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Full Length Research Paper

Thymus kotschyanus and *Carum copticum* essential oils as botanical preservatives for table grape

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In the present study, essential oils extracted from *Thymus kotschyanus* and *Carum copticum* has been evaluated for efficacy in the control of postharvest fungal decay in Thompson seedless table grape. Results showed that the tested oils exhibited good inhibitory activity against fungal decay in oil-treated grapes. In addition, the essential did not show a negative impact on the sensory quality of the grape. The profile of the oil components showed that carvacrol (28.54%) and thymol (63.18%) were the main compounds available in *T. kotschyanus* and *C. copticum* respectively. Results of this research showed that the evaluated essential oils may be used as a preservative for reduction of postharvest losses.

Key words: Essential oil, table grape, *Thymus kotschyanus, Carum copticum,* antifungal.

INTRODUCTION

Table grape is a perishable fruit and several factors such as mechanical, physiological and phytopathogenical are responsible for its postharvest losses. *Botrytis cinerea* Pers. Fr., *Aspergillus niger* Tiegh, *Rhizopus stolonifer* (Ehrenb: Fr.) *Vuill*, *Penicillium* spp. and *Mucor* sp. are prevalent postharvest pathogens of table grapes (Snowdon, 1990). The use of synthetic fungicides such as SO_2 is the prime method to control of postharvest diseases caused by fungal phytopathogens in table grape (Deng et al., 2005).

In recent years, consumers have become more concerned about application of chemicals in food products because synthetic preservatives release residues on foods that have negative effects on human health and environment. Besides, the use of synthetic compounds have significant drawbacks such as bleaching, discoloration, hairline on the berries, sulfurous taste and browning of the rachis of grape (Zoffoli et al., 2008; Smilanick et al., 1990). Therefore, researchers are looking for the control of postharvest spoilage fungi using natural substances from botanical sources such as oils, extracts, among others. Plant products, especially essential oils, are one of the most promising groups of natural compounds for the development of antimicrobial agents and their use in plant protection. Generally, essential oils are complex mixtures of hydrocarbon monoterpenes, oxygenated monoterpenes, hydrocarbon sesquiterpenes, oxygenated sesquiterpenes, and related compounds that derive from the secondary metabolism of plants (Reverchon, 1997).

The antimicrobial activity of essential oils or their constituents on postharvest fungi have been quite extensively examined (Regnier et al., 2010). Control of storage pathogen *Alternaria alternata* and *Penicillium*

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digitatum on tomato fruits by essential oils of *C. copticum* and *Foeniculum vulgare* under *in vitro* and *in vivo* conditions has been studied (Abdolahi et al., 2010a). Antifungal activities of *Mentha spicata* and *Lippia scaberrima* essential oils to maintain postharvest quality of citrus and avocado fruits has been investigated (Regnier et al., 2010; Plooy et al., 2009). The effect of *Thymus vulgaris, Satureja hortensis*, *F. vulgare* (Abdolahi et al., 2010b) and *Ocimum sanctum*, *Prunus percica* and *Zingiber officinale* against fungal disease on table grape reported previously (Tripathi et al., 2008). In addition, the application of essential oil constituents such as thymol, carvacrol, eugenol and menthol to enhance the shelf life of sweet cherry (Serrano et al., 2005), lettuce (Martínez-Romero et al., 2008) and table grape (Martínez-Romero et al., 2007; Valero et al., 2006; Valverde et al., 2005) has been tested.

Therefore, based on the above-mentioned points, the aim of this study was to evaluate the potential use of essential oils from *T. kotschyanus* and *C. copticum* in order to control the fungal decay on table grape and enhance the fruit quality and marketability by reducing the application of synthetic fungicides.

Experimental

Plant material and essential oils

Bunches of table grapes (*Vitis vinifera* L. cv. Thomson seedless) were harvested from commercial vineyards and selected for uniformity in size, appearance, ripeness and the absence of physical defects. The aerial parts of thyme (*T. kotschyanus* L.) at flowering stage and ajowan (*C. copticum* L.) fruits at ripening stage were harvested, air dried and then submitted to hydrodistillation in a Clevenger-type apparatus for 3 h. The extracted essential oils dried over anhydrous sodium sulfate and stored at 4ºC until use and analysis.

Essential oil analysis

GC analyses were performed using a Shimadzu GC-9A gas chromatograph equipped with DB-5 fused silica column (30 m × 0.25 mm; film thicknesses 0.25 µm). Oven temperature was held at 60ºC for 5 min then programmed to 210ºC at a rate of 3ºC/min. Injector and detector (FID) temperature were 300 and 280ºC, respectively. Helium was used as carrier gas with a linear velocity of 32 cm/s. Percentages were calculated by electronic integration of FID peak areas without the use of response factor correction. GC/MS analysis were carried out on a Varian 3400 GC/MS system equipped with a DB-5 fused silica column (30 m × 0.25 mm; film thickness 0.25 µm); oven temperature program was 60 to 210ºC at a rate of 3ºC/min, transfer line temperature 240ºC, carrier gas helium with a linear velocity of 31.5 cm/s, split ratio 1/60, ionization energy 70eV; scan time 1 s; mass range 40 to 340 amu.

The constituents of the oils were identified by calculation of their retention indices under temperature-programmed conditions for identification of individual n-alkanes (C6 to C24) and the oil on DB-5 capillary column. Compounds were made by comparison of their mass spectra with those of the internal reference mass spectra library (NIST 98 and Wiley 5.0) or with authentic compounds or with those reported in the literature (Adams, 2001; Davies, 1990).

Quantitative data were obtained from flame ionization detector (FID) area percentages without the use of correction factors.

Treatment of bunches with essential oils

Six uniform grape bunches (150 to 200 g) sprayed with different concentrations of *T. kotschyanus* and *C. copticum* essential oils (0, 250 and 500 µL L^{-1}). Then they placed in plastic boxes with polyethylene cover and stored in cold storage (0 to 1ºC and 90% RH) for 45 days. Each treatment was replicated four times with six clusters per replicate.

Evaluation of fungal decay on sprayed bunches

Fungal decay was assayed after 45 days storage (0 to 1ºC), and scored by using the following scoring system: (0) bunch without rots; (1) 1 to 5% of rotted berries; (2) 6 to10% of rotted berries; (3) 11 to 25% of rotted berries; (4) 26 to 50% of rotted berries; (5) 51 to75% of rotted berries; (6) more than 75% of rotted berries (Nigro et al., 2006).

Evaluation of quality parameters on treated fruits

At the end of storage period, boxed grapes from each treatment were removed from storage, and overall visual appearance, berry and rachis colour were evaluated. The overall visual appearance of the grapes was evaluated for intensity on a 9-point scale: (1) extremely poor or soft in case of texture; (3) poor or soft; (5) moderate; (7) good; (9) excellent. Berry and rachis browning development were evaluated on a 5-point intensity scale of damage by using the following scoring system: (1) none; (2) slight; (3) moderate; (4) severe; (5) extreme (Artés-Hernández et al., 2004). Berry shrinkage of fruits was evaluated on a 5-point scale: (1) very shrinkage; (2) low shrinkage; (3) normal (medium); (4) smooth; (5) very smooth (Bourne, 1980).

Weight loss was calculated by weighing the fruit at harvest and reweighing at the end of storage period. Weight loss percentage was calculated as percentage loss of initial weight. At the end of storage period plus 2 d at 20°C, flavor analysis to compare the quality of treated and control table grapes were carried out by 10 trained panelists. A questionnaire was used to record the data; each judge evaluated five berries for each treatment for flavor analysis, on a scale of 1 to 5 (ranked), where (1) very low; (2) low; (3) medium; (4) high and (5) very high (Valero et al., 2006).

A random sample of berries (10 berries) was sampled per replicate, juiced, and filtered to get a clear sample. Total soluble solids content (TSS) was determined by means of digital refractometer (Atago, Tokyo, Co. Ltd, Japan) and results were expressed in ºBrix. Titrable acidity (TA) content was determined with phenolphthalein as indicator using 0.1 mol L^{-1} NaOH and expressed as mmol H^{+} per 100 g fresh weight. MI was expressed as the ratio between TSS and TA.

Statistical analysis

Statistical analysis of the data were performed with MSTATC statistical software (Freed et al., 1991) using a completely randomized design (CRD) with 4 replicates. Data were subjected to one-way ANOVA and mean differences were established by Tukey's test ($P < 0.05$).

Table 1. The chemical compositions (%) of essential oils extracted from *T. kotschyanus* and *C. copticum.*

Component	RI a	*T. kotschyanus*	*C. copticum*
α-thujene	936	-	0.21
α-pinene	942	1.46	0.09
Camphene	950	3.27	-
Sabinene	963	2.35	0.61
β-pinene	987	1.56	0.33
Myrcene	999	2.91	-
α-terpinene	1022	0.68	-
ρ-cymene	1035	11.45	21.4
Limonene	1040	7.1	-
β-phellandrene	1043	-	0.02
(Z)-β-Ocimene	1047	0.67	-
γ-terpinene	1061	14.66	13.8
Cis-sabinene hydrate	1065	2.01	-
Trans linalool oxide	1076	0.42	-
Linalool	1107	1.2	-
Trans pinocarveol	1149	0.6	-
Terpinene-4-ol	1174	3.16	-
ρ-cymene-8-ol	1182	0.92	-
α-terpineol	1199	2.9	0.18
Thymol	1311	3.31	63.18
Carvacrol	1332	28.54	-
E-caryophyllene	1416	2.57	-
β-bisabolene	1510	0.29	-
Spathulenol	1570	0.46	-
Total		92.49	99.82

a. Retention indices.

RESULT AND DISCUSSION

Essential oil analysis

The chemical composition of the essential oils was analyzed using a GC-MS technique. Qualitative and quantitative analytical results are shown in Table 1. In the *T. kotschyanus* essential oil, 22 constituents, representing 92.49% of the total components. The major compounds were carvacrol (28.54%) followed by γ-terpinene (14.66%), ρ-cymene (11.45%) and limonene (7.1%). At the same time, *C. copticum* oil contained 9 constituents, representing 99.82% of the total components in the oil and thymol (63.18%), ρ-cymene (21.4%) and γ-terpinene (13.8%) were the main components of C. *copticum* oil.

Effect of *T. kotschyanus* and *C. copticum* oils in control of fungal decay

In this study, the effect of spraying different concentrations of *T. kotschyanus* and *C. copticum* oils treatment on control of fungal decay in table grape tested. Evaluation of fungal decay after 45 days in oil-treated bunches showed that essential oil treatment had good inhibitory effect and reduced fungal decay in oil-treated bunches in comparison with controls (Figure 1). But no significant differences were observed in both used essential oil (Table 2) and with increase of essential oil concentration the antifungal activity of essential oils increased (Figure 1). These results were in accordance with those of Abdolahi et al. (2010b) who stated that *T. vulgaris* and *S. hortensis* oils showed antifungal activity against *B. cinerea* under *in vitro* condition. Moreover, they showed that these oils had a good inhibitory effect in reduction of fungal decay in table grape after 60 days storage. In another report, they stated that *C. copticum* oil reduced alternaria and penicillium rots in inoculated tomato fruits (Abdolahi et al., 2010a).

On the other hand, thymol and carvacrol, as the main components available in tested essential oils, showed high antimicrobial activity against fungal decay on sweet cherry (Serrano et al., 2005) and table grape (Valero et al., 2006; Valverde et al., 2005). Previous reports indicated that treatment of several horticultural crops including mango (Dubey et al., 2008), strawberry (Tzortzakis, 2007) and apple (Shahi et al., 2003) by volatile oils extracted from *Amomum subulatum*,

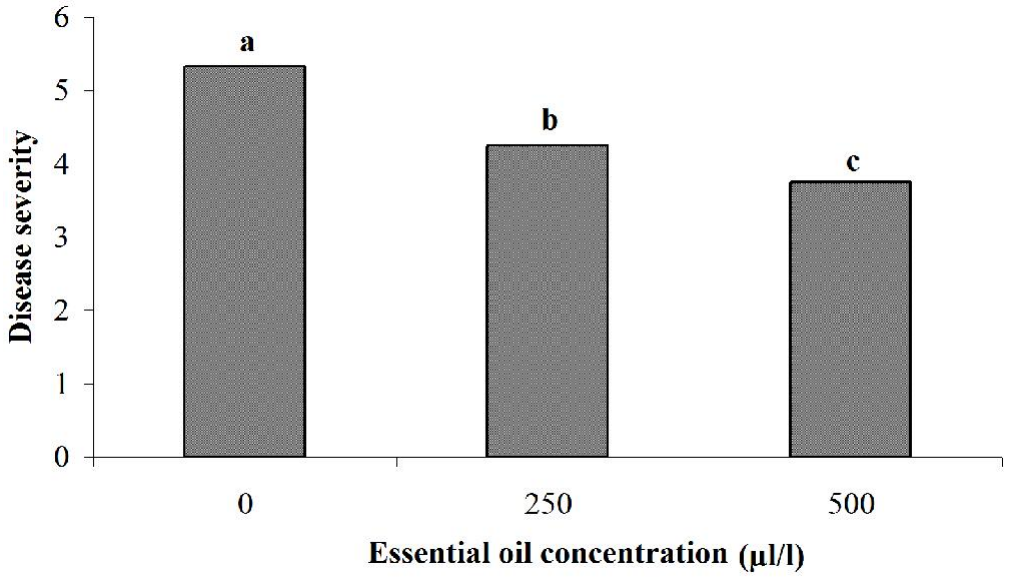

Figure 1. The effect of different concentration of essential oils on disease severity of table grapes. Means of treatments with the same letter are not significantly different according to Duncan's multiple range test at (P< 0.01).

Table 2. Means squares for the variance of the effects of essential oils on disease severity and quality parameters of treated table grape.

Significance	Disease severity	Weight loss (%)	Appearance	Berry browning	Rachis browning	Berry shrinkage	Flavour	TSS	TA	MI
EO	ns	ns	ns	ns	ns	*	ns	ns	ns	ns
Con	**	ns	**	**	ns	**	ns	ns	ns	ns
EO * Con	ns	ns	*	ns	ns	ns	ns	ns	ns	ns

O: Essential oil, Con: Essential oil concentration. *, ** and ns: Significant at (*P< 0.05*, *P< 0.01*)and not significant, respectively. TSS: Total Soluble solids, TA: Titrable acidity and MI: Maturity index

Cinnamomum zelanicum and *Cymbopogon flexuosus* reduced fruit decay during postharvest. In the case of antifungal activity of essential oils several papers published. Their data however show much variation between the same oils. There has been correlation between chemical composition and biological activity of essential oils. On the other hand, the chemical composition of essential oils could be affected by several factors including genetic, geographical location, harvest time, plant part used and method of isolation (Rasooli, 2007). Therefore, if we study all the factors influencing the chemical composition of essential oils we can understand the reason of this variability. It is important to mention here that even if the variability is high, the genetic factor surpass all other agronomic and environmental conditions, which means that an essential oil produced in Brazil from *A. subulatum* will present similar constituents if the same oil is produced in Japan. Also, the mode of action of essential oils is different for microorganisms (Soković and Griensven, 2006).

Expression of antifungal activity of essential oils often very clear, but the mechanism(s) of antifungal action is not completely understood. It is evident that the site of action of terpenoid compounds available in essential oil composition is the cell membrane. Rasooli and Owlia (2005) showed that thyme oil, which is rich in thymol, caused severe damage to cell walls, cell membranes and cellular organelles such as mitochondria of fungi tested, which seem to be destroyed. In addition, the essential oils from *A. subulatum* and *Eupatorium cannabinum* showed inhibitory effect on pectinase and cellulase enzymes (Dubey et al., 2008; 2007). Pectinase and cellulase enzymes produced by fruit rotting fungi play a prominent role in disease development during host pathogen interaction (Yakoby et al., 2000). Therefore, essential oils can inhibits the fungi growth by acting on enzymes related to an early stage pathogenesis in the fruits. Some authors related the antifungal property of essential oils to their major compounds especially phenolic compounds such as thymol and carvacrol (López-Malo et al., 2006; Rassoli and Mirmostafa, 2003; Nychas, 1995). López-Malo et al. (2006) distinguished that antimicrobial activities of phenolic compounds was related to their concentration.

Table 3. Effect of different concentrations of essential oils on quality parameters of essential oil treated bunches.

Essential oils (µL L-1)	Weight loss (%)	Appearance	Berry browning	Rachis browning	Berry shrinkage	Flavour	TSS	TA	MI
C. copticum	1.39	1.4	4.33	2.78	3.67 b	6.33	21.53	0.54	39.43
T. kotschyanus	1.43	2.56	3.89	2.78	4.11 a	6.56	20.93	0.56	37.19
0	1.46	1 b	5 a	3.17	3.5 b	5.67	20.52	0.53	38.41
250	1.37	1.67 ab	4 b	2.5	3.83 b	6.67	21.82	0.55	39.16
500	1.4	3.33 a	3.33 b	2.67	4.33 a	7	21.37	0.57	37.36
C. copticum * 0	1.45	1 b	5	3	3.33	5.67	20.53	0.53	38.26
C. copticum * 250	1.32	1.67 ab	4.33	2.33	3.67	6.33	21.33	0.54	39.03
C. copticum * 500	1.4	1.67 ab	3.67	3	4	7	22.73	0.55	41
T. kotschyanus * 0	1.48	1 b	5	3.33	3.67	5.67	20.5	0.53	38.57
T. kotschyanus * 250	1.42	1.67 ab	3.67	2.67	4	7	22.3	0.57	39.28
T. kotschyanus * 500	1.39	5 a	3	2.33	4.67	7	20	0.59	33.72

Thus, at lower concentration these compounds affected enzymes associated with energy production, whereas at higher concentrations they caused protein denaturizing. Also, phenolic compounds could affect the enzymes responsible for spore germination and interfere with amino acids that were necessary in germination processes (Nychas, 1995). Therefore, the antifungal activity of tested oils in our study could be related to thymol (63.18%) and carvacrol (28.54%) as major components available in *T. kotschyanus* and *C. copticum*.

Effect of *T. kotschyanus* and *C. copticum* essential oils on table grape quality parameters

The effect of essential oils treatment on quality parameters related to table grape is presented in Table 2. These results indicated that oil treatment had no significant efficacy on weight loss (%), TSS, TA, MI, flavour and rachis browning. These results were in accordance with Ranasinghe et al. (2003) who stressed that *C. zeylanicum* oil had not efficacy on physico-chemical properties of banana fruit such as weight loss, fruit firmness, TSS, TA and flavour. Also, it is showed that oils from eucalyptus have a great and negative impact on sensory features of food products, especially fruits.

The strawberries treated with *Eucalyptus globulus* and *C. zeylanicum* oils did not differ in weight loss, organic acid content and sweetness compared with untreated fruits (Tzortzakis, 2007). On the other hand, our results do not agree with Abdolahi et al. (2010b) who reported that *T. vulgaris*, *S. hortensis*, *F. vulgare* and *O. basilicum* essential oils reduced weight loss (%) and rachis browning. They showed that TSS level was lower in oil-treated grapes rather than controls and TA level increased in oil-treated clusters compared to the control and the level of MI in treated grapes were lower than control. Also it is showed that oil treatment had a good effect on maintenance of cluster appearance and with increase of essential oil concentration cluster appearance improved and *T. kotschyanus* oil at 500 µL L-1 concentration had greater effect on cluster appearance (Table 3). These results were in accordance with those reported by Abdolahi et al. (2010b) who showed that *S. hortensis* and *T. vulgaris* oils had a significant effect on the maintenance of cluster appearance in comparison with controls.

In addition, with increase of oil concentration berry browning in oil-treated grapes decreased but had not significant difference between both tested oils (Table 3). The positive effect of eugenol, thymol, and menthol in reduction of weight loss, fruit firmness and non change in stem colour in essential oil treated sweet cherries was reported by Serrano et al. (2005). In addition, carvacrol, thymol and eugenol delayed weight loss, colour changes, MI and fruit firmness in the table grape (Guillén et al., 2007; Valero et al., 2006; Valverde et al., 2005). Evaluation of berry shrinkage showed that essential oil treatment had inhibitory effect on berry shrinkage and with increase of oil concentration the level of berry shrinkage decreased. These results were accordance with previous studies (Abdolahi et al., 2010b). Also results showed that *T. kotschyanus* oil had better effect on maintenance of berry shrinkage in comparison with *C. copticum* oil (Table 3).

Although several works for investigation of mechanism(s) action of essential oils have been carried out but the effect of these products on quality sensors of fruits reported in a few reports. As a result their possible mechanism action in change of quality factors is ambiguous and need to further studies on the efficacy of essential oils on quality parameters of fruits should be practiced.

Conclusion

In our study, *T. kotschyanus* and *C. copticum* essential

oils did not affect the flavour and rachis browning. Also, essential oils had positive effect on cluster appearance, berry shrinkage and berry browning and proved its potency to control the development of fungal decay on table grapes during storage. Therefore, it can be stated that the use of plant essential oils during storage may be named as a new key for preservation of grape quality. In this regard, further *in vivo* studies on different types and concentrations of essential oils need to be assessed to evaluate a safe concentration of essential oil used in each type of food product. Moreover, sensory evaluation should always be used as an important and demanding tool to assess the fruit acceptability. If the efficiency is proven using key-pathogens and no negative impact on sensory attributes are found using an essential oil on a fruit, then this oil may be considered potential.

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