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The Effects of Zataria Multiflora Essential Oil on Some Characteristics of Sultana Table Grapes Contaminated with *Botrytis cinerea*

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ABSTRACT: The object of this research was to find an alternative to fungicide for the prevention of post-harvest fruits. Therefore the effect of Zataria multiflora essential oil on the quality of Sultana grapes following the harvest was investigated. The essential oil was extracted by hydro-distillation and analyzed using a combination of GC and GC/MS. The results indicated a high percentage of anti-fungal components such as Thymol (44.4%) and Carvacrol (26.3%) in the extracted essential oil. The grapes were contaminated with *Botrytis cinerea* spores suspension at the concentration of 5×10^5 per ml sterile distilled water. The samples were treated with suspension of 0 to 200 and 400 mgL^{-1} Zataria multiflora essential oil and stored at 4 °C. Samples were examined and experimental design in a completely randomized design with three replications were employed. The results revealed that by increasing the concentrations of the essential oil, the anti-fungal activity against *Botrytis cinerea* was increased.

Keywords: *Botrytis cinerea, Essential Oil, Sultana Grapes, Zataria multiflora.*

Introduction

Fruits and vegetables are the most important sources for the healthy nutrition. Due to the ascending population growth, there is a need to increase the agricultural products with reductions of the wastes. However, a huge amount of wastes are created by birds, rodents and fungi that are also the major cause of lesions in vegetables and fruits (Scora & Scora, 1998). Besides fungus diseases, can cause mycotoxin contamination in fruits, vegetables, cereals, and other products (Tripathi *et al*., 2008).

Up to now, over 100,000 species of fungus have been detected which less than 10% of them as pathogenic fungi and 100 species are responsible for the major part of wastes production and the post harvest rotting in agricultural products. These factors cause remarkable damages on different food products in tropical and mild countries. The application of chemical materials like fungicides has a great role in controlling such wastes. The major wastes of fresh grape is due to the weight reduction, changes in color and firmness of berries, brown bunches of grape and large dispersion of fungus disease in berries. *Botrytis cinerea* is considered as the most important agent in table grape's disease (Elad *et al*., 2004). Considerable quantities of fungicides are used for fruits and vegetables treatments annually. The application of these materials will cause some major problems such as fungal resistance against fungicides, pesticide residues in food, water, air, soil and also ecosystem's pollution (Shahi *et al*., 2003).

Recently studies have been carried out on the anti-microbial properties of the natural

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ingredients such as essential oils and herbal extracts. Presently, the use of these materials for the prevention and reducing the growth of pathogenic and toxigenic fungi and postharvest contamination which has caused a major part of agricultural wastes have been studied (Caccioni *et al*., 1998). Essential oils contain volatile organic components like monoterpenes, sesquiterpenes (Jaimand *et al*., 2000). Some essential oils have been studied to determine their antifungal effects (Tripathi *et al*., 2008). Using the combination of essential oils, has more fungal inhibitory effect than each one of their components. Through activated mechanisms, fungi can easily increase it's resistance to one component, and also, applying the combination of essential oils, might be regarded more economic than using purified ingredients. (Romero, 2010).

Therefore, this study is concerned with the effect of natural essential oils on some characteristics of Sultana grapes.

Materials and Methods

- *Plant materials and extraction of essential oil*

Cinnamon was provided from Eram botanical garden of Shiraz. After Zataria multiflora had been authenticated, 100g portion of this plant was subjected to hydrodistillation for 3 hours in a clevenger type apparatus. The resulting oils were dried over anhydrous Na_2SO_4 and preserved in sealed vials at 4°C for future analysis. The yield from Zataria multiflora extraction was 2.1% (w/w).

- *Identification of components*

The essential oils were analyzed by combination of GC and GC/MS equipments. The results (Table 1), showed high percentage of anti fungal components like Thymol (44.4%) and Carvacrol (26.3%) in Zataria multiflora essential oil.

- *Provision of fungi strain*

Fungi pure culture (*Botritis cinerea*) was provided from Iranian research institute of plant protection.

- *Fruits*

Sultana grapes were harvested from a vine yard located in Shahriyar, Baghdasht. Healthy grapes were selected for experiment.

Table 1. Ingredients of Zataria multiflora essential oil-analyzed by a combination of GC and GC-MS equipments

No	Name of Compound	R Time	Content (%)
1	alpha-pinene	5:17	1.400
2	3-octanone	6:38	0.755
3	myrcene	6:48	0.776
4	alpha-terpinene	7:38	1.777
5	p-cymene	7:53	10.478
6	limonene	8:02	0.365
7	1/8-cineole	8:07	0.243
8	gamma-terpinene	9:05	5.450
9	linalool	10:38	2.415
10	terpinene	13:47	0.364
11	alpha-terpineol	14:22	0.241
12	methyl thymol	16:14	1.178
13	methyl carvacrol	16:38	1.702
14	thymol	18:55	44.415
15	carvacrol	19:19	26.360
16	e-caryophyllene	24:15	2.090

- *Invivo experiment*

The culture was maintained on PDA at 4°C. Fresh cultures were grown on PDA plates before use. Spore suspention was prepared by removing spores from the spourlation edges of 7 to 8 day –old culture with a bacteriological loop and suspending them in sterile distilled water. Spore concentration was determined with a hemocytometer and adjusted as required with sterile distilled water (5×10^5 spores mL^{-1}). Before being contaminated, fruits were treated with sodium hypochlorite (1%). Healthy grapes were immersed in spore suspentions for 1 minute. The contaminated grapes were stored at room temperature for 2 h in order to fix the fungal inoculation (Asghari et al., 2009). In this phase, fruits were treated with different concentrations (0, 200, 400 mgL^{-1}) of Zataria multiflora essential oil. Treated and control fruits were placed in cold storage at 4°C. when signs of corruption in the control samples were observed, all the samples were examined.

- *Measurments Titrable acidity* (TA)

Titrable acidity, was determined by titration with 0.1 N mol L^{-1} NaOH to pH 8.3 and reported as tartaric acid per 100 g fresh weight (Sams, 1999).

- *Total soluble solid* (TSS)

Total soluble solid were determined at 20°C using a refrctometer (Pancherng *et al*., 2003).

- *Berries abscission and crushing*

In order to determine crushing berries and their abscission during storage, fallen and crushed berries both in the treated and control samples were weighed then the percentage of this agent to the total were measured.

- *Experimental design and statistical analysis*

Experimental design was factorial in a completely randomized design with three replications.

Results and Discussion

Statistically, different concentrations of this essential oil, had significant effects in 1% confidence level with 99% probibility on TSS, berries abscission and berries crushing, but had no significant effects on TA (Table 2).

By increasing the concentrations of Zataria multiflora essential oil, titrable acidity increased slightly that wasn't statistically significant (Table 3). Different concentrations of this essential oil statistically have no significant effects on titrable acidity. Total soluble solid, berries abscission and crushing were decreased by increasing the concentration of Zataria multiflora essential oil. There are not significant differences between concentrations.

Table 2. Variance analysis of Zataria multiflora essential oil treatment on some characteristics in Sultana grapes

		Mean square			
source	DF	TA%	TSS%	Berries abscission%	Berries crushing%
Zataria multiflora essential oil	2	1.46×10^{-5}ns	131.12**	401.92**	398.44**

**،*significant in 1% and 5% confidence level.

Table 3. Average mean squared comparison of characteristics after treatment with different concentration of Zataria multiflora essential oil with Duncan method

Zataria multiflora essential oil mgL^{-1}	TA%	TSS%	Berries abscission%	Berries crushing%
0	0.07a	20.07a	15.88a	20.13a
200	0.07a	19.19b	13.88b	17.90b
400	0.079a	17.44c	3.44c	7.65c

Figure 1 indicates that different concentrations of Zataria m. essential oil has affected the titrable acidity of sultana grapes contaminated with *Botrytis cinerea,* but there are not significant differences between 0, 200 and 400 mgL^{-1}. Titratable acidity in the grapes is mainly due to the presence of tartaric acid. Different studies carried out by Valero *et al*., 2006; Valverde *et al*., 2005; Martinez and Romero, 2007 have shown that the essential oils increase ethylene production during fruit maturation andstorage, therefore, delaying the increase of the acidity. In this experiment titrable acidiy was increased but there were not significant differences between the control and other samples.

As indicated in Figures 2, 3 and 4 the minimum amounts of crushing berries, TSS and berries abscission were measured at essential oil concentrations of 400 mgL^{-1}.

Water loss in fruits is usually observed that has a reflection on the TSS (Jaimand *et al*., 2000). In this research work the increase in TSS of control is higher than the treated samples with the essential oil that is due to water loss.

Similar works have been carried out by Vesaltalab *et al*. (2012) and (2009), indicating that there are significant differences between water losses in samples particularly in samples that have been treated with different concentrations of clove essential oil. Some researchers have

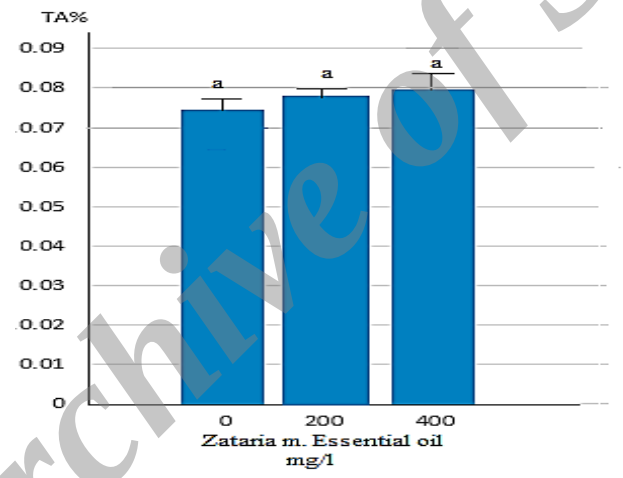

Fig. 1. The effect of Zataria m. essential oil on TA Sultana grapes contaminated with *Botrytis cinerea*

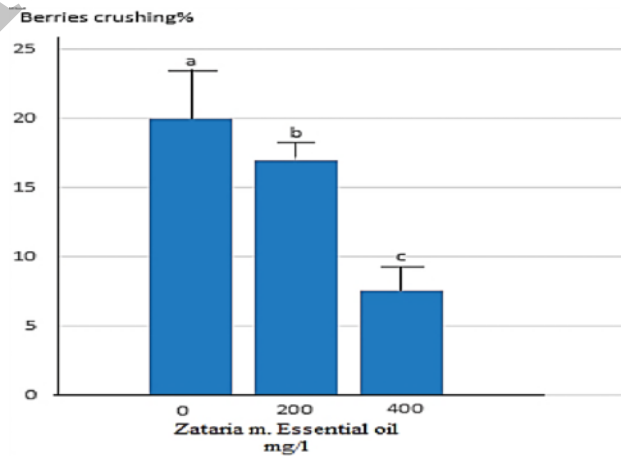

Fig. 2. The effect of Zataria m. essential oil on crushing berries of Sultana grapes contaminated with *Botrytis cinerea*

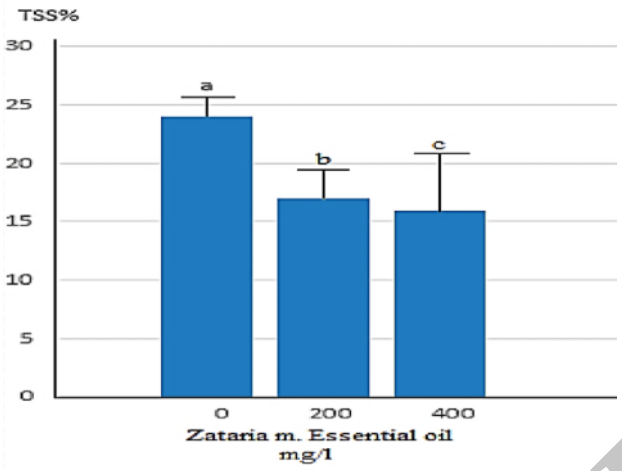

Fig. 3. The effect of Zataria m. essential oil on TSS of Sultana grapes contaminated with *Botrytis cinerea*

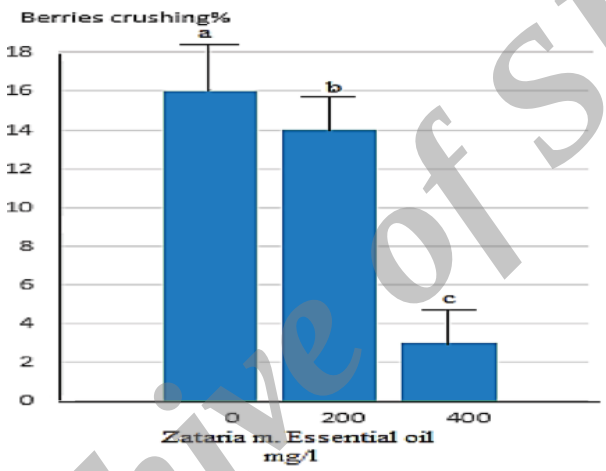

Fig. 4. The effect of Zataria m. essential oil on berries abscission of Sultana grapes Contaminated with *Botrytis cinerea*

investigated that applying natural components such as essential oils and herbal extracts; thyme and clove extracts on Valensia oranges (Rahemi, 2009), thyme, clove and cinnamon extracts on peaches (Artez, 2000), thyme and satureja extracts on tomatoes (Carris *et al*., 2006), grapefruit seed extract on grapes (Guillen *et al*., 2007) have positive effects on preventing or reducing post-harvest fruits decay. In this study, most fungal inhibitory effects were observed by using higher levels of Zataria m. concentrations.

Conclusion

The results revealed that the use of essential oil of Zataria multiflora has positive effect on the inhibition of *Botrytis cinerea's* growth on grapes. Significant differences were observed when different concentrations of the essential oil were applied in this study. By increasing the concentrations of the essential oil, anti-fungal activity against *Botrytis cinerea* was increased and also berries decay were improved. Further study is recommended to eliminate the undesirable effects of essential oil's aroma and flavor.

References

Artez, F., Villaescusa, R. & Tudela, J. A. (2000). Modified atmosphere packaging of Pomegrante. J. of Food Sci. Toxicology, 65, 1112-1116.

Asghari Marjanlo, A., Mostofi, Y., Shoeibi, Sh. & Fattahi, M. (2009). Effect of

cumin essential oil on postharvest decay and some quality factors of strawberry. Journal of Medicinal Plants, 8, 25-43.

Caccioni, D. R. L. & Guizzardi, M. (1998). Inhibition of germination and growth of fruit and vegetable postharvest pathogenic fungi by essential oil compounds. J.Esse.Oil Res., 6, 173-179.

Carris, L. M., Castlebury, L. A. & Goates, B. J. (2006). Non systemic bunt fungi Tilletia indicia and T. horrida: a review of history, systematic, and biology. Annu Rev Phytopathol. 44, 113–133.

Elad, Y., Williamson, B., Tadzynki, P. & Delen, N. (2004). *Botrytis* Biology pathology and control. Kluwer Academic Publishers Dordrecht the Netherlands. PP. 4-6.

Guillen, F., Zapata, P. J., Martinez-Romero, D., Castillo, S., Serrano, S. & Valero, D. (2007). Improvement of the overall quality of table grapes stored under modified atmosphere packaging in combination with natural antimicrobial compounds. J. Food Sci. 72(3), 185 – 190.

Jaimand, K., Rezaei, M. B. & Barazandeh, M. M. (2000). Investigation on essential oil composition of Achilles mille folium L. ssp.Millefolium'. J. of Pajouhesh and Sazandegi. 48(2), 68-69.

Martinez - Romero, D., Alburqueerqne, N., Valerdo, J. M., Guitillos, S., Valero, D. & Serrano, M. (2007). Postharvest sweet cherry quality and safety main tenance by Aloevera treatment: A new edible coating. Postharvest Biol Technol., 39, 93 – 100.

Pancherng, A. & Ouyang, F. (2003). A Firmness Index for fruit of Ellipsoidal shape. Biosystems Eng. 86(1), 35-46.

Rahemi, M. (2009). Physiology of postharvest, introduction on physiology on handling fruits and vegetables. Shiraz uni. Pp 253.

Romero, D. (2010). Antifungal efficacy of Aloe vera in vitro and its use as a preharvest treatment to maintain postharvest table grape quality. pp. 450.

Sams, C. E. (1999). Preharvest factors of affecting Post harvest textual.Post harvest Biology and Technology, 15, 244-254.

Scora, M. & Scora, W. (1998). Effect of volatiles on mycelium growth of Penecillium digitatum, P.italicum and P.ulaiense.J.of Basic Microbiology, 38(6), 405-413.

Shahi, S. K., Patra, M. & Shukla, P. A. C. (2003). Use of essential oil as botanical - pesticide against post harvest spoilage in Malhs pumilo fruits. Biocontrol. 48, 223-232.

Tripathi, P. & Dubey, N. K. (2004). Exploitation of natural products as an alternative strategy to control postharvest fungal rotting of fruit and vegetables. Review article Postharvest Bio and Techno. 32(3), 235-245.

Tripathi, P., Dubey, N. K. & Shukla, A. K. (2008).Use of some essential as postharvest botanical fungicides in the management of gray mould of grapes caused by Botrytis cinerea J. of Micro and Bio technol., 34 (2), 235-250.

Valero, D., Valverde, J. M., Martinez-Romero, D., Guillen, F., Castillo, S. B. & Serrano. M. (2006). The combination of modified atmospHere packaging white eugenol or thymol maintain quality safety and functional properties of table groups. Postharvest Bio and Technol. J., 40,514-523.

Valverde, J. M., Culles, F., Martinez Romero, D., Castillo, S., Serano, M. & Valero, D. (2005). Improvement table grapes quality safety by the combination of modified atmosphere packaging (MAP) and eugenol, menthol or thymol J. Agri. Food chem., 53, 7458 – 7464.

Vesaltalab, Z. & Gholami, M. (2012). The effect of essential oil and extract of clove buds on some quality characteristics of table grapes during storage. Iranian J. of horticultural sci., 3(2), 255-265.

Vesaltalab, Z. & Gholami, M. (2009). The effect of clove buds extract on rot control and some quality characteristics of Sultana grapes during storage. The 6th Horticulture Iranian Conference. Guilan uni.