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# Assessment of the Preservative Activity of Some Essential Oils to Reduce Postharvest Fungal Rot on Kiwifruits (*Actinidia deliciosa*)

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**Abstract:** The aim of this study was to find a natural alternative for synthetic agrochemicals currently used in preservation of kiwifruits during postharvest stage. Different concentrations (0, 250, 500 and 750 µl/l) of thyme (*Thymus vulgaris* L.), ajowan (*Carum copticum* L.), fennel (*Foeniculum vulgare* Mill.) and summer savory (*Satureja hortensis* L.) essential oils were sprayed on kiwifruits. Treated fruits were kept in storage (0 - 1°C) for 90 days. Accordingly, *T. vulgaris* and *S. hortensis* oils showed a good antifungal activity compared with *C. copticum* and *F. vulgare*. Evaluation of sensory parameters showed that total soluble solids content (TSS), titrable acidity (TA) and vitamin C content were decreased under essential oil treatment while, fruit firmness and weight loss were not affected significantly. In addition, TSS, TA, TSS/TA and vitamin C content in kiwifruits treated with *T. vulgaris* oil were higher than other oils. GC and GC-MS analysis showed that β-ocimene (12.6 %), thymol (63.0 %), *trans*-anethole (64.7 %) and carvacrol (54.1 %) were the main compounds identified in *T. vulgaris*, *C. copticum*, *F. vulgare* and *S. hortensis* oils, respectively.

**Key words:** Essential Oil, Kiwi fruit, Antifungal, Postharvest, Fungal decay.

**Introduction:** The storage period and marketing life of fruits and vegetables are generally lost via some fungal diseases. Kiwi fruit [*Actinidia deliciosa* (A. Chev.) C.F. Liang et A.R. Ferguson var *deliciosa*] is a climacteric and susceptible fruit sensitive to decay caused by several phytopathogenic fungi, including *Botrytis cinerea, Botryosphaeria dothidea*, *Colletotrichum*, *Alternaria alternata*, *Sclerotinia*, *Phoma* and *Diaporthe actinidiae*. Stem-end rot caused by *B. cinerea* is the most important postharvest disease of kiwifruit in many areas of the world [1].

Application of synthetic fungicides is a primary way to reduce postharvest fungal diseases in kiwifruit [2]. However, postharvest use of synthetic fungicides is becoming ineffective due to development of fungicide resistant strains of postharvest fungi and also consumers awareness on the side-effects of chemical fungicides [3]. In addition, recently, postharvest use of chemical fungicides considered forbidden

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in European Union countries [1]. Therefore, in response to these challenges, several biological and physical approaches such as application of plant and animal derived substances i.e. table grape volatiles [1] salicylic acid [2] and chitosan [5]; biological control agents [6], modified atmosphere [7], controlled atmosphere [8] and ozone [9] were evaluated for preservation of postharvest quality parameters in kiwifruit. But, researches on the application of plants derived essential oils for this purpose are limited.

Essential oils are complex natural mixtures of volatile secondary metabolites, commonly isolated from plants by steam distillation and cold expression. The main constituents of essential oils (mono and sesquiterpenes), along with carbohydrates, alcohols, ethers, aldehydes and ketons are responsible for fragrant and biological properties of aromatic and medicinal plants. Essential oil bearing plants have widely been used as flavouring and pharmaceutical agents in food and drugs since recorded history and it is established that many of essential oils have a wide range of antifungal, antiviral, antibacterial and insecticidal actions [10, 11, 12, 13].

The literature review showed several examples of previous trials conducted on the antifungal properties of various essential oils against postharvest fungi. Antifungal activity of essential oils has mostly been tested in *in-vitro* conditions. For example, the antifungal activity of thyme (*Thymus vulgaris*), ajowan (*Carum copticum*), fennel (*Foeniculum vulgare*) and summer savory (*Satureja hortensis*) against *B. cinerea* was reported previously [14, 15, 16]. But only a few studies conducted on the antifungal/preservative activities of essential oils on fruits and vegetables. Thanassoulopoulos and Yanna [17] studied the antifungal activity of essential oils from origanum, sweet basil and thyme on gray mold rot in kiwifruits. Antifungal activity of *T. vulgaris* essential oil has been investigated against *B. cinerea* and *A. alternata* rots in strawberry and cherry tomatoes as well [18, 19]. Furthermore, antifungal activity of *S. hortensis* essential oil has been confirmed against *Aspergillus flavus* grown on lemon fruits [20]. Similarly Tripathi *et al*. [21] showed that essential oils from *Prunus persica*, *Ocimum sanctum* and *Zingiber officinale* inhibited the growth of *B. cinerea* on table grapes and increased the storage life of oil treated grapes by 4, 5 and 6 days, respectively. Plant essential oils posses a number of highlighted advantages over traditional chemical fungicides which make their future applications promising. In general, they are considered safe to human, biodegradable and environmentally friendly, non phytotoxic and very low risk that pathogens will develop resistance to the mixture of components that make up the oils with their apparent diversity of antifungal mechanisms [15]. The objective of our research was to apply the essential oils obtained from *T. vulgaris*, *C. copticum*, *F. vulgare* and *S. hortensis* as antifungal/preservative agents to maintain fresh kiwifruit quality through storage.



## Experimental

**Essential oil preparation:** Four locally available aromatic plants were selected in order to extract their essential oils. Fruits of ajowan (*Carum copticum* L.), fennel (*Foeniculum vulgare* Mill.) and aerial parts of thyme (*Thymus vulgaris* L.) and summer savory (*Satureja hortensis* L.) harvested from plants growing in the Research Garden of the Agricultural College of Urmia University and identified by Dr. Abbas Hassani from Department of Horticultural Sciences, Urmia University were used for extraction of essential oils by hydrodistillation during 3 hrs using a Clevenger-type apparatus. The oils were separated, dried over anhydrous sodium sulphate and kept in air tight sealed dark glasses at 4°C until used.

**GC and GC-MS analysis:** The GC analyses were carried out on a Shimutzu 17A gas chromatograph equipped with a non-polar DB-5 (95 % dimethyl polysiloxane) capillary column (30 m × 0.25 mm; 0.25 µm film thickness). The oven temperature was held at 30°C for 3 min then programmed at 280°C. Other operating conditions were as follows: carrier gas He, with a flow rate of 2.1 ml/min; injector temperature 230°C; detector temperature 250°C; split ratio, 50:1 GC-MS analyses were performed on a Shimadzu 17A GC coupled to a Shimutzu QGD5050 Mass Spectrometer. The operating conditions were the same as described above. Mass spectra were taken at 70 eV. Mass range was from

*m/z* 50-450 amu.

The constituents of the oils were identified by calculation of their retention indices under temperature-programmed conditions based on co-injection of homologous *n-alkanes* ($C_6$-$C_{24}$) on DB-5 capillary column. Compounds were identified 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 [22, 23]. Percentage amounts of individual components were obtained from FID area without using the correction factors.

**Treatment of kiwifruits with essential oils: Kiwifruits** (*Actinidia deliciosa* (A. Chev.) C.F. Liang and A.R. Ferguson var. *deliciosa* 'Hayward') were obtained from Urmia wholesale market in Urmia, Iran. Fruits were selected for uniformity in size, appearance, ripeness and the absence of physical defects. The selected fruits were randomized before being used for treatment with essential oils. Fruits (100-125 g) were randomly distributed into plastic boxes (1.5 L) with four replicates of eight fruits per treatment. The different concentrations (0, 250, 500 and 750 µl/l) of essential oil (Tween 80 was used as surfactant) were sprayed on fruits by using a hand-sprayer until fruits were enough wet to runoff. Treated fruits were placed on absorbent pad in plastic boxes, and then were sealed immediately to minimize vaporization. Boxes were stored in a cold room at 0-1ºC and 90% RH (90 days).

**Assessment of quality parameters**

**Evaluation of fruits decay, fruit flavour, fruit firmness and weight loss:** At the end of storage period, disease severity on total fruits evaluated, the surface of fruit divided to 10 part and percentage of decayed fruits were estimated. Flavour analyses were carried out to compare the flavour quality of treated and control kiwifruits by 6 trained panellists. A questionnaire was used to record the data for each treatment for the following characteristics: visual aspect (general aspect), firmness, sweetness, juiciness, sourness and crunchiness, on a 5-point scale: (1) very low; (2) low; (3) medium; (4) high and (5) very high. Flesh firmness of all fruits was destructively measured. Firmness reading was taken by using a penetrometer (FT 327, International Ripening Company, Alfonsine, Italy) fitted with a flat-8 mm diameter tip. The tip was pushed toward pulp after skin removal, at the fruit equator, in opposite sides to a depth of 7 mm. Weight loss was calculated by weighting the fruit before treatment and re-weighting at the end of the storage period. Weight loss percentage was calculated as percentage loss of initial weight.

**Evaluation of TSS, TA, TSS/TA and vitamin C content:** A random sample of fruits (8 fruit) 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 Brix. Titrable acidity (TA) content was measured by titration of fruit juice with 0.1 N sodium hydroxide (NaOH) to an end point of 8.1 and expressed as percentage of citric acid. The ratio between TSS and TA was calculated. Ascorbic acid concentration (Vitamin C) was determined using the 2, 6 dichlorophenol indophenol titration method [24].

**Statistical analysis:** Data were analyzed based on completely randomized design (CRD) with 4 replications representing 8 fruits per replication. Statistical analysis of the data was performed with MSTATC version 4.00/EM program [25]. Data were subjected to ANOVA analysis. Mean differences were separated by Duncan's multiple range test ($P < 0.05$).

**Results**

**Essential oil analysis:** The chemical compositions of essential oils used in the present study

are listed in table 1. The major compounds found in *T. vulgaris* oil were β-ocimene (12.6 %), thymol (10.6 %) and carvacrol (6.9 %). The oil of *C. copticum* was particularly rich in thymol (63.2 %), ρ-cymene (21.4 %) and γ-terpinene (13.8 %). *trans*-anethole (64.7 %), fenchone (14.6 %) and methyl chavicol (6.7 %) were the main components of *F. vulgare*. At the same time, *S. hortensis* oil contained carvacrol (54.1 %), terpinolene (20.6 %) and α-phellandrene (5.3 %) as main components.

**Effect of essential oils on fruits decay, flavour, firmness and weight loss:** Evaluation of disease severity at the end of storage period showed that *T. vulgaris* oil had the highest influence on the prevention of disease incidence followed by *S. hortensis*, *F. vulgare* and *C. copticum*, respectively (Fig. 1). But there was no significant difference between several essential oil concentrations (Table 2). Assessing the effect of essential oils on flavour of kiwifruits showed that *T. vulgaris* and *S. hortensis* oils had significant effect on fruits flavour compared with control. On the other hand, *F. vulgare* oil treated fruits had off-flavour followed by *C. copticum* (Table 3). Essential oil treatments had no effect on the firmness and weight loss percentage of kiwifruits (Table 2).

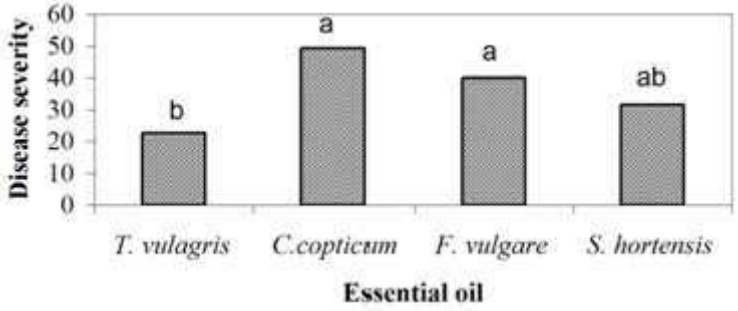


**Figure 1.** Effect of *Thymus vulgaris*, *Carum copticum*, *Foeniculum vulgare* and *Satureja hortensis* essential oils on disease severity in treated kiwifruits * Different letters on bars shows significant difference based on Duncan's multiple range test ($P < 0.05$).

**Effect of essential oil treatments on TSS, TA, TSS/TA and vitamin C content in fruits:** Assay of essential oil treatment impacts on TSS, TA and TSS/TA showed that the highest and the lowest amounts for those traits belonged to kiwifruits treated with *T. vulgaris* and *C. copticum*, respectively (Figs. 2-4). However, no significant differences were found among different essential oil concentrations (Table 2). In addition, the vitamin C content in fruit treated with *T. vulgaris* was the highest, and the other oils had no significant effect on its content (Fig. 5).

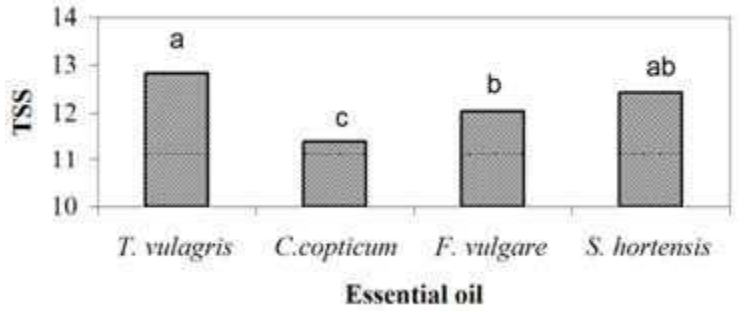


**Figure 2.** Effect of *Thymus vulgaris, Carum copticum, Foeniculum vulgare* and *Satureja hortensis* essential oils on total soluble solids (TSS) in treated kiwifruits. Different letters on bars shows significant difference based on Duncan's multiple range test ($P < 0.05$).

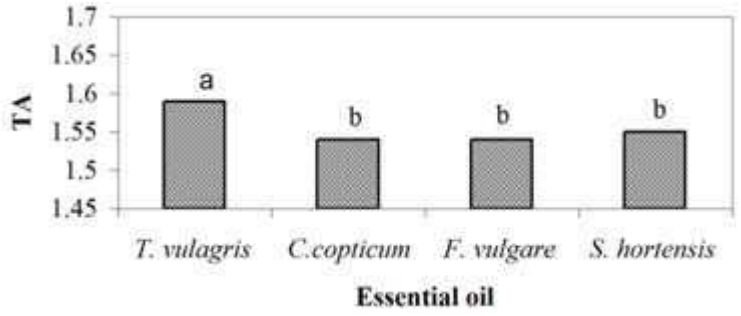


**Figure 3.** Effect of *Thymus vulgaris, Carum copticum, Foeniculum vulgare* and *Satureja hortensis* essential oils on titrable acidity (TA) in treated kiwifruits. Values followed with unlike letters differ significantly according to Duncan's multiple range test ($P < 0.05$)

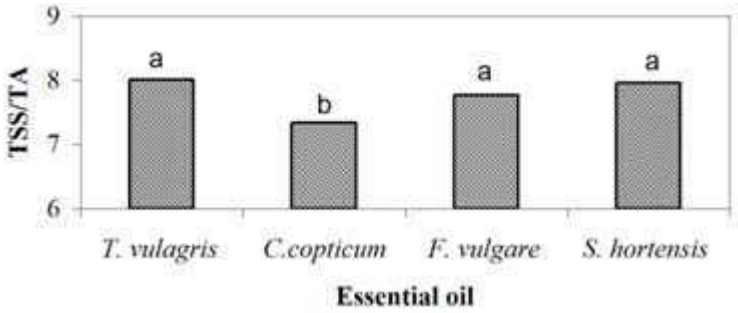


**Figure 4.** Effect of *Thymus vulgaris, Carum copticum, Foeniculum vulgare* and *Satureja hortensis* essential oils on TSS/TA in treated kiwifruits. Values followed with unlike letters differ significantly according to Duncan's multiple range test ($P < 0.05$)

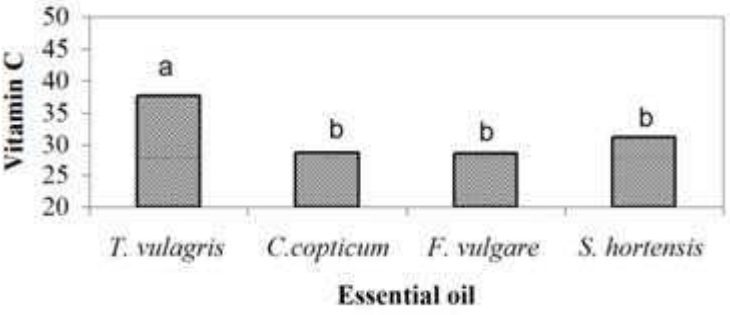


**Figure 5.** Effect of *Thymus vulgaris, Carum copticum, Foeniculum vulgare* and *Satureja hortensis* essential oils on vitamin C in treated kiwifruits. Values followed with unlike letters differ significantly according to Duncan's multiple range test ($P < 0.05$).

**Discussion:** The results of our experiment revealed that the essential oil of *T. vulgaris* followed by *S. hortensis* had appreciable antifungal activity compared with *C. copticum* and *F. vulgare* oils. The antifungal property of *T. vulgaris* oil has been reported on some important postharvest diseases such as Alternaria and Penicillium rot in tomato fruits [16] as well. In addition, essential oils from two clonal types of *T. vulgaris* (Laval-1 and Laval-2) were reported to be highly effective against *B. cinerea* and *Rhizopus stolonifer* in strawberry fruits [18]. Furthermore, *S. hortensis* oil has showed high antifungal activity against *Aspergillus flavus* in lemon fruits [20]. However, Thanassoulopoulos and

Yanna [17] stated that thyme and oregano oils had no efficiency in reduction of gray mold rot in kiwifruits. Expression of antifungal activity of essential oils is often very clear, but their mechanism(s) and action(s) are not completely understood. There is overwhelming consensus that prevalent components of essential oils such as phenols, aldehydes and alcohols exert most antifungal activity and among those compounds, special attention has been focused on phenolics such as carvacrol, thymol and their related precursors ρ-cymene and γ-terpinene [26]. It is confirmed that cell wall and cell membrane are the main target of essential oils [27]. An important characteristic of essential oils is their lipophilic nature which enables them to interact with lipids of the fungi cell membrane and mitochondria and to disturb their structure and function [28]. Other authors attributed this function of essential oils to especially phenolic compounds and their deteriorative interaction with bio-membranes [29]. In addition, essential oils may affect the essential metabolic pathways in microorganisms. Conner and Beuchat [30] suggested that the antimicrobial activity of essential oils could be a result of disturbance in enzymes involved in energy production and synthesis of structural components of microorganisms.

Although, the antimicrobial property of essential oils is attributed mainly to their principal constituents however, the synergistic effect of some minor components commonly available in essential oil composition has to be considered as well. For example, *T. vulgaris* oil contains lower than 1/3 phenolic compounds thymol (10.6 %) and carvacrol (6.6 %). However in the present work it was proved to be the most effective in reduction of fungal diseases in kiwifruits. According to these observations, it can be speculated that the high occurrence of antifungal activity of *T. vulgaris* could be related to thymol itself and /or its synergistic effects with other essential oil components. These finding are in well accordance with previous reports [31].

Evaluation of the effect of essential oil treatment on sensory parameters in kiwifruits showed that essential oil treatment decreased the amount of TA, TSS and vitamin C content compared to controls. These results were in agreement with previous works [32, 33]. Tzortzakis [33] showed that eucalyptus and cinnamon essential oils increased the TSS in cherry tomato. On the other hand, these essential oils had not affected the TA, pH, TSS/TA, firmness and weight loss in strawberry and main crop tomatoes. Also, in the present study it was determined that *F. vulgare* essential oil treatment decreased the flavour of kiwifruits, but Ranasinghe *et al.* [32] reported that cinnamon and clove essential oil treatment had no affect on the physicochemical and organoleptic attributes of banana fruits. In contrast with many reports on the antifungal property of essential oils under *in vitro* and *in vivo* conditions, only a few studies have been carried out to evaluate the effect of essential oil treatment on sensory parameters of fruits and the reason of differences among several essential oils is indeterminate.

**Conclusion:** It can be concluded that the plant essential oils especially *T. vulgaris* oil may be promising natural agent in postharvest disease control of kiwifruits. On the other hand, increasing consumer demand for naturally preserved fruits and vegetables attracts the special attention on essential oils as natural inexpensive natural preservative tools for storage of fruit crops. However further investigations are needed to elucidate the preservative action of these phytochemicals. This will be the subject of future study.

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**Table 1. The chemical compositions (%) of essential oils extracted from *Thymus vulgaris, Carum copticum, Foeniculum vulgare* and *Satureja hortensis***

| No | Compound [a] | RI [b] | *T. vulgaris* | *C. copticum* | *F. vulgare* | *S. hortensis* |
|---|---|---|---|---|---|---|
| 1 | α-Pinene | 936 | 5.2 | - | - | - |
| 2 | Limonene | 964 | - | - | 3.4 | - |
| 3 | β-Pinene | 971 | 3.8 | - | 1.5 | - |
| 4 | Camphene | 988 | 2.6 | - | - | - |
| 5 | γ-Terpinene | 996 | - | 13.7 | 1.4 | - |
| 6 | α-Phellandrene | 997 | 8.5 | - | - | 5.3 |
| 7 | β-Ocimene | 1030 | 12.6 | - | - | - |
| 8 | ρ-Cymene | 1040 | - | 21.4 | - | 3.6 |
| 9 | Fenchone | 1051 | - | - | 14.6 | - |
| 10 | Terpinolene | 1075 | - | - | - | 20.6 |
| 11 | α-Terpinolene | 1080 | 1.2 | - | - | - |
| 12 | Camphor | 1083 | - | - | 1.5 | - |
| 13 | Linalool | 1090 | 4.2 | - | - | - |
| 14 | Nerol oxide | 1103 | 3.2 | - | - | - |
| 15 | Borneol | 1107 | 2.3 | - | - | - |
| 16 | *trans*-Anethole | 1240 | - | - | 64.4 | - |
| 17 | Linalyl acetate | 1253 | 1.3 | - | - | - |
| 18 | Carvacrol | 1285 | 6.9 | - | - | 54.1 |
| 19 | Thymol | 1289 | 10.6 | 63.2 | - | - |
| 20 | Geranyl acetate | 1352 | 2.0 | - | - | - |
| 21 | α-Copaene | 1358 | 1.4 | - | - | - |
| 22 | β-Caryophyllene | 1420 | 6.1 | - | - | - |
| 23 | Germacrene D | 1462 | 1.9 | - | - | - |
| 24 | δ-Cadinene | 1517 | 2.1 | - | - | - |
| 25 | Spathulenol | 1550 | 1.7 | - | - | - |
| 26 | Widdrol | 1569 | 2.1 | - | - | - |
| 27 | Caryophyllene oxide | 1586 | 5.7 | - | - | - |
| 28 | Docosane | 2300 | 2.8 | - | - | - |

[a] The compounds that present lower than 1% are not presented; [b] Retention indices

**Table 2. Means squares for the effects of *Thymus vulgaris, Carum copticum, Foeniculum vulgare* and *Satureja hortensis* essential oils on quality parameters of treated kiwifruits**

| Treatments | Disease severity | Flavor | Firmness | Weight loss | TSS | TA | TSS/TA | Vitamin C content |
|---|---|---|---|---|---|---|---|---|
| EO [a] | * * | * | ns | ns | ** | * | ** | ** |
| Con [b] | ns | ** | ns | ns | ** | ** | ns | ** |
| EO×Con | ns | * | ns | ns | ns | ns | ns | ns |

[a] EO: Essential oil; [b] Con: Essential oil concentration.
Treatments with an associated asterisk were statistically significant (*, **, and ns = $P < 0.05$, 0.001, and not significant, respectively).

**Table 3. Effect of different concentration of *Thymus vulgaris, Carum copticum, Foeniculum vulgare* and *Satureja hortensis* essential oils on flavor of kiwifruits**

| Essential oil | Flavor | | | |
|---|---|---|---|---|
| | 0 | 250 | 500 | 750 (µl $l^{-1}$) |
| *T. vulgaris* | 4.15 $^{a}$ * | 3.62 $^{abc}$ | 4.12 $^{a}$ | 3.03 $^{a-d}$ |
| *C. copticum* | 3.68 $^{ab}$ | 1.21 $^{e}$ | 1.56 $^{e}$ | 2.09 $^{de}$ |
| *F. vulgare* | 4.27 $^{a}$ | 2.45 $^{cde}$ | 2.11 $^{de}$ | 2.31 $^{de}$ |
| *S. hortensis* | 4.28 $^{a}$ | 3.28 $^{ad}$ | 2.09 $^{de}$ | 2.84 $^{bcd}$ |

* Different letters in columns shows significant difference based on Duncan's multiple range test ($P < 0.05$).

**Table 4. Effect of different concentrations of *Thymus vulgaris, Carum copticum, Foeniculum vulgare* and *Satureja hortensis* essential oils on titrable acidity (TA), total soluble solids (TSS) and vitamin C content of kiwifruit**

| Concentration (µl $l^{-1}$) | TA | TSS | Vitamin C content |
|---|---|---|---|
| Control (0) | 1.63 $^{b}$ * | 12.94 $^{b}$ | 37.84 $^{b}$ |
| 250 | 1.51 $^{a}$ | 11.78 $^{a}$ | 29.41 $^{a}$ |
| 500 | 1.54 $^{a}$ | 11.87 $^{a}$ | 29.52 $^{a}$ |
| 750 | 1.54 $^{a}$ | 12.14 $^{a}$ | 29.48 $^{a}$ |

* Different letters in columns shows significant difference based on Duncan's multiple range test ($P < 0.05$).