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Antioxidant enzyme activities and lipid peroxidation as biomarker for potato tuber stored by two essential oils from Caraway and Clove and its main component carvone and eugenol

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ABSTRACT

Objective: To evaluate two essential oils from Caraway and Clove and its main component carvone and eugenol as sprout inhibitors on germination of potato tubers. **Methods:** The enzymes activities: catalase, glutathione-S-transferase, peroxidase, polyphenol oxidase and superoxide dismutase, in addition to lipid peroxidation level were tested in potato tubers stored for 3, 6 and 9 weeks. Essential oils; Caraway, Clove, carvone and eugenol with three concentration (100, 200 and 300 ppm) were used to control germination process of potato tubers. **Results:** The results of enzyme activities varied depending on the function of enzymes involved. As general trend the activities of the enzymes recorded are significantly found on the range of enzyme control or less, which prevent of potato tuber from germination. Glutathione-S-transferase activity was significantly increased after treatment with essential oils and the activity of enzyme reached (23.3±5.15) (100 ppm) for Caraway, (18.8±0.00) (100 ppm) for carvone, (10.4±0.00) (100 ppm) for colve, and (14.1±0.0) (100 ppm) for eugenol respectively compared to control (7.86±3.26). **Conclusions:** Polyphenol oxidase and peroxidase activity increased in its activity and recovered to the level of control after treatment with essential oils which maintain potato tuber for 9 weeks. The pure essential oils especially carvone have more potent as suppressor of potato tuber germination.

1. Introduction

Potato tuber (*Solanum tuberosum* L.) is the fourth most important food crop in the world mainly due to its starch content and high quality protein, substantial amounts of essential vitamins, minerals, and very low fat content[1]. Potato suffers from undesirable sprouting during storage for the fresh market, prior to industrial processing, this serious problem occurs when dormancy is broken and sprouting is activated. In particular, dormancy release was accompanied by a transient but remarkable increase in H_2O_2 content. Catalase inhibitor (thiourea) or of exogenous H_2O_2 application on tuber sprouting behaviour was assessed. Both treatments resulted in a reduction of the dormancy period and in rapid and synchronised sprouting of the treated tubers when compared to the control as well as in increased sprout number per tuber[2]. Sprouting represents a loss of material to the tubers and causes an accelerated loss of water through the permeable surface of the sprout. Due to increasing concern for consumer health and safety, there is considerable interest in finding effective potato sprouting suppressants that have a negligible environmental impact effect. Potato genotypes (Tigoni, Asante and Dutch Robyjn) were evaluated for quality under three light intensities, 612.2 kW (diffused), 1376 kW (direct) and 8 kW (dark) for 12 weeks. Tuber stored in dark conditions had a lower weight loss than tubers stored in direct light conditions. Dutch Robyjn lost the lowest weight (4.49%) while Asante lost the highest (13.90%) mean weight. Tigoni in the dark had the highest number (9.25) of sprouts. Tubers in the dark developed long (46.25 mm) etiolated sprouts as opposed to the short (10.50 mm) firm sprouts observed in tubers subjected to the diffused light. High sprout vigor score (2.42) was observed in Asante while Dutch Robyjn had the lowest (1.00) score[3]. It's very important to use natural products compounds such as essential oils as well as the pure compound derived from essential oils or alcoholic extracts [4-7]. On the same time it's not advisable to use pesticides because of its bad impact to the health of consumer [8, 9].

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Oils of some herbs and spices, essential oils, have been shown to reduce sprouting in potatoes and can be applied to certified organic crops. These compounds are volatile plant derivatives including spearmint oil, peppermint oil, and clove oil. Lipid oxidation is a major cause for the deterioration of fat-containing food [10-13]. It initiates other undesirable changes in food, affecting its nutritional quality, color, flavor and texture especially when it exposed to light, ionizing radiation, roasting, metal ions or metaloprotein catalysts [14-18]. Auto-oxidation of polyunsaturated lipids involves a free radical chain reaction, generally initiated by exposure of the lipids to, Lead toxicity, nematode infection, organisms and micro-organisms [19-23]. Therefore, the inhibition of free radical oxidation by antioxidants such as α-tocopherol is of great practical importance in preserving polyunsaturated lipids from deterioration [24]. Carvone was obtained from the essential oil of caraway seed (*Cuntm carvi* L.) and showed antisprouting activity of potato tuber [25]. Caraway is one of several ancient cultivated known for its characteristic aromatic small seeds. The essential oil of caraway seed consists mainly of two monoterpenes: S(+)-carvone (50-60%) and S(+)-limonene (35-45%). Carvone has been shown to be the biologically active component to prevent sprouting, whereas limonene did not exhibit sprout suppression. Until now sprout suppression effects of carvone were tested in small-scale experiments with different application rates for short storage periods (max. 3 to 4 months). Clove oil is also being investigated as a potential sprout suppressant and disease control agent found that treatments with caraway with 50 g, 100 g and 150 g had anti- sprouting activity on potato tuber at 8 ℃ , until day 105 [26]. Respiration of tubers during storage and breakdown of dormancy during storage result in sprouting and loss of nutritive value of tubers [27]. Breakdown of dormancy also leads to physiological aging of tubers resulting in weight and quality losses for fresh market tubers and also leads to yield losses if such tubers are used as seed tubers for planting [28]. Therefore, carnation, caraway, thyme oils and the chemical fungicide Ridomil MZ 72 at various concentrations on mycelial growth of *A. solani* was tested [29]. Essential oils and methanol extract of *O. stamineus* and the derived fractions of hexane, chloroform, and ethyl acetate were tested for anti-fungal activity as a mycelial growth inhibitor against the tested phytopathogenic fungi such as *Botrytis cinerea, Rhizoctonia solani, Fusarium solani, Colletotricum capsici* and *Phytophthora capsici*, in the range of 49.3-70.3% and minimum inhibitory concentration ranging from 500 to 1000 g/ml [30]. Use of commercial S-(+)-Carvone may not be practical due to its cost and effects on the taste of stored tubers. In addition, usage of S-(+)-Carvone in store rooms requires special arrangements [31]. Use of S-(+)-Carvone containing natural sources rather than commercial S-(+)-Carvone could be an alternative way to prevent sprouting during the storage. While these natural products are likely to be less potent than the commercially available compounds, they are also cheaper and more readily accessible. The natural sources of these compounds slowly release active compounds during the storage and they may also contain a wider range of useful bioactive compounds [25]. The aim of the present investigation is to study the capability of two essential oils and its main components (Caraway, Carvone, Clove and Eugenol) for inhibiting sprouting of potato tuber (*Solanum tuberosum* L.) variety Diamont during storage and extending its shelf life instead of using pesticides chemical which have its bad impact factor to human[32-33]. Therefore one of the main biomarker for validity of potato tuber during storage is studying antioxidant enzyme activities (Peroxidase, Polyphenol oxidases Glutathione-S-transferase, Superoxide dismutase and Catalase) as well as lipid peroxidation of potato tubers during storage.

2. Materials and methods

Potato tubers (*Solanum tuberosum*) cv. Diamont of a uniform size (60-65 mm) was obtained at harvest time from the farm of faculty of Agriculture Cairo University. They were washed and allowed to dry at room temperature then divided to groups according to the different treatments.

2.1. Experimental design

Stored boxes (13) with small iterance door (32.5X32.5X39.5cm) were manufactured, 12 for samples treated by essential oils, in addition to control.

2.2. Essential oils treatments

Twenty potato tubers were put into air tight stored boxes (32.5×32.5×39.5 cm), 20 per cage and were kept in dark conditions. The essential oil to be tested (caraway and carvone) was placed in a beaker, inside boxes quantities of 10.4 μl, 20.8 μl and 31.25 μl corresponding to 100 ppm, 200 ppm and 300 ppm of vapor, respectively. Clove and Eugenol were placed in a beaker, inside the cage in quantities of 9.4 μl, 18.9 μl and 28.3 μl corresponding to 100 ppm, 200 ppm and 300 ppm of vapor, respectively. Experiments lasted for 9 weeks. Throughout the experimental period, the temperature in the dark boxes, varied from 27 ℃ to 36.5 ℃.

2.3. Biochemical analysis

2.3.1. Preparation of enzyme extracts

Ground samples, (10.0 g each) were homogenized in 10 ml of 50 mM phosphate buffer pH 7.0, 1% PVP (Sigma), 1 mM ascorbate (Sigma) at 4 ℃. After centrifugation at 15 000×g for 15 min the supernatant was collected according toPukacka S[34].

2.3.2. Determination of soluble proteins

Soluble proteins were measured by the Bio-Rad micro assay modification of the[35] procedure using crystalline bovine serum albumin as a standard.

2.3.3. Peroxidase activity (POD; EC 1.11.1.7)

Peroxidase activity was assayed by monitoring the increase in absorbance at 430 nm due to the oxidation of pyrogallol (2.6 mM^{-1} cm^{-1}), as described by[36]. The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7.0), 20 mM pyrogallol, 5 m H_2O_2 and 20 μL of enzyme extract. POD activity was expressed as U/100g tuber f.w. One unit of enzyme was the amount necessary to decompose 1 μmol of substrate per minute at 25 ℃.

2.3.4. Polyphenol oxidases (PPOs; EC 1.14.18.1)

Polyphenol oxidases activity was determined by spectrophotometer at 20 ℃ in triplicate. The reaction mixture consisted f 0.1 M sodium phosphate buffer pH 6.8, 20 mM, 4-dihydroxy L-phenylalanine (L-DOPA, Merck) and 50 μl of the sample. The increase in absorbance was measured in a 1 cm light path cuvette at 475 nm, in a final volume of 1 ml. PPO activity was calculated considering molar extinction coefficient for dopaquinone of 3600 M^{-1} cm^{-1} [37]. Polyphenol oxidases activity was expressed as (U/g tuber f.w.).

2.3.5. Glutathione-S-transferase (GST; EC 2.5.1.18)

Glutathione-S-transferase activity was measured according to the method of [38] by following the changes in the absorbance at 340 nm in a mixture containing 0.17 mM sodium phosphate buffer, pH 6.5, 1 mM GSH, 1 mM 1-chloro-2, 4 dinitrobenzene (CDNB) in ethanol and enzyme extract. EU = the amount of enzyme that catalyses the formation of 1 μmol of S-2, 4- dinitrophenylglutathione min^{-1}. Glutathione-S-transferase was expressed as (U/100 g tuber f.w.).

2.3.6. Superoxide dismutase (SOD; EC 1.15.1.1)

Superoxide dismutase activity was measured by the photochemical method as described [39]. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the rate of nitro blue tetrazolium (NBT) reduction at 560 nm in the presence of riboflavin and light. The reaction mixture contained 45 mM potassium phosphate buffer, pH 7.0, containing 0.1 mM EDTA and 13 mM methionine, 0.17 mM NBT in ethanol, 0.007 mM riboflavin and enzyme aliquot. Blanks were kept in the dark and the others were illuminated for 15 min. One unit of SOD is the amount of extract that gives 50% inhibition to the rate of NBT reduction. Superoxide dismutase was expressed as (U/100 g tuber f.w.).

2.3.7. Catalase (CAT; EC 1.11.1.6)

Catalase activity was determined as H_2O_2 consumption measured as the decrease in absorbance at 240 nm according to the method of [40]. The assay contained 50 mM KH_2PO_4/ K_2HPO_4 (pH 7.0), 10 mM H_2O_2 in phosphate buffer. Extinction coefficient of 39.4 $mM^{-1}cm^{-1}$ was used to calculate activity. Enzyme activity was ex pressed in μM H_2O_2 min^{-1}. Catalase activity was expressed as (U/100g tuber f.w.)

2.3.8. Lipid peroxidation (MDA)

The lipid peroxidation was measured in terms of malonyldialdehyde (MDA) content by thiobarbituric acid (TBA) reaction. The level of lipid peroxidation is expressed as mmol of MDA formed using an extinction coefficient of 155 mM^{-1}/cm^{-1}[41]. Malonaldialdehyde was expressed as (nmol/g tuber f.w.).

2.4. Statistical analysis

All analysis were performed in triplicate (n=3). Statistical analysis was done using SPSS (version 10) program. Mean and standard error were descriptive measures of quantitative data using the analysis of variance test (ANOVA) for independent samples. P-values <0.05 were considered significant.

3. Results

At harvest and for a definite period thereafter, potato tubers will not sprout and are considered as dormant. Dormancy is defined as (the physiological state of the tuber in which autonomous sprout growth will not occur, even when placed under ideal conditions for sprouting). The length of this dormant period is dependent on the genotype as well as on both pre- and post-harvest conditions. Tuber dormancy is desirable when potatoes must be stored (industrial processing); however, excessively long dormancy poses a problem in sprouting of seed tubers (early crop installation). Accelerated or delayed sprouting of the harvested tubers may be favored depending on the intended purpose. Controlling the length of dormancy period could therefore be of considerable economic importance. Unfortunately, the underlying mechanisms regulating the maintenance and breakage of tuber dormancy are still poorly understood. Therefore essential oils Caraway and Clove and its main component Carvone and Eugenol were used as trial to suppress and control of potato sprouting processing . Since the ROS removal rate is controlled by antioxidant enzymes activity and by a variety of low molecular weight antioxidants, it is of interest to determine the global change of antioxidant activity present in potato tubers during different time of storing. Enzyme activities include Peroxidase, Polyphenol oxidases, Glutathione-S-transferase, Superoxide dismutase Catalase and MDA of lipid profiles were investigated.

3.1. Peroxidase activity

Data presented in Table 1 revealed that non-treated potato tubers were decayed progressively during the different storage periods and the decay percent reached its maximum value at the end of experiment. The data in table 1 proved that Caraway (*Carum carvi*) seed essential oils and its main components carvone inhibit sprouting for nine weeks by keeping the activity of the antioxidant enzyme in the range of enzyme activity in control. The purified and the main components of Caraway seed essential oils represented by carvone showed the most potent inhibition of antioxidant activity and inhibit sprouting comparing to the control, Caraway (200 ppm) with peroxidase activity (10.12±0.16) compared Carvone (100 ppm) with peroxidase activity 7.70±0.13. On the same time Clove (100 ppm) as well as Eugenol (100 ppm) with peroxidase activity 23.89±1.86, 38.46 ±1.40 respectively after nine week were observed.

3.2. Polyphenol oxidases activity

Treatments with two essential oils Caraway and Clove and its main components Carvone and Eugenol increased the

Table 1
Influence of different treatments for sprouts suppression effects during storage period (weeks) on peroxidase (U/100g tuber f.w.) activity.

Treatments (ppm)		Storage period (Weeks)			
		0	3	6	9
Control		19.03±0.39	–	–	–
Caraway	100	–	33.3±0.30^{a}	42.4±0.30^{a}	18.8±0.28^{e}
	200	–	17.0±0.23^{e}	27.0±0.24^{e}	10.1±0.16^{f}
	300	–	17.4±0.26^{e}	29.7±0.27^{d}	18.5±0.24^{e}
Carvone	100	–	13.4±0.17^{g}	24.7±0.31^{f}	7.70±0.13^{g}
	200	–	12.2±0.21^{h}	29.6±0.21^{d}	10.5±0.26^{f}
	300	–	10.9±0.13^{f}	23.1±0.18^{f}	33.3±0.28^{c}
Clove	100	–	15.9±0.25^{f}	41.8±0.27^{a}	23.9±0.26^{d}
	200	–	21.5±0.21^{d}	23.9±0.25^{ef}	39.7±0.31^{a}
	300	–	26.9±0.27^{b}	33.2±0.23^{b}	39.3±0.32^{a}
Eugenol	100	–	25.1±0.16^{c}	30.8±0.19^{c}	38.5±0.28^{a}
	200	–	32.8±0.21^{a}	31.2±0.32^{c}	34.0±0.20^{c}
	300	–	26.90±0.15^{b}	22.67±0.20^{f}	36.17±0.27^{ab}
LSD at 0.05		–	0.28	0.35	0.38

Each value is expressed as mean±SE. Data with different superscript letters were significantly different ($P \leq 0.05$).

Table 2
Influence of different treatments for sprouts suppression effects during storage period (weeks) on Polyphenol oxidases (U/g tuber f.w.) activity.

Treatments (ppm)		Storage period (Weeks)			
		0	3	6	9
Control		39.6±0.36	–	–	–
Caraway	100	–	75.7±0.35^{c}	38.7±0.22^{g}	36.0±0.21^{g}
	200	–	29.6±0.34^{j}	27.2±0.23^{i}	60.7±0.34^{c}
	300	–	31.1±0.29^{i}	54.6±0.39^{b}	32.7±0.26^{h}
Carvone	100	–	59.1±0.30^{e}	45.3±0.28^{e}	40.0±0.25^{f}
	200	–	11.0±0.19^{k}	80.1±0.39^{a}	44.0±0.19^{d}
	300	–	75.9±0.34^{c}	46.7±0.26^{d}	21.0±0.13^{j}
Clove	100	–	73.3±0.38^{d}	49.3±0.25^{c}	27.3±0.20^{j}
	200	–	77.1±0.35^{b}	41.1±0.29^{f}	30.7±0.16^{i}
	300	–	44.1±0.24^{f}	37.8±0.18^{h}	76.0±0.37^{b}
Eugenol	100	–	34.7±0.24^{h}	16.0±0.13^{j}	42.0±0.29^{e}
	200	–	80.1±0.40^{a}	12.1±0.15^{k}	26.7±0.19^{ij}
	300	–	36.5±0.21^{g}	10.0±0.11^{l}	80.0±0.36^{a}
LSD at 0.05		–	0.45	0.42	0.39

Each value is expressed as mean ± SE. Data with different superscript were significantly different ($P \leq 0.05$).

Table 3
Influence of different treatments for sprouts suppression effects during storage period (weeks) on Glutathione-S-transferase (U/100g tuer f.w) activity.

Treatments (ppm)		Storage period (Weeks)			
		0	3	6	9
Control		7.86±0.26	–	–	–
Caraway	100	–	10.7±0.28^{d}	17.7±0.18^{e}	23.3±0.15^{d}
	200	–	9.40±0.13^{e}	5.90±0.14^{i}	12.5±0.20^{h}
	300	–	20.3±0.26^{ab}	16.3±0.17^{f}	28.1±0.28^{c}
Carvone	100	–	7.26±0.17^{g}	7.26±0.11^{g}	18.8±0.15^{f}
	200	–	6.87±0.18^{h}	6.56±0.05^{h}	10.4±0.08^{i}
	300	–	6.81±0.22^{h}	5.52±0.03^{d}	5.10±0.05^{k}
Clove	100	–	15.1±0.25^{c}	24.7±0.21^{c}	10.4±0.17^{i}
	200	–	8.3±0.08^{fg}	19.34±0.20^{d}	42.0±0.23^{b}
	300	–	20.8±0.16^{a}	47.2±0.25^{a}	63.0±0.33^{a}
Eugenol	100	–	7.03±0.13^{g}	25.0±0.27^{c}	14.1±0.19^{g}
	200	–	16.5±0.11^{b}	6.56±0.17^{h}	6.46±0.13^{j}
	300	–	8.78±0.09^{f}	26.6±0.19^{b}	20.3±0.21^{e}
LSD at 0.05		–	0.30	0.28	0.35

Each value is expressed as mean ± SE. Data with different superscript letters were significantly different ($P \leq 0.05$).

Table 4
Influence of different treatments for sprouts suppression effects during storage period (weeks) on Superoxide dismutase (U/100g tuber f.w.) activity

Treatments (ppm)		Storage period(Weeks)			
		0	3	6	9
Control		920±1.40	–	–	–
Caraway	100	–	862.5±1.03^{d}	770.8±1.29^{f}	525.0±1.46^{e}
	200	–	716.2±1.18^{g}	787.5±1.25^{e}	508.3±1.70^{g}
	300	–	658.3±1.21^{j}	775.0±1.79^{f}	487.5±1.15^{i}
Carvone	100	–	920.8±1.39^{a}	745.8±1.68^{h}	533.3±1.57^{d}
	200	–	908.3±1.57^{b}	758.3±1.36^{g}	516.7±1.71^{f}
	300	–	637.5±1.91^{k}	837.5±1.25^{d}	450.0±0.83^{k}
Clove	100	–	875.0±1.14^{c}	875.0±1.01^{b}	554.2±1.68^{b}
	200	–	841.7±2.12^{e}	883.3±1.21^{a}	500.0±1.14^{h}
	300	–	708.3±1.40^{h}	858.3±1.88^{c}	466.7±1.74^{j}
Eugenol	100	–	866.7±1.63^{d}	591.7±0.89^{i}	466.7±1.14^{j}
	200	–	866.7±1.93^{d}	579.2±1.68^{j}	545.8±1.89^{c}
	300	–	825.0±1.56^{f}	529.2±1.89^{k}	640.7±1.37^{a}
LSD at 0.05		–	2.35	2.18	1.85

Each value is expressed as mean ± SE. Data with different superscript letters were significantly different ($P \leq 0.05$)

Table 5
Influence of different treatments for sprouts suppression effects during storage period (weeks) on Catalase (U/100g tuber f.w.) activity.

Treatments (ppm)		Storage period (Weeks)			
		0	3	6	9
Control		191.4±1.80			
Caraway	100	–	119.8±1.17^{g}	28.40±0.29^{l}	50.0±0.28^{g}
	200	–	170.4±1.07^{c}	55.6±0.31^{g}	35.6±0.24^{j}
	300	–	112.3±0.67^{h}	70.4±0.37^{e}	44.4±0.22^{h}
Carvone	100	–	214.8±1.10^{a}	107.4±0.43^{c}	38.9±0.36^{i}
	200	–	101.2±1.32^{i}	46.9±0.28^{i}	38.8±0.34^{i}
	300	–	33.3±0.31^{j}	59.3±0.42^{f}	21.5±0.18^{d}
Clove	100	–	150.6±0.55^{d}	129.6±0.45^{a}	55.6±0.32^{f}
	200	–	135.8±0.40^{f}	111.1±0.40^{b}	158.1±0.39^{c}
	300	–	200.0±0.52^{b}	100.0±0.41^{d}	244.4±0.42^{b}
Eugenol	100	–	142.0±0.57^{e}	54.3±0.32^{h}	61.6±0.35^{e}
	200	–	216.7±0.60^{a}	44.1±0.20^{j}	103.7±0.37^{d}
	300	–	197.5±0.80^{bc}	38.9±0.36^{k}	253.9±0.46^{a}
LSD at 0.05		–	1.40	0.52	0.48

Each value is expressed as mean ± SE. Data with different superscript letters were significantly different ($P \leq 0.05$).

Table 6
Influence of different treatments for sprouts suppression effects during storage period (weeks) on MDA (nmol.g^{-1} tuber f.w.).

Treatments (ppm)		Storage period (Weeks)			
		0	3	6	9
Control		23.5±0.33	–	–	–
Caraway	100	–	14.0±0.17^{fg}	55.2±0.29^{b}	20.0±0.19^{g}
	200	–	34.4±0.22^{e}	27.4±0.28^{h}	70.0±0.22^{b}
	300	–	14.4±0.25^{f}	20.0±0.23^{k}	15.0±0.27^{i}
Carvone	100	–	8.15±0.13^{h}	40.1±0.34^{d}	25.1±0.17^{f}
	200	–	8.04±0.14^{h}	60.0±0.35^{a}	18.1±0.23^{d}
	300	–	9.80±0.20^{g}	30.0±0.22^{f}	65.8±0.26^{c}
Clove	100	–	42.0±0.28^{d}	29.1±0.26^{g}	61.0±0.20^{d}
	200	–	34.2±0.21^{e}	24.6±0.21^{i}	77.5±0.32^{a}
	300	–	50.8±0.24^{c}	21.2±0.28^{j}	40.6±0.28^{e}
Eugenol	100	–	97.0±0.25^{a}	41.1±0.37^{c}	25.1±0.17^{f}
	200	–	57.5±0.31^{b}	19.3±0.17^{l}	18.1±0.21^{h}
	300	–	34.4±0.24^{e}	33.8±0.27^{e}	65.8±0.24^{c}
LSD at 0.05		–	0.35	0.38	0.36

Each value is expressed as mean ± SE. Data with different superscript letters were significantly different ($P \leq 0.05$).

activity of Polyphenol oxidases and maintain the activity of the enzymes to the normal control (39.6±0.36), therefore the treatment help potato to be stored for nine weeks. The activity of polyphenol oxidase after treatment with Caraway (100 ppm) was (75.70±0.35) after three weeks and the activity reached (36.00±0.21) after nine weeks. The same trend could be seen in the remaining treatment of the essential oils and its main components. Clove essential oils represents the major inhibitor one with concentration of 100 ppm with enzyme of polyphenol oxidases and reached (27.30±0.20) after nine weeks of potato storage (Table 2).

3.3. Glutathione-S-transferase

Data in Table 3 showed that treatment of potato tuber with vapor of essential oils as well as with main components of the essntial oils increseaed the activity of Glutathione-S-transferase with different levels according to the concentration used .From the results above we could conclude that the concentration of 100 ppm as pure and crude essential oils reached the maximum activity of Glutathione-S-transferase with 100 ppm concentration (Caraway (23.30±0.15), Carvone (18.80±0.15), Clove (24.70± 0.21), Eugenol (25.00±0.27). The results proved that the pure essential oils carvone have more potency as inhibitor of potato tuber germination compared to the crude Caraway.

3.4. Superoxide dismutase enzyme

It is very important to note that the activity of Superoxide dismutase in potato tubers after treatment with essential oils vapor was small decreased in all treatment even potato maintained for nine weeks. These results proved that activity of Superoxide dismutase enzyme is not a major factor to inhibit potato sprouting. The activity of superoxide dismutase showed a significant decrease compared to the control potato tubers. Superoxide dismutase reached its minimum after nine weeks of storage (525.0±1.46) (Caraway, 100 ppm), (533.3±1.57) (Carvone, 100 ppm), (554.2±1.68) (Clove, 100 ppm), (466.7±1.14), (Eugenol, 100 ppm). From the results Eugenol with concentration of 100 ppm showed a significant decrease in superoxide dismutase enzymes which subsequently inhibit potato tuber germination (Table 4).

3.5. Catalase

Data in Table 5 showed that treatments with two essential oils Caraway and Clove and its main components Carvone and Eugenol increased the activity of Catalase and maintain the activity of the enzymes to the normal control (191.4± 1.80), therefore the treatment help potato to be stored for nine weeks. The activity of catalase after treatment with Caraway (100 ppm) was (119.8±1.17) and the activity reached (50.0±0.28) after nine weeks. The same trend could be seen in the remaining treatment of the essential oils and its main components.

3.6. MDA

Data in Table 6 showed that MDA which represents the oxidation process was decreased after three weeks and reached to (14.0±0.17) Caraway with 100 ppm and increased after 6 weeks and reached the normal amount after nine weeks (20.00±0.19) Caraway with 100 ppm.

4. Discussion

4.1. Peroxidase activity

Our results in Table 1 in agreement with results showed that increased volatility of essential oils enhanced phytotoxicity to sprouts and plant growth inhibiting activities and to possess antiviral and antifungal activity exhibited fungicidal activity for protecting the potato tubers against sprouting without altering taste or quality of the treated commodity [26, 42, 43]. Collectively, these results suggest that a gradual build-up of FRs leads to peroxidative damage of membrane lipids during aging of potato seed-tubers [44]. cited that when Potato tubers (cv. Bintje) (*Solanum tuberosum* L.) were stored under extreme conditions at 20 ℃ for 350 days without sprout inhibitors in order to assess whether aging- and/or senescence-related processes occurred. Antioxidant enzyme activity peroxidase was enhanced during the advanced phase of aging.

4.2. Polyphenol oxidases activity

Sprouting occurs in potato tubers at the end of the rest period (also known as end dormancy), when exogenous conditions like temperature or photoperiod are favorable [45]. The duration of the rest period and its termination are primarily regulated by plant hormones but little is known about physiological pathways or metabolic factors involved in this transition. Moreover, the metabolic alterations connected to the inhibition of sprouting are scarcely understood [46]. Knowledge of the processes occurring in potato tubers in which this physiological event is restrained could be useful in order to reveal the stages critical for commitment to sprouting. Polyphenol oxidases (PPOs) are metalloenzymes, which catalyze the oxygen- dependent oxidation of phenols to quinones. Quinones can polymerize and cross-link many cellular nucleophilic compounds through 1,4 addition mechanism: these secondary reactions lead to the formation of brown or black pigments, which are responsible for tissues alteration of different fruits and vegetables [47]. Since these reactions decrease the vulnerability of plants or plant organs, different authors have suggested a close connection between the expression of polyphenol oxidases and plant defense against wound and pathogens [48]. In particular, the induction of polyphenol oxidases in response to wounding was studied at the physiological level showing that the expression of these enzymes triggered by wounding is regulated via the octadecanoid signal trasduction pathway [49]. This observation suggests that the physiological regulation of polyphenol oxidases expression is part of the plant defense system and is related to other metabolic pathways; moreover, it suggests that these enzymes are involved in the response of plants to different stressing conditions.

4.3. Glutathione-S-transferase

Exposure of potato tuber to S-Carvone vapor lead to an inhibition of the longitudinal sprout growth[50]. The

inhibition of elongation of potato sprouts following S-carvon treatment correlate strongly with decreasing of 3-hydroxy-3methylglutaryl coenzyme A reductase a key enzyme in the mevalonate pathway . This effect seems rather specific since enzyme of the citric acid cycle such as isocitrate dehydrogenase and malate dehydrogenase were not affected whereas the activity of glutathione related enzymes was stimulated. On the other hand activity of several enzymes was induced in tuber tissue following an S-carvone treatment, probably together with an increased synthesis of enzymes, e.g. glutathione reductase, glutathione-S-transferase, dehydroascorbate reductase, S-carvone converting enzymes. Therefore Carvone proved to be an effective in inhibiting sprouting and its effect was concentration depending[51].

4.4. Superoxide dismutase enzyme

The main reduction in enzyme activity was noticed below the normal potato control level of superoxide dismutase activity[52]. On the other hand[53] cited that decreased germination ability of the aged legume seeds were well correlated with the increase in lipid peroxidation levels and the decreased in the antioxidants .During germination, SOD activity did not show any significant differences between the aged and non-aged beech[54]. Collectively, these results suggest that a gradual build-up of free radicals leads to peroxidative damage of membrane lipids during aging of potato seed-tubers.

During tuber dormancy, Mn/SOD and cytosolic Cu/ZnSOD activity was relatively constant in both Desiree and Bintje varieties while catalase activity decreases. Moreover, tuber dormancy breakage did not involve significant changes in the activity of these enzymes. Antioxidant enzyme activities superoxide dismutase was enhanced during the advanced phase of aging [55]. Therefore use the two essential oils as well as its main component with concentration of 100 ppm will advisable. While for economic circumstances it is preferable to use the crude essential oils with high concentration of the main component by using tissue culture technique [26].

4.5. Catalase activity

Catalase antioxidant enzyme activity, was enhanced during the advanced phase of aging[45]. Their storage was under extreme conditions at 20 ℃ for 350 days without sprout inhibitors. The involvement of hydrogen peroxide (H_2O_2) metabolism in dormancy release and sprouting of potato tuber changes occurred at the (bud /sprout) level was accompanied by a transient but remarkable increase in H_2O_2 content. Catalase and H_2O_2 resulted in a reduction of the dormancy period and in rapid and synchronised sprouting of the treated tubers when compared to the control. It thus appears that tuber dormancy and sprouting can be controlled in potato by the manipulation of H_2O_2 metabolism via the inhibition of CAT activity. The possible mechanisms whereby CAT inhibitors or H_2O_2 overcome dormancy and promote sprouting in the potato tuber. There is evidence that endogenous plant hormones play a pivotal role in the initiation, maintenance and release of potato tuber dormancy[55].

4.6. MDA content

The results concluded that accelerate ageing due to increased lipid peroxidation, decreased activities of several free radical and peroxide scavenging enzymes as approved by previous study who stated that the main reasons for loss of storability, occurs due to decreased levels of antioxidants, reduced activity of free radical and peroxide scavenging enzymes, and increased lipid peroxidation through malondialdehyde content [56]. Seed aging and leaf senescence are characterized by a decrease in SOD, APX and CAT activities [45]. Activities of superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxidase (POD) and catalase (CAT) showed uniform increases from young floret through to the mature stage and thereafter, declined. Among the SOD isoforms, Fe-SOD and Cu/Zn-SOD were induced during the onset of senescence. Similarly different isoforms of APX and glutathione reductase (GR) also appeared during the senescence process [57]. Level of MDA, a product of the lipid peroxidation, was significantly (P<0.01) high in the aged dry seeds, compared to controls. High lipid peroxidation and oxidative stress have been observed during storage of various seeds and have been widely proposed as the major cause of deterioration during seed aging [58]. Our results show that high lipid peroxidation is one of the major results of the natural aging of the long-term stored potato tubers. Therefore, the lipid peroxidation degree can be stable between the germinating aged seeds and control seeds (non-aged). It has been known that the reactive oxygen species considered major cause of lipid peroxidation in cell membranes, can be generated not only in metabolism during stress and aging, but also in metabolism of a plant under normal conditions.

In conclusion, treatment with the essential oils and its main component reduced the germination capability, and caused a delay in the germination speed the of potato tuber. In addition, from antioxidant enzymes, CAT, POX, and SOD activities were also low in stored potato tuber. The decrease in germination capability of the aged potato tuber was well correlated with the increased levels of lipid peroxidation and the decreased activities of POX, CAT and SOD and increseaed the activity of Glutathione-S-transferase. However, the most noticeable result was high POX and low CAT activity in long-term during the germination.

Conflict of interest statement

We declare that we have no conflict of interest.

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