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Evaluation of some Fungicide Alternatives against Alternaria Fruit Rot on Tomato

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Mineral salts, natural oils and some natural extracts were evaluated for its efficiency as potential fungicides against *Alternaria alternata*, the causal of tomato fruit rot. Obtained results showed that potassium sulphate (K_2SO_4) at 3% inhibited *A. alternata* growth to 66.6%, followed by magnesium sulphate ($MgSO_4$) which reached 52.2%. *In vitro* studies indicated that ethanol extract of clove at concentration of 100 µl/100ml caused fungal growth inhibition reached 92.4%, while jojoba oil caused 44.8% growth inhibition. Moreover, when chitosan applied at 6mg/ml, it caused reduction in *A. alternata* growth reached 66.12%. Moreover, results of the *in vivo* studies indicated that applying of K_2SO_4, clove extract, chitosan solution and thyme oil reduced the diameter of lesions caused by *A. alternata* on infected tomato fruits. On the other hand, storage of tomato fruits at 13°C increased the efficacy of tested treatments. Obtained results showed the possibility of using K_2SO_4, clove extract, thyme and chitosan to control tomato postharvest fruit rot caused by *A. alternata*.

Keywords: *Alternaria alternata*, chitosan, clove, fruit rot, mineral salts, thyme and tomato fruits.

Tomato (*Solanum lycopersicum* Mill.) is one of the most widely grown vegetable in Egypt. Its fruits are vulnerable to attack by many pathogenic diseases. Black mould rot of tomato fruit, caused by *Alternaria alternata* (Fr.) Keissl., is the most common tomato diseases in Egypt. The causal agent of this disease is frequently causes substantial postharvest losses (Reddy *et al*., 2000). The application of postharvest fungicides is prohibited, due to the several normative restrictions for fungicide usage. Also, the appearance of pathogens resistant to fungicides has dissuaded their repeated usage (Mari *et al*., 2009).

Recently, a worldwide trend was concerned to explore new alternatives that manage postharvest pathogenic diseases, giving priority to methods that reduce disease incidence and avoid negative and side effects on human health as a result of the excessive application of synthetic fungicides (Johnson and Sangchote, 1994 and Oliveira *et al.*, 2012). Thus, research efforts have led to the development of novel control tools, as alternatives to synthetic fungicide treatments. For schematic reasons, these can be grouped into four main categories: (i) natural compounds (ii) compounds generally recognized as safe (iii) biological control agents; and (iv) physical methods alone or the combination of all four groups (Mari *et al.*, 2009; Romanazzi *et al.*, 2012).

The main objective of this study is to evaluate the antifungal activity of some fungicide alternatives, such as mineral salts, essential oil and chitosan on *A. alternata*, the causal of tomato fruit rots.

Materials and Methods

The pathogen:

Alternaria alternata was originally isolated from decayed tomato fruits. The pathogen was maintained in potato dextrose agar (PDA) slant in test tubes and stored at 4°C. Fungal pathogenicity and virulence were maintained by inoculating tomato fruit with the fungus and re-isolating it.

Fruit samples:

Tomato fruits (cv. GS) were harvested at maturity stage and kept at 5±0.5°C. Tested fruits were dipping in 3% sodium hypochlorite solution for 30 second and rinsed three times with sterile distilled water and left on folds of tissue papers to remove the access of water.

In vitro studies:

Inhibition of A. alternata mycelial growth:

Tested microorganism was performed on PDA medium in Petri dishes (9-cm-diam.). Procedures for growth inhibition measurements were done according to the technique described by Ambroziak (2012). Mycelial discs (5-mm-diam.), taken from the periphery of an actively *A. alternata* culture growing on PDA were transferred onto the centre of the prepared Petri dishes, then incubated at 25±0.5°C for 15 days. Three dishes were used as replicates for each treatment, *i.e.* mineral salts, seed extracts, essential oils and chitosan. Fungal growth diameter was measured and growth reduction was calculated in relative to check (control) treatment. This experiment was repeated twice.

Effect of mineral salts on fungal growth:

Tested mineral salts, *i.e.* calcium carbonate ($CaCO_3$), potassium monohydrogen phosphate (K_2HPO_4), potassium sulphate (K_2SO_4), magnesium sulphate ($MgSO_4$), were individually applied into Conical flasks containing sterilized PDA medium to obtain the proposed concentrations, *i.e.* 0.25; 0.5; 1; 2 and 3g/l, then mixed gently and dispensed in sterilized Petri dishes (9-cm-diam.). Another set of flasks containing sterilized PDA medium free of tested salts, was used as check treatments. Petri dishes were individually inoculated at the centre with equal disks (5-mm-diam.) of tested fungal cultures. The fungal linear growth in each treatment was measured 7 days after incubation, and the growth reduction was calculated in relative to check treatment according to Fokemma (1973) using the following equation:

$$\text{Reduction percentage} = (C- T) /C \times 100$$

Whereas; C = Maximum linear growth in control.
T = Maximum linear growth in treatment.

Effect of seed extracts on fungal linear growth:

Seed extracts of clove (*Eugenia caryophyllata* Thunb.) and jojoba (*Simmondsia chinensis* (Link) Schneid.), were prepared in the Agric. Botany Dept., Fac. Agric., Suez Canal Univ., by adding grinded seeds to different tested solvents, *i.e.* ethyl ether, acetone, ethyl alcohol 98% and water (1:1; w:w) at room temperature for 48h. The inhibitory effect of tested extracts, at concentrations of 50 and 100 µl, was determined against the linear growth of *A. alternata*. Fungal inoculation, incubation conditions, growth measurements and calculations were followed as stated above.

Effect of chitosan on mycelial growth of A. alternata:

Chitosan [poly-β-(1→4) N-acetyl-D-glucosamine], is the N-deacetylated form of chitin, a high molecular weight polysaccharide is currently obtained from the outer shell of crustaceans (Sandford, 1989). Chitosan at concentrations of 1, 2, 3, 4, 5 and 6 mg/ml were added to PDA medium to evaluate their effect on *A. alternata* growth (Du *et al.*, 1997). Fungal inoculation, incubation conditions, growth measurements and calculations were followed as mentioned before.

Effect of essential oils on A. alternata growth:

Antifungal activity of two essential oils of rosemary (*Rosmarinus officinalis* L.) and thyme (*Thymus vulgaris* L.) was determined at two concentrations, *i.e.* 50 and 100 µl/ml, against *A. alternata* growth on PDA medium. Twenty millilitres of media containing tested concentration of essential oils amended with 0.5% Tween-80 was poured into each Petri plate and then inoculated with the tested fungus and incubated at 25±0.5°C for 14 days. Growth measurements and calculations were followed as mentioned before.

In vivo antifungal assay:

Apparently healthy tomato fruits, collected from Ismailia Governorate markets, were surface sterilized using 3% sodium hypochlorite solution followed by immersing in sterile distilled water for 2 min., then left at room temperature on folds of tissue paper to remove the excess of water. Tested fruits were then randomly divided into groups; each one consists of 20 fruits, and then transferred into 1.5 litres plastic boxes. Under aseptic conditions, tested fruits were wounded (4-mm-long and 2-mm-deep) using a sterile stainless steel scalpel, each wound was inoculated with 20 µl of *A. alternata* spore suspension (2.5×10^5 conidia/ml) using a micropipette. A group of fruits was treated with sterile distilled and kept as check (control). Two hours later, each wound was treated with a pre-determined concentration of each tested materials. A layer of water was placed at the bottom of the plastic boxes, to maintain high humidity, and then boxes were closed with perforated lids to eliminate any accumulation of CO_2. Two boxes were used as replicates for each treatment. Tested boxes were stored at 13, 23 and 28°C with 90% relative humidity for two weeks, and then the fruits were classified into four categories, whereas 1= No rind blemishes; 2= Slight blemishes present; 3= Moderate blemishes present and 4= Severe rind injury. This experiment was repeated twice with each particular treatment. Tested materials were evaluated as fungicide alternatives against *A. alternata* according to Reddy *et al.* (2000). Fruit stem scars were treated individually either with chitosan solution or sterile deionized water (as check). On each stem scar, 100 µl of chitosan solution (6 gl^{-1}) or sterile water was applied.

Treated tomatoes were incubated at 13, 23 and 28°C for 7 and 14 days. Infected fruit percentages were recorded at the end of incubation periods. Each treatment was replicated three times with 20 fruits/replicate, and the entire experiment was repeated twice. Different volumes of the thyme, rosemary, clove and jojoba extract (50 and 100 µl/l) and sterile distilled water (check) were added into each wound site. To evaluate pathogenesis and disease resistance, fruits were observed daily and diameter of formed lesions were recorded on the 7^{th} and 14^{th} day. This experiment was repeated twice.

Statistical analysis:

All experiments were set up in a complete randomized design. Analysis of variance (ANOVA) for unequal sample sizes and means was separated by least significant differences (LSD) at $p \leq 0.05$ as described by Song and Keane (2006).

Results

Inhibition fungal growth studies:

The inhibitory activity of inorganic salts (K_2SO_4, $MgSO_4$, $CaCO_3$, K_2HPO_4) against *A. alternata* was evaluated *in vitro*. The mycelial growth of the causal agent of tomato fruit rot was suppressed to the highest degree (66.6%) in a medium containing K_2SO_4 at a rate of 3% (Table 1). Increasing salt concentrations from 0.25% to 3% inhibited mycelial growth. Different tested salts at 3% concentration such as K_2SO_4 and $MgSO_4$ strongly reduced mycelial growth of the fungus whereas K_2HPO_4 and $CaCO_3$ inhibited mycelial growth to a lesser extent (41.6 and 29.4%, respectively). The differences in the inhibitory effects of the salts were in general statistically significant ($P \leq 0.05$).

Table 1. Effect of different salt concentrations on *A. alternata* linear growth

Treatment	Salt concentration (%)									
	0.25		0.5		1		2		3	
	Linear (mm)	GR*	Linear (mm)	GR	Linear (mm)	GR	Linear (mm)	GR	Linear (mm)	GR
K_2SO_4	67.5	25.0	62.5	30.5	50.0	44.4	46.0	48.8	30.0	66.6
$MgSO_4$	67.3	25.2	66.1	26.5	60.1	33.2	58.1	35.4	43.1	52.1
$CaCO_3$	74.6	17.1	68.6	23.7	65.6	27.1	64.1	28.7	63.5	29.4
KH_2PO_4	76.5	15.0	74.8	16.8	60.6	32.6	60.1	33.2	52.5	41.6
Control	90.0	-	90.0	-	90.0	-	90.0	-	90.0	-
LSD at 5%	2.52		2.41		1.95		2.33		1.74	

* GR= Growth reduction (%)

Regarding the effect of different solvent extracts of clove and jojoba on the biomass of *A. alternata*, results (Table 2) show that only ethyl ether extracts exhibited the highest antifungal activity, with gradual reduction in fungal growth with increasing the extract concentration. Moreover, ethyl ether extract of clove at concentration of 100 µl/100ml reduced the fungal growth by 92.4%. Meanwhile, the other tested solvents didn't have any antifungal activity against the tested fungus.

Table 2. Effect of clove and jojoba extracts from different solvents on growth of *A. alternata*

Treatment	Solvent	Solvent concentration (µl/100 ml)			
		50 µl		100 µl	
		Linear growth (mm)	GR*	Linear growth (mm)	GR
Clove	Water	90	0.0	90	0.0
	Ethyl alcohol	90	0.0	90	0.0
	Acetone	90	0.0	90	0.0
	Ethyl ether	29	67.8	6.8	92.4
Jojoba	Water	90	0.0	90	0.0
	Ethyl alcohol	90	0.0	90	0.0
	Acetone	90	0.0	90	0.0
	Ethyl ether	58.3	35.2	49.6	44.8
Control	-	90	-	90	-
LSD at 5%		1.29		1.49	

* GR= Growth reduction (%)

Antifungal activity of chitosan compounds against *A. alternata* was *in vitro* investigated. Results presented in Table (3) show that in general, all the tested concentrations exhibited a good fungicidal activity against the tested fungus compared to the check treatment. Also, results indicate that applying chitosan at concentration of 6 mg/ml caused the highest growth reduction (being 66.1%) of the tested fungus. Meanwhile, concentration of 1mg/ml recorded the least effect (37.3%) in this concern.

Table 3. Effect of different chitosan concentrations on *A. alternata* growth

Chitosan concentration (mg ml^{-1})	*A. alternata*	
	Linear growth (mm)	Reduction (%)
1.0	56.4	37.4
2.0	51.1	43.2
3.0	48.9	45.7
4.0	43.8	51.4
5.0	33.5	62.8
6.0	30.5	66.1
Control	90.0	-
LSD at 5%	4.1	-

Efficacy of thyme and rosemary oil on fungal growth reduction was evaluated. Results presented in Table (4) show that the highest degree of antifungal activity caused by thyme oil followed by rosemary oil. Thyme oil exhibited most pronounced antifungal potentials against *A. alternata* as it produces 53.4% reduction in the mycelial growth at concentration of 50µl/100ml after 7 days of incubation. Whereas, at concentration of 100µl/100ml the inhibition growth of *A. alternata* was

Table 4. Effect of two concentrations of thyme and rosemary oils on linear growth of *A. alternata*

Treatment	50 µl/100 ml		100 µl/100 ml	
	Linear growth (mm)	GR*	Linear growth (mm)	GR
Thyme	42.0	53.4	22.6	74.9
Rosemary	57.5	36.2	37.7	58.2
Control	90.0	-	90.0	-
L.S.D. at 5%	3.46		2.82	

* R= Growth reduction (%)

recorded 74.9% and growth started at 3ed day after inoculation. Increase in concentration of rosemary oil from 50 and 100µl/100ml, increased fungal inhibition up to 36.2 and 58.2%, respectively. They had a statistically significant negative impact on mycelium growth of tested fungus compared with the control treatment.

Effect of tested materials in disease reduction:

Results presented in Table (5) indicate that all treatments were effective in reducing the tomato fruit rot incidence under different storage conditions. Results also indicate the positive correlation between the decrease in thermal storage degree with K_2SO_4, $MgSO_4$ and the disease reduction. In general, K_2SO_4 was more effective than $MgSO_4$ in reducing the fruit rot infection with *A. alternata*. Treated tomato fruits with K_2SO_4 at 13°C storage condition caused the best control of disease incidence and suppressed the lesion diameter 7 days and 14 days after inoculation at 0.28 and 0.95 mm, respectively. In this regard, negative correlation was noticed between the shelf-life of tomato fruits and lower disease incidence during storage period and the disease incidence on tomato fruits under storage conditions.

Table 5. Effect of two salts on the development of tomato fruit rot caused by *A. alternata* under different storage temperatures

Treatment	Temp. (°C)	Lesion diameter (mm)		Rot incidence (%)	
		7-day	14-day	7-day	14-day
Potassium sulphate (K_2SO_4)	13	0.3	0.9	11.2	22.3
	23	1.0	5.3	22.3	33.4
	28	1.9	7.4	22.3	33.4
Magnesium sulphate ($MgSo_4$)	13	0.4	1.3	11.2	22.3
	23	1.6	7.0	22.3	33.4
	28	2.5	8.2	22.3	44.4
Control	13	5.8	11.6	44.5	66.7
	23	21.3	33.7	88.9	100.0
	28	48.4	52.7	100.0	100.0
LSD at 5%		2.93	2.39	4.14	5.1

Results presented in Table (6) show that the essential oil substances indirectly affect the growth of *A. alternata* and significantly prevented its development. The black mould rot incidence of artificially inoculated tomato fruit reduced at 3 and 22.3 after 7 and 14 days after inoculation at 23°C as compared with control. No disease symptom was observed on infected tomato fruit treated with clove extract at 13°C as compared with the control. However, the inhibition by clove extract in tomato was not as dramatic as that in plates. In general, levels of essential oils and their compounds necessary to inhibit microbial growth were higher in foods than in culture media.

Table 6. Effect of clove extract on the tomato fruit rot development caused by of *A. alternata* at different storage temperatures

Treatment	Temp. (°C)	Lesion diameter (mm)		Rot incidence (%)	
		7-day	14-day	7-day	14-day
Clove extract	13	0.0	0.0	0.0	0.0
	23	0.7	3.0	11.2	22.3
	28	0.8	4.8	11.2	33.4
Control	13	5.8	11.6	44.5	66.7
	23	21.3	33.7	88.9	100.0
	28	48.4	52.7	100.0	100.0
LSD at 5%		2.1	1.7	2.4	2.9

Effect of chitosan on tomato fruit black mould rot was obtained in Table (7). Black mould rot developed at a higher rate ($P < 0.05$) in the inoculated control fruit compared with chitosan-treated fruit. In the inoculated control fruit, lesions were visible within 7 days after inoculation and increased significantly with increasing storage time under different storage temperature. While, in the chitosan-treated fruit no visual symptoms were observed at 13°C after 7 and 14 days. The lesions were visible only after 7 days of storage at moderate or high storage temperature (23 and 28°C). It is also clear, from the results in Table (7), that the lesion development in chitosan-treated fruit at the same storage temperature was lower than other symptoms at the same temperature in the control fruits. Control of lesion development in chitosan-treated fruit indicated that chitosan had an inhibitory effect on pathogenic fungus understudy. Overall, a general trend was found that the concentration of extracted incubation period was increased in the contrary lesion diameter and disease incidence was decreased.

Results presented in Table (8) indicate that all tested treatments had a good inhibitory effect on mycelial growth of *A. alternata* when tested on the tomato fruits. Results also show that thyme essential oil exhibited most pronounced antifungal potentials against *A. alternata* as it inhibited the mycelial growth at concentration of 100µl/100ml 14 days after incubation period. It is also clear that, thyme oil reduced the lesion diameter followed by rosemary oil compared with the control at 7 days after inoculation (0.95, 1.84 and 21.28 mm, respectively). Whereas, no significant difference was recorded in inhibitory effects caused by both the thyme and rosemary

Table 7. Effect of chitosan on the tomato fruit rot development caused by of *A. alternata* at different storage temperatures

Treatment	Temp. (°C)	Lesion diameter (mm)		Rot incidence (%)	
		7-day	14-day	7-day	14-day
Chitosan	13	0.0	0.0	0.0	0.0
	23	0.5	2.6	11.2	22.3
	28	0.7	5.2	22.3	33.4
Control	13	5.8	11.6	44.5	66.7
	23	21.2	33.7	88.9	100.0
	28	48.4	52.7	100.0	100.0
LSD at 5%		2.83	3.47	4.0	4.9

Table 8. Effect of thyme and rosemary oils on the development of tomato fruit rot caused by of *A. alternata* 7 and 14 days after inoculation

Treatment	Lesion diameter (mm)		Rot incidence (%)	
	7-day	14-day	7-day	14-day
Thyme	0.95	3.73	95.5	88.9
Rosemary	1.84	7.80	91.3	76.8
Control	21.28	33.73	---	---
LSD at 5%	2.73	2.23	3.11	2.91

essential oils 7 days after inoculation. Furthermore, at 14 days, the tested oil cause best disease reduction when applied on tomato fruit compared with the control treatment.

D i s c u s s i o n

Alternaria fruit rots considered as the most tomato common diseases in Egypt. Some mineral salts, natural oils and natural extracts were used in estimating its efficiency as potential fungicides against *Alternaria alternata*. In this research, inhibitory effect of inorganic salts, *i.e.* K_2SO_4, $MgSO_4$, $CaCO_3$, K_2HPO_4 against *A. alternata* was *in vitro* observed by varying degrees. The obtained results are in line with those reported by previous studies. Mills *et al.* (2004) determined that mycelial growth and spore germination of *A. alternata*, *Botrytis cinerea* and *Fusarium solani*, were strongly limited by sodium metabisulfite and propyl-paraben. During *in vitro* trials, the mycelial growth of *A. alternata* was strongly inhibited by aluminium chloride and copper sulphate. Blachinski *et al.* (1996) found that KNO_3, KCl, K_2SO_4, and KH_2PO_4 had an inhibitory effect on the mycelial growth of *A. solani* and *A. macrospora*, as well as spore germination of *A. solani*. Moreover, Feng and Zheng (2006) demonstrated that potassium chloride and sodium chloride suppressed the growth of *A. alternata*.

Observed results regarding the effect of different solvents for clove and jojoba extract on the biomass of *A. alternata*. Among the various solvents of the plant materials, ether solvent exhibited the highest antifungal effect on the fungal pathogen. The obtained results are in agreement with thus Abd-El-Khair and Haggag (2007) and Aslam *et al.* (2010). In this regard, Huang and Chung (2003) described

possible mechanism for antifungal activity of phenolics includes swelling of hyphal tip followed by seeping and leaking of plasma due to distortion of cell wall resulting in abnormal branching, fusion and wrinkling of hyphae. According to this result, it is possible that essential oils could be used in plant disease control as the main or as adjuvant antimicrobial compounds. Montes-Belmont and Carvajal (1998) reported that clove oil showed the presence of eugenol, alpha-terpineol, isoeugenol and beta-terpinene as its major components. Eugenol has been reported by different workers to be the most effective component of the clove and cinnamon EOs against various pathogens.

Antifungal activity of chitosan compounds towards the *A. alternata* was *in vitro* investigated. In general, all the tested concentrations were more active than the control and exhibited a good fungicidal activity against the tested fungus especially with the higher concentrations compared with the control treatment. This finding is in harmony with those of Allan and Hadwiger (1979) and Saharan *et al.* (2013) who suggested that chitosan showed the maximum growth inhibitory effects on *in vitro* mycelial growth of *A. alternata* at 0.1% concentration. In this respect, Reddy *et al.* (1997) proved that chitosan significantly affected both growth and toxin production of *A. alternata* at higher concentrations. However, at lower concentrations, toxin production was affected more than the growth as evidenced by minimum inhibitory concentrations of chitosan derived for toxin production and mycelial growth.

Efficacy of thyme and rosemary oil on fungal growth reduction was obtained. Presented results showed that the highest degree of antifungal activity caused by thyme oil followed by rosemary oil was recorded and thyme oil exhibited most pronounced antifungal potentials against *A. alternata*. Those results are in accordance with the strong toxic properties of thyme oil and its active compounds, such as thymol and carvacrol, against a large number of microorganisms described by Soliman and Badea (2002), Edris and Ferrag (2003) and Ćosić *et al.* (2010). In addition, according to Šegvić *et al.* (2007) the thyme essential oil, which contains p-cymene (36.5%), thymol (33.0%) and 1,8-cineole (11.3%) as main components, and pure thymol exhibited antifungal activities. *In vitro* tests showed that fungi such as *A. alternata* were controlled 100% with thyme essential oil incorporated into nutrient agar at 500 mg L−1 (Plotto *et al.*, 2003). On the contrary, in the present research, rosemary oils showed moderate inhibitory effect on *A. alternata*. In this regard, Bartyńska and Budzikur-Ramza (2001) described high toxicity of eucalyptus, lavender and rosemary (1-8 cineole 44.40%) against *Fusarium* spp.

The effect of tested materials against disease incidence, K_2SO_4 was the more effective salt than $MgSO_4$ in reducing the fruit rot infection with *A. alternata*. Recorded results also indicated the positive correlation between the decreases in thermal storage degree with K_2SO_4, $MgSO_4$ and the disease reduction. Concerning the tested salts, K_2SO_4 and $MgSO_4$ were more effective on decreasing the disease incidence and lesion diameter where these were highly significance in their efficacy compared with the check treatment under inoculation with *A. alternata*. Our results are in agreement with Olivier *et al.* (1999), which stated that some organic and inorganic salts are active antimicrobial agents and have been widely used in the food industry. Many of these salts are effective against a range of microorganisms; most

have low mammalian toxicity and therefore have potential for postharvest disease control. Salt treatments can inhibit plant pathogens or suppress mycotoxin production.

Oil substances indirectly affect the growth of *A. alternata* and significantly prevent its development. Disease was not observed on infected tomato fruit which treated with clove extract at 13°C as compared with the control. Levels of essential oils and their compounds necessary to inhibit microbial growth were higher in foods than in culture media. This was due to interactions between phenolic compounds and the food matrix (Nychas and Tassou, 2000). Essential oils are made up of many different volatile compounds; the residual levels would be low after storage. Clove extract possessed antifungal activity inhibiting the growth of *A. alternata* and leading to deleterious cellular morphological modifications. Moreover, it did not affect the flavour or the appearance of the tomato fruits.

Chitosan-treatment for controlling tomato fruit rot indicated that chitosan had an inhibitory effect on pathogenic fungus understudy. In the inoculated control fruit, lesions were visible within 7 days after inoculation and increased significantly with increasing storage time under different storage temperature. Inhibition of fungal growth as evidenced by reduced lesion size in chitosan treatments showed that chitosan has directly antifungal effect. In addition, chitosan interfered with production of fungal virulence factors such as cell wall degrading enzymes, organic acids, and host specific toxins. Previous studies confirmed direct antifungal action of chitosan (Allan and Hadwiger, 1979 and El-Ghaouth *et al.*, 1992). Although information is available on the antimicrobial effects of chitosan, it is stable to know the actual mechanism and its impediment in pathogenesis of the causing organism in plant. Several studies have confirmed the antimicrobial effect of chitosan when it is in direct contact with the target organism. Chitosan is unusually susceptible to a variety of enzymes such as proteases, cellulases, pectic enzymes and lipases (Pantaleone *et al.*, 1992). At the same trend, the finding of Doares *et al.* (1995) indicated that chitosan oligosaccharides activate plant defence genes by signal transduction involving jasmonic acid similar to wound response, sustains this hypothesis.

Presented results showed that thyme essential oil exhibited most pronounced antifungal potentials against *A. alternata* as it inhibited the mycelial growth at concentration of 100µl/100ml. Tested oil cause best disease reduction when applied on tomato fruit compared with the control treatment. Other investigators have also reported the inhibitory effect of some essential oils which have potential antifungal properties also have the possibility for use as alternatives to synthetic fungicides (Caccioni and Guizzardi, 1994; Gorris *et al.*, 1994 and Lis-Balchin *et al.*, 1998).

Essential oils provide a wide variety of compounds as alternatives to synthetic fungicides; however, they have not been developed into products for postharvest treatments. Their potential as alternatives for disease control resides precisely in that their antimicrobial activity is not attributable to one specific mechanism so it is difficult to create resistance in the microorganisms (Burg, 2004).

Conclusion

This study has shown the potential of using mineral salts, essential oils, chitosan and some plant extracts in reducing this most important tomato postharvest disease of tomato. The results obtained from both *in vitro* and *in vivo* experiments indicated that K_2SO_4, clove extract, chitosan solution and thyme essential oils inhibited the mycelial growth of postharvest pathogen (*A. alternata*). In all experiments, the antifungal effect was increased by increasing the concentration of the used materials. Therefore, it could be used in treatment of fruits and vegetables against postharvest pathogens.

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تقييم فعالية بعض بدائل المبيدات الفطرية فى مكافحة عفن الألترناريا فى ثمار الطماطم

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الطماطم أكثر محاصيل الخضر انتشاراً فى مصر ويتاثر محصولها سنوياً بعدد من الامراض الفطرية ومنها عفن ثمار الالترناريا أكثر الامراض انتشاراً فى مصر. فى هذا البحث تم استخدام بعض المستخلصات و الزيوت الطبيعية والاملاح المعدنية فى تقدير كفائتها كبدائل طبيعية وامنة بدلا من المبيدات الفطرية. بعض الزيوت النباتية استخدمت لتقييم فاعليتها كبدائل طبيعية وآمنة للمبيدات ضد الفطر *Alternaria alternata*. أظهرت النتائج أن من بين أربعة أملاح معدنية مختبرة استطاع ملح كبريتات البوتاسيوم (K_2SO_4) عند تركيز ٣% تثبيط النمو الفطرى بمعدل ٦٦.٦% يلية ملح كبريتات الماغنسيوم $MgSO_4$. كما أظهر الاختبار المعملي للمستخلص الكحولي للقرنفل عند تركيز ١٠٠ميكروليتر /١٠٠ مل القدرة على تثبيط النمو الفطرى الى ٩٢.٤% كما أن مستخلص بذور الجوجوبا أدى إلى تبيط النمو بمعدل ٤٤.٨% عند نفس التركيز السابق. من ناحية اخرى, أظهر الكيتوزان قدرة على تثبيط نمو فطر *A. alternata* . وكان أكبر معدل للتثبيط عند التركيز الاعلى من الكيتوزان وهو ٦ملجم/مل. أشارت نتائج دراسة تاثير المركبات السابقة فى مقاومة مرض عفن الثمار الالترنارى إلى أن كبريتات البوتاسيوم, ومستخلص القرنفل والكيتوزان وزيت الزعتر استطاعت السيطرة بشكل ملحوظ على تقدم مرض العفن الأسود فى الطماطم المتسبب عن فطر *A. alternata* كما أشارت النتائج أيضاً إلى أن تخزين الثمار أثناء بعض هذه المعاملات عند درجة حرارة ١٣°م ساهم بشكل كبير فى السيطرة على عفن الثمار وإطالة فترة التخزين بشكل امن. مما سبق من النتائج المتحصل عليها فى هذه الدراسة نستنتج أنه يمكن استخدام كل من ملح كبريتات البوتاسيوم ومستخلص القرنفل وزيت الزعتر والكيتوزان فى مقاومة أعفان ثمار الطماطم بعد الحصاد.