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Clove Essential Oil for Controlling White Mold Disease, Sprout Suppressor and Quality maintainer for Preservation of Jerusalem Artichoke Tubers

Ghoneem[1], K. M.; W. I. A. Saber[2]; Aml, A. El-Awady[3]; Y. M. Rashad[4] and A. A. Al-Askar[5]

[1]Seed Pathology Research Dept., Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.
[2]Microbial Activity Unit, Microbiol. Dept., Soils, Water and Environ. Res. Ins., Agric. Res. Cen.r, Giza, Egypt.
[3]Vegetable Research Dept., Horticulture Research Institute, Agricultural Research Center, Giza, Egypt.
[4]Plant Protec. and Biomolecular Diagnosis Dept., Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Applications, Alexandria, Egypt, younesrashad@yahoo.com.
[5]Botany and Microbiology Dept., Faculty of Science, King Saud University, Riyadh, Saudi Arabia.
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ABSTRACT

White mold, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, is the most globally significant disease of Jerusalem Artichoke (JA) causing considerable losses during storage periods. The aim of this study was to evaluate the use of a natural essential oil for control of white mold disease, sprouting suppression and quality maintaining of JA tubers under storage periods. *In vitro* fungitoxicity was investigated using two natural essential oils; clove and sweet wormwood at concentrations of (2, 3, 4 and 5%). Clove oil at 2% inhibited completely the fungal growth of *S. sclerotiorum*. Chemical composition of clove essential oil was studied using GLC-MS analysis and resulted in identification of 12 compounds. The major components were eugenol and eugenol acetate (81.6 and 9%, respectively). Along 120 day of storage, treatment of JA tubers with clove oil led to a significant decrease in the disease severity, sprouting percentage and weight loss. On the other hand, this treatment enhanced the dry matter and contents of carbohydrates, protein, inulin and total phenols as well as the activity of peroxidase and polyphenol oxidase enzymes of JA tuber compared with the untreated-infected tubers. Based on the obtained results, use of clove oil and peat moss when storing JA tubers at room temperature can be recommended due to its eco-safety and saving of the cooling energy.

Key words: *Sclerotinia sclerotiorum*, Clove essential oil, Eugenol, inulin, storage, sweet wormwood.

INTRODUCTION

Jerusalem artichoke (JA) (*Helianthus tuberosus* L.), also known as sunchoke, is a perennial plant belongs to Asteraceae family (Compositae). It has received increased attention as a biorefinery/green chemical crop. Its tubers can be utilized for feeding, production of inulin, biogas, ethanol or platform chemicals, *e.g.*, succinic acid. Moreover, it has been used in folk medicine for pharmaceutical purposes. Aerial parts of JA have shown antimicrobial activities (Yang *et al*., 2015).

A thin, easily penetrated surface layer and high sugar content make JA tubers more susceptible to infection by a wide range of pathogenic fungi. White mold, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, is the most globally significant disease of JA causing considerable losses for its production. Whit mold, also called *Sclerotinia* stem rot, is prevalent in temperate, subtropical and tropical regions of the world (Kays and Nottingham, 2008). In the field, chemical fungicides as herbicides containing lactofen, can be a component of an integrated management system for white mold, although none offer complete control (Peltier *et al*., 2012). For storage, tubers can be harvested in the fall or left in the ground for winter storage and spring harvest. Some synthetic chemicals used as preservatives have been reported to cause harmful effects to consumers and the environment (Tesio *et al.*, 2011).

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. They are composed of a number of different components such as terpenes, aromatic phenols, oxides, ethers, alcohols, esters, aldehydes and ketones in different composition or combinations. These active compounds provide less chance to development of resistance in fungi (Mishra *et al.*, 2013).

Clove (*Syzygium aromaticum* (L.) Merr. and Perr.) is an evergreen tree in the family Myrtaceae. Its flower buds have been used in folk medicine as powerful germicidal, antimicrobial, anti-inflammatory, carminative, antioxidant, antiseptic, and for skin disorders. It is also used in many therapeutic fields such the treatment of kidney and intestinal diseases, against impotence, genital-pain and infertility and is reported as stomachic and has smooth muscle relaxant property (Cortés-Rojas *et al.*, 2014).

Sweet wormwood (*Artemisia annua* L.) is a highly aromatic annual herb in the family Asteraceae. It is of Asiatic and eastern European origin and widely dispersed throughout the temperate region. It has been used for many centuries as an anti-malarial, anti-cancer, antibacterial and antifungal (Garcia, 2015).

This study aimed to investigate *in vitro* antifungal activity of clove and sweet wormwood essential oils

against the phytopathogenic fungus *S. sclerotiorum*, also to evaluate the application of the most potent essential oil as an alternative preservative to control the white mold disease of JA tubers under storage conditions, as well as enhance their storability and maintain their quality characteristics.

MATERIALS AND METHODS

Causal organism

The pathogenic fungus *S. sclerotiorum* was isolated from naturally diseased JA tubers exhibiting typical symptoms of white mold disease obtained from the refrigerated storage. The fungus was isolated on potato dextrose agar (PDA) plates (Difco, USA), supplemented with chloramphenicol (5mg/L) and streptomycin sulphate (5 mg/L) and incubated at 25±2°C for 5-7 days. Hyphal tip technique was used to obtain pure cultures of the isolated fungal pathogens. The recovered isolates were maintained onto slants of potato carrot agar medium and kept at 4°C for further studies. The isolated fungi were identified according to their cultural, morphological and microscopical characteristics as described by Domsch *et al*. (1980).

Essential oils extraction

Essential oils were extracted separately from (200 gm) of dry clove flower buds and dry sweet wormwood stems and leaves. Extraction was done by hydro-distillation of the plant materials for 150 min using Clevenger apparatus as described by Charles and Simon (1990). The purified extracted essential oils were then stored in clean dark glass bottles at 4°C until used.

In vitro assessment for antifungal activity

Antifungal activity of the two essential oils was evaluated according to Mohana and Raveesha, (2007) with modifications. Certain volumes of the plant essential oils were separately added to 100 ml Erlenmeyer flasks containing 20 ml sterilized PD broth and 0.5% Tween-20 to obtain the proposed concentrations of 2, 3, 4 and 5% (v/v). Discs (5 mm diameter) from 5-days-old culture of *S. sclerotiorum* were used to inoculate the flasks, and then incubated at 25±2ºC for 5 days. Untreated medium was used as control. Triplicate flasks were used for each treatment. At the end of the incubation period, the mycelial mats were harvested, washed several times with distilled water, and oven dried to constant weight at 80°C. The antifungal activity was expressed as the reduction percentage in the mycelial dry weight.

Gas-liquid chromatography – Mass Spectrometry (GLC-MS)

After extraction, the most efficient antifungal essential oil was analyzed to identify and quantify its basic constituents. Identification of the constituents of the essential oil was performed by gas-liquid chromatography coupled to mass spectrometry (Schimadzu QP-5000 GC/MS), using an Autosystem XL equipped with a DB-1 fused silica column (30 m x 0.25 mm film thickness 0.25 m; J&W Scientific Inc.) connected to a Perkin-Elmer Turbomass. The oven temperature was programmed from 50 to 200°C in increments of 3°C min^{-1}. On reaching 200°C, the temperature was kept isothermal for 10 min, temperature of the transfer line, 230°C; temperature of the ionization chamber 200°C, helium carrier gas, adjusted to a linear velocity of 30 cm s^{-1}; split-flow ratio 1:40, ionization energy 70 eV, ionization current, 60 A; mass range, 40- 300 U, scan time 1 s. A standard solution of *n*-alkanes (C9-C21) was used to obtain the retention indices. The compounds were identified by comparison of their retention indices with those reported in literature and also to the Wiley Registry of Mass Spectral Data, 6th Edition (Wiley Interscience, New York) (Andrade *et al*., 2015).

Evaluation of clove essential oil and peat moss application under storage conditions

Healthy JA tubers (~20 g each) were washed by water, surface disinfected in 0.5% (v/v) sodium hypochlorite for 3 min, then rinsed in sterilized water and left to dry. For essential oil application, JA tubers were soaked in clove essential oil at 2% concentration for 20 min, then left to dry about 2 hours before the storage. For inoculum preparation, 250 ml Erlenmeyer flask containing 50 ml of sterilized PD broth medium and inoculated with a disc (5 mm diameter) from 5-days-old culture of *S. sclerotiorum* was incubated in the dark at 25±2ºC for 10 days. The mycelial mat was then harvested and washed gently with sterile distilled water. Twenty grams of the fungal mat were taken, mixed thoroughly and blended in 1000 ml of distilled water to produce a homogenized suspension and adjusted at concentration of 5×10^4 cfu mL^{-1}. Artificial infection was done by spraying the tubers with the fungal suspension until run-off. The experiment was divided into two groups according to the storage method. In the first group, the tubers were kept in 10-kg capacity perforated polyethylene bags (0.075 mm thickness), and stored at 4°C at R.H. of 90-95%. In the other group, the tubers were stored at 25±2°C in carton boxes (50×30×20 cm) with moistened peat moss layers (SAB Syker Agrarberatungs und Handels GmbH & Co., Germany, pH 3.5 and RH 75%) at the rate of peat moss: JA tubers (1.5: 1, Kg/Kg). The treatments applied for each group can be summarized as follows: untreated control (C), infected with *S. sclerotiorum* (P), treated with clove oil (O), and treated with clove oil and infected with *S. sclerotiorum* (O + P). For each treatment, 30 kg of tubers were used. Monthly and along 120 days of

storage, disease severity, percentage of sprouting, weight losses and dry matter of tubers were evaluated. The severity (%) of the white mold disease was evaluated based on our proposed scale; where: 0 = no symptom of mold, 1 = 1-10% of tuber mold, 2 = 11-20% of tuber mold, 3 = 21-30% of tuber mold, 4 = 31- 40% of tuber mold, 5 = 41-50% of tuber mold, 6 = 51-60% of tuber mold, 7 = 61-70% of tuber mold, 8 = 71-80% of tuber mold and 9 ≥ 81% of tuber mold. The severity was then calculated according to Bdliya and Dahiru (2006) with modification;

$$S = \frac{\sum n}{N \times 9} \times 100$$

Where: S = severity of tuber mold (%); Σn = sum of the individual ratings; N = total number of JA tubers assessed and 9 is the highest score on the severity scale.

The stored tubers were also analyzed for carbohydrate, inulin and protein contents according to Dubois *et al.* (1956), Winton and Winton (1958) and Robinson (1973), respectively. The defense related enzymes (peroxidase and polyphenoloxidase) were assessed according to the methods described by Maxwell and Bateman (1967) and Maria *et al*. (1981). Total phenol was determined according to Diaz and Martin (1972).

Statistical Analysis

All experiments were arranged in randomized blocks design. Statistical analysis, including, analysis of variance and Duncan test, as well as the standard deviation were performed using CoStat (CoHort Software, U.S.A) version 6.4 at level of probability (P) ≤ 0.05.

RESULTS AND DISCUSSION

Effect of the essential oils on mycelial growth of *S. sclerotiorum*

The *in vitro* effects of clove and wormwood essential oils on mycelial dry weight of *S. sclerotiorum* are shown in fig. (1). Obtained data showed a higher antifungal potency (complete inhibition) by clove essential oil at concentration of 2% than sweet wormwood oil even at its highest concentration (5%). These results are in accordance with that of Al-Askar and Rashad (2010) against *Rhizoctonia solani* and Al-Askar *et al*. (2014) against *Alternaria radicina, Fusarium oxysporum, F. solani, Macrophomina phaseolina, Nigrospora oryzae, Phoma destructiva* and *Sclerotium rolfsii*. The main constituent of clove oil is eugenol which is known as a strong enzyme inhibitor. The antagonistic property of clove oil can be attributed to the presence of an aromatic nucleus and a phenolic OH group that can reacts with the target enzyme, resulting in the deactivation of enzymes in fungi, and reacts with cell membrane phospholipids changing their permeability (Nuzhat and Vidyasagar, 2013).

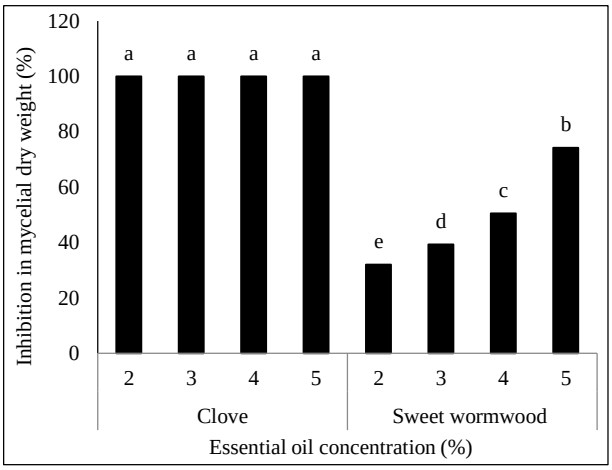

Fig. (1): Mean Inhibition in mycelial dry weight (%) of *Sclerotinia sclerotiorum* after 5 d incubation at 25°C, when exposed to clove or sweet wormwood essential oils at different concentrations.

*Columns superscripted with the same letter are not significantly different at P≤0.05 (Duncan test).

Chemical composition by GLC-MS analysis

Twelve components, representing about 99.17% of the clove essential oil, were identified. The major components were eugenol (81.6%) and eugenol acetate (9%). In addition, 10 compounds were identified in the clove oil in small amounts (*β*-caryophyllene oxide (1.7%), *p*-cymene (0.9%), 5-hexene-2-one (0.67%), thymol (0.87%), guail (0.9%), benzene-1-butylheptyl (0.6%), nootkatin (1.1%), *trans* isolongifolanone (0.9%), hexadecanoic acid (0.5%) and vitamin E acetate (0.43%). These results are in agreement with that obtained by Razafimamonjison *et al*. (2014). They reported that the percentage of eugenol, eugenyl acetate and *β*-caryophyllene can be used to characterize the essential oil although these percentages are varied according to the geographic origins of sample.

Evaluation of clove essential oil application on JA tubers under storage conditions

Disease severity

Effects of application of clove oil on JA tubers infected with *S. sclerotiorum* on mold severity are presented in table (1). As the experiment continued during 120 day of storage, the disease severity increased with the time increase in the two storage methods. Storage in peat moss layers at 25°C reduced the disease severity of the infected-non treated tubers during the first 60 day compared with the storage using the cooling method, after which, the tubers were fully deteriorated. Although deterioration in the control tubers was achieved due to reasons other than the target pathogen (physiological changes and infection by decay organisms), but treatment of these

Table (1): Rot severity (%) of JA tubers treated with clove essential oil and infected with *Sclerotinia sclerotiorum*

Storage method*	Treatment**	Storage period (day)			
		30	60	90	120
1	C•	28.5 b ***	63.2 a	100.0 a	NA
	P	100.0 a	NA	NA	NA
	O	0.0 d	6.6 c	16.1 c	39.9 b
	O + P	15.6 c	28.9 b	82.4 b	100 a
2	C	17.4 a	32.9 b	40.1 b	55.3 a
	P	20.0 a	48.5 a	100.0 a	NA
	O	0.0 b	0.0 c	0.0 d	0.0 c
	O + P	0.0 b	4.5 c	8.7 c	11.5 b

* 1 = storage in polyethylene bags at 4°C and 2 = storage in peat moss layer at 25°C.
** C = untreated control, P = infected, O = treated with clove essential oil and O + P = infected and treated with clove essential oil. NA = not applicable due to full deterioration. *** Means in each column followed by the same letter(s) are not significantly different at $P \leq 0.05$.
• Tubers rot in the untreated control (C) treatment was due to reasons other than the target fungus.

tubers with clove oil significantly reduced their deterioration compared with the non treated control. In this regard, in the second storage method, treatment of the control JA tubers with clove oil recorded no deterioration. Meanwhile, treatment of the infected JA tubers with clove oil led to a significant reduction in the disease severity when compared with the infected-non treated tubers. In this connection, tubers stored at 25°C in peat moss layers after treatment with clove oil showed a less disease severity than that stored using the cooling method. These results are in agreement with that obtained by Sameza *et al*. (2016) against *Rhizopus stolonifer* and *F. solani*, tuber rot causing fungi in yam. In addition, Hong *et a*l. (2015) showed that clove oil can protect green pepper fruit from anthracnose cause by *Colletotrichum gloesporioides*.

The antifungal property of clove oil is mainly depends on their chemical composition and synergistic interactions between the components, mainly, phenolic components, followed by aldehydes, ketones, and alcohols. Particularly, the bioactive terpene eugenol that was identified in this study as the major component has strong antifungal potency (Nuzhat and Vidyasagar, 2013). It may inactivate essential enzymes, react with the cell membrane or disturb the genetic material functionality. However, this activity could also be due to the other trace constituents that may act in synergistic interactions (Nuzhat and Vidyasagar, 2013). On the other hand, peat moss has been used during the last centuries in food preservation (Taskila *et al*., 2016) due to its antimicrobial characteristics against many pathogenic fungi and bacteria. This activity can be attributed to its antimicrobial constituents like sphagnan, phenolics, sterols and polyacetylenes (Borsheim *et al*., 2012).

Sprouting, weight loss and dry matter of JA tubers

Effects of application of clove oil on sprouting, weight losses and dry matter weights of JA tubers infected with *S. sclerotiorum* are presented in table (2). Obtained results indicated that sprouting and weight loss of JA tubers increased while, dry mater weight percentage decreased with the increase in the storage period. Treatment of the uninfected JA tubers with clove oil completely inhibited tuber sprouting, reduced weight loss and recorded the highest dry mater weight percentages compared with the control treatment. Moreover, treatment of the infected tubers with clove oil led to a lower sprouting and weight loss and higher dry matter weight compared with the untreated-infected tubers. These effects of clove oil were more in case of tubers stored in peat moss at 25°C than that stored in polyethylene bags at 4 °C. Storage of the control tubers using peat moss at 25°C increased the sprouting, decreased the weight loss and lowered the reduction in the dry matter weight of the tubers compared with the storage in polyethylene bags at 4°C. These results are in accordance with that obtained by Abbasi *et al.* (2015) on potato tubers. Storage of JA tubers usually results in high losses in quality, caused mainly by desiccation, rotting, sprouting, freezing, and inulin degradation (Danilcenko *et al*. 2008). So, there is a high demand for an effective and eco-safe preservative. In this regards, the bioactive compound eugenol in clove oil is known as a sprouting suppressor via physical or chemical damaging of the developing buds affecting the lipid peroxidation and the enzymes activities of catalase, glutathione-S-transferase, peroxidase, polyphenol oxidase and superoxide dismutase (Afify *et al*., 2012). On the other hand, peat moss layers provided a relative humidity around the tubers and blocking heat transfer within the peat leading to a decrease in water loss and keep the dry matter content due to its relatively high water retention capacity (Taskila *et al.*, 2016).

Nutritional content of JA tubers

Effects of application of clove oil on carbohydrates, inulin and protein of JA tubers infected with *S. sclerotiorum* are presented in

Table (2): Mean sprouting, weight loss and dry matter weight (%±2SD) of JA tubers treated with clove oil and infected with *Sclerotinia sclerotiorum*

Criterion	Storage method*	Treatment**	Storage period (day)			
			30	60	90	120
Sprouting	1	C	40.7 [a]	70.7 [a]	91.3 [a]	NA
		P	NA***	NA	NA	NA
		O	0.0 [b]	0.0 [c]	0.0 c	0.0 [b]
		O + P	3.7 [b]	4.9 [b]	6.7 [b]	8.4 [a]
	2	C	49.0 [a]	79.3 [a]	93.7 [a]	100 [a]
		P	3.0 [b]	4.7 [b]	NA	NA
		O	0.0 [c]	0.0 [c]	0.0 [b]	0.0 [b]
		O + P	0.0 [c]	0.0 [c]	0.0 [b]	0.0 [b]
Weight loss	1	C	7.2 [a]	35.8 [a]	51.3 [a]	NA
		P	NA	NA	NA	NA
		O	2.0 [c]	3.7 [b]	4.3 [b]	5.6 [a]
		O + P	3.8 [b]	4.0 [b]	5.0 [b]	6.1 [a]
	2	C	10.7 [b]	21.8 [b]	60.2 [a]	71.5 [a]
		P	46.1 [a]	50.4 [a]	NA	NA
		O	0.0 [c]	0.0 [c]	0.0 [b]	0.0 [b]
		O + P	0.0 [c]	0.9 [c]	1.1 [b]	1.6 [b]
Dry matter weight	1	C	17.4 [b]	18.7 [b]	19.5 [c]	NA
		P	NA	NA	NA	NA
		O	22.4 [a]	22.8 [a]	23.3 [a]	24.7 [a]
		O + P	22.0 [a]	22.2 [a]	22.3 [b]	22.6 [b]
	2	C	22.9 [b]	23. 3 [ab]	24.3 [c]	24.8 [c]
		P	17.0 [c]	16.6 [c]	NA	NA
		O	24.2 [a]	25.5 [a]	26.8 [a]	27.0 [a]
		O + P	22.6 [b]	22.7 [b]	22.9 [b]	23.0 [b]

*1 = storage in polyethylene bags at 4°C and 2 = storage in peat moss layer at 25°C.
**C = untreated control, P = infected, O = treated with clove oil and O + P = infected and treated with clove oil.
***NA = not applicable due to full deterioration.

Table (3): Mean contents of carbohydrates, inulin (mg/g ± 2SD) and protein (% ± 2SD) of JA tubers treated with clove oil and infected with *Sclerotinia sclerotiorum*

Criterion	Storage method*	Treatment**	Storage period (day)			
			30	60	90	120
Carbohydrates	1	C	42.8 [b]	41.9 [b]	38.7 [c]	NA
		P	NA***	NA	NA	NA
		O	44.8 [a]	44.0 [a]	43.9 [a]	43.6 [a]
		O + P	42.7 [ab]	42.2 [ab]	41.7 [ab]	41.0 [ab]
	2	C	42.3 [b]	39.2 [c]	37.9 [c]	36.3 [c]
		P	35.4 [c]	34.6 [d]	NA	NA
		O	45.6 [a]	46.6 [a]	46.7 [a]	47.8 [a]
		O + P	44.0 [ab]	42.7 [b]	41.5 [b]	39.9 [b]
Inulin	1	C	16.5 [b]	15.9 [c]	14.3 [c]	NA
		P	NA	NA	NA	NA
		O	17.5 [a]	17.0 [a]	16.6 [a]	16.4 [a]
		O + P	16.9 [b]	16.3 [b]	15.7 [b]	14.9 [b]
	2	C	14.6 [c]	14.3 [c]	14.2 [c]	13.3 [c]
		P	12.4 [d]	12.0 [d]	NA	NA
		O	18.9 [a]	18.8 [a]	18.5 [a]	17.0 [a]
		O + P	16.8 [b]	16.7 [b]	15.0 [b]	14.5 [b]
Protein	1	C	11.2 [b]	10.5 [b]	10.3 [b]	NA
		P	NA	NA	NA	NA
		O	12.8 [a]	12.7 [a]	12.7 [a]	12.4 [a]
		O + P	12.6 [a]	12.5 [a]	12.3 [a]	11.3 [b]
	2	C	12.8 [a]	12.6 [a]	12.3 [ab]	12.2 [a]
		P	10.6 [b]	10.0 [b]	NA	NA
		O	12.9 [a]	12.7 [a]	12.7 [a]	12.7 [a]
		O + P	12.8 [a]	12.6 [a]	11.5 [b]	11.3 [b]

* 1 = storage in polyethylene bags at 4°C and 2 = storage in peat moss layer at 25°C.
** C = untreated control, P = infected, O = treated with clove essential oil and O + P = infected and treated with clove essential oil.
*** NA = not applicable due to full deterioration.

Table (4): Mean activities of peroxidase, polyphenoloxidase enzymes and phenol content (%±2SD) of JA tubers treated with clove oil and infected with *Sclerotinia sclerotiorum*

Criterion	Storage method*	Treatment**	Storage period (day)			
			30	60	90	120
Peroxidase	1	C	0.35 [b]	0.30 [b]	0.29 [b]	NA
		P	NA***	NA	NA	NA
		O	0.39 [b]	0.38 [b]	0.32[b]	0.30 [b]
		O + P	2.75 [a]	2.69 [a]	2.63 [a]	2.60 [a]
	2	C	0.23 [c]	0.21 [c]	0.21 [b]	0.20 [b]
		P	1.75 [b]	1.75 [b]	NA	NA
		O	0.25 [c]	0.24 [c]	0.23 [b]	0.23 [b]
		O + P	2.55 [a]	2.51 [a]	2.47 [a]	2.46 [a]
Polyphenoloxidase	1	C	0.38 [b]	0.36 [b]	0.35 [b]	NA
		P	NA	NA	NA	NA
		O	0.46 [b]	0.46 [b]	0.42 [b]	0.42 [b]
		O + P	1.59 a	1.59 [a]	1.58 [a]	1.57 [a]
	2	C	0.40 [b]	0.39 [b]	0.36 [b]	0.35 [b]
		P	1.53 [a]	1.60 [a]	NA	NA
		O	0.40 [b]	0.39 [b]	0.38 [b]	0.37 [b]
		O + P	1.66 [a]	1.79 [a]	1.88 [a]	2.11 [a]
Total phenol	1	C	0.28 [b]	0.28 b	0.27 [b]	NA
		P	NA	NA	NA	NA
		O	0.59 [a]	0.59 [a]	0.56 [a]	0.54 [b]
		O + P	0.63 [a]	0.63 [a]	0.63 [a]	0.62 [a]
	2	C	0.35 [c]	0.35 [c]	0.34 [b]	0.34 [b]
		P	0.69 [a]	0.68 [a]	NA	NA
		O	0.25 [d]	0.24 [d]	0.24 [c]	0.24 [c]
		O + P	0.56 [b]	0.58 [b]	0.59 [a]	0.59 [a]

* 1 = storage in polyethylene bags at 4°C and 2 = storage in peat moss layer at 25°C. *** NA = not applicable due to full deterioration.
** C = untreated control, P = infected, O = treated with clove essential oil and O + P = infected and treated with clove essential oil.

table (3). Obtained results showed that carbohydrates, inulin and protein contents of JA tubers decreased with the increase in storage period. Treatment of the uninfected control tubers with clove oil significantly reduced the degradation in their carbohydrate, inulin and protein contents compared with the untreated control.

Moreover, treatment of the infected JA tubers with clove oil led to a reduction in the carbohydrate, inulin and protein degradation compared with the infected-untreated tubers along 120 days of storage. Studies on nutritive value of JA tubers showed that they contain 20% dry matter, from which carbohydrates are the main component. Most of these carbohydrates consist of inulin (50% of dry matter) (Brkljaca *et al.*, 2014). Long term storage of JA tubers usually results in significant alterations in their carbohydrate and protein chemistry which affect their marketability. Clove oil and its major bioactive constituent, eugenol, have the ability to slow down carbohydrates and protein degradation by interfering with the hydrolysis and energy enzymes. As reported by Solgi and Ghorbanpour (2014) the hydroxyl group on eugenol is thought to bind to proteins, preventing the enzyme activity.

Peroxidase, polyphenoloxidase enzymes and phenol content in JA tubers

Data presented in table (4) show the effects of application of clove oil on activities of peroxidase, polyphenoloxidase enzymes and phenol content of JA tubers infected with *S. sclerotiorum*. An increase in the total phenol and activity of peroxidase and polyhenoloxidase enzymes of JA tubers was recorded as a result of infection with *S. sclerotiorum* compared with the control treatment. Although the treatment of JA tubers with clove oil did not affect the activities of the two enzymes, but it led to an increase in the total phenol in the tubers compared with the untreated control.

Moreover, treatment of the infected JA tubers with clove oil led to a significant increase in the activities of peroxidase, polyphenoloxidase enzymes and phenol content in the tubers compared to the untreated-infected tubers in both methods. These results are in agreement with that obtained by Afify *et al*. (2012) on potato tubers. Peroxidase is a key enzyme in defense related processes including the accumulation of lignin and phenolic compounds and suberization. Polyphenol oxidase enzyme catalyzes the oxidation of phenols to quinines which is highly toxic substances against the pathogen, while phenolic compounds contribute to resistance through their antimicrobial properties against the pathogen (Martínez, 2012). The increase in these oxidative enzymes and phenol content is associated with the increase in plant resistance against infection by the fungal pathogen.

In conclusion, results indicated an effective role of clove essential oil in prolongation of the storage period for JA tubers. Treatment of JA tubers with clove oil and preserving them in between peat moss layers could provide an effective protection against tuber mold for 120 days of storage even at 25°C, as well as maintains their quality parameters. This eco-safe and cooling energy saving method is suitable for preserving JA tubers up to 120 days of storage.

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REFERENCES

Abbasi, K. S., Masud, T., Ali, S., Khan, S. U., Mahmood, T. and Abdul Qayyum 2015. Sugar-starch metabolism and antioxidant potential in potato tubers in response to different antisprouting agents during storage. Potato Res., 58:361-375.

Al-Askar, A. A. and Rashad, Y. M. 2010. Efficacy of Some Plant Extracts against *Rhizoctonia solani* on Pea. J. Plant Protec. Res., 50(3): 239-243.

Al-Askar, A. A., Rashad, Y. M. and Abdulkhair, W. M. 2014. Evaluation of the antimicrobial potential of selected medicinal plant extracts against some plant and human pathogens. J. Pure App. Microbiol., 8(1): 159-168.

Afify, A. M. R., El-Beltagi, H. S., Aly, A. A., and El-Ansary, A. E. 2012. 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. Asian Pacif. J. Trop. Biomed., 2012: S772-S780.

Andrade, M. A., Cardoso, M. d., Gomes Mde, S., de Azeredo, C. M., Batista, L. R., Soares, M. J., Rodrigues, L. M., Figueiredo, A. C. 2015. Biological activity of the essential oils from *Cinnamodendron dinisii* and *Siparuna guianensis*. Braz. J. Microbiol., 46(1): 189-194.

Bdliya, B.S. and Dahiru, B. 2006. Efficacy of some plant extracts on the control of potato tuber soft rot caused by *Erwinia carotovora* ssp. *carotovora*. J. Plant Prot. Res. 46(3): 285-294.

Borsheim, K. Y., Christensen, B. E., and Painter, T. 2012. Preservation of fish by embedment in Sphagnum moss, peat, or holocellulose: experimental proof of the oxopolysaccharidic nature of the preservative substance and its antimicrobial and tanning action. Innov. Food Sci. Emerg. Technol. 2(1):63-74.

Brkljaca, J., Bodroza-Solarov, M., Krulj, J., Terzic, S., Mikic, A., Marjanovic-Jeromela, A., 2014. Quantification of inulin content in selected accessions of Jerusalem artichoke (*Helianthus tuberosus* L.). Helia, 37(60): 105-112.

Charles, D. J. and Simon, J. E. 1990. Comparison of extraction methods for the rapid determination of essential oil content and composition of basil. J. Amer. Soc. Hort. Sci., 3:458-462.

Cortés-Rojas, D. F., de Souza, C. R. F., and Oliveira, W. P. 2014. Clove (*Syzygium aromaticum*): a precious spice. Asian Pacif. J. Trop. Biomed. 4(2): 90-96.

Danilcenko, H., Jariene, E., Aleknaviciene, P. and Gajewski M. 2008. Quality of Jerusalem artichoke (*Helianthus tuberosus* L.) tubers in relation to storage conditions. Not. Bot. Horti Agrobo., 36 (2): 23-27.

Diaz, D. H. and Martin, G. C. 1972. Peach seed dormancy in relation to endogenous inhibitors and applied growth substances. J. Amer. Soc. Hort. Sci., 97: 651-654.

Domsch, K. H., Gams, W., Anderson, T. H. (*eds*). 1980. Compendium of Soil Fungi. Vols. 1, 2. Academic Press, New York.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem., 28, 350-356.

Garcia, L. C. 2015. A Review of *Artemisia annua* L.: Its Genetics, Biochemical Characteristics, and Anti-Malarial Efficacy. Int. J. Sci. Technol., 5(2): 38-46.

Hong, K. J., Yang, J. H., Jung, H., Sang, K. M. and Yong-Chull, J. 2015. Application of volatile antifungal plant essential oils for controlling Pepper fruit anthracnose by *Colletotrichum gloeosporioides*. Plant Pathol. J., 31: 269-277.

Kays, S. J. and Nottingham, S. F. 2008. Biology and Chemistry of Jerusalem Artichoke: *Helianthus tuberosus* L. - CRC Press, Taylor and Francis Group, Boca Raton - Abingdon - Oxon - New York.

Maria, A., Galeazzi, M., Valdemo, C., Garbieri, S. and Spiros, M. 1981. Isolation, purification and physicochemical of polyphenol oxidase (PPO) from a dwarf variety of banana. J. Food Sci. 46(1): 150-5.

Martínez, J. A. 2012. Natural Fungicides Obtained from Plants. In: Dhanasekaran, D., Thajuddin, N. and Panneerselvam, A. (*eds*) Fungicides for Plant and Animal Diseases. InTech Open, Rijeca, Croatia.

Maxwell, D. P. and Bateman, D. F. 1967. Changes in the activities of some oxidases in extracts of *Rhizoctonia*-infected bean hypocotyl in relation to

lesion maturation. Phytopathology 57: 132.

Mohana, D. C. and Raveesha, K. A. 2007. Anti-fungal evaluation of some plant extracts against some plant pathogenic field and storage fungi. J. Agric. Technol. 4(1): 119-137.

Mishra, P. K., Singh, P., Prakash, B., Kedia, A., Dubey, N. K. and Chanotiya, C. S. 2013. Assessing essential oil components as plant-based preservatives against fungi that deteriorate herbal raw materials. Int. Biodeterior. Biodegrad. 80: 16- 21.

Nuzhat, T. and Vidyasagar, G. M. 2013. Antifungal Investigations on Plant Essential Oils: A Review. Int. J. Pharm. Pharm. Sci. 5(2): 19-28.

Peltier, A. J., Bradley, C. A., Chilvers, M. I., Malvick, D. K., Mueller, D. S., Wise, K. A., et al 2012. Biology, yield loss and control of sclerotinia stem Rot of soybean. J. Integr. Pest Manage. 3(2): 1-7.

Razafimamonjison, G., Jahiel, M., Duclos, T., Ramanoelina, P., Fawbush, F. and Danthu, P. 2014. Bud, leaf and stem essential oil composition of *Syzygium aromaticum* from Madagascar, Indonesia and Zanzibar. Int. J. Basic App. Sci., 3: 224-233.

Robinson, R. G. 1973. Element composition and response to nitrogen of sunflower and corn. Agron. J., 66: 313.

Sameza, M. L., Nguemnang Mabou, L. C., Tchameni, S. N., Boat Bedine, M. A., Tchoumbougnang, F., Jazet Dongmo, P. M. and Boyom Fekam, F. 2016. Evaluation of Clove Essential Oil as a Mycobiocide Against *Rhizopus stolonifer* and *Fusarium solani*, Tuber Rot Causing Fungi in Yam (*Dioscorea rotundata* Poir.). J. Phytopathol. doi: 10.1111/jph.12468

Solgi, M. and Ghorbanpour, M. 2014. Application of essential oils and their biological effects on extending the shelf-life and quality of horticultural crops. Trakia J. Sci., 2: 198-210.

Taskila, S., Särkelä, R. and Tanskanen, J. 2016. Valuable applications for peat moss. Biomass Conv. Bioref. 6:115-126.

Tesio, F., Weston, L. A. and Ferrero, A. 2011. Allelochemicals identified from Jerusalem artichoke (*Helianthus tuberosus* L.) residues and their potential inhibitory activity in the field and laboratory. Sci. Hortic. Amsterdam, 129: 361-368.

Winton, A. L. and Winton, K. B. 1958. The Analysis of Foods. John Wiley & Sons. Inc. London, 857p.

Yang, L., He Q. S., Corscadden K. and Udenigwe, C. C. 2015.The prospects of Jerusalem artichoke in functional food ingredients and bioenergy production. Biotechnol. Rep., 5: 77–88.