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NATURAL TREATMENTS FOR EXTENDING STORAGE LIFE AND INHIBITION FUNGI DISEASE OF JERUSALEM ARTICHOKE FRESH TUBERS

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ABSTRACT

Two storage experiments were carried out at Mansoura Horticultural Research Station, Agriculture Research Center, Egypt, during of 2009 and 2010 seasons, to study the effect of natural treatments of plant extracts for prolonging life of *Jerusalem artichoke* fresh tubers during storage and to control common fungi diseases, i. e., clove (*Syzygium aromaticum* L. Merr & Perry.), cumin (*Cuminum cyminum* L.) and garlic (*Allium sativum* L.) at different temperature.

Results of experiments can be summarized as follows:

Tubers treated with cumin or clove extracts and stored at 2 ºC or ambient temperature (average: 25/10 ºC day/night) suppressed the incidence of sprouting percentage, and decreased weight loss, decay percents, compared with the control (2 ºC). Tubers treated with!garlic extract and stored at 2 ºC slightly increase dry matter content. However, mncrease"of carbohydrates and inulin were found as a result of use garlic and cumin extract at ambient temperature.

Tuber rots caused by microorganisms during the growing season or during refrigerated or field storage were the most émportant diseases of Jerusalem artichokes in Egypt. *Mucor* sq., *Fusarium oxysporum* Schlecht, *Fusarium solani* Mart Sacc. Desm., *Rhizoctonia solani* Kühn, *Rhizopus stolonifer* Ehrenb ex Link and *Sclerotium rolfesii* Sacc. were!isolatud örom votted tissues and found to cause rots of Jerusalem tubers. *In vitro* antifungal acti~ity of íuthanol-water extracts of three medicinal plants, clove, garnic and cumin was investigatmd against Jerusalem tuber rots pathogenic fungi. *In vitro* antifungal agtivity test shïwed a high grïwth inhibition at concenôration (¸4 %) of each°qlant(extract. • The study6óuggmsts using plant extracts as natural treatments fïr(kogreasing storability and quality of Jerusalem artichoke fresh tubers and inhibition of fungi diseases.

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Keywords: Jerusalem artichoke; natural tveatments; plant extract; antifungi; inulin

INTRODUCTION

Jerusalem artichoke (*Helianthus tuberosus*), also known as sunchoke is a tuber of a variety of xerennial flowers in the aster family (Pan *et al.*, 2009).(Jerusalem artichoke is a rich source on fructooligosaccharides, e.g. inulin (ca 20% fresh, ca 50% dried) (Roberfroid, 2005). Dried Jerusalem artichoke powder ãontains also other valuable substances, such as fructose, glucose, sucrose, proteins, lipids, nucleic acids (Gedrovica and Karklina, 2009). Interest in the crop ias stemmed from the fact that the storage form of carbon in the Jerusalem artichoke is inulin, a straight chain fructan that is poorly digested by humans. Inulin can be used as a bu|king agent in foods wèen sugar is replaced with an artificial sweetener.!

Jerusalem artichoke is one of the most suitable materials for ethanol production. The increased oil price for nonrenewable oil resources has stimulated worldwide interest in the utilization of fermentation ethanol as a potential liquid fuel (Margaritis and Bajpai, 1983).

Storage losses are due primarily to desiccation, rotting, sprouting, freezing, and inulin degradation. Desiccation remains a significant storage problem even though losses can be fairly easily circumvented with proper storage conditions. Storage at high RH is essential (Stepanets *et al.*, 1992) because tubers lack of a corky surface layer similar to that found on potatoes to reduce water loss, and have a thin, easily damaged surface that permits rapid water loss.

Jerusalem artichoke tubers are difficult to store outside the soil because of the rapid onset of rotting. Therefore, the crop must be harvested according to the daily capacity of processing facilities (Frese, 1993). McCarter and Kays (1984) first reported Jerusalem tuber rots caused by either *Sclerotium rolfesii* during the growing season or by *Fusarium* and *Pseudomonas* spp. during refrigerated or field storage were the most important diseases in Georgia. Barloy (1988) recorded approximately 20 organisms causing storage rots have been isolated from Jerusalem tubers. The organisms most frequently isolated were *Botrytis cinerea* Pers. and *Rhizopus stolonifer* (Ehernb.: Fr.) Vuill., though R. stolonifer and *Sclerotium rolfesii* (Lib.) de Bary are the most serious organisms causing rots at low storage temperatures.

Chemical fungicides such as Dichloronitroanline are commonly used to protect tubers against *Rhizopus* sof rot (Clark and Moyer, 1988). However, their field application may not always be desirable. The persistent, injudicious use of chemicals was discouraged owing to their toxic effects on non-target organisms, the undesirable changes they inflict upon the environment (Arcury and Quandt, 2003) and due to development of resistant strains of pathogens against various chemical fungicides (Deising *et al*., 2008).

Garlic, cumin and clove extracts as an alternative to chemical preservatives for improving the shelf life of Jerusalem artichoke tubers products and antifungal also, plants extract have effectively inhibited sprouting relative to control (El-Sharkawy, 1998). In recent reports (Avato *et al*., 2000) suggested that volatile compounds of garlic such as diallylmonosulfide, diallyldisulfide, and diallyltrisulfide were also found to have antimicrobial properties. Similarly, the pharmacologically active principles in the volatile oil of cumin thymoquinone, dihydrothymoquinone, p-cymene, carvacrol, α-thujene, thymol, α-pinene, β-pinene and t-anethole as major constituents (Velluti *et al.*, 2003) were identified as: thymoquinone, dithymoquinone, thymol and thymohydroquinone. The inhibitory activity of clove is due to the presence of several constituents, mainly eugenol, eugenyl acetate, beta-caryophyllene, 2-heptanone (Chaieb *et al.*, 2007), acetyl-eugenol, alpha-humulene, methyl salicylate, iso-eugenol, methyl-eugenol (Yang *et al.*, 2003), phenyl propanoides, dehydrodieugenol, trans-confireryl aldehyde, biflorin, kaempferol, rhamnocitrin, myricetin, gallic acid, ellagic acid and oleanolic acid (Cai and Wu, 1996).

Storage of Jerusalem artichoke tubers at low temperature (4 ºC) for 34 days also increases the fructo-oligosaccharide content (Kang *et al*., 1993). Stepanets *et al*. (1992) reported that tubers of JA were stored in vegetable boxes (control), polyethylene bags, or containers with or without polyethylene wrapping. Weight losses showed the order: polyethylene bags < wrappings << containers < boxes. They discuss the mechanism where by polyethylene films that have been activated by minimal catalyst water excite nitrogen in the air; this, in turn, keeps foodstuffs fresh by protecting them from attack by oxygen. Ben Chekroun *et al*. (1997) found that storage at 4 ºC for the two varieties ('Kharkov' and "Violet de Rennes') beyond 7 weeks would cause a decrease of general reserves in tubers of JA. Modler *et al.* (1993) mentioned that of the five storage treatments tested (2 ºC, 5 ºC, - 10 ºC, program cooled to - 10ºC, and ambient), the 2ºC treatment yielded the best quality tuber at the end of 12 months of storage. El-Sharkawy (1998) found that adding peatmoss to storage containers as well as cold storage decreased decay, weight loss and total losses percentage in JA tubes.

The objectives of the performed research work were to:-

1. Evaluate the efficiency of using three plant extract in prolonged the storability and keeping quality properties of Jerusalem tubers in storage. Also, to determine changes in inulin, carbohydrate content, weight losses, decay, sprouting and dry matter at different period of storage.
2. Determine the fungi responsible for tuber rots in Egypt and to investigate the antifungal activity of methanol-water extracts of clove, garlic and cumin *in vitro* on growth of phytopathogenic fungal isolates.

MATERIALS AND METHODS

Jerusalem artichoke source and treatments

Jerusalem artichokes (*Helianthus tuberosus* L.) of Fuseau cv. were planted on April 15, 2009 and 2010 at Mansoura Horticultural Research Station and the yield were harvested at October 15, 2009 and 2010 seasons. Tubers were soaked with plant extracts 80% (clove, garlic and cumin) for 20 min and were repeated at 45 days intervals. Some tubers were packed in sealed polyethylene bags (0.075 mm thickness) and each bag contents of 50 tubers (ca 5 kg tubers) and were stored at cold storage (2 ºC, 90-95 % RH). The other tubers were kept in carton boxes (50 x 30 x 20 cm) all one content of 50 tubers (5 kg tubers) with moist peat moss at the rate of 1.5 kg per 1 kg tuber, and were stored at ambient temperature (25/10º C day/night). The storage period was 5 months for all treatments.

The experimental design was complete randomized blocks with three replicates. Each replicate included 11 treatments, which were as follows:

1. Control, storage tubers at ambient temperature; 2. cold storage; 3. storage in the soil (Baramoon Experimental Farm), 4: wrapping with polyethylene; 5: treating tubers with clove extract and wrapping with polyethylene; 6: treating tubers with garlic extract and wrapping with polyethylene; 7: treating tubers with cumin oil and wrapping with polyethylene; 8: storing tubers in moist peatmoss; 9: treating tubers with clove extract and storing in peatmoss; 10:

treating tubers with garlic extract and storing in peatmoss; 11: treating tubers with cumin oil and storing in peatmoss. Treatments from 4 to 7 were stored at cold storage, whereas treatments from 8 to 11 were kept in ambient temperature.

The plant extracts

Plant materials were dried in the shade (clove peels, garlic bulbs and cumin seeds) were washed with distilled water and dried in shade. They were finely grinded to powder. Fifty grams of each plant material in powder form was homogenized by laboratory blender in 200 ml of methanol (96%) and distilled water (20:80 v/v) for 10 min, and then left dark glass bottles for 72 h for complete extraction. The extracts were filtered through thin cheesecloth sheets. The final extracts were collected separately in other dark glass bottles and exposed to 60 °C in water bath for 30 min for methanol evaporation. The collected extracts were then stored in a refrigerator at 5 °C until needed. Plant extracts was subjected to GC-MS analysis using a Gas Chromatograph (Singh *et al*., 2005) (Table 1).

Studied traits

Sprouting, Wight loss and decay percentage of tuber were recorded at 30days intervals. Dry matter of the tuber was determined by drying in a hot-air oven at 105 °C for 24 h (AOAC, 1990). Samples of tubers were analyzed for inulin and carbohydrate composition at 30 days intervals according to Winton and Winton (1958) and Dubois *et al*. (1956), respectively.

Source of rotted Jerusalem tubers

Rotted Jerusalem tubers were collected from different fields in Egypt. Isolation was also made from tubers held in refrigerated storage. Plant materials (seeds, bulb, cloves and peels) were collected from locations in Egypt. The scientific names and families of these plants were confirmed by the Department of Plant Pathology, Faculty of Agriculture, Mansoura University.

Isolation of fungi associated with rotted Jerusalem tubers:

Rotted Jerusalem tubers were washed in tap water, and cut into sections with sterilized scalpel. The sections were surface sterilized in 1% sodium hypochlorite and rinsed with several changes of sterile distilled water. Ten sections of the sterilized tubers were plated out on potato dextrose agar incorporated with streptomycin. The plated Petri dishes were incubated at room temperature (25±2°C) for 2-5 days. They were examined daily for presence of fungal growth. The fungi isolated were put into pure culture and stored in slants in the refrigerator at 4 °C.

Determination of percentage of fungal occurrence:

This was done to determine the frequency of occurrence of the different fungal isolates. Isolations were made from ten different rotted Jerusalem tubers and were cultured differently. The number of occurrence for each of the isolates in the eight different samples were recorded and calculated as a ratio of the total number of occurrence and was then expressed as a percentage (Table 2). It was given by the formula below;

$$\text{Percentage occurrence} = \frac{X}{N} X \frac{100}{1}$$

X= Total number of each organism in all the samples.
N = Total number of the entire organism in all the samples screened.

Table 1: Functional components of clove, garlic and cumin extracts by GC-MS analysis.

Clove extract parameters	(%)	Garlic extract parameters	(%)	Cumin extract parameters	(%)
Eugenol	88.6	diallyl sulfide	46.67	Thymoquinone	23.25
Eugenyl acetate	5.6	diallyl disulfide	20.32	Dihydrothymoquinone	3.84
Beta-Caryophyllene	17.4	allyl methyl sulfide	8.96	ρ-Cymene	32.02
Alpha-Humulene	2.1	3-vinyl-1,2-dithiole-5-cyclohexene	49.19	Carvacrol	10.38
Cadenine	1.05	vinyl-1,2-thia-4-cyclohexene	17.26	α-Thujene	2.40
2-Heptanone	0.9	diallyl disulfide	3.37	Thymol	2.32
(E)-ß-Ocimene	0.3	methyl sulfide	8.96	α-Pinene	1.48
ρ-Allyl phenol	0.2	diallyl disulfide	20.31	β-Pinene	1.72
A-Copaene	0.1			t-Anethole	2.10
Caryophyllene oxide	0.1			Minor components	23.81
gallic acid	0.09			**Cumin extract parameters (mg/kg-oil**)	
ellagic acid	0.08			α-Tocopherol	182.56
oleanolic acid	0.03			β-Tocopherol	18.56
				γ- Tocopherol	142.97
				δ-Tocopherol	17.62
				Total tocopherols	361.71
				Carotenoids	88.95
				Tocopherols and carotenoids	450.66

Table 2: Occurrence of fungi isolated from diseased Jerusalem tubers

Fungi	Occurrence %
Alternaria alternata	6.70 gh
Aspergillus flavus	15.73 f
Aspergillus glaucus	4.50 gh
Aspergillus niger	20.50 e
Aspergillus ochraceus	5.0 gh
Cladosporium sp.	7.60 g
Cunninghamella sp.	5.0 gh
Fusarium incarnatum	37.30 b
Fusarium oxysporum	8.20 g
Fusarium solani	30.85 c
Fusarium verticillioides	3.20 h
Gliocladium rosum	4.60 gh
Mucor sp.	23.10 de
Penicillium sp.	15.38 f
Rhizoctonia solani	46.0 a
Rhizopus stolonifer	6.50 gh
Sclerotium rolfesii	25.10 d
Stemphylium sp.	5.0 gh
Trichoderma harzianum	8.69 g

Identification of fungi

Fungi were identified according to their cultural properties, morphological and microscopical characteristics as described by Raper and Fennel (1965); Ellis (1971); Domsch *et al*. (1980); Booth (1977); Burrges *et*

al. (1988). For determination of morphological structures, portions of fungal growth were mounted in lacto-phenol cotton blue stain on clean slides. The prepared slide was examined under a light microscope using the 40X and 100X objectives for vegetative mycelium; septation, diameters, conidiophores (sporangiophores) and the reproductive structures: conidia, sporangiospores etc. Fungal colonies were examined under the 10X (low power) objective of the microscope. The colonial characteristics of size, texture and color of the colony were investigated.

Pathogenicity test

Pathogenicity tests were conducted to determine rotting potential of the fungal isolates. Disease-free tubers were washed and surface sterilized with 70% ethanol. Inoculations with fungi were made by removing a cylinder from tubers with a sterile 8-mm cork borer and inserting a mycelia plug of the tested fungus or by cutting tubers in half and placing a mycelia plug on the cut surface. Inoculated tubers were placed in 9-cm glass or 25-cm plastic containers with covers. Tuber sections inoculated with fungi were kept moist during incubation by placing water or moist filter paper in the bottom of the dishes and incubated for 14 days at 25±2°C. The same procedure was used for the control except that discs of uninoculated PDA were placed in the holes created in the tubers (Amienyo and Ataga, 2006). After incubated period, the tubers were examined for infection and disease development. The inoculated fungi were re-isolated from the diseased Jerusalem tubers to prove the Koch's postulates.

In vitro antifungal activity of the Plant extracts

The plant extracts were added to conical flasks (100 ml) containing sterilized potato dextrose brother (PDB) medium (20 ml foe each) to obtained the proposed concentrations of 40,60 and 80% (v/v) and another PDB flasks, was used control. Flasks were inoculated with two discs of fungal culture (0.5 cm diameter) grown on PDA and incubated under dark conditions at 26±2°C for 7 days. For each treatment, 4 replicates (flasks) were used. The cultures were filtered through pre-weighed Whatman No.1 filter paper, washed and dried in an oven at 80°C for 48 hours, and then the dry weight was recorded. The antifungal activity of a tested extract was expressed as a percent inhibition of fungus growth calculated according to the following equation:-

$$\% \text{ Inhibition} = \frac{A-B}{A} X100$$

Where:

A=Fungal dry weight of the control

B= Fungal dry weight of the treatment

Statistical Analysis:

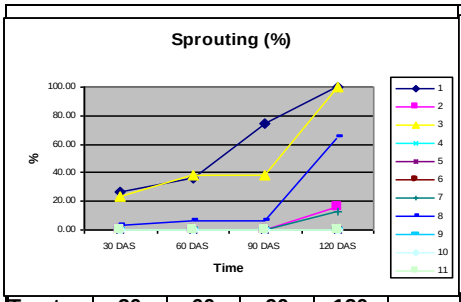
Data were subjected to statistical analysis of variance of randomized complete block design by Gomez and Gomez (1984). Mean values of treatments were compared using the last significant differences (LSD) at 5 % level of probability as described by Steel and Torrie (1980).

RESULTS AND DISCUSSION

Sprouting, weight loss and decay

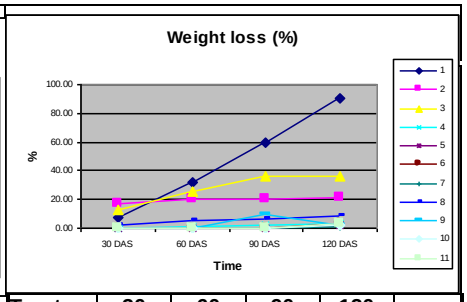
Tuber treated with plant extract and stored at 2ºC or ambient temperature (25/10ºC day/night) were still healthy and good appearance, exhibited no sign of spoilage or sprouting (Fig. 1 and photo 5). The storing in moist peatmoss (without application of plant extract) gave good results, but we observed occurrence of swelling in the sprouts with roots formation (photo 6). Wrapping Jerusalem artichoke tubers with polyethylene without any application of natural treatments and storing at cold storage gave medium results (Fig. 2 and photo 3)

A decrease in sprouting, weight loss and decay were seen in tubers stored at 2ºC and treated with cumin extract and wrapped with polyethylene or clove extract and stored their tubers in peatmoss at ambient temperature, respectively (Figs 1, 2 and 3). A gradual increase in sprouting was shown in tubers stored at soil, and reached 100% sprouting at the end of storage (120 days) (Fig. 1 and photo 4). In this respect, Stepanets *et al*. (1992) reported that tubers of Jerusalem artichoke were stored in vegetable boxes (control), polyethylene bags, or containers with or without polyethylene wrapping. Weight losses showed the order: polyethylene bags < wrappings << containers < boxes. They discuss the mechanism where by polyethylene films that have been activated by minimal catalyst water excite nitrogen in the air; this, in turn, keeps foodstuffs fresh by protecting them from attack by oxygen. Tuber, stored at 2 ºC for 120 days were still firm and crisp, and no sign of sprouting or spoilage. Most enzymatic and chemical reactions were drastically reduced or stopped at freezing temperature, while Jerusalem artichoke tissue metabolism could be continue at a slow rate, even at 2ºC storage temperature. But high temperature stressful factor induce generation of elevated components for the level of the toxic and degradable reactive oxygen species ROS within stressed tissues (Bowler *et al*., 1992). Also, they found that high storage temperature accelerate respiration rate and other metabolic activities such as carbohydrate reserves degraded to sugars to provide energy and structural rapidly developing sprout tissues, sprouts serves as a powerful sink for the mobilized sugars. The plant extracts (garlic, cumin and clove) contain basic compounds such as volatile and fixed oils. The inhibitory activity of plant extracts is due to the presence of several constituents such as antioxidant and monoterpene, mainly eugenol 88.6%, eugenyl acetate 5.6%, beta-caryophyllene 17.4%, alpha-humulene 2.1%, carvacrol, thymol and cinnamaldehyde (clove oil). The main constituents of cumin extract are thymoquinone 23.25%, p-cymene 32.02%, carvacrol 10.38%, thymo l2.32%. Garlic extract contains diallyl sulfide 46.67%, 3-vinyl-1, 2- dithiole-5-cyclohexene 49.19%, vinyl-1,2-thia-4- cyclohexene1 7.26% (Table 1). These constituents preserved stored tubers, keeping the internal biochemical enzymatic activities in minimum levels. Also, these treatments proved that they were highly effective in protection of their tubers against oxidative stressful storage conditions at higher temperature (Davies, 1990). The carvone completely inhibited 3-hydroxy-3 methylglutrayl coenzyme A reductase (HMGR, the key enzyme of mevalonate pathway in tuber at low concentration (Oosterhaven *et al*., 1993). The mevalonate, known to be main pathway of gibberellins biosynthesis.



Treat-ments	30 DAS	60 DAS	90 DAS	120 DAS	Mean
1	26.68	35.89	75.00	100.00	59.39
2	0.00	0.00	0.00	15.80	3.95
3	23.86	38.65	39.86	100.00	50.59
4	0.00	0.00	0.00	0.00	0.00
5	0.00	0.00	0.00	0.00	0.00
6	0.00	0.00	0.00	0.00	0.00
7	0.00	0.00	0.00	12.33	3.08
8	2.86	6.88	7.02	64.50	20.32
9	0.00	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00	0.00
11	0.00	0.00	0.00	0.00	0.00
Mean	4.85	7.40	11.08	26.60	12.48
L.S.D.	0.92	0.80	3.09	2.42	------

Fig. 1: Sprouting of Jerusalem artichoke tubers as affected by plant extract (average two seasons).



Treat-ments	30 DAS	60 DAS	90 DAS	120 DAS	Mean
1	7.75	31.52	59.86	90.85	47.50
2	17.51	19.75	20.28	21.65	19.80
3	13.22	25.14	35.67	36.56	27.65
4	0.00	1.55	2.57	3.05	1.79
5	0.00	0.00	0.00	2.53	0.63
6	0.00	0.00	0.00	2.81	0.70
7	0.00	0.00	0.00	1.55	0.39
8	2.57	4.86	6.38	8.75	5.64
9	0.00	0.00	10.00	1.68	2.92
10	0.00	0.00	0.00	2.65	0.66
11	0.00	0.00	0.00	2.85	0.71
Mean	3.73	7.53	12.25	15.90	9.85
L.S.D.	0.80	2.04	2.42	1.91	------

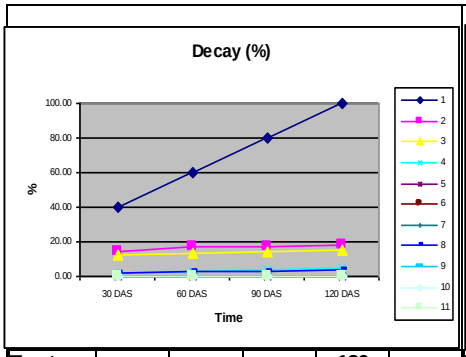
Fig. 2: Weight loss of Jerusalem artichoke tubers as affected by plant extract (average two seasons).

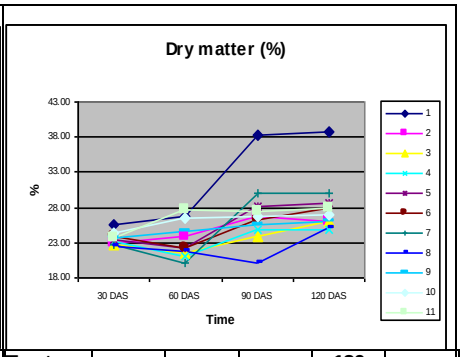
Treatments: 1: control, storing tubers at ambient temperature (25/10 ºC day/night); 2: cold storage, storing tuber at zero ° C, RH 90-95%; 3: storing tubers in the soil; 4: wrapping with polyethylene x cold storage; 5: treating tubers with clove extract x wrapping with polyethylene x cold storage; 6: treating tubers with garlic extract x wrapping with polyethylene x cold storage; 7: treating tubers with cumin oil x wrapping with polyethylene x cold storage; 8: storing tubers in moist peatmoss at ambient temperature; 9: treating tubers with clove extract x storing in peatmoss x ambient temperature; 10: treating tubers with garlic extract x storing in peatmoss x ambient temperature; 11: treating tubers with cumin oil x storing in peatmoss x ambient temperature.

Dry matter, carbohydrate and inulin

During the storage period for tubers were stored at 2ºC and treated with garlic extract, we observed a slightly increase the content of dry matter. On the other hand, a fast increases of dry matter due to control treatment (Fig. 1), suggesting principally to the loss of a certain quantity of water of the tuber (Fig. 4 and Photo 1). Moreover, during long term at cold storage conditions (without application of plant extract), shrinking and water losses were happened in tubers (photo 2).

The structure of Inulin depends upon many factors, such as the plant source from which it is extracted, the climate and growing conditions, the harvesting maturity and the storage time after harvest (De Leenheer and Hoebregs, 1994; Coussement, 1999).





Treat-ments	30 DAS	60 DAS	90 DAS	120 DAS	Mean
1	40.00	60.00	80.00	100.00	70.00
2	14.50	16.81	17.00	18.00	16.58
3	12.00	13.26	14.58	15.65	13.87
4	1.50	2.50	4.00	5.00	3.25
5	0.00	0.00	0.00	0.00	0.00
6	0.00	0.00	0.00	0.00	0.0
7	0.00	0.00	0.00	0.00	0.00
8	1.83	1.83	2.68	3.98	2.58
9	0.00	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00	0.00
11	0.00	0.00	0.00	0.00	0.00
Mean	6.35	8.58	10.75	12.97	9.66
L.S.D.	5.16	2.62	2.83	1.03	------

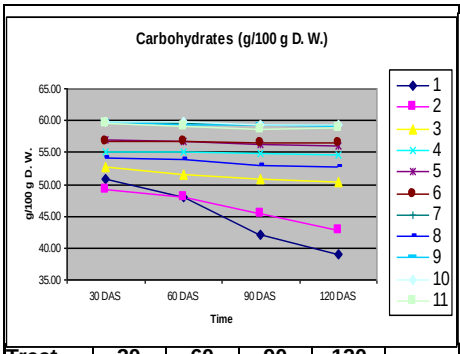
Fig. 3: Decay of Jerusalem artichoke tubers as affected by plant extract (average two seasons).

Treat-ments	30 DAS	60 DAS	90 DAS	120 DAS	Mean
1	25.65	26.68	38.35	38.66	32.34
2	22.85	23.93	26.73	26.00	24.88
3	22.60	21.61	24.00	25.98	23.55
4	23.28	21.06	24.80	24.95	23.52
5	23.50	22.20	28.22	28.60	25.63
6	23.80	22.32	26.14	27.80	25.02
7	22.68	20.19	30.05	30.00	25.73
8	22.58	21.80	20.10	25.00	22.37
9	23.72	24.52	25.50	26.00	24.94
10	24.28	26.50	26.80	27.00	26.15
11	23.61	27.60	27.50	28.00	26.68
Mean	23.50	23.49	27.11	28.00	25.53
L.S.D.	1.72	1.78	3.56	3.01	------

Fig. 4: Dry matter of Jerusalem artichoke tubers as affected by plant extract (average two seasons).

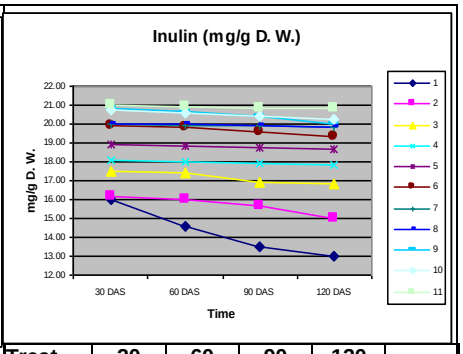
Treatments: 1: control, storing tubers at ambient temperature (25/10 ºC day/night); 2: cold storage, storing tuber at zero º C, RH 90-95%; 3: storing tubers in the soil; 4: wrapping with polyethylene x cold storage; 5: treating tubers with clove extract x wrapping with polyethylene x cold storage; 6: treating tubers with garlic extract x wrapping with polyethylene x cold storage; 7: treating tubers with cumin oil x wrapping with polyethylene x cold storage; 8: storing tubers in moist peatmoss at ambient temperature; 9: treating tubers with clove extract x storing in peatmoss x ambient temperature; 10: treating tubers with garlic extract x storing in peatmoss x ambient temperature; 11: treating tubers with cumin oil x storing in peatmoss x ambient temperature.

Increase of carbohydrates and inulin were found as a result of use garlic and cumin extract at ambient temperature (Figs 5 and 6) .These changes corresponded with the increase in dry matter (Fig. 4). Molder *et al*. (1993) found that higher storage temperature encouraged breakdown of inulin and utilization of monosaccharide formed from breakdown, presumably due to higher respiration and other metabolic activities (tuber stored at 2ºC or ambient temperature without treated with plant extract).



Treat-ments	30 DAS	60 DAS	90 DAS	120 DAS	Mean
1	50.84	48.08	42.18	39.13	45.06
2	49.26	48.07	45.44	42.75	46.38
3	52.68	51.54	50.83	50.24	51.32
4	55.11	55.00	54.92	54.72	54.94
5	56.97	56.83	56.20	56.03	56.51
6	56.78	56.65	56.54	56.49	56.62
7	59.81	59.60	59.31	59.25	59.49
8	54.12	53.87	53.00	52.68	53.42
9	59.79	59.40	59.34	59.12	59.41
10	59.88	59.70	59.40	59.25	59.56
11	59.60	59.14	58.69	58.86	59.07
Mean	55.89	55.26	54.17	53.50	54.71
L.S.D.	2.34	3.01	3.41	3.40	------

Fig. 5: Carbohydrates of Jerusalem artichoke tubers as affected by plant extract (average two seasons).



Treat-ments	30 DAS	60 DAS	90 DAS	120 DAS	Mean
1	16.00	14.59	13.51	12.97	14.27
2	16.16	16.00	15.64	15.02	15.71
3	17.52	17.40	16.91	16.86	17.17
4	18.09	18.00	17.89	17.83	17.95
5	18.89	18.82	18.74	18.64	18.77
6	19.91	19.85	19.62	19.32	19.68
7	20.00	19.95	19.89	19.83	19.92
8	20.00	20.00	19.95	19.86	19.95
9	20.80	20.65	20.40	20.00	20.46
10	20.75	20.62	20.40	20.24	20.50
11	21.00	20.95	20.85	20.81	20.90
Mean	19.01	18.80	18.53	18.31	18.66
L.S.D.	0.40	0.49	0.22	0.63	------

Fig. 6: Inulin of Jerusalem artichoke tubers as affected by plant extract (average two seasons).

Treatments: 1: control, storing tubers at ambient temperature (25/10 ºC day/night); 2: cold storage, storing tuber at zero º C, RH 90-95%; 3: storing tubers in the soil; 4: wrapping with polyethylene x cold storage; 5: treating tubers with clove extract x wrapping with polyethylene x cold storage; 6: treating tubers with garlic extract x wrapping with polyethylene x cold storage; 7: treating tubers with cumin oil x wrapping with polyethylene x cold storage; 8: storing tubers in moist peatmoss at ambient temperature; 9: treating tubers with clove extract x storing in peatmoss x ambient temperature; 10: treating tubers with garlic extract x storing in peatmoss x ambient temperature; 11: treating tubers with cumin oil x storing in peatmoss x ambient temperature.

Storage of Jerusalem artichoke tubers at low temperature (4 ºC) for 34 days also increases the fructo-oligosaccharide content (Kang *et al*., 1993). The study made by Ben Chekroun *et al*. (1994) on the Fuseau variety has shown that after the third week of storage, the decrease in the content of total carbohydrate was evaluated at 0.98% of the fresh weight/week of storage. On the other hand, use of plant extract reduced starch degradation and sugar changes (Daniels *et al*., 1996). Moreover, Davies (1990) who indicated that basic constituents (monoterpenes and antioxidants) trended to slow down the activity of carbohydrates and protein breakdown associated enzymatic systems as well as respiration and energy metabolism enzyme.

Photo 1: Control, storing tubers at ambient temperature (25/10 ºC day/night), after 5 months.

Photo 2: Jerusalem artichoke tubers during cold storage conditions (2 ºC, 90-95 RH), after 5 months.

Photo 3: Jerusalem artichoke tubers without application of essential oil or plant extract and wrapped with polyethylene at ambient temperature.

Photo 4: Jerusalem artichoke tubers after 5 months of storage in the soil.

Photo 5: Jerusalem artichoke tubers treating with clove extract x storing in peatmoss x ambient temperature. Photos were taken after 5 months of storing.

Photo 6: Jerusalem artichoke tubers storing in peatmoss x ambient temperature. Photos were taken after 5 months of storing.

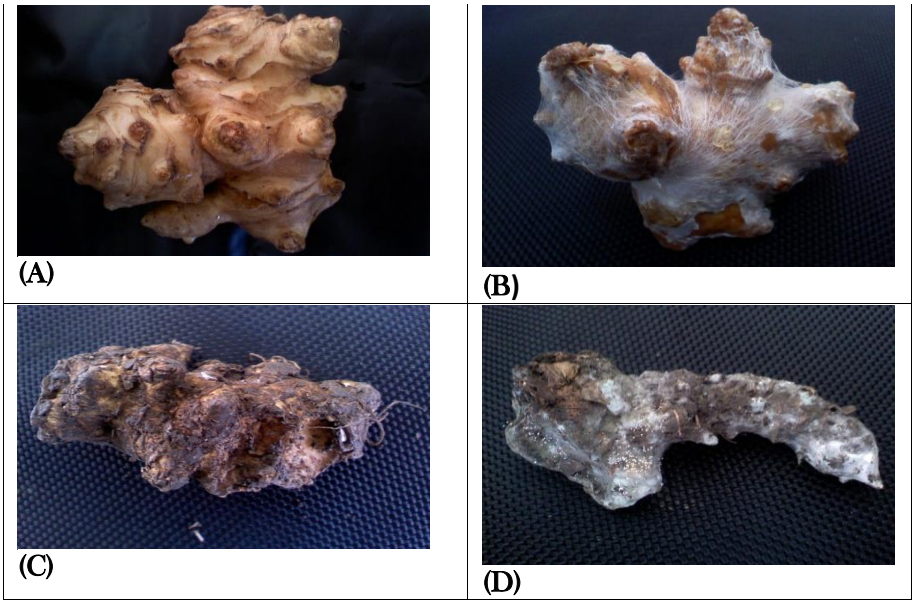


Photo 7: Jerusalem artichoke tubers naturally infected by tuber rots fungi. A), healthy tuber, B), tuber rot caused by *Sclerotium rolfesii*, C) dry tuber rot caused by *Rhizoctonia solani* (note the mummy tuber symptom) and D) tuber rot complex (caused by *Apergillus* spp., *Penicillum* spp*., Fusarium* spp. and *Sclerotium rolfesii*).

Isolation of fungi associated with rotted Jerusalem tubers:

Many different fungi were successfully isolated from different Jerusalem tuber rots. A total of 13 genera and 19 species of fungi were successfully isolated from different Jerusalem tubers rots. Of all the isolated fungi, *Rhizoctonia solani*, followed by *Sclerotium rolfesii*, *Muor* spp. and *Penicillium* spp. had the highest percentage frequency of occurrence (Table 2). *F. incarnatum* (37.3%) was most frequently isolated *Fusarium* species, followed by *F. solani* (30.85%), while *F. oxysporum* and *F. verticillioides* (8.2 and 3.2%, respectively) were detected at low percentages. Others fungal species including *Aspergillus niger* and A*. flavus* were recorded at moderate percentages (15.73 and 15.38%, respectively), where *Trichoderma harzianum*, *Cladosporium* sp., *Alternaria alternate*, *Cunninghamella* sp., *A. ochraceus*, *Aspergillus glaucus*, *Stemphylium* sp. and *Gliocladium rosum* were detected at low percentages.

Tubers rot studies:

Data obtained from the pathogencity tests with fungal isolates, revealed that *S. rolfesii* (Photo 7B) was the most important isolates caused rot of tubers. It was easily recognized by presence of profuse growth of robust white of mycelium that caused a white to light brown soft rot, which covered the entire tuber surface (Photo 7A). Other types of rots were observed as

moderate dry rot symptoms caused by the two *Fusarium* species e.g., *F. oxysporum* and *F. solani* in all tubers (3.5 and 2 cm^3, respectively). The pathogens were then recovered from the inoculated tubers (Photo 7D). The symptoms of rotted tubers caused by *R. solani* appears as dark brown to black sclerotia on the surface of the tuber. Infected tuber surface showed small, flat, barely visible blotches to large area (Photo 7C). In compassion, no lesions were observed in tubers inoculated with sterile agar plugs, whereas, none of the other isolated fungi was pathogenic.

In vitro antifungal activity of the plant extracts:

The growth reduction of *S. rolfesii*, *F. oxysporum*, *F. solani* and *R. solani* in response to the tested plant extracts is presented in Table 3. All tested plant extracts had an antifungal activity against fungi radial growth of fungi decreased significantly with increasing the concentration of plant extracts. High growth inhibition was observed at concentration 80% and 60% of each plant extract. The highest antifungal activity was recorded for clove, garlic and cumin those caused complete inhibition (100%) at concentration 80%. Our results indicated that all tested plant extracts showed an antifungal activity against *R. solani* with the highest effect of clove extract. This finding is in agreement with that of Beg and Ahmad (2002). Eugenol is main component of clove oil. Pepeljnjak *et al*. (2003) pointed out that eugenol is one of the strongest inhibitors of enzyme processes and related compounds as methyle- or actyleugenol could change this property.

Table 3: Growth of Jerusalem tuber rot fungi as affected by different extracts concentration.

Rot fungi	Clove extract			Cumin extract			Garlic extract		
	40%	60%	80%	40%	60%	80%	40%	60%	80%
Alternaria alternata	60.33 g	84.67 de	100.00 a	57.33 g	80.67 de	100.00 a	63.33 g	87.67 cd	100.00 a
Aspergillus flavus	70.67 e	89.67 c	100.00 a	67.67 e	85.67 c	100.00 a	73.67 e	92.67 b	100.00 a
Aspergillus glaucus	65.33 f	85.33 de	100.00 a	62.33 f	81.33 de	100.00 a	68.33 f	88.33 cd	100.00 a
Aspergillus niger	80.33 bc	100.00 a	100.00 a	77.33 bc	96.00 a	100.00 a	83.33 bc	100.00 a	100.00 a
Aspergillus ochraceus	65.33 f	90.33 c	100.00 a	62.33 f	86.33 c	100.00 a	68.33 f	93.33 b	100.00 a
Cladosporium sp.	75.00 d	85.00 de	100.00 a	72.00 d	81.00 de	100.00 a	78.00 d	88.00 cd	100.00 a
Cunninghamella sp.	60.00 g	89.00 c	100.00 a	57.00 g	85.00 c	100.00 a	63.00 g	92.00 b	100.00 a
Fusarium incarnatum	78.00 cd	66.00 h	100.00 a	75.00 cd	62.00 h	100.00 a	81.00 cd	69.00 g	100.00 a
Fusarium oxysporum	76.00 d	79.67 g	100.00 a	73.00 d	75.67 g	100.00 a	79.00 d	82.67 f	100.00 a
Fusarium solani	65.00 f	82.67 ef	100.00 a	62.00 f	78.67 ef	100.00 a	68.00 f	85.67 de	100.00 a
Fusarium verticillioides	85.00 a	88.67 c	100.00 a	82.00 a	84.67 c	100.00 a	88.00 a	91.67 b	100.00 a
Gliocladium rosum	85.00 a	88.33 c	100.00 a	82.00 a	84.33 c	100.00 a	88.00 a	91.33 b	100.00 a
Mucor sp.	80.00 bc	90.67 c	100.00 a	77.00 bc	86.67 c	100.00 a	83.00 bc	93.67 b	100.00 a
Penicillium sp.	82.00 abc	94.67 b	100.00 a	79.00 abc	90.67 b	100.00 a	85.00 abc	97.67 a	100.00 a
Rhizoctonia solani	75.00 d	85.33 de	100.00 a	72.00 d	81.33 de	100.00 a	78.00 d	88.33 cd	100.00 a
Rhizopus stolonifer	82.00 abc	81.00 fg	100.00 a	79.00 abc	77.00 fg	100.00 a	85.00 abc	84.00 ef	100.00 a
Sclerotium rolfesii	75.00 d	84.67 de	100.00 a	72.00 d	80.67 de	100.00 a	78.00 d	87.67 cd	100.00 a
Stemphylium sp.	83.00 ab	85.67 d	100.00 a	80.00 a	81.67 d	100.00 a	86.00 ab	88.67 c	100.00 a
Trichoderma harzianum	80.00 bc	84.00 de	100.00 a	77.00 bc	80.00 de	100.00 a	83.00 bc	87.00 cd	100.00 a

Each of the data is a mean of three replicates. Each data followed by the same letter (s) along the columns not significantly different at *p*= 0.05. Using Duncan Multiple Range Test to separate the means.

Antimicrobial activity of this oil can be attributed to the presence of an aromatic nucleus and a phenolic OH group that are known to be reactive and can form hydrogen bonds with-SH group in the active sites of target enzymes, resulting in deactivation of enzymes in fungi (Velluti *et al*. 2003; Alma *et al*., 2007).The sites and number of hydroxyl groups on the phenol group are thought to be related to the irrelative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity.

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معـاملات طبيعيــة لإطالــة العمـر التخزينــي ومقاومــة الأمـراض الفطريــة لـدرنات الطرطوفة الطازجة

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أجريت تجربتان تخزين بمحطة بحوث البساتين بالمنصورة- معهد بحوث البساتين- مركز البحوث الزراعية فى موسمين متتاليين ٢٠٠٩-٢٠١٠م لدراسة تأثير المعاملات الطبيعية باستخدام المستخلصات النباتية (القرنفل، الكمون، والثوم) بتركيز ٨٠% لأطالة فترة التخزين ومقاومة الأمراض الفطرية لدرنات الطرطوفة الطازجة المخزنة على ٢ درجة مئوية ومغلفة بأكياس البولى ايثلين ودرجة حرارة الغرفة ٢٥ درجة مئوية نهارا /١٠ درجة مئوية ليلا ، والموضوعة مع طبقات متبادلة من البيت موس فى صناديق كرتون ولمعرفة الفطريات المسببة لأعفان درنات الطرطوفة أثناء التخزين.

تم تجميع الدرنات من حقول مختلفة فى مصر وتم عزل هذه الفطريات فى معمل الأمراض - قسم امراض النبات - كلية زراعة - جامعة المنصورة، وتم التعرف على هذه الفطريات ونسبة تركيزها ثم عوملت الفطريات بالمستخلصات السابقة بتركيز ٤٠، ٦٠ و٨٠%.

وكانت أهم النتائج كمايلى:-

١- أعطت الدرنات المعاملة بمستخلص القرنفل والكمون والمخزنة على درجة حرارة ٢ درجة مئوية أو المخزنة على درجة حرارة الغرفة أقل القيم فى نسبة التزريع والفقد فى الوزن ونسبة التالف، في موسمي الدراسة.

٢- أدت معاملة الدرنات بمستخلص الثوم والمخزنة على درجة حرارة ٢ درجة مئوية الى زيادة محتوى الدرنات من المادة الجافة، بينما تفوقت الدرنات المعاملة بمستخلص الكمون والثوم والمخزنة على درجة حرارة الغرفة معنويا علي باقي المعاملات في محتوى الدرنات من المادة الجافة والكربوهيدرات والأنولين في كلا الموسمين.

٣- تم التعرف على الفطريات المسببة للأعفان التى تصيب الطرطوفة، وكان من أهمها سلكورتيم رولفيسى، فيوزاريم سولانى، فيوزاريم اوكسبورم، ريزوكتونيا سولانى.

٤- أعطت جميع معاملات المستخلصات النباتية بتركيز ٨٠% تثبيط كامل لنشاط الانواع المختلفة للفطريات.

توصي هذه الدراسة باستخدام المستخلصات النباتية لنباتات القرنفل والكمون والثوم بتركيز ٨٠% لتخزين درنات الطرطوفة الطازجة مع التغليف بالبوليثيلين علي درجة ٢ مئوية أو في طبقات البيتموس علي درجة حرارة الغرفة لمدة ٥ أشهر مع الحفاظ علي المكونات الغذائية وصفات الجودة وتثبيط كامل لنمو الفطريات المسببة للأعفان أثناء التخزين.

قام بتحكيم البحث

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