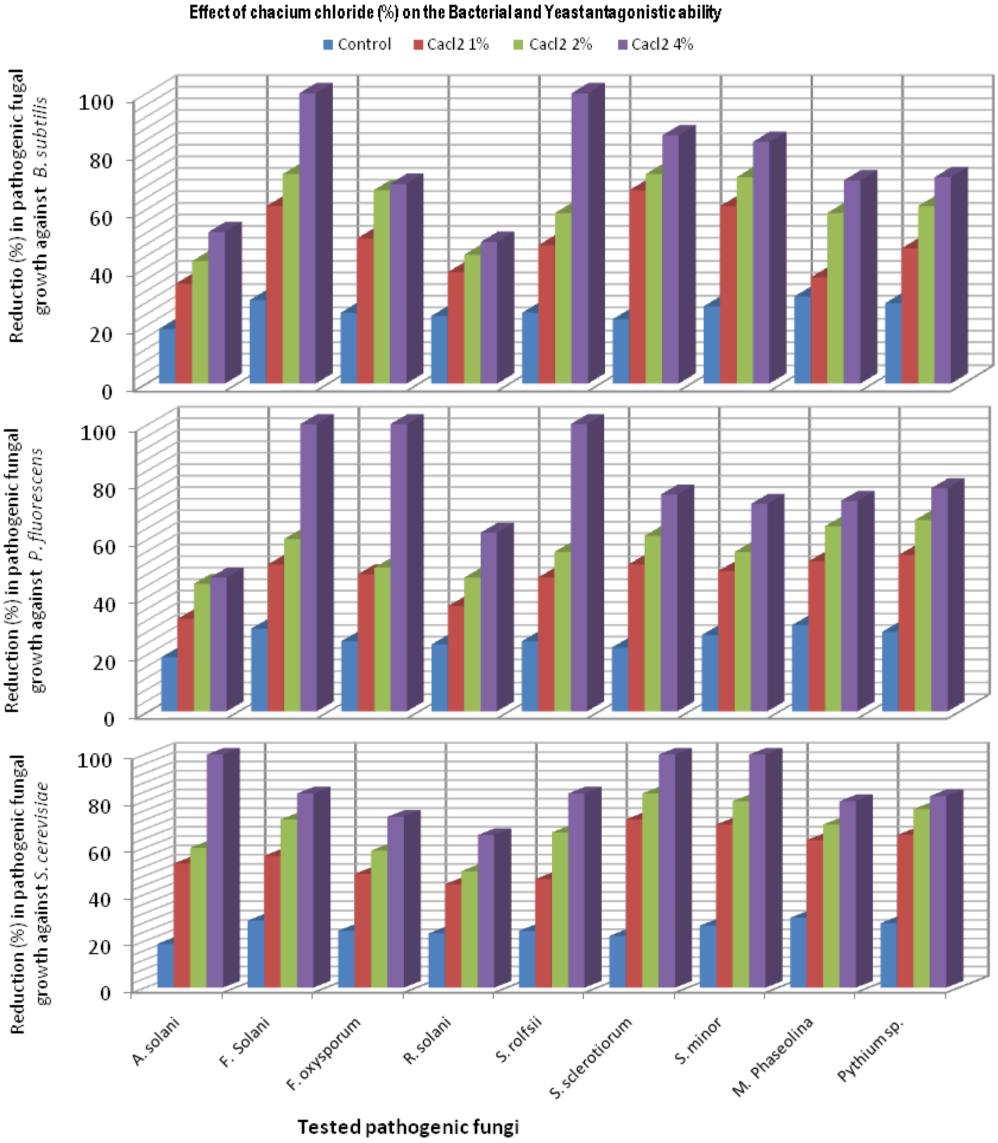


Fig. 1: Effect of Calcium chloride on the antagonistic ability of bacterial and yeast isolates against some soilborne pathogenic fungi *in vitro.*
Reduction in pathogenic fungal growth calculated relatively to their growth in control (free of Calcium chloride)

The effect of Humic and folic acids as a mixture on the antagonistic ability of bacteria and yeast against some soilborne pathogenic fungi was evaluated *in vitro*. The obtained results were tabulated in Table (2) and Fig (2). Presented data 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, complete inhibition in pathogenic fungal growth of *A. solani*, *F. solani, F. oxysporum, R. solani, S. rolfsii, 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. rolfsii.* 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 fluorescens* showed a lower response to all concentrations of Humic and folic acids mixture for increasing their antagonistic ability.

Table 2: Effect of the mixture of Humic and Folic acids mixture on the antagonistic ability of bacteria and yeast against some soilborne pathogenic fungi *in vitro*

Pathogenic Fungi	Antagonistic bacteria and yeast											
	B. subtilis				*P. fluorescence*				*S. sevisease*			
	Humic and folic acids concentration mixture (%)											
	0	0.2	0.4	0.6	0	0.2	0.4	0.6	0	0.2	0.4	0.6
A. solani	73[*] a	65 b	55 c	0 h	76 a	65 b	57 c	49 d	72 a	48 d	39 e	23 f
F. solani	64 b	35 e	15 g	0 h	74 a	50 c	45 d	30 e	70 a	41 d	25 f	0 h
F. oxysporum	68 b	45 d	20 f	0 h	73 a	48 d	43 d	28 f	74 a	45 d	30 e	0 h
R. solani	69 b	55 c	44 d	0 h	69 b	58 c	51 c	45 d	69 b	35 e	25 f	0 h
S. rolfsii	68 b	38 e	24 f	0 h	68 b	48 d	36 e	30 e	76 a	45 d	27 f	0 h
S. sclerotiorum	70 a	43 d	30 e	15 g	71 a	46 d	34 e	29 f	71 a	40 d	29 f	15 g
S. minor	66 b	46 d	37 e	18 g	72 a	47 d	41 d	30 e	70 a	35 e	24 f	18 g
M. phaseolina	63 b	57 c	36 e	0 h	70 a	48 d	32 e	12 g	72 a	41 d	33 e	14 g
Pythium sp.	65 b	55 c	34 e	0 h	68 b	47 d	29 f	22 f	73 a	43 d	34 e	18 g

Mean values within columns followed by the same letter are not significantly different ($P \leq 0.05$). [*] Linear fungal growth (mm)

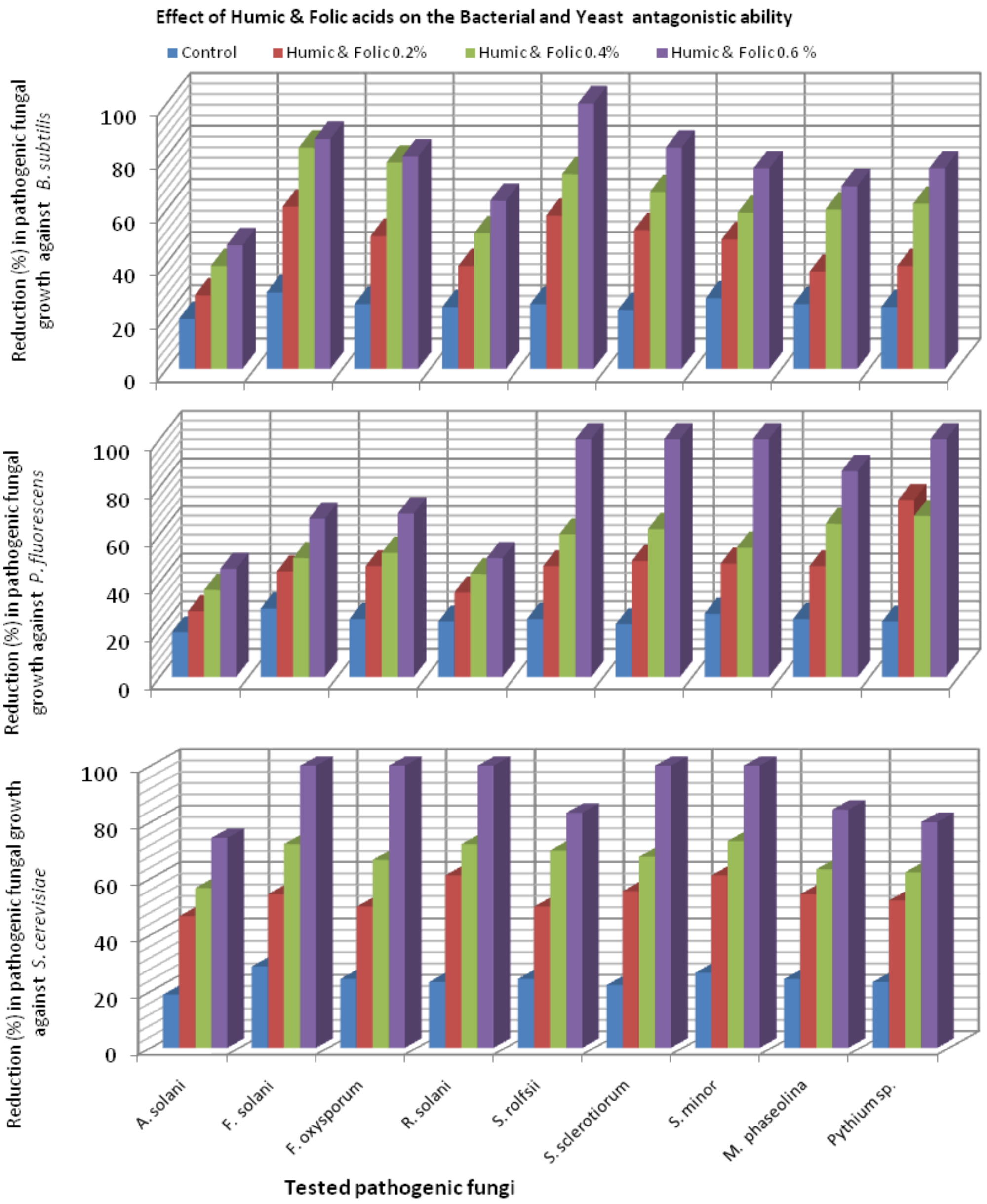


Fig. 2: Effect of Humic & Folic acids on the antagonistic ability of bacterial and yeast isolates against some soilborne pathogenic fungi *in vitro*.

Reduction in pathogenic fungal growth calculated relatively to their growth in control (free of Humic & Folic acids).

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 that biosolubilization of humic acid enhances plant growth and biocontrol efficacy against phytopathogenic organism.

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*. Data in Table (3) and Fig (3) revealed that different concentrations of chemicals used have positive effect for enhancing the efficacy of antagonistic ability of tested bio-agents. Potassium mono-hydrogen phosphate showed superior effect in this regard followed by Potassium bicarbonates and Sodium bicarbonate, respectively. Data also showed 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 bioagents for enhancing the biological activity. Sodium bicarbonate has been successfully used in combination with bacterial and yeasts biocontrol 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; Gamagae *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 biocontrol 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 biocontrol 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).

Table 3: Effect of some plant resistance inducers on the antagonistic ability of bacteria and yeast against some soilborne pathogenic fungi *in vitro*

Antagonistic Bacteria And yeast	Pathogenic fungi	Chemical concentration (%)									
		Control	Potassium bicarbonate			Sodium bicarbonate			Potassium mono-hydrogen phosphate		
		0.0	1	2	4	1	2	4	1	2	4
B. subtilis	*A. solani*	58* c	55 c	48 d	42 d	43 d	39 e	33 e	48 d	39 e	28 f
	F. solani	63 b	55 c	47 d	43 d	57 c	52 c	44 d	52 c	43 d	32 e
	F. oxysporum	52 c	45 d	43 d	40 d	50 c	46 d	41 d	46 d	37 e	28 f
	R. solani	68 b	60 b	52 c	48 d	62 b	57 c	50 c	58 c	48 d	35 e
	S. rolfsii	59 c	50 c	44 d	40 d	55 c	50 c	44 d	51 c	46 d	38 e
	S. sclerotiorum	58 c	50 c	45 d	41 d	55 c	51 c	43 d	50 c	41 d	36 e
	S. minor	66 b	58 c	52 c	47 d	61 b	57 c	52 c	59 c	48 d	39 e
	M. phaseolina	70 a	60 b	57 c	52 c	66 b	61 b	54 c	62 b	50 c	41 d
	Pythium sp.	42 d	38 e	33 e	29 f	41 d	36 e	32 e	34 e	27 f	24 f
P. fluorescens	*A. solani*	53 c	50 c	45 d	41 d	48 d	41 d	34 e	45 d	36 e	29 f
	F. solani	48 d	45 d	42 d	37 e	44 d	38 e	31 e	40 d	31 e	22 f
	F. oxysporum	72 a	62 b	58 c	54 c	67 b	60 b	52 c	61 b	53 c	41 d
	R. solani	73 a	65 b	62 b	58 c	68 b	62 b	57 c	60 b	52 c	40 d
	S. rolfsii	72 a	65 b	61 b	57 c	67 b	61 b	54 c	69 b	51 c	42 d
	S. sclerotiorum	78 a	71 a	69 b	64 b	72 a	67 b	60 b	68 b	50 c	40 d
	S. minor	72 a	56 c	52 c	48 d	68 b	62 b	56 c	62 b	46 d	38 e
	M. phaseolina	70 a	65 b	61 b	56 c	68 b	63 b	57 c	60 b	51 c	43 d
	Pythium sp.	62 b	57 c	51 c	47 d	58 c	54 c	50 c	56 c	51 c	47 d
S. cevisease	*A. solani*	58 c	55 c	48 d	40 d	52 c	47 d	41 d	50 c	42 d	37 e
	F. solani	56 c	52 c	47 d	41 d	50 c	44 d	39 e	51 c	46 d	41 d
	F. oxysporum	52 c	50 c	47 d	41 d	50 c	46 d	41 d	48 d	42 d	38 e
	R. solani	54 c	49 d	42 d	38 e	50 c	44 d	37 e	51 c	46 d	41 d
	S. rolfsii	55 c	51 c	46 d	40 d	48 d	42 d	37 e	50 c	44 d	40 d
	S. sclerotiorum	61 b	56 c	51 c	48 d	55 c	51 c	47 d	57 c	52 c	47 d
	S. minor	59 c	53 c	48 d	44 d	52 c	49 d	42 d	53 c	47 d	41 d
	M. phaseolina	62 b	57 c	52 c	48 d	57 c	51 c	47 d	55 c	47 d	40 d
	Pythium sp.	57 c	47 d	41 d	36 e	44 d	40 d	35 e	48 d	42 d	35 e

Mean values within columns followed by the same letter are not significantly different (P ≤ 0.05).

* Linear fungal growth (mm)

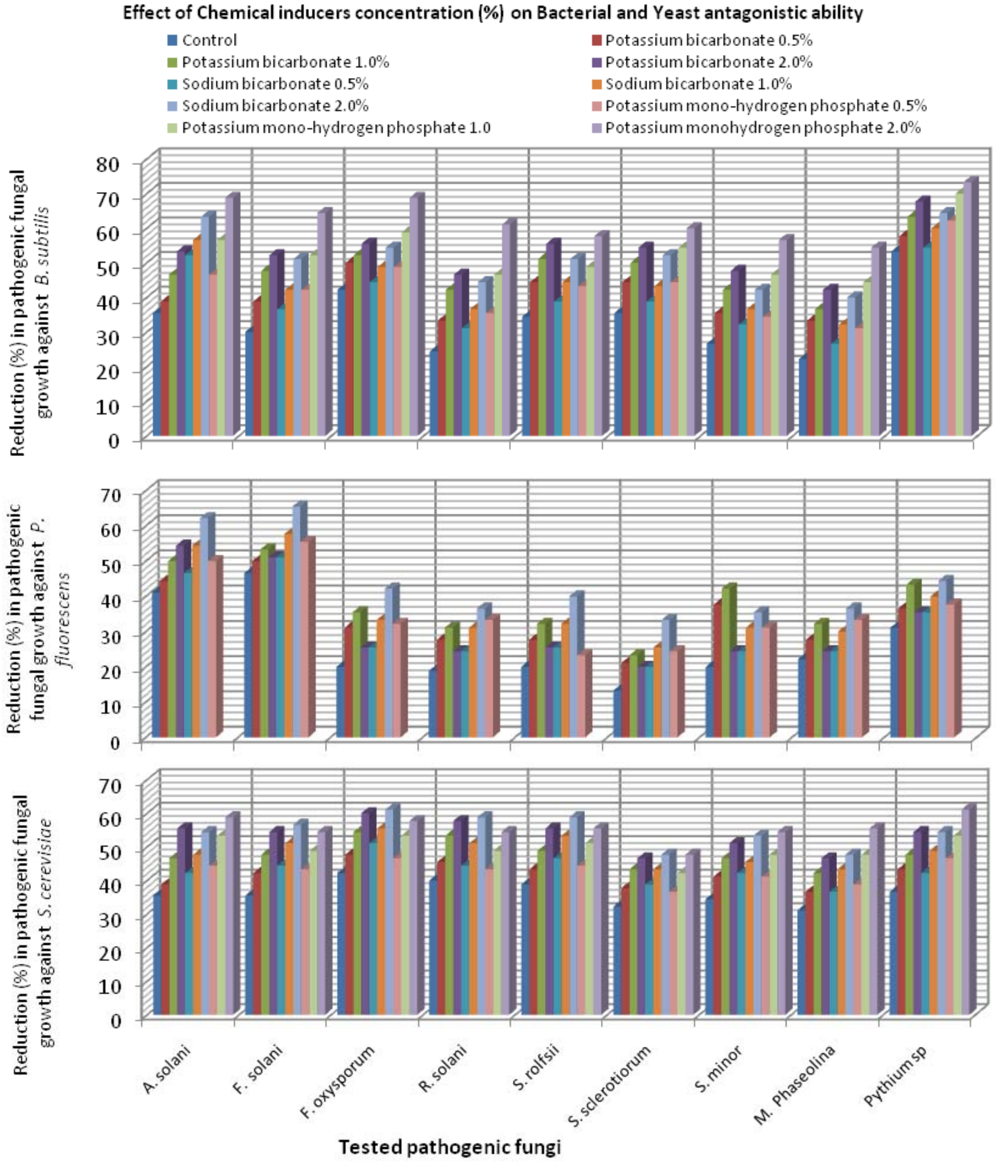


Fig. 3: Effect of some chemical inducers on the antagonistic ability of Trichoderma spp. against some soilborne pathogenic fungi *in vitro.*
Reduction in pathogenic fungal growth calculated relatively to their growth in control (free of chemical inducers)

Results presented in Table (4) and Fig (4) showed the effect of some essential oils on the antagonistic ability of bacteria and yeast against pathogenic fungi *in vitro.* The obtained results revealed that in general Thyme oil have more enhancing effect on the antagonistic ability than that of both Cinnamon 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 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%. In this concern, interest has been shown in combining microbial biocontrol agents with other chemical components to increase their activity against post-harvest pathogens (Droby *et al*., 1998). Essential oils are also considered a promising alternative with many having antifungal properties. However, very high concentration is needed when applied to real food systems (Hammer *et al*., 2003; Ahmet *et al*., 2005). Application of essential oil is a very attractive method for controlling postharvest 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 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 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.

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).

Finally, the effect of some plant extracts on the antagonistic ability of bacteria and yeast against pathogenic fungi was evaluated *in vitro*. Data in Table (5) and Fig (5) revealed that all tested concentrations of plant extracts could enhance the efficacy of antagonistic ability of bacterial and yeast bio-agents.

Table 4: Effect of some essential oils on the antagonistic ability of bacteria and yeast against some soilborne pathogenic fungi *in vitro*

Antagonistic Bacteria and yeast	Pathogenic fungi	Essential oils concentration (%)									
		Control	Cinnamon			Clove			Thyme		
		0.0	0.25	0.5	1.0	0.25	0.5	1.0	0.25	0.5	1.0
B. subtilis	*A. solani*	38[*] g	35 g	20 h	10 i	30 g	25 h	22 h	32 g	28 h	20 h
	F. solani	73 c	70 c	50 e	25 h	72 c	60 d	55 e	65 d	50 e	20 h
	F. oxysporum	52 d	45 ef	35 g	20 h	50 e	42 ef	40 ef	50 e	35 g	28 h
	R. solani	68 d	65 d	50 e	35 g	65 d	55 e	46 ef	35 g	22 h	10 i
	S. rolfsii	69 d	65 d	40 ef	30 g	60 d	55 e	46 ef	50 e	40 ef	25 h
	S. sclerotiorum	68 d	40 ef	25 h	15 i	65 d	60 d	50 e	65 d	50 e	25 h
	S. minor	71 c	65 d	50 e	20 h	66 d	55 e	45 ef	60 d	50 e	30 g
	M. phaseolina	70 c	65 d	50 e	35 g	65 d	50 e	25 h	35 g	22 h	10 i
	Pythium sp.	67 d	54 e	46 ef	27 h	61 d	52 e	38 g	58 e	42 ef	34 g
P. flourescence	*A. solani*	53 d	50 e	38 g	23 h	50 e	42 ef	31 g	50 e	35 g	20 h
	F. solani	48 ef	40 ef	35 g	20 h	45 ef	33 g	27 h	40 ef	34 g	20 h
	F. oxysporum	82 b	25 h	18 i	15 i	45 ef	30 g	15 i	43 ef	28 h	15 i
	R. solani	83 b	50 e	40 ef	25 h	80 b	55 e	30 g	60 d	45 ef	30 g
	S. rolfsii	82 b	72 c	65 d	52 e	80 b	55 e	30 g	40 ef	28 h	15 i
	S. sclerotiorum	78 c	60 d	45 ef	25 h	75 c	55 e	35 g	67 d	42 ef	31 g
	S. minor	73 c	62 d	51 e	34 g	66 d	51 e	38 g	64 d	48 ef	29 h
	M. phaseolina	70 c	50 e	40 ef	25 h	80 b	80 b	80 b	61 d	42 ef	31 g
	Pythium sp.	74 c	61 d	44 ef	28 h	68 d	48 ef	32 g	54 e	48 ef	26 h
S. servisease	*A. solani*	68 d	40 ef	28 h	15 i	75 c	67 d	43 ef	60 d	46 ef	31 g
	F. solani	66 d	50 e	40 ef	22 h	78 c	71 c	58 e	58 e	43 ef	31 g
	F. oxysporum	82 b	40 ef	30 g	15 i	80 b	65 d	50 e	48 ef	33 g	28 h
	R. solani	84 b	80 b	65 d	50 e	60 d	45 ef	35 g	45 ef	35 g	26 h
	S. rolfsii	85 b	80 b	55 e	30 g	85 b	73 c	55 e	50 e	35 g	35 g
	S. sclerotiorum	71 c	50 e	35 g	20 h	65 d	50 e	30 g	65 d	50 e	35 g
	S. minor	73 c	62 d	43 ef	33 g	60 d	44 ef	30 g	58 e	36 g	24 h
	M. phaseolina	82 b	80 b	65 d	50 e	60 d	45 ef	35 g	65 d	50 e	35 g
	Pythium sp.	76 c	70 c	62 d	54 e	43 ef	68 d	50 e	61 d	44 ef	28 h

Mean values within columns followed by the same letter are not significantly different ($P \leq 0.05$).

[*] Linear fungal growth (mm)

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. 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 study 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, 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*. Radha and Padma (2011) reported that it was clear that the methanolic extract of *Majorana hortensis* (majoram) leaves significantly increases the cell viability of the yeast *Saccharomyces cerevisiae*.

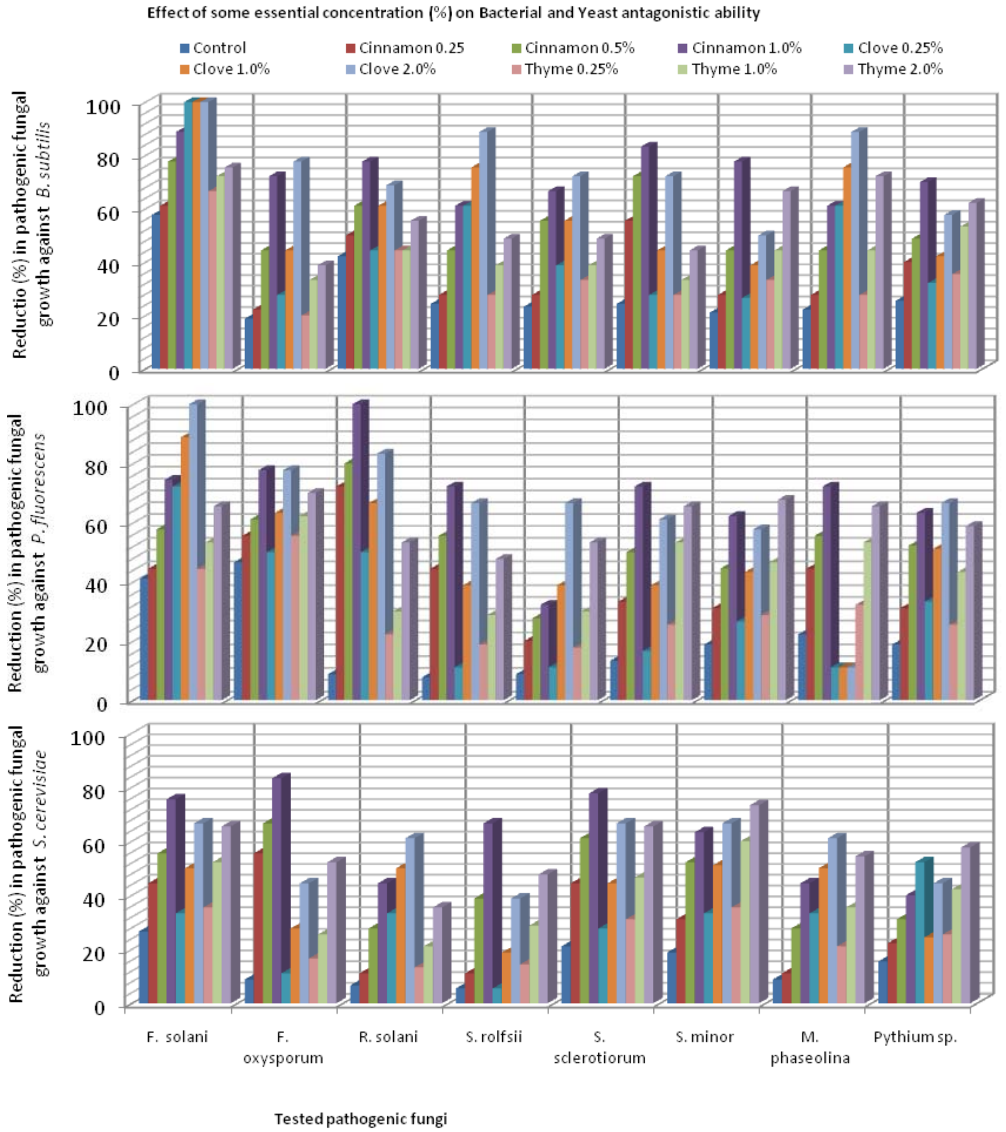


Fig. 4: Effect of essential oils on the antagonistic ability of bacterial and yeast isolates against some soilborne pathogenic fungi *in vitro.*
Reduction in pathogenic fungal growth calculated relatively to their growth in control (free of essential oils).

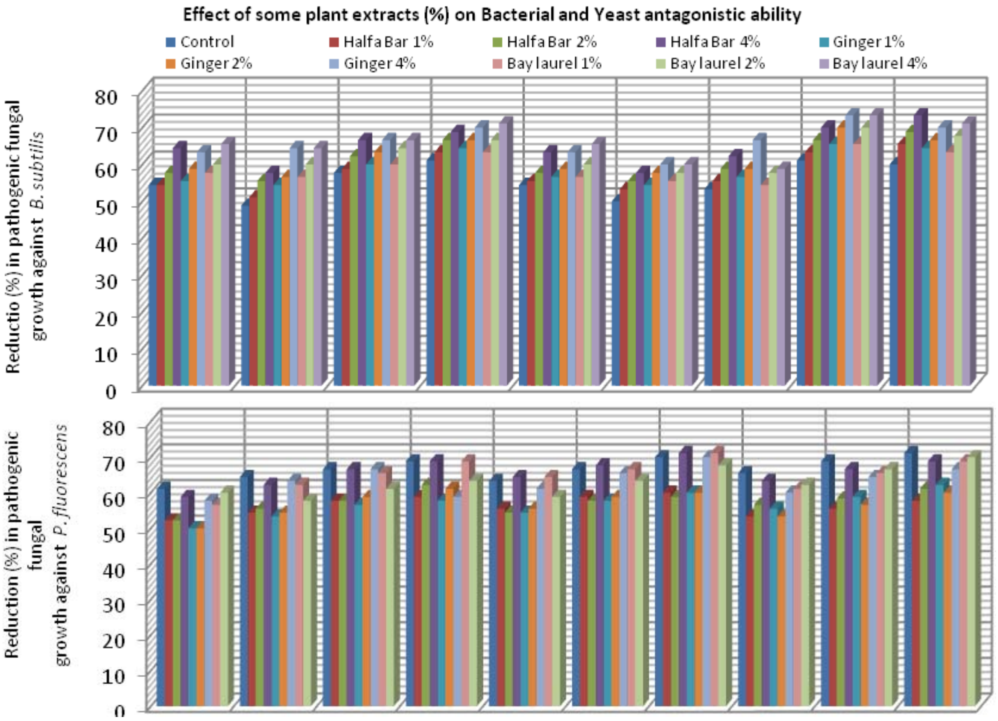
The present results may lead to the conclusion that since application of plant resistance inducers, essential oils and plant extracts is proved to be applicable, safe and cost-effective method for controlling plant diseases. Also, the use of them in agriculture could be a suitable alternative for inclusion 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, further greenhouse experiments to test 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.

Table 5: Effect of some plant extracts on the antagonistic ability of bacteria and yeast against some soilborne pathogenic fungi *in vitro*

Antagonistic Bacteria and yeast	Pathogenic fungi	Plant extracts (%)									
		control	Halfa Bar *C. Proximus*			Ginger *Z. officinale*			Bay laurel *L. nobilis*		
		0.0	1	2	4	1	2	4	1	2	4
B. subtilis	*A. solani*	42 * a	40 a	39 ab	34 b	40 a	38 ab	36 b	38 ab	36 b	34 b
	F. solani	47 a	45 a	42 a	39 ab	42 a	39 ab	35 b	39 ab	36 b	36 b
	F. oxysporum	39 ab	37 ab	35 b	33 b	37 ab	34 b	32 b	36 b	33 b	31 b
	R. solani	35 b	33 b	32 b	28 bc	32 b	30 b	29 c	33 b	31 b	30 b
	S. rolfsii	41a	40 a	38 ab	36 b	39 ab	38 ab	36 b	39 ab	36 b	35 b
	S. sclerotiorum	45 a	43 a	42 a	39 ab	41 a	39 ab	37 ab	42 a	38 ab	36 b
	s.minor	42 a	40 a	39 ab	36 b	39 ab	37 ab	36 b	41 a	38 ab	37 ab
	M. phaseolina	35 b	33 b	31 b	30 b	31 b	28 bc	26 c	32 b	28 bc	26 c
	Pythium sp.	36 b	33 b	29 bc	26 b	32 b	30 b	28 bc	33 b	29 bc	26 c
P. flourescence	*A. solani*	35 b	32 b	31 b	28 bc	33 b	32 b	29 bc	31 b	28 bc	26 c
	F. solani	43 a	41 a	38 ab	37 ab	40 a	37 ab	36 b	42 a	40 a	38 ab
	F. oxysporum	43 a	40 a	38 ab	34 b	41 a	38 ab	37 ab	39 ab	37 ab	35 b
	R. solani	37 b	34 b	32 b	28 bc	32 b	29 bc	26 c	33 b	31 b	28 bc
	S. rolfsii	45 a	41 a	39 ab	38 ab	41 a	38 ab	34 b	40 a	37 ab	35 b
	S. sclerotiorum	45 a	41 a	38 ab	36 b	40 a	37 ab	36 b	42 a	39 ab	35 b
	s. minor	38 ab	35 b	32 b	30 b	35 b	32 b	30 b	36 b	32 b	30 b
	M. phaseolina	39 b	35 b	33 b	30 b	32 b	30 b	29 bc	35 b	32 b	29 bc
	Pythium sp.	41 a	38 ab	36 b	34 b	37 ab	35 b	32 b	34 b	33 b	30 b
S. servisease	*A. solani*	37 ab	33 b	31 b	29 bc	31 b	29 bc	26 c	34 b	30 b	28 bc
	F. solani	40 a	36 b	32 b	31 b	37 ab	35 b	32 b	36 b	34 b	31 b
	F. oxysporum	41 a	38 ab	36 b	32 b	38 ab	35 b	32 b	36 b	33 b	30 b
	R. solani	35 b	32 b	30 b	28 bc	31 b	28 bc	26 c	30 b	26 c	24 c
	S. rolfsii	42 a	38 ab	35 b	33 b	37 ab	34 b	31 b	37 ab	35 b	29 bc
	S. sclerotiorum	43 a	40 a	37 ab	33 b	39 ab	35 b	32 b	38 ab	35 b	30 b
	s. minor	45 a	41 a	38 ab	35 b	42 a	39 ab	36 b	39 ab	37 ab	34 b
	M. phaseolina	36 b	33 b	30 c	28 bc	31 b	28 c	25 c	32 b	28 c	26 b
	Pythium sp.	41 a	37 b	34 c	31 b	38 ab	35 b	32 b	36 b	34 b	30 b

Mean values within columns followed by the same letter are not significantly different ($P \leq 0.05$).

* Linear fungal growth (mm)



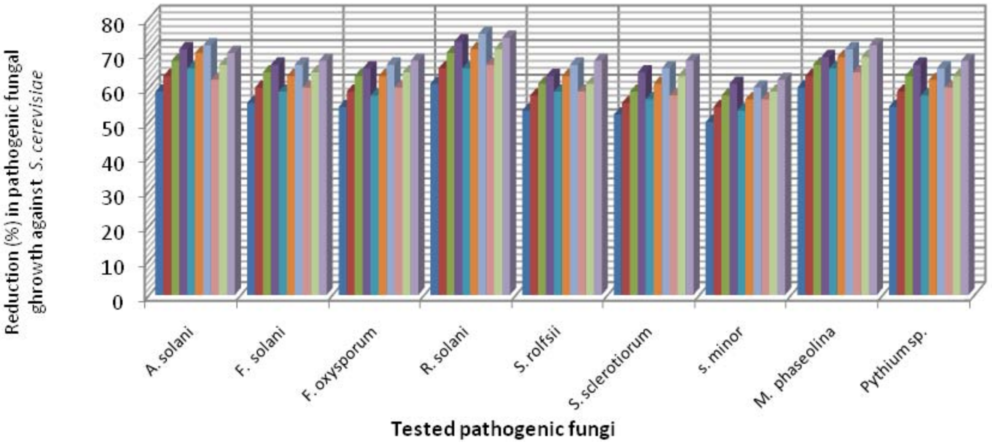


Fig. 5: Effect of some plant extracts on the antagonistic ability of bacterial and yeast isolates against some soilborne pathogenic fungi *in vitro.*

Reduction in pathogenic fungal growth calculated relatively to their growth in control (free of plant extracts).

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