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Effect of Essential Oils on Storability and Preservation of Some Vegetable Crops

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Abstract

Essential oils, as natural sprout inhibitor and safe fungicides, are a promising tool and good alternative compounds otherwise synthetic due to their high efficacy, biodegradability, eco-safety and volatile nature. They are consisting of a number of various components, i.e., terpenes, phenols, alcohols, esters, aldehydes and ketones in different composition or combinations. These effective compounds supply excess to prevent sprouting in potatoes and Jerusalem artichoke (JA) and less chance to development of resistance in fungi in JA, strawberry and broccoli with low concentrations. On contrary, high concentration of these oils induce the germination of seeds like broccoli and carob. This chapter explains the practical application of using essential oils as natural antisprouting, inducing quality, preserving fungal diseases, eco-friendly compounds, alternating synthetic chemicals, giving high benefits and easy to apply. The foliar application with essential oils increases the productivity, quality and marketable yield and storability and reduces weight losses and decay. Moreover, the essential oils increase broccoli seed germination, antioxidant content and other phytochemical parameters. The chapter provides a novel anti-sprouting agent for inhibiting growth of processing potato tubers and identification of terpenoids that use to inhibit tuber sprouting as well as application of Chloroprotham (CIPC) isopropyl-N-(3-chlorophenyl) carbamate as a conventional chemical inhibitor.

Keywords: essential oils, constituents, anti-sprouting agent, antifungal, sprout growth, postharvest, vegetables, structure, extraction, application

1. Introduction

Recently, the natural alternatives such as plant essential oils provide a promising control of plant diseases and anti-sprout agent because they virtually constitute a rich source of bioactive chemicals such as phenols, flavonoids, quinones, tannins, alkaloids, saponins, sterols terpenes, aromatic and aldehydes [1]. Moreover, these natural alternatives can also maintain the biochemical constituents of tubers during storage, they are biodegradable to nontoxic products, and are potentially suitable for use in integrated pest management programs.

Jerusalem artichoke JA or sun choke (*Helianthus tuberosus* L.) is a perennial plant which has a high economic value. JA used traditionally for human food and livestock feed due to its high nutritive value. JA tubers used for production of biofuels

(ethanol) and some functional food like inulin, fructooligosaccharides and fructose. Moreover, some bioactive metabolites from its leaves and stems have been used in some pharmaceutical industries [1]. Storage JA tuber, controlled rots can be done by various techniques including; cold temperature, removal of diseases in tubers and minimizing mechanical injuries or application of synthetic fungicides. Another simple applied method in developing countries is keeping the tubers in the soil. Unfortunately, many fungi diseases can grow at cold storage temperatures and lead to damage, especially in extending long storage [2], However, storage of the harvested tubers usually results in high losses in quality, caused mainly by desiccation, rotting, sprouting, freezing and inulin degradation. A common solution is the use of synthetic chemical fungicides, however, their use is accompanied by threatening human health and the environment by supporting the emergence of resistant pathogens and by contamination of food with the pesticide deposits [3]. Essential oils, as green fungicides, are emerging as a better alternative of synthetic fungicides due to their high efficacy, biodegradability, eco-safety and volatile nature.

Respiration of potato tubers during storage and breakdown of dormancy during storage result in sprouting and loss of nutritive value of tubers [4]. Sprouting reduces the weight, the nutritional and processing quality of tubers and the number of marketable potatoes, being responsible for important economic losses during potatoes storage [5]. These physiological changes affect the internal composition of the tuber and destruction of edible material and changes in nutritional quality [6]. Various methods are available to control sprouting during storage. The primary method to control sprouting in storage is with postharvest application of isopropyl N-(3-chlorophenyl) carbamate (chlorpropham; CIPC). CIPC inhibits sprout development by interfering with cell division [7]. Therefore, a pressing need exists to find other, more environmentally acceptable sprout inhibitors for tubers. Nowadays it's very important to use natural products compounds such as essential oils.

Broccoli sprouts are considered as a functional food. Essential nutrient content provides diverse secondary metabolites and phytochemicals [8]. The phenolic compounds, especially flavonoids and anthocyanin, show a great ability capture free radical that leading to oxidative stress, to these compounds are attributed a beneficial effect in the prevention of cardiovascular diseases, circulatory problems, neurological disorders and cancer [9]. Broccoli has been identified as a vegetable with potential anti-cancer activity due to high levels of glucosinolates. The use of essential oil treatments rich in antioxidant to stimulate broccoli seed germination should be considered. Application of thyme and basil oil at 4% reduced the pathogenic fungi from seed to seedling and had a positive effect on the seed germination of infected seeds [10]. Aromatic plants especially essential oils are well known for their antioxidant and antimicrobial properties that prevent food degradation and alteration [11], as they are rich in phenolic substances, usually referred to as polyphenols, which are ubiquitous components of plants and herbs.

2. Application of essential oils

2.1 Alternative preservation method against sclerotium tuber rot of Jerusalem artichoke using natural essential oils

2.1.1 Methodology

Two experiments were conducted in Mansoura laboratory for vegetable crop handling and postharvest according to the storage method. In the first experiment, the tubers were kept in perforated polyethylene bags (0.075 mm thickness), and

stored at 4°C and 90–95% relative humidity RH. In the second experiment, the tubers were stored in carton boxes (3 m³) at 25 ± 2°C with moistened peat moss layers at the rate of peat moss: JA tubers (1.5: 1, kg/kg). The treatments applied for each experiment can be summarized as follows: Control (C), infected with fungal pathogen *S. rolf sii* (P), treated with caraway essential oil (O) and treated with caraway oil and infected with pathogen *S. rolf sii* (O + P). About 30 kg of tubers was used for each treatment.

2.1.2 Important results

2.1.2.1 Antifungal activity of the essential oils

Assessment of antifungal activity in vitro of caraway and spearmint essential oils was evaluated against *S. rolf sii* (**Figure 1**). Caraway essential oil completely inhibited the growth of the fungal pathogen even at the lowest concentration (2%). On the other hand, spearmint essential oil showed slight reduction in the fungal pathogen growth. The antifungal activity of caraway essential oil may be attributed to some antifungal phytochemicals that constitute a large fraction of the oil like carvone, limonene, carveol, pinene and thujone [12].

2.1.2.2 Evaluation of the caraway essential oil and peat moss application under storage conditions

2.1.2.2.1 Severity of disease

Data presented in **Table 1** show the rot fungal disease severity of JA tubers exposed to caraway oil and infected with fungal pathogen *S. rolf sii* under the two storage methods. The disease severity increased with the increasing the storage period over to the storage methods. JA tubers infected with *S. rolf sii* and exposed to emulsion of caraway essential oil (O + P) in peat moss layer at 25°C significantly reduced the disease severity compared to the cold storage method after 4 months storage. Infected-control JA tubers (P) and storage in peat moss layer at 25°C significantly reduced the disease severity for 2 months of storage compared with cold storage method, after which, the tubers were fully decadent. On the other hand, control-uninfected JA tubers (C) and storage in peat moss layer at 25°C significantly reduced the disease severity (caused by reasons other than *S. rolf sii*) compared with the storage under cold storage method. Caraway essential oil had the antimicrobial effects due to its content of basic constituents of monoterpenes, carvone and limonene. The basic constituents had a permeability effect on fungal

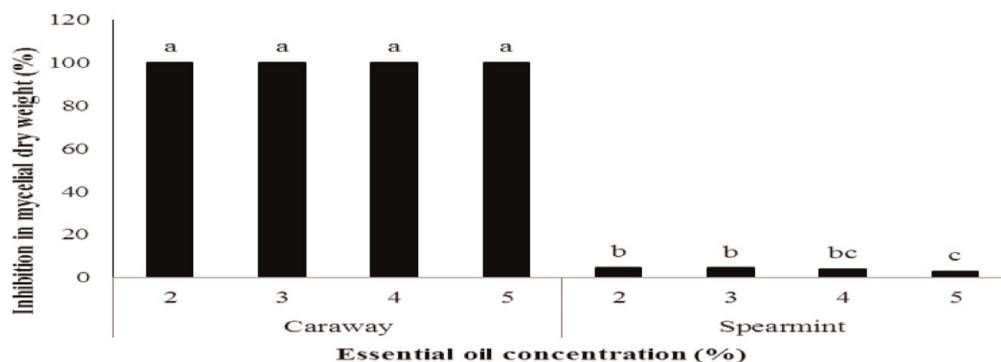


Figure 1. Inhibition in mycelial dry weight of *S. rolf sii* as a function of the tested oils. Columns superscripted with the same letter are not significantly different at $P \leq 0.05$ (Duncan test).

Storage method*	Treatment**	Storage period (day)			
		30	60	90	120
1	C	28.5 ^{b,***}	73.3 ^a	100.0 ^a	NA
	P	100.0 ^a	NA	NA	NA
	O	0.0 ^e	0.0 ^e	0.0 ^d	0.0 ^d
	O + P	8.9 ^d	24.4 ^d	37.5 ^b	53.6 ^b
2	C	20.1 ^c	33.6 ^c	40.0 ^b	66.5 ^a
	P	20.0 ^c	47.2 ^b	100.0 ^a	NA
	O	0.0 ^e	0.0 ^e	0.0 ^d	0.0 ^d
	O + P	0.0 ^e	0.0 ^e	8.9 ^c	13.3 ^c

*1 = storage JA tubers in polyethylene bags at 4°C and 2 = storage JA tubers in peat moss layer at 25°C.

**C = untreated control, P = infected tubers with pathogen, O = treated JA tubers with caraway essential oil and O + P = infected tubers with pathogen and treated with caraway essential oil. NA = not applicable due to full decay.

***Means in each column followed by the same letter(s) were not significant at P= 0.05; where, (a) refer to the highest mean values, and (e) refer to the lowest mean values according to Duncan multiple range test.

Tubers rot in the untreated control (C) treatment was due to many reasons rather than the fungal pathogens.

Table 1.

Mean rot severity (%) of JA tubers treated with caraway essential oil and infected with *S. rolfisii* using two storage methods.

cell membrane, inactivation of some organic compounds and enzymes and/or the inhibition of conidial germination, eventually, the death of fungal pathogen [13]. Moreover, the use of essential oils in storage of JA may have many benefits, including, they are natural-ecofriendly products, biodegradable and multifunctional purposes.

Moreover, the activity of essential oils against may tend to reduce pathogenic fungi resistance reinforcement against chemical fungicide because they contain two or more stereo-isomers that may be located on multi-sites on the pathogen's plasma membrane. One of the valuable applications for peat moss is the traditional use in food preservation [14]. The antifungal effect of the use of peat mosses has been reported by many investigators against *Aspergillus niger*, *A. flavus*, *Candida albicans*, *Cryptococcus albidus* and *Trichophyton rubrum* [15]. The antifungal effect of peat moss may be related to some of its contains of extranutritional constituents or bioactive components like a pectin-like polymer and sphagnum, that inhibit fungal mycelium growth via electrostatic immobilization of extracellular enzymes and/or nitrogen deprivation, phenolics that inhibit the activity of extracellular enzymes of microbes or other constituents like sterols and polyacetylenes [16].

2.1.2.2.2 Sprouting, weight loss and dry matter percentages of JA tubers

Table 2 show the mean data of weight loss and dry matter percentages of JA tubers exposed to emulsion of caraway essential oil and infected with fungal pathogenic *S. rolfisii* under using the two different storage methods. Results indicated that, the treatment of healthy JA tubers with emulsion of caraway essential oil completely inhibited the tubers sprouting and weight loss, but recorded the highest dry matter weight percentage along the storage period compared with the untreated-uninfected control treatment over the use of the two different methods. Even after 120 days of storage period, the treatment of the use of JA tubers with caraway oil and infected with pathogenic fungi significantly decreased sprouting and weight loss percentages and increased dry matter content for JA tubers that stored in peat moss layers at 25°C than those stored in polyethylene bags at 4°C when compared

Criterion	Storage method [*]	Treatment ^{**}	Storage period (day)			
			30	60	90	120
Sprouting	1	C	40.0 ± 10.0	70.0 ± 4.0	90.0 ± 4.0	NA
		P	NA ^{***}	NA	NA	NA
		O	0.0	0.0	0.0	0.0
		O + P	3.8 ± 0.1	3.9 ± 0.1	4.0 ± 0.4	4.3 ± 0.2
	2	C	50.0 ± 10.0	80.0 ± 14.0	95.0 ± 6.0	98.0 ± 2.0
		P	3.6 ± 0.4	4.8 ± 0.4	NA	NA
		O	0.0	0.0	0.0	0.0
		O + P	0.0	0.0	0.0	0.0
Weight loss	1	C	20.5 ± 2.9	35.7 ± 5.3	59.9 ± 8.0	NA
		P	NA	NA	NA	NA
		O	0.0	0.0	0.0	0.0
		O + P	2.9 ± 0.2	3.0 ± 0.2	3.8 ± 0.6	4.6 ± 0.5
	2	C	7.1 ± 0.2	21.7 ± 3.3	50.9 ± 5.8	70.9 ± 8.2
		P	30.7 ± 4.0	39.5 ± 9.9	NA	NA
		O	0.0	0.0	0.0	0.0
		O + P	0.0	1.0 ± 0.1	1.0 ± 0.2	1.7 ± 0.4
Dry matter weight	1	C	17.2 ± 0.5	18.0 ± 0.7	19.2 ± 1.5	NA
		P	NA	NA	NA	NA
		O	22.5 ± 0.4	22.9 ± 0.6	23.6 ± 0.3	24.9 ± 0.6
		O + P	22.0 ± 0.2	22.3 ± 0.4	23.3 ± 0.9	23.5 ± 0.8
	2	C	22.6 ± 0.4	23.0 ± 0.3	21.0 ± 0.5	21.5 ± 1.1
		P	18.6 ± 0.6	17.0 ± 0.5	NA	NA
		O	23.6 ± 0.5	24.6 ± 0.8	24.9 ± 0.5	25.8 ± 0.5
		O + P	23.5 ± 0.4	23.7 ± 0.6	24.2 ± 0.4	25.5 ± 0.3

^{*} 1 = storage JA tubers in polyethylene bags at 4°C and 2 = storage JA tubers in peat moss layer at 25°C.

^{**} C = untreated control, P = infected tubers with pathogen, O = treated JA tubers with caraway essential oil and O + P = infected tubers with pathogen and treated with caraway essential oil.

^{***} NA = not applicable due to full decay.

Table 2.

Mean sprouting, weight loss and dry matter weight (% ± 2SD) of JA tubers treated with caraway essential oil and infected with *S. rolfisii* using two storage methods.

with the control (infected-untreated) tubers. On the other hand, storage of the untreated-uninfected JA tubers in peat moss layers at 25°C increased the sprouting, and dry matter content and decreased the weight of the tubers compared to the storage of tubers in polyethylene bags at 4°C. The bioactive components like limonene and carvone, in caraway essential oil are known to inhabit sprouting percentage of JA tubers by the suppressing of mitochondrial respiration and reducing carbohydrate deterioration sugar content. Carvone had a specific tool for inhibition of sprout growth of potato tubers, such as the repression of key enzyme in the mevalonate acid pathway, which is the main precursor of gibberellin biosynthesis [17]. On the other hand, peat moss has a relatively high water retention capacity; their cells can hold 16–25 times their dry weight of water [14]. This in turns

encourages such amendment for its use in the preservation of JA tubers by increasing a relative humidity around the tubers and preventing heat transfer within the peat moss layer leading to the decrease of the water loss from fresh tubers depends on the difference between the *water* vapor pressure within the tubers and the *water* vapor pressure of the surrounding air, with *moisture* passing from the higher pressure to the lower even at 25°C. Cabezas et al. [18] reported that dry matter content in JA tubers decreased significantly depends on many factors, such as storage conditions, storage periods and keeping tubers for 30 days at 18°C, this leads to losing water above 20%.

Criterion	Storage method*	Treatment**	Storage period (day)			
			30	60	90	120
Carbohydrates	1	C	42.5 ± 1.4	41.7 ± 1.5	38.4 ± 4.3	NA
		P	NA***	NA	NA	NA
		O	44.6 ± 1.3	44.2 ± 1.3	43.3 ± 1.3	43.0 ± 1.0
		O + P	42.7 ± 1.7	42.0 ± 0.7	41.6 ± 1.1	41.3 ± 0.3
	2	C	42.0 ± 0.3	40.2 ± 1.4	37.7 ± 1.0	36.0 ± 0.5
		P	36.7 ± 1.4	36.0 ± 0.3	NA	NA
		O	46.0 ± 0.3	46.9 ± 0.5	47.0 ± 0.8	47.7 ± 0.9
		O + P	42.0 ± 0.8	41.9 ± 0.7	41.5 ± 1.2	41.0 ± 1.4
Inulin	1	C	14.2 ± 0.6	13.6 ± 0.7	12.8 ± 1.2	NA
		P	NA	NA	NA	NA
		O	15.6 ± 0.8	15.0 ± 0.3	16.7 ± 0.6	15.0 ± 0.2
		O + P	14.9 ± 0.6	14.0 ± 0.1	14.0 ± 0.0	13.9 ± 0.4
	2	C	14.3 ± 0.5	14.0 ± 0.4	13.8 ± 0.6	13.0 ± 0.2
		P	13.0 ± 0.4	12.0 ± 0.3	NA	NA
		O	18.9 ± 0.5	18.0 ± 0.4	17.9 ± 0.2	17.6 ± 0.6
		O + P	17.9 ± 0.5	17.6 ± 0.8	17.0 ± 0.2	17.0 ± 0.0
Protein	1	C	12.2 ± 0.4	12.0 ± 0.2	11.9 ± 0.4	NA
		P	NA	NA	NA	NA
		O	12.8 ± 0.3	12.7 ± 0.3	12.7 ± 0.4	12.6 ± 0.4
		O + P	12.6 ± 0.5	12.6 ± 0.2	12.4 ± 0.1	12.0 ± 0.1
	2	C	12.5 ± 0.3	12.3 ± 0.4	12.0 ± 0.4	11.9 ± 0.2
		P	9.9 ± 0.2	9.0 ± 0.4	NA	NA
		O	13.0 ± 0.3	13.0 ± 0.6	12.7 ± 0.5	12.7 ± 0.2
		O + P	12.6 ± 0.5	12.4 ± 0.5	12.3 ± 0.4	12.0 ± 0.4

*1 = storage JA tubers in polyethylene bags at 4°C and 2 = storage JA tubers in peat moss layer at 25°C.

**C = untreated control, P = infected tubers with pathogen, O = treated JA tubers with caraway essential oil and O + P = infected tubers with pathogen and treated with caraway essential oil.

***NA = not applicable due to full decay.

Table 3.

Mean contents of carbohydrates, inulin (mg/g ± 2SD) and protein (% ± 2SD) of JA tubers treated with caraway essential oil and infected with *S. rolfisii* using two storage methods.

2.1.2.2.3 Biochemical constituents of JA tubers

Table 3 show the data of carbohydrates content, inulin and protein in JA tubers exposed to emulsion of caraway essential oil and then infected with fungal pathogenic *S. rolf sii* over the use of two storage methods. The application of caraway essential oil and uninfected JA tubers had significant effects on total carbohydrates, inulin and protein contents compared with the untreated-uninfected control in both storage methods. Along 4 months of storage, the treatment of infected JA tubers with pathogen and treated with caraway essential oil effectively decreased the carbohydrate, inulin and protein contents compared with the infected-untreated control JA tubers in both methods of storage. A fresh JA tuber contains 80% water, 15% carbohydrates, mainly in the form of inulin and about 2% protein in dry matter [19]. There are many changes in fresh JA tubers with long term storage, i.e., physical, biochemical, microbiological and enzymatic and which may lead to tuber decay. To inhibit these biochemical activities, natural or artificial drying products are widely used [20]. Davies [21] reported that the basic constituents of caraway oil (monoterpenes) tend to delay and the deterioration of carbohydrates and protein contents associated with the enzymatic system as well as respiration and energy metabolism enzyme keeping the internal biochemical enzymatic activities in minimum level.

2.1.2.2.4 Peroxidase, polyphenoloxidase enzymes and phenol content in JA tubers

The mean activities data of peroxidase, polyphenoloxidase and phenol contents of JA fresh tubers treated with caraway essential oils and infected with pathogenic fungi over the use of two different two storage methods are presented on **Table 4**. Results revealed that infection with *S. rolf sii* had significant effects on total phenol and the activity of peroxidase and polyphenoloxidase enzymes in JA tubers than those of the uninfected JA tubers control in the two different storage methods. On the contrary, the application of caraway essential oil to infected/uninfected JA tubers increased peroxidase and polyphenoloxidase and phenol content compared with the untreated-uninfected JA tubers in both methods. These results are in a line with those obtained by [22] who reported an increasing in peroxidase and polyphenoloxidase enzymes in potato fresh tubers when treated with caraway essential oil. Although regulatory mechanisms of plant *enzyme* complexes and the most enzymatic reactions are reduced at low temperature degree, JA tubers metabolism could continue at a slow rate even at minimum temperature (2°C) during cold storage. The enzymatic activation due to the exogenous application of caraway essential oil treatment could be directly related to its content of bioconstituents like carvone.

2.2 Inhibition of sprout growth and increase storability of processing potato by antisprouting agent

2.2.1 Methodology

2.2.1.1 Tuber material

Fresh local potato cv. Fridor and uniformly size of 60–80 mm in diameter (weighing 180–250 g) were selected without any sprouting in eyes and no anti-sprouting treatment was used. Each treatment was treated with natural and safe

Criterion	Storage method ¹	Treatment ^{**}	Storage period (day)			
			30	60	90	120
Peroxidase	1	C	0.40 ± 0.02	0.30 ± 0.0	0.28 ± 0.05	NA
		P	NA ^{***}	NA	NA	NA
		O	0.40 ± 0.03	0.38 ± 0.01	0.38 ± 0.01	0.37 ± 0.02
		O + P	2.67 ± 0.16	2.69 ± 0.26	2.73 ± 0.04	2.74 ± 0.03
	2	C	0.23 ± 0.01	0.22 ± 0.02	0.21 ± 0.01	0.20 ± 0.06
		P	1.90 ± 0.02	1.92 ± 0.03	NA	NA
		O	0.33 ± 0.02	0.34 ± 0.02	0.34 ± 0.02	0.34 ± 0.03
		O + P	1.77 ± 0.03	1.86 ± 0.04	1.87 ± 0.02	1.87 ± 0.26
Polyphenoloxidase	1	C	0.39 ± 0.01	0.36 ± 0.01	0.35 ± 0.02	NA
		P	NA	NA	NA	NA
		O	0.47 ± 0.03	0.47 ± 0.02	0.45 ± 0.03	0.42 ± 0.06
		O + P	1.46 ± 0.02	1.57 ± 0.05	1.47 ± 0.02	1.57 ± 0.03
	2	C	0.40 ± 0.04	0.40 ± 0.03	0.37 ± 0.03	0.35 ± 0.03
		P	1.49 ± 0.03	1.50 ± 0.04	NA	NA
		O	0.46 ± 0.02	0.43 ± 0.01	0.41 ± 0.06	0.40 ± 0.01
		O + P	1.46 ± 0.02	1.57 ± 0.02	1.57 ± 0.03	1.67 ± 0.02
Total phenol	1	C	0.29 ± 0.02	0.28 ± 0.01	0.27 ± 0.01	NA
		P	NA	NA	NA	NA
		O	0.32 ± 0.03	0.32 ± 0.03	0.32 ± 0.02	0.31 ± 0.02
		O + P	0.52 ± 0.02	0.52 ± 0.02	0.51 ± 0.02	0.52 ± 0.01
	2	C	0.35 ± 0.02	0.35 ± 0.02	0.35 ± 0.02	0.34 ± 0.03
		P	0.57 ± 0.04	0.57 ± 0.04	NA	NA
		O	0.26 ± 0.01	0.26 ± 0.01	0.26 ± 0.02	0.26 ± 0.02
		O + P	0.55 ± 0.02	0.55 ± 0.02	0.55 ± 0.01	0.56 ± 0.01

¹ 1 = storage JA tubers in polyethylene bags at 4°C and 2 = storage JA tubers in peat moss layer at 25°C.

^{**} C = untreated control, P = infected tubers with pathogen, O = treated JA tubers with caraway essential oil and O + P = infected tubers with pathogen and treated with caraway essential oil.

^{***} NA = not applicable due to full decay.

Table 4.

Mean activities of peroxidase, polyphenoloxidase enzymes and phenol content (% ± 2SD) of JA tubers treated with caraway essential oil and infected with *S. rolfisii* using two storage methods.

antisprouting agent and stored at ambient temperature (average: 35/15°C day/night and 70% RH) in Laboratory for 4 months.

2.2.1.2 Treatments

The experiment included seven treatments, which were as follows: *Cymbopogon martini* (rich in geraniol and geranyl acetate), *C. flexuosus* (rich in citral), *C. winterianus* (rich in rich in citronellal and citronellol), *Ocimum sanctum* (rich in rich in ketone and camphor), *Carum carvi* (rich in rich in carvone), *Artemisia annua* (rich in ketone camphor) and *Lavendula officinalis* (rich in linalool). The isolated terpenoids were purified by HPLC. Essential oils were purified by column chromatography and substantially pure compounds were used. Tubers dipped in emulsions

of 8 mm concentration of each compound in distilled water and Tween 20 (6%) for 30 min after 1 month of harvest or at such time that the tubers begin to sprout.

2.2.2 Results and discussion

2.2.2.1 Sprouting, weight loss and dry matter content

All control tubers had significant values of sprouting and weight loss percentages at the end of storage period (**Table 5**). Geraniol and citral completely inhibited sprouting by 100%, decreased weight loss and increase tuber dry matter content in both seasons. Application of geranyl acetate inhibited sprouting by 95%. On the other hand, linalool and L-carvone had no significant effect on tuber sprouting. It has been reported that L-carvone and D-carvone displayed little or no inhibition of sprouting in potatoes [17]. Geraniol and citral have a high content in monoterpenes such as benzaldehyde, eugenol and thymol [23]. CIPC inhibited sprouting over 98.5%.

Under this study condition, the beneficial effect of the applied anti-sprouting agent (geraniol and citral) on controlling tubers sprouting and increasing dry matter content could be associated with their similar advantages effect in preservation of their tubers starch, carbohydrates, sugars and amino acid content (**Table 6**). Suppression of sprouting and weight loss logically associated with maintenance of dry matter. Furthermore, monoterpenes acts as antioxidant and had a protective role against oxidative stress under normal conditions of storage.

2.2.2.2 Reducing sugars, amino acids and peroxidase POD activity

All storage treatments gave significant lower values on reducing sugars and amino acids content during two seasons of study as compared to the control (**Table 6**). In the ambient temperature, the lowest significant values of reducing

Treatments	Sprouting (%)		Weight loss (%)		Dry matter (%)	
	2012	2013	2012	2013	2012	2013
1. Control	100.0 ^a	96.00 ^a	25.12 ^a	26.18 ^a	21.65 ^f	22.80 ^e
2. CIPC	2.49 ^e	1.20 ^c	4.33 ^e	2.80 ^{ef}	23.60 ^{a-d}	23.66 ^d
3. Geranyl acetate	4.68 ^d	4.33 ^c	3.41 ^f	4.65 ^d	22.50 ^{ef}	24.55 ^{ab}
4. Geraniol	0.00 ^f	0.00 ^c	2.19 ^h	1.45 ^g	24.56 ^a	25.30 ^a
5. Camphor	6.92 ^c	5.98 ^c	2.88 ^g	2.95 ^{ef}	23.33 ^{b-e}	24.38 ^{bc}
6. Citral	0.00 ^f	0.00 ^c	1.51 ⁱ	1.26 ^g	24.00 ^{ab}	24.95 ^{ab}
7. Linalool	100.00 ^a	72.00 ^b	9.50 ^b	8.00 ^b	22.66 ^{de}	23.60 ^d
8. L-Carvone	70.58 ^b	62.00 ^b	9.50 ^b	6.25 ^c	22.80 ^{c-e}	23.70 ^{cd}
9. D-Carvone	72.00 ^b	76.98 ^b	8.03 ^c	3.45 ^e	22.90 ^{c-e}	24.89 ^{ab}
10. D-Citronellol	2.89 ^e	2.00 ^c	6.75 ^d	5.73 ^c	23.60 ^{a-d}	24.68 ^{ab}
11. L-Citronellol	0.00 ^f	0.00 ^c	2.25 ^{gh}	2.10 ^{fg}	23.80 ^{a-c}	24.55 ^{ab}

Means followed by the same letter(s) within each column do not significantly differ using Duncan's multiple range test at the level of 5%; where, (a) refer to the highest mean values, and (h) refer to the lowest mean values according to Duncan Multiple Range Test.

Table 5. Sprouting behavior characters and dry matter of potato tubers as affected by anti-sprouting agent during 2012 and 2013 seasons (after 4 months of storage period).

Treatments	Reducing sugars (%)		Total free amino acids (%)		Peroxidase activity POD (%)	
	2012	2013	2012	2013	2012	2013
1. Control	4.29 ^a	4.52 ^a	0.352 ^a	0.348 ^a	56.77 ^g	55.51 ^g
2. CIPC	2.05 ^c	3.18 ^d	0.307 ^{ab}	0.301 ^{ab}	95.81 ^b	94.63 ^b
3. Geranyl acetate	1.39 ^{cd}	3.93 ^b	0.084 ^{bc}	0.047 ^c	79.75 ^e	79.33 ^e
4. Geraniol	1.24 ^d	1.51 ^f	0.030 ^c	0.028 ^c	97.33 ^a	96.29 ^a
5. Camphor	3.41 ^b	3.48 ^c	0.152 ^{a-c}	0.153 ^{a-c}	80.68 ^e	80.26 ^e
6. Citral	1.25 ^d	1.52 ^f	0.045 ^c	0.045 ^c	97.68 ^a	96.46 ^a
7. Linalool	4.07 ^{ab}	4.13 ^b	0.106 ^{bc}	0.108 ^{bc}	80.67 ^e	79.06 ^c
8. L-Carvone	3.81 ^{ab}	1.83 ^e	0.084 ^{bc}	0.151 ^{a-c}	81.67 ^e	80.50 ^e
9. D-Carvone	1.45 ^{cd}	1.68 ^{ef}	0.146 ^{a-c}	0.157 ^{a-c}	77.55 ^f	76.77 ^f
10. D-Citronellol	1.76 ^{cd}	1.54 ^f	0.186 ^{a-c}	0.187 ^{a-c}	84.50 ^d	83.62 ^d
11. L-Citronellol	1.29 ^d	1.58 ^f	0.147 ^{a-c}	0.059 ^c	87.67 ^c	86.65 ^c

Means followed by the same letter(s) within each column do not significantly differ using Duncan's multiple range test at the level of 5%; where, (a) refer to the highest mean values, and (g) refer to the lowest mean values according to Duncan Multiple Range Test.

Table 6.

Reducing sugars, amino acids and peroxidase enzyme of potato tubers as affected by anti-sprouting agent during 2012 and 2013 seasons (after 4 months of storage period).

sugars and amino acids content were found in tubers exposed to emulsion of geraniol and citral, without significant difference between the two treatments.

The monoterpenes rich in compounds had a potential role in preservation and maintenance of the stored tubers reserves, keeping the enzymatic activities in a minimal level and in more stable case thereby prolonged their dormancy period. Also, application of these treatments were highly effective in tuber protection against the degradable effects of oxidative stressful during high temperature storage conditions and accordance to the findings of [20] who indicated that monoterpenes and antioxidants tended to slow down the activity of carbohydrates, breakdown of protein and enzymatic activity as well as reduce respiration rate and metabolism enzyme. The role of POD in sprouting of potatoes was widely reported, particularly its degrading activity of IAA, and cytokinin which is considered an effective promote oxidative stress is of great importance and depending on the activation degree of peroxidase as affected by storage treatments.

2.2.2.3 Processing quality of potato fries and chips

All storage treatments and CIPC treatment at ambient temperature had significant differences on quality characters of potato chips and French fries, i.e., color, crispiness and taste in comparison with the control treatment (**Table 7**).

The same treatments prevented and blocked the accumulation of total sugars, and kept the reducing sugars and amino acids in optimize levels in the stored tubers at ambient temperature. This is true in the end of storage (4 months). Thus, we noticed the worst processing quality (dark potato chips and crispness with bad taste) of storage treatments due to the appearance of Millard reaction during frying process and the accumulation of reducing sugars and amino acids [23]. The same processing quality parameters were correlated with dry matter content (**Table 8**) and with amino acids content (**Table 9**) in both seasons. These results are in harmony with those previously obtained by [24]. Meanwhile, we also noticed that

Treatments	Chips						French fries					
	Color		Taste		Crispness		Color		Taste		Crispness	
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
1. Control	3.00 ^e	3.33 ^c	3.00 ^d	3.33 ^{bc}	4.33 ^{a-c}	4.33 ^{a-c}	3.33 ^{de}	3.00 ^d	3.33 ^{cd}	4.00 ^{b-d}	4.67 ^{ab}	4.67 ^{ab}
2. CIPC	3.33 ^{de}	3.33 ^c	4.33 ^{a-c}	4.33 ^{ab}	4.33 ^{a-c}	4.67 ^{ab}	3.67 ^{cde}	3.33 ^{cd}	4.00 ^{a-c}	4.33 ^{a-c}	4.67 ^{ab}	4.67 ^{ab}
3. Geranyl acetate	4.67 ^{ab}	4.67 ^{ab}	5.00 ^a	4.67 ^a	5.00 ^a	5.00 ^a	4.67 ^{ab}	4.67 ^{ab}	5.00 ^a	4.67 ^{ab}	5.00 ^a	4.33 ^{abc}
4. Geraniol	5.00 ^a	5.00 ^a	5.00 ^a	4.67 ^a	5.00 ^a	5.00 ^a	4.67 ^{ab}	4.67 ^{ab}	5.00 ^a	4.67 ^{ab}	5.00 ^a	4.33 ^{a-c}
5. Camphor	4.67 ^{ab}	4.67 ^{ab}	5.00 ^a	4.67 ^a	5.00 ^a	5.00 ^a	4.67 ^{ab}	4.67 ^{ab}	4.67 ^{ab}	4.67 ^{ab}	5.00 ^a	5.00 ^a
6. Citral	5.00 ^a	5.00 ^a	5.00 ^a	4.67 ^a	5.00 ^a	5.00 ^a	5.00 ^a	5.00 ^a	4.67 ^{ab}	5.00 ^a	5.00 ^a	5.00 ^a
7. Linalool	4.67 ^{ab}	4.67 ^{ab}	4.64 ^{ab}	4.67 ^a	5.00 ^a	5.00 ^a	4.67 ^{ab}	4.67 ^{ab}	4.67 ^{ab}	4.67 ^{ab}	5.00 ^a	5.00 ^a
8. L-Carvone	4.67 ^{ab}	4.67 ^{ab}	4.67 ^{ab}	4.67 ^a	5.00 ^a	5.00 ^a	4.67 ^{ab}	4.67 ^{ab}	5.00 ^a	5.00 ^a	5.00 ^a	5.00 ^a
9. D-Carvone	5.00 ^a	5.00 ^a	5.00 ^a	4.67 ^a	5.00 ^a	5.00 ^a	5.00 ^a	5.00 ^a	4.67 ^{ab}	5.00 ^a	5.00 ^a	5.00 ^a
10. D-Citronellol	4.00 ^{b-d}	4.67 ^{ab}	4.67 ^{ab}	4.67 ^a	4.67 ^{ab}	4.67 ^a	4.00 ^{b-d}	4.00 ^{a-c}	4.33 ^{a-c}	4.33 ^{a-c}	4.67 ^{ab}	5.00 ^a
11. L-Citronellol	4.33 ^{a-c}	4.67 ^{ab}	4.67 ^{ab}	4.67 ^a	4.67 ^{ab}	4.67 ^a	4.00 ^{b-d}	4.33 ^{a-c}	3.67 ^{b-d}	4.33 ^{a-c}	4.67 ^{ab}	4.67 ^{ab}

Means followed by the same letter(s) within each column do not significantly differ using Duncan's multiple range test at the level of 5%; where, (a) refer to the highest mean values, and (d) refer to the lowest mean values according to Duncan Multiple Range Test.

Table 7. Quality processing of potato tubers as affected by anti-sprouting agent during 2012 and 2013 seasons (after 4 months of storage period).

Treatment	Seed germination index [%]		Seed germination [%]		Seedling length [cm]		Seedling vigor index [cm]		Yield [g] container/242 cm ²	
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
	1 Water (control)	13.36 ^e	12.96 ^d	86.67 ^c	86.0 ^a	4.67 ^c	4.00 ^b	4.03 ^c	3.44 ^c	36.40 ^e
2 Hot water	14.61 ^{de}	13.02 ^d	93.78 ^b	90.44 ^{bc}	5.00 ^c	4.80 ^b	4.71 ^c	4.33 ^c	40.88 ^{de}	37.21 ^d
3 Fennel oil	22.01 ^a	23.01 ^a	97.33 ^{ab}	97.33 ^a	7.33 ^{ab}	7.67 ^a	7.13 ^{ab}	7.47 ^{ab}	56.90 ^b	49.17 ^c
4 Caraway oil	21.94 ^a	22.88 ^a	97.33 ^{ab}	99.00 ^a	8.00 ^{ab}	8.33 ^a	7.79 ^a	8.25 ^a	54.97 ^{bc}	67.75 ^a
5 Basil oil	20.22 ^{ab}	21.82 ^{ab}	94.67 ^b	92.33 ^b	7.00 ^b	7.67 ^a	6.63 ^b	7.07 ^b	64.87 ^a	68.17 ^a
6 Thyme oil	18.81 ^{bc}	20.14 ^b	100.00 ^a	100.0 ^a	8.20 ^a	8.30 ^a	8.20 ^a	8.30 ^a	66.54 ^a	67.75 ^a
7 Sage oil	16.91 ^{cd}	17.76 ^c	100.00 ^a	100.0 ^a	7.83 ^{ab}	7.83 ^a	7.83 ^a	7.83 ^{ab}	47.83 ^{cd}	49.17 ^c

Means followed by the same letter(s) within each column do not significantly differ using Duncan's multiple range test at the level of 5%; where, (a) refer to the highest mean values, and (e) refer to the lowest mean values according to Duncan Multiple Range Test.

Table 8.

Vegetative characters of treated broccoli seeds with different essential oils before cold storage.

the best processing quality of basic constituents of essential oils produced chips, the optimization of reducing sugars and amino acids of their tubers thereby, the prevention of Millard reaction occurrence during frying processes and thus it turn reflects on best color, crispiness and taste.

2.3 Increasing antioxidant content of broccoli sprouts using essential oils during cold storage

2.3.1 Methodology

2.3.1.1 Plant material and germination condition

(*Brassica oleracea* L. var. italica and the variety name is F1 Hybrid Sakura) from Tokita Seeds CO., LTD (Saitama, Japan). The seeds (1000 seeds, nearly 5 g) for each treatment were soaked in a sodium hypochlorite solution at 0.5% v/v for 15 min then were dipped in 50 ml of deionized water for 5½ h with shaking every 30 min and washed with deionized and sterilized water. On 15th of September, broadcast the seeds were done over absorbent medical cotton in sprouting plastic containers (220 × 110 mm). The emulsions of various natural essential oil at the concentration of 0.05% were emulsified in tween 80 (1.5 ml/l) in the cotton media and the containers were getting closed immediately. The containers were maintained at 25 ± 2°C with and 16 h light/8 h darkness, 80–90% relative humidity and 7.4 lmol/m²/s light intensity to give the best germination conditions. All sprouts in containers were cut above their root mats after 3 days from sowing. The sprouts were weighed for 20 g for each placed container and stored at 4°C in the dark to simulate a domestic refrigerator for 15 days. The sprouts of best treatment with control were stored only.

2.3.1.2 Application and extraction of essential oils

The essential oils of fennel seeds (*Foeniculum vulgare*), caraway seeds (*Carum carvi*), thyme herbs (*Thymus vulgaris*), basil herbs (*Ocimum basilicum*) and sage leaves (*Salvia officinalis*) (200 g from each one) were used for oil extraction by

Treatment	Total phenolic acid (mg/100 g F.W.)		Total flavonoids (mg/100 g F.W.)		Anthocyanin (mg/100 g F.W.)		Ascorbic acid (mg/100 g F.W.)		DPPH (Mmol TE/g F.W.)	
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
1 Water (control)	83.33 ^d	84.11 ^e	91.99 ^d	95.18 ^e	7.13 ^d	7.70 ^d	70.58 ^e	81.23 ^d	23.66 ^a	24.66 ^a
2 Hot water	88.71 ^c	88.56 ^c	100.95 ^c	101.03 ^d	8.62 ^c	8.77 ^c	86.81 ^c	86.81 ^c	23.54 ^b	23.66 ^a
3 Fennel oil	88.46 ^c	88.90 ^c	107.66 ^b	107.72 ^c	8.86 ^c	8.87 ^{bc}	87.66 ^c	88.00 ^c	21.98 ^d	21.98 ^c
4 Caraway oil	87.90 ^c	88.13 ^{cd}	104.66 ^b	104.73 ^c	9.84 ^{bc}	9.84 ^{bc}	77.33 ^d	85.80 ^c	21.96 ^{de}	21.96 ^c
5 Basil oil	122.06 ^b	122.29 ^b	113.00 ^a	113.00 ^b	11.71 ^a	12.05 ^a	94.67 ^b	94.67 ^b	21.94 ^{de}	21.94 ^c
6 Thyme oil	131.66 ^a	131.60 ^a	115.66 ^a	116.24 ^a	12.09 ^a	12.14 ^a	102.33 ^a	103.33 ^a	21.86 ^e	20.03 ^d
7 Sage oil	87.9 ^c	84.74 ^{de}	104.33 ^{bc}	104.59 ^c	10.38 ^b	10.38 ^b	82.33 ^{cd}	86.69 ^c	22.79 ^c	22.79 ^{bc}

Means followed by the same letter(s) within each column do not significantly differ using Duncan's multiple range test at the level of 5%; where, (a) refer to the highest mean values, and (e) refer to the lowest mean values according to Duncan Multiple Range Test.

Table 9.
Phytochemical screening by GLC for 3-days-old broccoli sprouts produced from treated seeds with essential oils before cold storage.

hydro-distillation for 2–3 h. After extraction, essential oils were analyzed by Gas Liquid Chromatography (GLC) to separate and identify their basic constituents.

2.3.2 Results and discussion

2.3.2.1 Vegetative characters of broccoli sprout

All essential oil treatments rich in antioxidant stimulate the germination of broccoli seeds. All essential oils treatments significantly increased germination, germination index, seedling length, seedling vigor index and container yield compared with the control (tap water) during the two seasons (**Table 8**). The essential oils of fennel, caraway and thyme increased the seed germination index by 171.43, 170.29 and 148.02%, respectively, compared to the control 100%. The increases of seed germination % over the control reached to 12.73, 13.74 and 15.82% for the effective treatments, respectively. The essential oils of thyme, caraway and fennel had significant increases in seedling vigor and yield container over the control to 50.25, 73.82 and 90.22%, respectively.

The allelochemical effects of essential oils for induce stimulatory or inhibitory of seed germination and other physiological process varied depending on the dose, tested species, concentration and basic components. Under our study, the lower doses of essential oils had a stimulatory effect [25]. The obtained results reveal that the applications of essential oils at a low level improve seed germination of broccoli. However, application of thyme oil reaches 100% of sprouts after seed germination (**Table 8**). Impact of essential oils on seed germination of other plant species was reported as 24 out of 47 tested terpenoids enhanced the seed germination of *Lactuca sativa* [26]. Also, the positive impact of thyme essential oil on broccoli seeds could be because of its active ingredients.

2.3.2.2 Phytochemical characters

All treatments significantly surpassed over the control in Broccoli sprout bio-constituents, i.e., total phenolic acid, total flavonoids, anthocyanin and ascorbic acid, while the control treatment gave the highest DPPH radical scavenging capacity (**Table 9**). Application of thyme oil treatment produced significant increases of total phenol, total flavonoids, anthocyanin and ascorbic acid content. Moreover, thyme and basil essential oils decreased significantly the DPPH free radical scavenging capacity. Accordingly, it has been chosen to study the storage behavior characters, in addition to control treatment. The majority of the antioxidant activity attributes to phenolic compounds, flavonoids and ascorbic acid in essential oils [27]. Moreover, the effect of antioxidant on DPPH free radical was due to the presence of hydroxyl groups in their chemical structure. In this respect, [28] found that the oregano essential oil inhibited hydro-peroxide formation and that the CHO fraction showed the highest antioxidants activity.

The thyme oil showed significant lowest radical scavenging capacity compared to the control and other treatments (**Table 9**). All other antioxidants/essential oils showed high and almost the same antioxidant capacity effect. It was known that the free radical scavenging DPPH intensity of some compounds can be influenced by their different kinetic behavior [29]. For slow reacting compounds the influence was attributed to the complex reacting mechanism. In our study, probably, the constituents from thyme essential oil involved one or more secondary reactions, which result the slower reduction of DPPH solutions [29].

2.3.2.3 Antioxidant activity during cold storage

2.3.2.3.1 Total phenolic compounds and DPPH radical scavenging capacity

Figure 2 illustrate that there was a gradual increase in the total phenolic acid content, and reaching a maximum value at day 5 and 10 (132.67 and 135.04 mg GAE/100 g F.W.) compared to the initial time. This concentration decreased in to 129.03 mg at day 15 due to thyme oil application (**Figure 2**). Keeping in view that the control treatment decreased to 73.84 GAE/100 g FW at day 5. On the 15th day, the old-sprout from storage, the control was reduced by 28.57% compared to thyme oil (1.98%). The control treatment of antioxidant capacity increased significantly until day 10 (29.43 mg/100 g F.W.), and finally decrease (28.46% mg 100/g F.W.) at day 15 increased from initial period (20.28%). While, application of thyme oil the change was not clear at the end of storage (1.98%) (**Figure 3**). During cold storage (**Figure 3**), the control was reduced DPPH by 28.57% compared to thyme oil at 15 day old-sprout (1.98%). Nath et al. [30] observed a constant decrease in the antioxidant capacity for 144 h of storage of broccoli inflorescences. This behavior in DPPH may be due to the steady changes in plant metabolism during storage period as a result of oxidative stress, which may include structural and chemical changes in synthesis or antioxidant content [31].

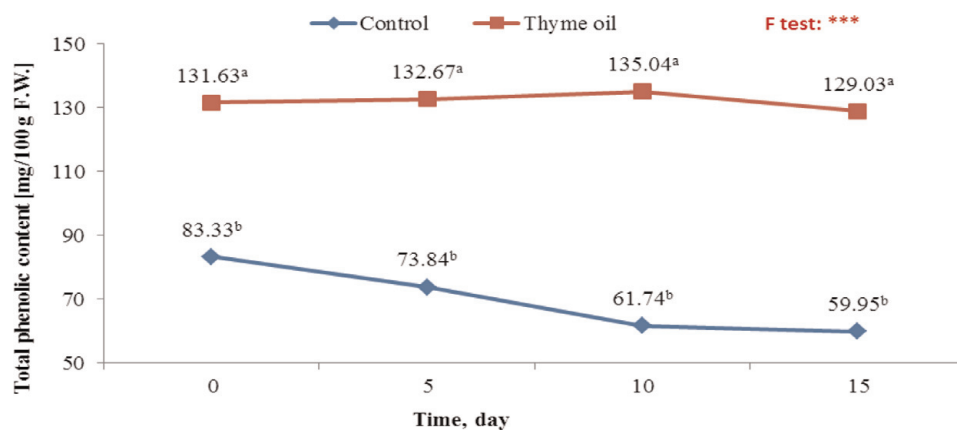


Figure 2. Total phenolic content as affected by thyme oil compared to control treatment at different storage period.

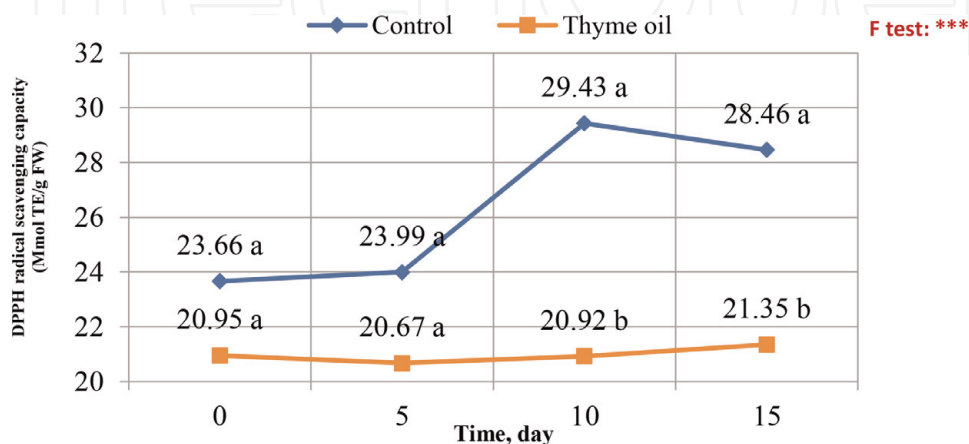


Figure 3. DPPH radical scavenging capacity as affected by thyme oil compared to control treatment at different storage period.

2.3.2.3.2 Total flavonoids

Total flavonoids (**Figure 4**) were found in a higher concentration in 3-day-old sprouts of thyme treatment, with values of 115.95 mg/100 g F.W., after 5 and

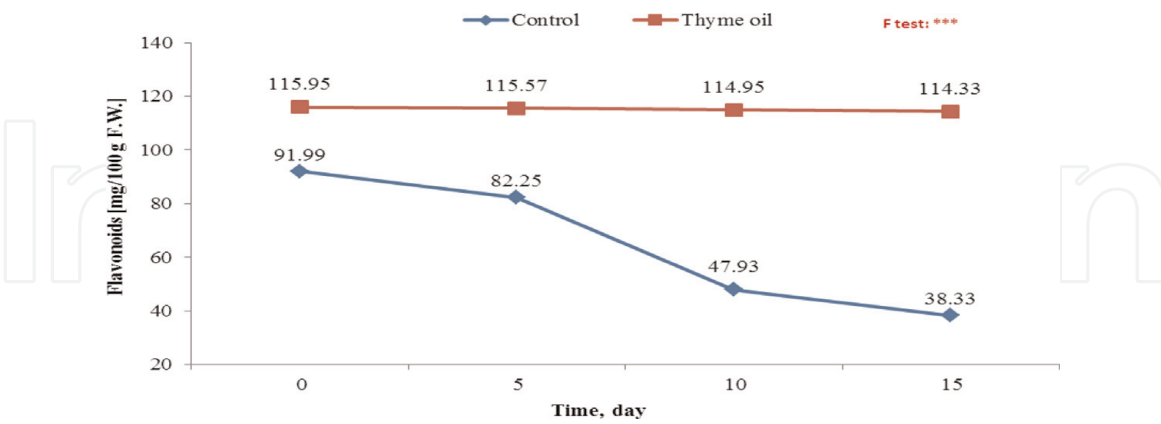


Figure 4. Total flavonoids content as affected by thyme oil compared to control treatment at different storage period.

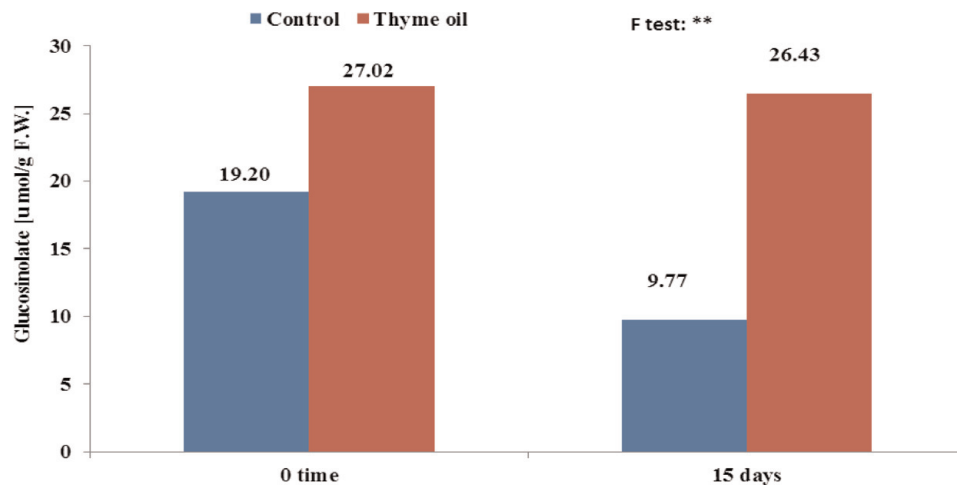


Figure 5. Total glucosinolates content as affected by thyme oil compared to control treatment at 0 time and 15 DAS.

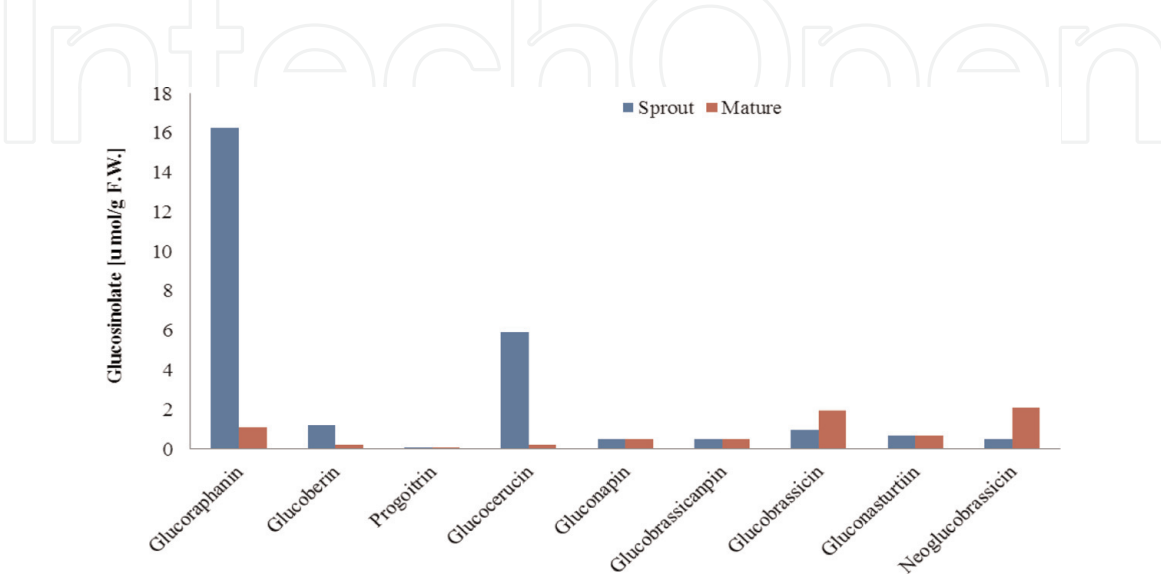


Figure 6. Total and individual aliphatic, aromatic/indole glucosinolates levels in broccoli in 3-days-old sprout and mature at harvest.

10 days of storage slight decrease to 0.021 and 0.086%, respectively, when compared with the initial value, and finally reduced by 1.39%. The high loss of flavonoids reached to 10.59 and 47.89%, after 5 and 10 days, respectively, and at 15 days the loss increased to 58.33% for control treatment (average two seasons).

2.3.2.3.3 *Glucosinolates content*

Storage time had significant differences in glucosinolates content of the samples analyzed. **Figure 5** illustrate that the thyme oil increased significantly glucosinolates content in 3-day-old sprouts, compared to control treatments. Moreover, thyme oil had a high value of total glucosinolates (27.02 $\mu\text{g/g}$ F.W.) and slightly decreased up to 26.43 $\mu\text{g/g}$ F.W. on day 15. At the end of storage, the decreasing changes percent was about 2.18%. In the control treatment, the highest decrease in total glucosinolates content was observed, where reached about 49.12% at the end of storage.

2.3.2.3.4 *Glucosinolates content of mature head versus sprout broccoli*

In sprout, the total glucosinolates level (27.02 $\mu\text{g/g}$ F.W.) is higher than in florets or heads (7.37) (**Figure 6**). Glucoraphanin is the powerful of antioxidant and the most abundant aliphatic glucosinolates present in sprout. The glucoraphanin reached the highest 16.24 followed by glucoerucin 5.9 and glucoiberm 1.2 $\mu\text{g/g}$ F.W. On the other hand, the florets/heads contain the highest level of aromatic/indolyglucosinolates, neoglucobrassicin (2.11) followed by glucobrassicin (1.67). Our results are in agreement with those obtained by [32].


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