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Essential oils, inorganic acids and potassium salts as control measures against the growth of tomato root rot pathogens *in vitro*

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Evaluation the inhibitor activity of some fungicides alternatives on the growth of some tomato root rot soil-borne pathogenic fungi (*Fusarium oxysporum radicis-lycopersici*, *F. oxysporum lycopersici*, *F. solani*, *Rhizoctonia solani*, *Sclerotium rolfsii*, *Macrophomina phaseolinae*, *Pythium* sp. and *Phytophthora* sp.) was studied under *in vitro* conditions. The evaluated chemicals were inorganic acids and salts, *i.e*. Salicylic, Sorbic acids, Potassium sorbate, Potassium carbonate, as well as essential oils were lemongrass, Thyme, citral and nerol. Linear growth of tested fungi decreased significantly by increasing the concentrations of both salicylic and sorbic acids. The fungal growth was completely inhibited at concentration of 12.5 mM of both salicylic and sorbic acids. Meanwhile, potassium sorbate and potassium carbonate had inhibitory effect against mycelia growth of all tested fungi *in vitro*. The inhibitory effect increased by the increase in concentration used to reach its maximum at the highest concentration of 4%. Furthermore, the inhibitor activity against mycelial growth of tested fungi was also observed at all concentrations of lemongrass, thyme, citral and nerol oils. The Mycelial growth decreased significantly with the increase in concentrations of essential oils and reached minimum mycelia growth at the highest concentration used. A complete inhibition of fungal growth was observed at 1.5% of all tested essential oils. The obtained results in the present study provide that fungicides alternatives could be effectiveness in management tomato soilborn pathogenic fungi.

Keywords: essential oils, fungal growth, inorganic acids, root rot pathogens, salts.

Introduction

Plant diseases caused by soil-borne plant pathogens considered the major problems in agricultural production throughout the world, reducing yield and quality of crops. Plant pathogens have caused an almost 20% reduction in the principal food and cash crops worldwide (Oerke *et al*., 1994). Root rot caused

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by soil-borne pathogenic fungi is one of the most serious diseases affected several cultivated plants worldwide. It results in poor production, poor quality, poor milling returns and reduced agriculture income. This has a negative impact on the livelihood of farmers. Fungal disease control is achieved through the use of fungicides which is hazardous and toxic to both people and domestic animals and leads to environmental pollution. Therefore, a more balanced, cost effective and eco-friendly approach must be implemented and adopted farmers. In order to overcome such hazardous control strategies, scientists, 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. The present research focuses on finding compounds that are safe to humans and the environment, *e.g.* chemical resistance inducers and essential oils. Inorganic acids and salts as food preservatives are recorded by several investigators to have antimicrobial inhibitor effect as well as they play important role to induce plant resistance against plant pathogenic fungi and bacteria either *in vitro* or in *vivo*.

Sodium benzoate and benzoic acid are employed in a wide range of preservative applications because of their combination of bactericidal and bacteriostatic action with their properties of being nontoxic and tasteless. They are the most effective preservatives against yeast and mould. Several inorganic salts and organic lipophilic acids and their salts, some of which are used, in the food-processing industry, have antimicrobial properties and could be useful as postharvest treatment for decay control. The food preservatives potassium sorbate or sodium benzoate, have antifungal activities against postharvest decaying fungi (Al-Zaemey *et al*., 1993; Olivier *et al*., 1999). Furthermore, some organic acids naturally found in or applied to fruits and vegetables behave primarily as fungistats, while others are more effective at inhibiting bacterial growth. Acetic, citric, succinic, malic, tartaric, benzoic and sorbic acids are the major organic acids that occur naturally in many fruits and vegetables (Foegeding and Busta, 1991).

On the other hand, essential oils are promising alternative compounds which have an inhibitory activity on the growth of pathogens. It is possible that essential oils could be used in plant disease control as the main or as adjuvant antimicrobial compounds (Kaur and Arora, 1999). 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).

The objective of the present work was to evaluate the inhibitor activity of some fungicides alternatives on the growth of some tomato root rot soil-borne pathogenic fungi *in vitro*. Inorganic acids and salts, *i.e*. Salicylic, Sorbic acids, Potassium sorbate, Potassium carbonate, as well as essential oils were lemongrass, Thyme, citral and nerol were evaluated.

Materials and methods

Pathogenic fungi

Virulent pathogenic fungal isolates *Fusarium oxysporum radicis-lycopersici*, *F. oxysporum lycopersici, F. solani*, *Rhizoctonia solani*, *Sclerotium rolfsii*, *Macrophomina phaseolinae*, *Pythium* sp. and *Phytophthora* sp. obtained from Plant Pathology Dept., National Research Centre, Egypt were used in the present work. These proved their pathogenic ability to induce root rot incidence to tomato seedlings at different degrees in pot experiment under greenhouse conditions at the same Department (unpublished data).

Inorganic Acids and Salts

Inorganic acids, salicylic acid ($C_7H_6O_3$) and sorbic acid ($C_6H_8O_6$) as well as potassium sorbate ($C_6H_7KO_2$) and potassium carbonate (K_2CO_3) purchased from Sigma-Aldrich were used in the present work.

Essential oils

Essential oils of lemongrass, thyme, were purchased from International Flavors and Plant oils Inc., Giza, Egypt. Meanwhile, citral and nerol were purchased from Delta Aromatic Co., Cairo, Egypt. These essential oils were stored in dark bottles at 4ºC for further studies.

Growth media

PDA (Difco Laboratories, Detroit, MI) was used for growing fungal isolates tested in the present work.

Effect of inorganic acids and salts on fungal growth

The inhibitor effect of different inorganic acids and salts on the linear growth of some pathogenic fungi was evaluated *in vitro* conditions. Salicylic and sorbic acids at concentrations of 0.0, 2.5, 5.0, 7.5, 10.0, 12.5 mM and 0.0, 1% .2%,4%,6%,8% respectively as well as potassium sorbate and potassium carbonate at concentrations of 0.0, 1% .2%,4%,6%,8% and 0.0, 0.5, 1.0, 2.0, 3.0 , 4.0% (w/v) respectively were tested for their inhibitory effect on the linear growth of *Fusarium oxysporum radicis-lycopersici*, *F. oxysporum lycopersici, F. solani, Rhizoctonia solani*, *Sclerotium rolfsii*, *Macrophomina phaseolinae*, *Pythium* sp. and *Phytophthora* sp.. Water salts or acid solutions were added to conical flasks containing sterilized PDA medium to obtain the proposed concentrations, then rotated gently and dispensed in sterilized Petri plates (10 cm Ø) about 20 ml each. The plates were individually inoculated at the center with equal disks (5 mm Ø) of 10-day old culture of the tested fungi. A set of inoculated plates free of tested chemical was used as controls. Five plates were used as replicates for each particular treatment. Inoculated plates were incubated at $25\pm1^{o}28C$. The average linear growth of fungi tested was calculated when controls reached the edge of the plate.

Effect of essential oils on fungal growth

Eessential oils, *i.e*., thyme, citral and nerol and lemongrass at concentrations of 0.0, 0.25, 0.5, 1.0 and 1.5% (v/v) and 0.0, 0.5 ,1.0,1.5,2.5% (v/v) respectively were evaluated for their inhibitory effect on fungal radial growth, through *in vitro* tests. Emulsified stocks at high concentrations of tested essential oils were prepared by dissolving in sterilized distilled water. A few drops of the emulsifier Tween 20 (Sigma Co.) were added to the essential oil volumes to obtain an emulsion feature. Different volumes of the essential oil emulsion were added to conical flasks containing 100 ml of sterilized PDA medium before its solidification, to obtain the proposed concentrations. The supplemented media were poured into Petri plates (10 cm Ø) about 20 ml each. The plates were individually inoculated at the center with equal disks (5 mm Ø) of 10-day old culture of the tested fungi. A set of inoculated plates free of tested essential oils was used as controls. Five plates were used as replicates for each particular treatment. Inoculated plates were incubated at $25\pm1^{o}28C$. The average linear growth of fungi tested was calculated when controls reached full growth.

Statistical analyses

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 $P < 0.05$ level was used for means separation (Winer, 1971).

Results and discussions

Effect of inorganic acids and salts on fungal growth

The inhibitory effect of salicylic and sorbic acids at different concentrations on the linear growth of various tomato root rot pathogenic fungi was studied *in vitro*. Soil-borne fungi, *i.e. Fusarium oxysporum radicis-lycopersici* (isolate ForlQ4), *Fusarium oxysporum lycopersici* (isolate FolG14), *Fusarium solani* (isolate FsG1), *Rhizoctonia solani* (isolate RsG1), *Sclerotium rolfsii* (isolate SrM2), *Macrophomina phaseolinae* (isolate MpB1), *Pythium* sp. (isolate Py H2) and *Phytophthora* sp. (isolate Ph MPC) were used in this study.

Presented results in Table (1) show the response of soil-borne fungi to the inhibitory effect of tested chemicals. It is clear that, linear growth of tested fungi decreased significantly by increasing the concentrations of both salicylic and sorbic acids. The tested fungi showed more sensitivity against sorbic acid comparing with salicylic acid at all concentrations used. Moreover, the fungal growth was completely inhibited at concentration of 12.5 mM and 8% of both salicylic and sorbic acids. Data also show that complete inhibition in the growth of *Pythium* sp. (isolate Py H2) and *Phytophthora* sp. (isolate Ph MPC) as well as *Fusarium solani* (isolate FsG1) at concentration of 10.0 mM of salicylic acid and sorbic acid, respectively.

The efficacy of salicylic acid as antifungal substances against various plant pathogenic fungi has been discussed in many investigations. In this concern, the results obtained by Ismail *et al.* (1988) clarify that among some phenolic compounds, salicylic acid has inhibitory effect on germ tube length and spores germination of *Fusarium oxysporum* f.sp. *lycopersic*i and *Aspergillus fumigatus* . Also, Shashi Chauhan *et al*. (1989) reported that salicylic acid was highly toxic to the mycelial growth of most of deterophytic fungi (*Trichophyton mentagrophytes, T. tonsuran, T. violaceum, T. rubrum, Microsporum gypseum* and *Epidermophyton floccosum*). They added that the toxicity increased with the increased concentration of the drug in the medium. In addition, Dwivedi (1990) recorded that *F. oxysporum* f. sp. *psidi* was completely checked by 0.15 % (10 mM) salicylic acid *in vitro*, while concentrations higher than 200 µg/ml (130 mM) inhibited mycelial growth of *Pythium aphanidermatum.*

Table 1. Reduction % on the linear growth of tomato fungal pathogens in response to different concentrations of Salicylic and Sorbic acids on PDA medium

Fungal isolates	**Salicylic acid**					**Sorbic acid**				
	2.5 mM	5.0 mM	7.5 mM	10 mM	12.5 mM	1%	2%	4%	6%	8%
Forl-Q4	46.6 e	57.7 e	74.4 c	88.8 b	100 a	52.2 c	66.6 c	80.0 b	94.4 ab	100 a
Fol-G14	51.1 d	61.1 d	75.5 c	91.1 b	100 a	55.5 c	66.6 c	78.8 b	91.1 b	100 a
Fs-G1	68.8 c	65.5 c	80.0 c	92.2 b	100 a	72.2 b	71.1 b	93.3b	100 a	100 a
Rs-G1	74.4 b	80.0 b	100 a	100 a	100 a	77.7 a	86.6 a	100 a	100 a	100 a
Sr-M2	35.5 f	46.6 f	64.4 d	84.4 b	100 a	42.2d	55.5 d	70.0 d	86.6 c	100 a
Mp-B1	71.1 b	66.6 c	75.5 c	84.4 b	100 a	75.5 b	70.0 b	77.7 c	88.8 c	100 a
Py-H2PC	77.7a	84.4a	90.0 b	100 a	100 a	80.0 a	86.6 a	100 a	100 a	100 a
Ph -MPC	72.2 b	80.0 b	100 a	100 a	100 a	77.7 a	86.6 a	100 a	100 a	100 a

Fusarium oxysporum radicis-lycopersici (isolate ForlQ4), Fusarium oxysporum lycopersici (isolate FolG14), Fusarium solani (isolate FsG1), Rhizoctonia solani (isolate RsG1), Sclerotium rolfsii (isolate SrM2), Macrophomina phaseolinae (isolate MpB1), Pythium sp (isolate Py H2) and Phytophthora sp (isolate Ph MPC).

Salicylic acid had been found to be active, as antimicrobia, in various trials as disease resistance inducers. These have been reported for inducting resistance against several soil-borne plant pathogens, *i.e*. fungal root rot and wilt pathogens (Chen-Chunquan *et al.*, 1999 and Mandavia *et al*., 2000), as well as fungal foliar diseases (Walters *et al.*, 1993; Srinivas *et al*., 1997; Sathiyabama and Balasubramanian, 1999; Cameron, 2000). Also, Guo *et al*. (1993) recorded that the toxicity of salicylic acid to *Fusarium oxysporum* f.sp. *vasinfectum* was 16.5 times that of normal fungicide Carbendazim. Moreover, salicylic acid could inhibit spore germination and mycelial growth of *Fusarium oxysporum* f. sp. *cumin*i (Mandavia *et. al*., 2000). Furthermore, Matthew and Alexander (1999) studied the direct effect of salicylic acid on the radial growth of *Alternaria solani*. They found that the fungal growth did not significantly differ when directly exposed to SA concentrations ranging from 0 to 200 µM, while the growth of *Pythium aphnidermatum*, the causal of cucumber root rot, was inhibited by salicylic acid at levels higher than 200 µg / ml (Chen-Chunquan *et al*., 1999).

Several inorganic salts and organic lipophilic acids and their salts, some of which are used, in the food-processing industry, have antimicrobial properties and could be useful as post-harvest treatment for decay control. The food preservatives potassium sorbate or sodium benzoate, have antifungal activities against post-harvest decaying fungi (Al-Zaemey *et al*., 1993; Olivier *et al*., 1999). Sorbic acid and its salt derivatives are the most widely used antimicrobial agents for food preservation worldwide. Using potassium sorbate or sodium benzoate against post-harvest diseases of tomato, apple, carrots and potato was reported (Ryu and Hold, 1993; Olivier *et al*., 1998). Food preservatives potassium sorbate or sodium benzoate when applied to citrus fruits inoculated with *Penicillium digitatum* had similar fungicidal activity and are equivalent to the traditional treatment used as a post-harvest fungicide for controlling citrus fruit decay (Hall, 1992). Furthermore, Fente *et al.* (1995) stated that *Aspergillus* was always more sensitive to potassium sorbate than *Penicillium*. *Aspergillus flavus, A. niger* and *Penicillium corylophilum* spoilage of bakery products was prevented by the use of weak acid derivatives such as potassium sorbate, calcium propionate and sodium benzoate. In the present study presented data in Table (2) show that potassium sorbate and potassium carbonate had inhibitory effect against mycelia growth of all tested fungi *in vitro*. The inhibitory effect increased by the increase in concentration used to reach its maximum at the highest concentration of 4%.and 8% Also, data (Table, 2) reveal higher inhibitor effect of potassium sorbate against the fungal growth comparing with potassium carbonate which resulted in observed complete growth inhibition (100%) at concentration 4% of potassium sorbate.

Table 2. Reduction % on the linear growth of tomato fungal pathogens in response to different concentrations of Potassium sorbate and Potassium carbonate on PDA medium

Fungal isolate	Potassium sorbate					Potassium carbonate				
	1%	2%	4%	6%	8%	0.5%	1.0%	2.0%	3.0%	4.0%
Forl-Q4	31.1 d	38.8e	22.2 f	91.1 b	100 a	33.3 b	44.4 b	61.1 d	81.1 b	100 a
Fol-G14	35.5 c	45.5 cd	70.0 c	88.8 b	100 a	31.1 b	43.3 b	83.8 a	77.7 c	88.8 b
Fs-G1	52.2 b	66.6 a	82.2 b	94.4 a	100 a	42.2a	50.0 a	57.7 c	75.5 c	91.1 b
Rs-G1	37.7c	47.7 c	60.0 d	91.1b	100 a	33.3 b	46.6 b	48.8 d	68.8 d	88.8 b
Sr-M2	38.8 c	50.0 c	22.2 f	88.8 b	100 a	31.1 b	38.8 c	51.1 d	68.8 d	80.0 c
Mp-B1	31.1 d	40.0 e	55.5 e	80.0 c	100 a	22.2 c	31.1 d	44.4 de	53.3 e	72.2 d
Py-H2PC	41.1 c	55.5 b	68.8 c	80.0 c	100 a	38.8 a	51.1 a	45.0 de	77.7 c	77.7 d
Ph -MPC	63.3 a	22.2 f	87.7a	100 a	100 a	26.6 h	38.8 c	66.6 b	91.1 a	100 a

Fusarium oxysporum radicis-lycopersici (isolate ForlQ4), Fusarium oxysporum lycopersici (isolate FolG14), Fusarium solani (isolate FsG1), Rhizoctonia solani (isolate RsG1), Sclerotium rolfsii (isolate SrM2) , Macrophomina phaseolinae(isolate MpB1) , Pythium spp (isolate Py H2) and Phytophthora spp (isolate Ph MPC).

There was a considerable interest in the use of sodium bicarbonate ($NaHCO_3$) and potassium bicarbonate ($KHCO_3$) for controlling various fungal diseases in plants (Karabulut *et al.*, 2003; Smilanick *et al.*, 2006). Bicarbonates are widely used in the food industry (Lindsay, 1985) and were found to suppress several fungal diseases of cucumber plants (Ziv and Zitter, 1992). Spraying plants with $NaHCO_3$ solution provided good control of several plant diseases (Palmer *et al.*, 1997; Horst *et al.*, 1999; Janisiewicz and Peterson, 2005). Also, spraying with KHCO3 solution provided the most effective protection against plant diseases (Fallilk *et al.*, 1996, 1997; Smilanick *et al.*, 1999, 2006). Sodium or potassium bicarbonate combined with oil were effective in controlling plant diseases (Ziv and Zitter, 1992; Horst *et al.,* 1999). Also, several studies dealt with the use of different salts to control various postharvest diseases of potato and other crops. Treatment of citrus fruit with sodium carbonate or sodium bicarbonate was shown to reduce the incidence of postharvest disease caused by *Penicillium digitatum* (Smilanick *et al.,* 1999, 2006). Postharvest application of ammonium bicarbonate, sodium bicarbonate, potassium carbonate, calcium propionate, and potassium sorbate reduced black root rot on carrots caused by *Chalara elegans* (Punja and Gaye, 1993). Recent studies suggest that salt application reduces postharvest potato diseases. Olivier *et al.* (1998) and Hervieux *et al.* (2002) showed that application of potassium sorbate, calcium propionate, sodium carbonate, sodium bicarbonate, potassium carbonate, potassium bicarbonate, and ammonium bicarbonate at 0.2 M reduced silver scurf lesion development and *Helminthosporium solani* sporulation on inoculated and naturally infected potato tubers. In general, organic acids and salts often have a broad spectrum of activity, even though the mechanisms by which they inhibit microorganisms are not well understood. Therefore, research is needed to better understand not only the mechanisms through which pathogens can contaminate raw fruits and vegetables but also the procedures for killing or removing pathogens once they are present, either on the surface or in internal tissues, and the analytical methods for their detection. In this regards, many investigations reported the use of potassium salts (K_2HPO_4 or KNO_3) as a chemical agent for induction of plant resistance (Stromberge and Brishammar, 1991; Yurina *et al.,* 1993). Furthermore, Abdel-Kader *et al.* (2012) reported that the inhibitory effect of the chemical plant resistance inducers, some essential oils and plant extracts against the linear growth of the root pathogenic fungi was evaluated *in vitro*. The chemical inducers were Sodium bicarbonate, Potassium bicarbonate, Potassium mono hydrogen phosphate, Calcium chloride and a mixture of Humic and Folic acids. They added that the obtained results indicate that all evaluated materials significantly reduced the linear growth of tested fungi *i.e. A. solani, F. solani, F. oxysporum* and *Pythium* sp.

Effect of essential oils on fungal growth

The inhibitory activity against mycelial growth of tested fungi was observed at all concentrations of lemongrass, thyme, citral and nerol oils (Table 3). The Mycelial growth decreased significantly with the increase in concentrations of essential oils and reached minimum mycelia growth at the highest concentration used. A complete inhibition of fungal growth was observed at 1.5% of all tested essential 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. Also, Ragab *et al*. (2012) reported that Thyme, Lemon grass, Peppermint, Clove and Mint oils had higher inhibitor effect on fungal mycelial growth than Limon, Cinnamon and Mustard oils. Fungal mycelial growth decreased significantly as the concentrations of essential oils were increased, to reach the fungal growth's minimum at the highest concentration used. Complete reduction (100%) in mycelial growth of two fungal isolates was recorded at concentration of 6% of all tested essential oils. Abd-Alla *et al.* (2011) studied the *in vitro* efficacy of the essential oils, lemongrass and thyme oils on linear growth and spores germination of *B. cinerea*. They found that rising concentration of tested chemicals reflected negatively on both linear growth and spores germination. Complete inhibition of linear growth and spore germination was observed with lemongrass and thyme oils vapors at concentration of 100.0 µl/L of each. Treated fungus with lemongrass and thyme oils vapors at concentration of 50.0 µl/L of each, resulted in reduction in the linear growth as 75.5 and 80.0% and spore germination as 82.1 and 84.2%, respectively. In this regard, vapors of thyme, oregano and lemongrass, and their respective major components showed completely growth inhibition of *Botrytis cinerea* and *Alternaria arborescens* as reported by Plotto *et al*. (2003). Also, Arrebola *et al*. (2009) recorded that Thyme and lemongrass oils showed over 50% and 25% inhibition of radial mycelia growth respectively. Tzortzakis and Economakis (2007) reported that lemongrass oil expressed antifungal activity against *Colletotrichum coccodes, B. cinerea, Cladosporium herbarium, Rhizopus stolonifer* and *A. nige*r *in vitro*.

They also reported that lemongrass oil at 25 ppm could inhibit spore production and at 500 ppm, the highest oil concentration employed, fungal sporulation was completely inhibited. Lemongrass oil could reduce spore germination and germ tube length of *C. coccodes, B. cinerea, C. herbarium* and *R. stolonifer*. Also, 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* (Alefyah and Avice, 1997). Furthermore, Essential oils of citrus or their constituents are shown to have fungicidal activities against postharvest pathogens of citrus (French *et al*., 1978; Rio Del *et al.*, 1998). In this regards, some constituents of essential oil from citrus fruits exhibited more toxicity against fungi than commercial fungicides (Singh *et al*., 1993). El-Gamal and El-Mougy (2002) recorded that Citral solutions at 8 ml/l and vapours at 40 µl/l caused complete inhibition to the growth of lime decaying fungi *Geotrihcum candidum*, *Penicillium digitatum* and *Penicillium italicum in vitro*. French *et al*. (1978) stated that citral is the most effective constituent of citrus essential oil. Furthermore, Rodov *et al*. (1995) reported that young mature–green lemon fruit manifests a significant lower level of postharvest decay compared with older yellow fruit. They added that, resistance of young fruit to decay development is related to citral level in lemon flavedo. They added that the flavedo of green lemon contained 1.5-2 times higher levels of citral comparing with the yellow fruit. Also, during long–term storage of lemon fruit, citral concentrations decreased in parallel with the decline of antifungal activity in the peel which was reflected in the increase of decay incidence. On the other hand, Aoudou *et al.* (2010) observed that nerol completely suppressed the growth of *A. flavus*, *A. niger* at 0.8 mg/ml; those of *A. parasiticus*, *Aspergillus* spp and *Penicillium* spp at 1 mg/ml. *Fusarium* species which are more susceptible were totally inhibited at 0.5 and 0.6 mg/ml. They added that fungi tested are all more susceptible to geraniol, citral, nerol and citronellol than others essential oils constituents.

Table 3. Reduction % on the linear growth of tomato fungal pathogens affected by some essential oils concentrations on PDA medium

Essential oils	**Con. %**	**Fungal isolates**							
		Forl-Q4	Fol-G14	Fs-G1	Rs-G1	Sr-M2	Mp-B1	Py-H2PC	Ph – MPC
Lemongrass	0.5	35.5 g	42.2 f	55.5 e	53.3 e	51.1 e	42.2 f	61.1 d	57.7 e
	1.0	50.0 e	57.8 e	62.2 d	66.6 d	55.5 e	60.0 d	73.3 c	70.0 c
	1.5	80.0 b	77.7 c	84.4 b	80.0 b	75.5 c	77.7 c	88.8 b	88.8 b
	2.5	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a
Thyme	0.25	40.0 f	44.4 f	50.0 e	57.8 e	35.5 g	40.0 f	64.4 d	55.5 e
	0.5	55.5 e	60.0 d	68.8 d	75.8 c	53.3 e	63.3 d	84.4 b	75.5 c
	1.0	82.2 b	82.2 b	90.0 b	91.1 b	80.0 b	82.2 b	88.8 b	90.0 a
	1.5	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a
Citral	0.25	42.2 f	46.6 f	57.7 e	66.6 d	36.6 g	46.6 f	70.0 c	66.6 d
	0.5	68.8 d	63.3 d	72.2 c	83.3 b	64.4 d	72.2 c	88.8 b	84.4 b
	1.0	86.6 b	88.8 b	86.6 b	100 a	83.3 b	88.8 b	100 a	100 a
	1.5	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a
Nerol	0.25	43.3 f	48.8 f	51.1 e	61.1 d	43.3 f	38.8 g	66.6 d	50.0 e
	0.5	57.7 e	53.3 e	58.8 e	80.0 b	55.5 e	64.4 d	86.6 b	72.2 c
	1.0	80.0 b	77.7 c	88.8 b	100 a	83.3 b	80.0 b	100 a	100 a
	1.5	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a

Fusarium oxysporum radicis-lycopersici (isolate ForlQ4), Fusarium oxysporum lycopersici (isolate FolG14), Fusarium solani (isolate FsG1), Rhizoctonia solani (isolate RsG1), Sclerotium rolfsii (isolate SrM2) , Macrophomina phaseolinae(isolate MpB1) , Pythium spp (isolate Py H2) and Phytophthora spp (isolate Ph MPC).

Also nerol oil has been reported as antifungal agent in several researches. Ćosić *et al.* (2010) stated that *e*leven essential oils (clove, rosemary, cinnamon leaf, sage, scots pine, neroli, peppermint, aniseed, caraway, lavander, common thyme) were tested for *in vitro* antifungal activity on twelve plant pathogenic fungi (*Fusarium graminearum, F. verticillioides, F. subglutinans, F. oxysporum, F. avenaceum, Diaporthe helianthi, Diaporthe phaseolorum var. caulivora, Phomopsis longicolla, P. viticola, Helminthosporium sativum,* *Colletotrichum coccodes, Thanatephorus cucumeris*). Their results indicated that all oils had antifungal activity against some or all tested fungi.

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, terpens, phenylpropannes, organic acids (Nychas, 1996). The aesthetic, medicinal and antimicrobial properties of plant essential oils have been known since ancient times.

It could be concluded that the obtained results in the present study provide insight into alternative antimicrobial compounds that can be manipulated in the production of fungal antiseptic substances that might be valuable for plant material disinfection.

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References

Abd-AllA M.A., M.M. Abdel-Kader, F. Abd-El-Kareem and R.S.R. El-Mohamedy (2011). Evaluation of lemongrass, thyme and peracetic acid against gray mold of strawberry fruits. Journal of Agricultural Technology 7(6):1775-1787.

Abdel-Kader, M.M., N.S. El-Mougy, N.G. El-Gammal, F. Abd-El-Kareem, M.A. Abd-Alla (2012). Laboratory Evaluation of Some Chemicals Affecting Pathogenic Fungal Growth. Journal of Applied Sciences Research 8(1):523-530.

Alefyah A. and Avice M.H. (1997). The fungicidal properties of plant extracts and essential oils. Plant Pathology-Global Perspectives of an Applied Science. E:\potato\The BSPP-Archives-BSPP Presidential Meeting 1997.htm (Offered Poster).

Al-Zaemey, A.B., Magan, N. and Thompson, A.K. (1993). Studies on the fruit coating polymers and organic acid on growth of *Colletotrichum musae in vitro* and postharvest control of anthracnose of bananas. Mycological Res., 97:1463–2468.

Aoudou, Y.; Léopold, T.N.; Michel, J.D.P.; Xavier, E.F. and Moses, M.C. (2010). Antifungal properties of essential oils and some constituents to reduce foodborne pathogen. Journal of Yeast and Fungal Research 1(1):001-008.

Arrebola, E., Sivakumar, D., Bacigalupo, R. and Korsten, L. (2009). Combined application of antagonist Bacillus amyloliquefaciens and essential oils for the control of peach postharvest diseases. Crop Protection: pp. 1-9.

Cameron, R.K. (2000). Salicylic acid and its rol in plant defense responses: what do we really know ? . Physiol. & Molecu. Pl Pathol., 56:91-93.

Chen-Chunquan S.; Belanger, R.R.; Benhamou, N.; Paulitz, T.C. and Chen, C.Q. (1999). Role of salicylic acid in systemic resistance induced by *Pseudomonas* spp. against *Pythium aphanidermatum* in cucumber roots . European J. Pl. Pathol., 105:477-486.

Ćosić, J., Vrandečić, K., Postić, J., Jurković, J. and Ravlić, M. (2010). In vitro antifungal activity of essential oils on growth of phytopathogenic fungi. Poljoprivreda 16(2):25-28.

Dwivedi, S.K. (1990). Antifungal activity of some phenolic compounds on *Fusarium oxysporum* f.sp. *psidi* causing guava wilt. Hindustan Antibiotics Bulletin, 32:33-35.

EL-Gamal, N.G. and El- Mougy, N.S. (2002). Safe, Easy and applicaple method for controlling Lime fruits postharvest diseases. Egypt. J. App. Sci., 17:27– 44.

Fente, C.A., Vazquez, B.B., Franco, A.C. and Quinto, F.E. (1995). Distribution of fungal genera in cheese and dairies: Sensitivity to potassium sorbate and natamycin. Arichiv fur Lebens-mitt Elhygiene, 46:62–65.

Foegeding, P.M. and Busta, F.F. (1991). Chemical food preservatives. In: Block SE, editor. Disinfection, sterilization and preservation. 4th edn. Philadelphia (PA): Lea & Febiger.

French, R.C., R.K. Long, F.M. Latterell, G.L. Granham, J.J. Smoot and P.E. Shaw (1978). Effect of nonnanel, citral and citrus oils on germination of conidia of *Penicillium digitatum* and *P. italicum.* Phytopathology, 68:877-882.

Guo, D.C., Wang, Q.F., Yan, S.Z. and Dai, K.S. (1993). A study on a new kind fungicide. zhiweiling Scientia Agricultura Sinica, 26:63-68.

Hall, D.J. (1992). Comparative activity of selected food pre-servatives as citrus postharvest fungicides. Horticultural Society, 101:184–187.

Hervieux, V., Yaganza E.S., Arul, J. and Tweddell, R.J. (2002). Effect of organic and inorganic salts on the development of *Helminthosporium solani*, the causal agent of potato silver scurf. Plant Dis. 86:1014–1018.

Horst, R.K., Kawamoto, S.O. and Porter, L.L. (1999). Effect of sodium bicarbonate and oils on control of powdery mildew and black spot of roses. Plant Dis.76:247–251.

Ismail, I.M.K.; Salama, A.M.; Ali, M.I.A. and Ouf, S.A. (1988). Effect of some phenolic compounds on spore germination and germ-tube length of *Aspergillus fumigatus* and *Fusarium oxysporum* f. sp. *lycopersici* . Egypt. J. Microbiol., 23:29-41.

Janisiewicz W.J. and Peterson D.L. (2005). Experimental Bin drenching system for testing biocontrol agents to control postharvest decay of apples. Plant Dis. 89:487–490.

Karabulut, O.A.; Bursa, G. and Mansour, M. (2003). Near-harvest applications of *Metschnikowia fructicola*, ethanol and sodium bicarbonate to control postharvest diseases of grape in central California. Plant Dis., 87:1384-1389.

Kaur J. and Arora D. (1999). Antimicrobial activities of species. Int. J. Antimicrob. Agents, 12:257–262.

Lindsay R.C. (1985). Food additives. In: "Food Chemistry" (O.R. Fennema, M. Dekker, eds.). Chapter 10, Inc., New York, USA, pp. 664–665.

Mandavia, M.K., Khan, N.A., Gajera, H.P., Andaria, J.H. and Parameswaram, M. (2000). Inhibitory effects of phenolic compounds on fungal metabolism in host- pathogen interactions in Fusarium wilt of Cumin . Allelopathy Journal 7:85-92.

Matthew, E.S. and Alexander, J.E. (1999). Salicylic acid induces resistance to *Alternaria solani* in hydroponically grown tomato. Phytopathol., 89:722-727.

Naqui, S.H.A., Khan M.S.Y. and Vohora S.B. (1994). Antibac-terial, antifungal and anthelmintic investigation on Indian medicinal plants. Fitoterapia 62:221–228.

Nascimento, G.G., Locatelli, J. and Freitas, P.C. (2000). Anti-bacterial activity of plants extracts and phytochemical on an-tibiotic resistant bacteria. Braz. J. Microbiol., 31:247–256.

Nychas, G.J.E. (1996). Natural antimicrobial from plantsIn: "New Methods of Food Preservation" (G.W Gould, ed.). Londres, CRC Press. . p. 235–258.

Oerke, C., H.W. Dehne, F. and Schonbeck, A. Weber (1994). Crop protection and crop production. Elsevier, Amsterdam.

Olivier C., Halseth D.E., Mizubuti E.S.G. and Loria R. (1998). Postharvest application of organic and inorganic salts for suppression of silver scurf on potato tubers. Plant Dis. 82: 213–217.

Olivier, C., Macneil, C.R. and Loria, J. (1999). Application of organic and inorganic salts to field-grown potato tubers can suppress silver scurf during potato storage. Plant Dis., 83:814–818.

Palmer C.L., Horst R.K. Langhans R.W. (1997). Use of bicarbonate to inhibit *in vitro* colony growth of *Botrytis cinerea*. Plant Dis. 81:1432–1438.

Plotto, A., Roberts, D.D. and Roberts, R.G. (2003). Evaluation of plant essential oils as natural postharvest disease control of tomato (*Lycopersicon esculentum*). Acta Horticulturae (ISHS) 628:737-745.

Punja Z.K. and Gaye M.M. (1993). Influence of postharvest handling practices and dip treatments on development of black root rot on fresh market carrots. Plant Dis. 77:989–995.

Ragab, M.M.M., A.M.A. Ashour, M.M. Abdel-Kader, R. El-Mohamady, A. Abdel-Aziz (2012). *In Vitro* Evaluation of Some Fungicides Alternatives Against *Fusarium Oxysporum* the Causal of Wilt Disease of Pepper (*Capsicum annum* L.). International Journal of Agriculture and Forestry 2(2):70-77.

Rio Del J. A.l., M.C. Arcas; O. Benaventa-Garcio; A. Ortuna and J.A. Rio Del. (1998). Citrus polymethoxylated flavones can confer resistance against *Phytophthora citrophthora, Penicillium digitatum* and *Geotrichum* species. J. Agric. & F. Chem., 46(10):4423-4428.

Rodov, V., S. Ben-Yehoshua; D.Q. Fang, J.J. Kim and R. Ashkenazi (1995). Pre-formed antifungal compounds of lemon fruit: citral and its relation to disease resistance. J. Agric. and F. Chem, 43:1057-1061.

Ryu, D., Hold, D.L. (1993). Growth inhibition of *Penicilium expansum* by several commonly used food ingredients. J Food Protection, 56:862–867.

SAS (1996). Statistical Analysis System. User‛s Guide: Statistics (PC-Dos 6.04). SAS Institute Inc., Cary, NC, USA.

Sathiyabama, M. and Balasubramanian, R. (19990. Treatment of groundnut leaves with salicylic acid controls the development of rust disease caused by *Puccinia arachidis* Speg . Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz, 106:166-173.

Shashi-Chauhan; Sharma, M. and Chauhan, S. 1989. *In vitro* efficacy of certain antifungal lotions on the growth of deratophytic fungi. National Academy of Science Letters, 12:11-13.

Singh, g.; R.K. Upadhyay; C.S. Narayanun; K.P. Padmkumorj and G.P. Rao (1993). Chemical and fungitoxic investigations on the essential of citrus sinensis (L.) pers. Zeitschrift fur pflanzenkranitan und pflanzenschutz, 100(1):69-74.

Smilanick J.L., Margosan D.A., Mlikota F., Usall J., Michael I.F. (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.

Smilanick, J.L.; Mansour, M.F. and Sorenson, D. (2006). Pre- and postharvest treatments to control green mould of citrus fruit during ethylene degreasing. Plant Dis., 90:89-96.

Srinivas, T.; Rao, K.C. and Chattopahayay, C. (1997). Effect of botanicals and chemicals on the management of blight (*Alternaria alternata; Alternaria helianthi*) of sunflower (*Helianthus annus*). Zeitschrift fur Pflanzenkrankheiten and Pflanzenschutz, 104:523-527.

Stromberge, A. and Brishammar, S. (1991). Induction of systemic resistance in potato (*Solanum tuberosum* L.) plants to late blight by local treatment with *Phytophthora infestans, Phytophthora cryptogea* or dipotassium phosphate. Potato Res., 34: =219-225.

Tzortzakis, N.G. and Economakis, C.D. (2007). Antifungal activity of lemongrass (*Cymbopogon citrates L*.) essential oil against Key postharvest pathogen. Innovation Food Science and Emerging Technologies 8(2):253-258.

Walters, D.R.; Mitchell, A.F.; Hampson, J. and McPherson, A. 1993. The induction of systemic resistance in barely to powdery mildew infection using salicylic acid and various phenolic acids . Ann. Appl. Biol., 122:451-456.

Winer B.J. (1971). Statistical Principles in Experimental Design. 2nd ed. MiGraw-Hil Kogakusha, LTD, pp. 596.

Yurina, T.P.; Karavaev, V.A. and Solntsev, M.K. (1993). Characteristics of metabolism in two cucumber cultivars with different resistance to powdery mildew. *Russian Plant Physiol.*, 40:197-202.

Ziv, O. and Zitter, T.A. (1992). Effects of Bicarbonates and film-forming polymers on cucurbit foliar iseases. Plant Dis. 76:513–517.

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