Efficiency of some Plant Extracts to Control Alternaria Rot of Tomato Fruits

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ABSTRACT

The effect of plant extracts from normal flora of Eljabel Alakhdar area in Libya on Alternaria decay of tomato fruits and maintaining storage quality of these fruits was investigated. Data proved that fruits treated with all plant extracts showed significant reduction in infection percentages compared with untreated ones. Thyme and Garlic significantly decreased the disease severity of decay. However, there were insignificant differences in the degrees of infection between untreated fruits and those treated with Rosemary extract at low concentration. Meanwhile, significant decrease in Titratble acidity values was detected in untreated fruits as compared with treated ones and at the same time, treated fruits gave the highest mean values of phenolic compound content.Whereas, fruits treated with plant extracts before inoculation with *A. alternata* showed a significant increase in total soluble sugars and lignin content compared with that of untreated fruits.Thus, these treatments might reduce the changes in biochemical properties of fruits inoculated with the tested fungus.

INTRODUCTION

Tomato (*Lycopersicon esculentum*) fruits is an abundant crop used by many in Libya and indeed all over the world for making salad or cooked food. Fresh-market tomatoes occupy an important position in producing markets in many countries. About 34% of world production comes from countries around the Mediterranean sea and about 14% from California and Mexico (FAO, 2002).

Fruits of tomato are susceptible to many diseases during harvesting, marketing and storage which cause serious losses in quantity or quality of the fruits. During storage and shipment of tomatoes, decay losses are mainly caused by pathogens such as *Botrytis cinerea, Alternaria alternate* and *Rhizopus stolonifer* (Akhtar et al., 1994). Extremely high losses in grains, vegetables and fruits are caused by fungal infection. As much as 30% of the tomato harvested crop may be lost due to postharvest diseases before it reaches the consumer (Boyette et al.,1994). About 80% of the total loss in prepackaged and loose tomato fruits were due to Alternaria rot and Rhizopus rot (Hahn, 2006). Recently, an increasing number of countries have demanded fresh products that are not treated

with agrochemicals, particularly those applied after harvest. Many reports (e.g. Dixit, *et al.*, 1987; El-Ghaouth, *et al.*, 1991 Ejechi, *et al.*, 1997 and Akpomedaye and Ejechi, 1998) show that some plant material possess antimicrobial activity. Many *in vitro* studies on the control of postharvest pathogens have been carried out and showed the fungicidal or fungistatic activity of some extracts and essential oils from various plants (Farina, et. al., 2007). Fawzi, *et. al.* (2009) revealed that plants extracts especially those performed with cold distilled water had a strong antifungal activity with significant inhibition on the growth of *A. alternata* and *F. oxysporum*. Some plants of Libyan flora may posses antimicrobial activity such as rosemary, garlic and thyme. Many investigators revealed that the extracts of these plants affected mycelial growth and spore germination of some isolated fungi and proved to be effective in controlling fungal decay of friuts (El-Jaly, 1996, Mohamed-Nawara, 1999 and Al-Awami, *et. al.*, 2009). The growth of the fungal *Alternaria alternata* was remarkably reduced by garlic extract (Muhsin, *et. al.*, 2000)

This study aimed to determine if some plant extracts from Libyan flora are beneficial in control of tomato postharvest decay caused by *A. alternate* and maintaining storage quality of fruits.

MATERISLS AND METHODS

Three plant materials from Eljabel Alakhdar area, libya, were tested for control studies of Alternaria rot on tomato fruits. The tested plants were presented in Table (1)

Table (1). The tested plants for antifungal activity studies

Arabic name	English name	Scientific name*
Ekleel	Rosemary	*Rosmarinus oficinalis* Linn.
Zatr	Thyme	*Thymus vulgaris* Linn.
Thoom	Garlic	*Allium sativum* Linn.

* The scientific names of these plant materials were confirmed by Kotb (1985).

Samples (300 g) of fresh leaves of rosemary, thyme and garlic cloves were washed under running tap water, rinsed with distilled water then chopped up and placed in distilled water (1L). Each sample was then processed in blender until a homogenous solution was obtained. The solution was allowed to stand for 30 min. The supernatant liquid was passed through whatman No. 1 filter paper, then through a membrane filter (0.2 µ) to avoid any bacterial or fungal contamination (Qasem, 1996).

An isolate of *Alernaria alternata* was supplied by plant protection department, Faculty of Agriculture, Omer Al-Mokhtar University, Elbieda, Libya. Inoculum suspensions were prepared and adjusted to 10^5 conidia/ml by hemocytometer. Tomato fruits (Rio Grand cultivar) were surface sterilized and wounded as mentioned by Hong *et al.*, (1998).. Into each wound, a small amount (20 µl) of the solution of the tested plant material was introduced and left there for 30 min. before inoculation with the tested fungi. In control treatment, 20 µl of sterile distilled water was applied instead of the solution of the tested plant material. The experiment was carried out twice with three replicates per treatment and four fruits per replicate. Fruits were stored for one week at room temperature. Disease development was recorded by measuring lesion diameter and the percendages of infection of decayed fruits were calculated as mentioned by Horsefall and Heuberger (1942). The samples were taken for determination of biochemical properties of fruits at the end of incubation period. Titratble acidity, using 0.1N NaOH was determinated (A.O.A.C.,1986). Total soluble sugars were determined according to Thomas and Dutcher(1924). Total phenolic compounds were determined according to Swain and Hillis (1959) and lignin to Ride (1975).

Statistical analysis

The disease severity of Alternaria rot on fruits was analyzed by analysis of variance (ANOVA). Mean values were separated using Duncan's multiple range test at 0.05 level of probability.

RESULTS

1. Effect on the development of tomato fruit rot

Effect of the tested plant extracts on the development of fruit rot caused by *Alternaria alternata* was studied. Degree of infection of each plant extract at different concentration was statistically analyzed and illustrated in Table 2. Data showed that fruits inoculated with *A. alternata* and untreated with extracts gave the most significant percentages of infection. However, mostly fruits treated with all plant extracts showed

significant reduction in infection percentages. Meanwhile the least significant values of infection were recorded at high concentrations of extracts. Generally, disease severity gradually decreased with the subsequent increase of concentration. Thyme and garlic significantly decreased the disease severity of decay. However, there were insignificant differences in the degrees of infection between untreated fruits and those treated with rosemary extract at low concentration.

2. Effect on titratble acidity (TA)

Titratable acidity was determined according to A.O.A.C. (1995) and expressed in percendage (as malic acid) of fruit juice. Obtained data were presented in Table 3. Results revealed that significant decrease in TA values was detected in untreated fruits as compared with treated ones. Fruits treated with thyme and garlic extracts at 75% showed the highest TA% values as it reached to 0.672%, whereas lower percentage of TA was noticed in untreated fruits (0.343%).

Table (2). Efficiency of some plant extracts at different concentrations to control Alternaria rot of tomato fruit (Disease severity %)

Plant extracts	Concentration of plant extract			
	:5%	50%	75%	100%
Rosemary	62.23 a	41.05 a	45.76 a	44.31 a
Thyme	42.90 b	30.11 b	32.72 a	26.00 b
Garlic	38.00 c	24.09c	28.01 b	28.22 b
Control (untreated inoculated fruits)	63.16 a	63.16 d	63.16 c	63.16 c

Different letiers in a column denote significant differences according to Duncan's multiple range test at 0.05 level of probability.

$$\text{*Disease severity (\%)} = \frac{\text{Sum of individual ratings}}{\text{No. of fruits assessed}} \times \frac{100}{5}$$

Table (3). Effect of plant extract treatments on titratble acidity (%) in fruits inoculated with *A. alternata* after one week of storage at room temperature

Plant extracts	Concentration of plant extract			
	25%	50%	75%	100%
Rosemary	0.362a	0.344a	0.531a	0.433a
Thyme	0.363a	0.485b	0.672b	0.550b
Garlic	0.421b	0.560c	0.640b	0.612c
Control (untreated inoculated fruits)	0.343a	0.343a	0.343c	0.343d

Different letters in a column denote significant differences according to Duncan's multiple range test at 0.05 level of probability.

3. Effect on total soluble sugars

Results presented in table 4 showed that treated fruits by plant extracts resulted a significant increase in total soluble sugar contents of fruits as compared with those of untreated control fruits. The highest significant amount of total sugars (70.44 mg/gm of fruit tissue) was detected in fruits treated by thyme extract at 100%, whereas untreated fruits or those treated with rosemary extract showed the lowest amount of total sugars (About 13.74 to 23.26 mg/gm of fruit tissue). Generally, it was noticed that amount of total sugars of treated fruits increased with the increase in of plant extracts concentration.

4. Effect on total phenolic compounds

Data presented in table 5 showed that total phenol content significantly increased in untreated fruits compared with treated fruits. Meanwhile, treated fruits with plant extracts gave the highest mean values of phenolic compound content (3.108 mg/gm of fruit tissue), whereas the lowest content was recorded in fruits treated with thyme extract at 100% (0.554 mg/gm of fruit tissue).

5. Effect on lignin content

Lignin content was determined in untreated fruits and those treated with the tested plant extracts at the end of storage period under room temperature conditions. Obtained results illustrated in table 5 indicated that there were significant differences in lignin content between untreated and treated fruits inoculated with *A. alternata*. We can concluded that fruits treated with plant extracts before inoculation with *A. alternata* showed a significant increase in the lignin content compared with that of untreated fruits. Moreover, untreated fruits exhibited the lowest significant mean values of lignin content (0.051 µg /gm of fruit tissue), whereas fruits treated by thyme and garlic extracts gave the highest content (0.218 and 0.209 µg /gm of fruit tissue, respectively)

Table (4). Effect of plant extract treatments on total soluble sugar (mg/gm fruit tissue) content in fruits inoculated with *A. alternata* after one week of storage at room temperature.

Plant extracts	Concentration of plant extract			
	25%	50%	75%	100%
Rosemary	16.52a	13.74a	16.15a	23.26a
Thyme	28.41b	39.51b	59.20b	70.44b
Garlic	23.71c	24.39c	30.58c	49.48c
Control (untreated inoculated fruits)	15.80a	15.80a	15.80a	15.80d

Different letters in a column denote significant differences according to Duncan's multiple range test at 0.05 level of probability.

Table (5). Effect of plant extract treatments on total phenol compounds (mg/gm fruit tissue) content in fruits inoculated with *A. alternata* after one week of storage at room temperature.

Plant extracts	Concentration of plant extract			
	25%	50%	75%	100%
Rosemary	1.974a	2.646a	2.478a	2.604a
Thyme	1.801a	1.932b	0.944b	0.554b
Garlic	0.966b	1.260c	0.630c	0.765b
Control (untreated inoculated fruits)	3.108c	3.108a	3.108d	3.108c

Different letters in a column denote significant differences according to Duncan's multiple range test at 0.05 level of probability.

Table (6). Effect of plant extract treatments on lignin content (µg/gm fruit tissue) in fruits inoculated with *A. alternata* after one week of storage at room temperature.

Plant extracts	Concentration of plant extract			
	25%	50%	75%	100%
Rosemary	0.093a	0.134ab	0.126a	0.152a
Thyme	0.109a	0.118a	0.176b	0.218b
Garlic	0.101a	0.151b	0.143a	0.209b
Control (untreated inoculated fruits)	0.051b	0.051c	0.051c	0.051c

Different letters in a column denote significant differences according to Duncan's multiple range test at 0.05 level of probability.

DISCUSSION

The present study showed that the aqueous extracts of the all tested plants reduced the disease severity of Alternaria rot, meanwhile extracts of thyme and garlic proved to be the most effective against the tested fungus, followed by rosemary. Similar results were found on different fungi by many investigators using the same plant extracts (El-Jaly, 1996, Mohamed-Nawara, 1999 and Al-Awami, 2009). Meanwhile, Hadizadeh, *et. al.* (2009) reported that oil of thyme exhibited a moderate to high antifungal *in vitro* activity against the pathogen *A. alternata*, ranging from 68.5-74.8% and significantly inhibited *A. alternata* on tomatoes stored at 20°C for 5 days. The previous data indicated that pretreatment of tomato fruits with extracts of any of the tested plants with subsequent inoculation with *A. alternata*, was effective in controlling tomato fruit rots. Concerning the antifungal mode of action of plant extracts, Singh, *et. al.*, (2010) showed that the increase in the production of phenolic compounds in the extract can be correlated with the induction of resistance in treated plants against phytopathogenic fungi .Rong and Ting (2000) found that the natural phenolic compounds carvac ol and thymol completely prevented spore germination and mycelial growth of *B. cinerea* and *M. fructicola*. In this respect, the antagonistic action of the tested plant extracts, in the present study, may be due to their phenolic compounds content. According to the available literature, garlic extract contains allistain which considered broad sepctrum against fungi, while thyme extract contains carvacrol, borneol, thymol and tannin compounds, whereas rosemary extract contains saponins, sterols and glycosidic compounds (Kotb, 1983). On the other hand, Muhsin, *et. al.*, (2000) repoted that the inhibitory action of garlic extract on fungal growth has been attributed to the presence of allicin, when inhibit enzyme production and activity in order to suppress fungal pathogenicity.

Concerning the effect of the tested plant extract on titratble acidity percentages, generally fruits treated with each of plant extract gave markedly higher mean value of weight loss than untreated fruits. From the present data, it can be concluded that titratble acidity increase with the increase in infection severity. This should be confirmed by the result of the present study which showed that untreated fruits with plant extract gave the highest significant degrees of infection as compared with treated fruits. Total soluble sugars in inoculated fruits treated with plant extract clearly affected as compared with untreated ones. The activity of fungal infection on carbohydrates uptake from the affected tissues could be the reason of

such reduction. Generally, it was noticed that amount of total sugars of treated fruits increased with the increase in concentration of plant extracts.

Generally fruits inoculated with the tested fungus previously treated by thyme or garlic extracts significantly decreased phenol compound content. These plant extracts also showed marked decrease in disease development. Meanwhile, the lignin content decreased as the disease development advanced. However, the highest significant values of infection were induced in untreated fruits, which gave the least significant content of lignin.

For these reasons, these treatments might reduce the changes in chemical properties of fruits inoculated with tested fungus. Several investigators studied the effect of disease development on chemical properties, including juice pH and titratable acidity (Hussin, 1976 and Fallik *et al.* 1993), total phenolic compounds (Hussin, 1976; Kaul and Munjal, 1980; Gaber, *et al.* 1990; Dijkstra and Walker, 1992 and Saring, *et al.* 1998), total soluble sugars (Abdel-Rehim *et al.* 1973; Gaber, *et al.* 1990 and Ekundayo and Okigbo, 1991).

REFERENCES

Abdel-Rehim, M.A., Wasfi, E.I. and Hassouna, M.S. (1973). Some changes in tomato fruits due to infection by *Alternaria alternata* and *Geotrichum candidum*. Egypt J. Phytopathol. 5: 55-64.

Akhtar, K.P., Matin, M., Mirza, J.H., Shakir, A.S., Rafique, M., (1994). Some studies on the postharvest diseases of tomato fruits and their chemical control. Pak. J. Phytopathol. 6, 125–129.

Akpomedaye, D.E. and Ejechi, B.O. (1998). The hurdle effect of mild heat and two tropical spice extracts on the growth of three fungi in fruit juices. Food Research International 31 (5): 339-341.

Alawami, A. M., El-samra,I.A., Hussein, A.M., Shama, S.M. (2009). Efficiency of some plant extracts to control fungal pathgenic agents of postharvest decay on peach fruits. Proceedings of the 5th national Congress of biotechnology in sabratha. Libya, 21 - 23 march 2009. (In Press)

A.O.A.C. (1995). Association of official analytical chemists. Official Methods of Analysis. Association of Official Analytical. Chemists, Washington, 4, D.C. U.S.A. PP 1899.

Boyette, M. D., Ritchie, D. F., Carballo, S. J., Blankenship, S. M. and Sanders, D. C. (1994). Chlorination and Posharvest Disease

Control. Publication AG-414-6. North Carolina Cooperative Extension Service, USA. PP 414-416.

Dijkstra, L. and Walker, J.R.L. (1992). Enzymatic browning in apricots (*Prunus armeniaca*). Journal of the Science of Food and Agriculture 54 (2): 229-234.

Dixit, K., Shukla, H.S. and Dubey, P. (1987). Fungitoxic properties of some seedling extracts. National Academy of Science Letters 9 (8): 219-221. (Rev. of Plant Path. 66: 4664).

Ejechi, B.O., Ojeata, A. and Oyeleke, S.B. (1997). The effect of extracts of some Nigerian spices on biodeterioration of okra (*Abelmoschus* (L) Moench) by fungi. Journal of Phytopathology 145: 469-472.

Ekundayo, C. A. and Okigbo, R.N. (1991). The effect of pathogenic fungi on the nutritional value of the black plum (*Vitex doniana*). Nigerian Journal of Botany 4: 61-68.

El-Ghaouth, A., Arul, J., Grenier, J. and Asselin, A. (1991). Glucanohydrolases and inhibitory activity to *Botrytis cinerea* in extracts from strawberry fruits. Canadian Journal of Plant Pathology 13 (4): 315-320.

El-Jaly, Z.I. (1996). Aflatoxin contamination of some seed crops in El-Jabal Al-Akhdar. M.Sc. Thesis, Faculty of Agriculture, Omar Al-Mukhtar University, El-Beida-Libya, 168 pp.

Fallik, E., Klein, J., Grinberg, S., Lomaniec, E., Lurie, S. and Lalazar, A. (1993). Effect of postharvest heat treatment of tomatoes on fruit ripening and decay caused by *Botrytis cinerea*. Plant Disease 77: 985-988.

FAO, 2002. Statistics. Available from: http://www.fao.org/.

Gaber, M.R., Saleh, O.I., Hussin, N.A. and Shehata, Z.A. (1990). *Botryodiplodia* fruit rot of pear fruits, some physiological and pathological studies. Annals Agriculture Science, Ain Shams Univ. 35 (1): 427-444.

Farina, G., Moretti, M., Saracchi, M. and Sardi, P. (2007). Growth inhibition of Mucor hiemalis and Rhizopua stolonifer by essential oils. Proceedings of Novel approaches for the control of postharvest diseases and disorders. 3 -5 May 2007. Bologna, Italy. Pp 423-43.

Fawzi, E. M., Khalil, A. A. and Afifi, A. F. (2009). Antifungal effect of some plant extracts on *Alternaria alternata* and *Fusarium oxysporum*. African Journal of Biotechnology 8 (11): 2590-2597

Hadizadeh, I. Peivastegan, B. and Hamzehzarghani, H. (2009). Antifungal Activity of Essential Oils from Some Medicinal Plants of

Iran against *Alternaria alternate*. American Journal of Applied Sciences 6 (5): 857-861.

Hahn, F. (2006). *Rhizopus stolonifer* Detection by sensing the tomato peduncle scar. Biosystems Engineering 95 (2), 171–179

Hong, C.X., Michailides, T.J. and Holtz, B.A. (1998). Effects of wounding, inoculum density and biological control agents on postharvest brown rot of stone fruits. Plant Disease 82: 1210-1216.

Horsfall, J.G. and Heuberger, J.W. (1942). Measuring of a defoliation disease of tomatoes. Phytopathology 32: 226-232.

Hussin, N.A. (1976). Studies on mango fruit rot. M.Sc. Thesis, fac. Agric., Ain Shams Univ. Egypt. 147 pp.

Kaul, J.L. and Munjal, R.L. (1980). Post infection biochemical changes in apple fruit due to rot causing through pathogens. Gartenau Wiss-enschaften 45 (4): 185-187. (C.F. Rev. Pl. Pathol. 60: 1520).

Kotb, F. (1985). Medicinal plants in Libya. Arab encyelopedia House. Beirut-Lebanon. 830 pp.

Mohamed-Nawara, A. (1999). Study on early blight disease of tomato in Jabal Al-Akhdar area, Libya. M.Sc. Thesis, Faculty of Agriculture, Omar Al-Mokhtar University, El-Bieda-Libya. pp123.

Muhsin, T. M., Al-Zubaidy, S. R. and Ali, E. T. (2000). Effect of garlic bulb extract on the growth and enzymatic activities of rhizosphere and rhizoplane fungi. *Mycopathologia* 152: 143–

Qasem, J.R. (1996). Fungitoxicity of weed extracts to tomato wilt pathogen (*Fusarium oxysporum* f.sp. *lycopersici*). Emir. J. Agric. Sci. 8: 103-112.

Ride, J. P. (1975). Lignification in wounded wheat leaves in response to fungi and its possible role in resistance. Physiological Plant Pathology 5: 125-134.

Rong, T. and Ting, Z. (2000). Antifungal activity of monoterpenoids against postharvest pathogens *Botrytis cinerea* and *Monilinia fructicola*. Journal of Essential Oil Research 12 (1): 113-121.

Saring, P., Zutkhi, Y., Lisker, N., Shkelerman, Y. and Ben-Arie, R. (1998). Natural and induced resistance of table grapes to bunch rots. Acta Horticulture 464: 65-70.

Singh, S., Srivastava, R. and Choudhary, S. (2010). Antifungal and HPLC analysis of the crude extracts of *Acorus calamus, Tinospora cordifolia* and *Celestrus paniculatus*. Journal of Agricultural Technology 6(1): 149-158.

Swain, T. and Hillis, W.E. (1959). The phenolic constituents of *Prunus domestica*. 1. The quantitative analysis of phenolic constituents. J.Sci Food Agri. 10: 63-68.

Thomas, W. and Dutcher, R.A. (1924). Picric acid method for carbohydrate. J. Amer. Chem. Soc. 46: 1662-1669.

الملخص العربى

فعالية بعض المستخلصات النباتية فى مكافحة عفن ثمار الطماطم الالترنارى

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استهدف هذا البحث دراسة تأثير مستخلصات بعض النباتات الطبيعية من منطقة الجبل الاخضرفي ليبيا على عفن ثمار الطماطم المتسبب عن الفطر الترناريا الترناتا والمحافظة على جودة الثمار اثناء تخزينها. اوضحت النتائج انخفاض شدة المرض على الثمار المعاملة بالمستخلصات النباتية مقارنة بالثمار غير المعاملة، وقد تميز كل من مستخلصي نباتي الزعترُ والثومُ باكثر فعالية في تقليل شدة العفن، في حين سجلت إختلافات بسيطة في درجات العدوى بين الثمار غير المعاملة وتلك المعاملة بمستخلص الاكليل عند التركيز المنخفض. من ناحية اخري، لوحظ انخفاض واضح في محتوي الثمار غير المعاملة من الحموضة الكلية مقارنة بالثمار المحقونة بالفطر وغير المعاملة بالمستخلصات. وفي نفس الوقت، ظهر اعلى محتوي من المواد الفينولية الكلية فى الثمار غير المعاملة. بينما، سجلت زيادة هامة في محتوي الثمار المعاملة من السكريات الذائبة الكلية واللجنين وذلك مقارنة بالثمار غير المعاملة بالمستخلصات النباتية. من هنا يمكن ان نستنتج ان معاملة الثمار بالمسخلصات النباتية قد قلل من التغيرات الكيموحيوية التي يمكن ان تحدث للثمار عند الاصابة بمسببات الامراض النباتية.