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Effect of storage on the physical and chemical ingredients of Siwa mint (*Mentha spicata* L. cv. Siwa) plants

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Abstract
This postharvest experiment was carried out in Egypt during the two successive seasons of 2015/2016 and 2016/2017 to study the effect of storage conditions on quantity and quality of dry leaves of *Mentha spicata* L. cv. Siwa plants. The design of experiment was split plot. The main plots included four storage periods:- 0, 4, 8 and 12 months at room temperature of 22-25ºC and a relative humidity of 50-60% whereas the sub plots included using of four packaging materials:- polyethylene bags, brown kraft paper bags (90 g), polypropylene bags and linen bags. Results showed that increasing of storage periods significantly decreased weight of leaves and essential oil percent as well as changed oil chemical composition. The significant lowest loss in weight and essential oil percent was recorded by packing in polyethylene bags followed by kraft paper bags, polypropylene bags and then linen bags. Due to environmental, health and commercial commitments it was concluded that under room temperature conditions, dry leaves should be stored for only 4 months in kraft paper bags followed by linen bags to have the minimum loss in quantitative and qualitative aspects.

Keywords: *Mentha spicata* L. cv. Siwa, storage periods, packaging materials.

1. Introduction
Storage of medicinal and aromatic plants is the transition between producer and consumer. Medicinal and aromatic plants are stored either for marketing or as raw material for manufacturing of medicines, cosmetics and food additives. The quality of stored parts is depending on storage conditions efficiency i.e. air temperature, light, humidity and packaging material. Therefore, it is important to examine the extent of changes in physical and chemical properties of stored medicinal and aromatic plants to avoid negative effects that can occur to humans as a result of changes in chemical active constituents during storage as well as to achieve highest possible economic return from storing process. Standardization of storage and packaging of raw herbal drugs result in highly safe, effective and high quality herbal products which would accelerate the global acceptance [1-4].
Siwa mint (*Mentha spicata* L. cv. Siwa, Family: Lamiaceae) is a distinguished geno and chemo type of spearmint grown in Siwa Oasis in the western desert of Egypt and is characterized by a higher essential oil content than other spearmint available in the local market and so, is highly valued by consumers for its strong aroma and sold for much higher prices than any other spearmint in the local market. It is used for preparing spicy tea besides its uses in folk medicine [5, 6].
Just as other foliage herbs the mint of Siwa Oasis cannot be kept for long periods after harvesting and its quantity and quality may deteriorate during storage. Until now, very little research was done in Egypt on postharvest treatments for dry medicinal and aromatic plants. In this concern, very little is known about the effect of storage conditions on properties of dry crude drugs. Thus, the objective of this work is to observe the effects of storage periods, packaging materials and their interaction on physical and chemical ingredients of stored dry Siwa mint to attain improvements for its current preserve processes.

2. Materials and Methods
The investigated raw material of the experiment was obtained from cultivated Siwa mint (*Mentha spicata* L. cv. Siwa) plants in the Agricultural Experimental Station of the Desert Research Center at Khamisa Village (29.21º N and 25.40º E), Siwa Oasis, Egypt.
Rhizomes of Siwa mint were cultivated in the field on March 11th 2015 and 2016, respectively under drip irrigation system in rows 75 cm apart and 30 between hills. The soil texture of the experimental station was sandy and the available irrigation water was from a well that had a

salinity level of 2824 ppm. All agricultural practices were carried out according to recommendations of the Egyptian Ministry of Agriculture and Land Reclamation. Plants were harvested twice per season on May 25th and July 15th for both seasons and the herbage of the second cuts were chosen for conducting this experiment.

Mint leaves were separated from remaining parts of herb and were subjected to open shade air drying for two weeks and then were ready for the next steps of the experiment.

The arrangement of experiment was split plot design with three replicates. The main plots included four storage periods:- 0, 4, 8 and 12 months at room temperature of 22-25ºC and a relative humidity of 50-60% whereas the sub plots included using of four packaging material types:- polyethylene bags, brown kraft paper bags (90 g), polypropylene bags and linen bags.

Samples of dry leaves were taken for analyses before storage and at the end of each storage period. Manner of sampling ensured its being representative of the entire lot of the raw material. L.S.D. test at 0.05 was used to compare the average means of treatments, according to [7].

The following data were recorded:-

A) Quantity parameters

Dry weight: Leaves samples of 100 g were randomly chosen from each treatment.

B) Quality parameters

i) Essential oil percentage

Essential oil percentage was determined in dry leaves by hydrodistillation for three hours using a Clevenger type apparatus. Essential oil content was calculated as a relative percentage (v/w) [8].

ii) Essential oil chemical composition

GC-MS analyses of extracted volatile oils of the second season were conducted using Gas Chromatography-Mass Spectrometry instrument stands at the Laboratory of Medicinal and Aromatic Plants, National Research Center, Egypt with the following specifications. Instrument: a Trace GC Ultra Gas Chromatographs (Thermo Scientific Corp., USA), coupled with a Thermo mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TR-5MS column (30 m x 0.32 mm i.d., 0.25 μm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.3 ml/min at a split ratio of 1:10 at the following temperature program: 80ºC for 1 min; rising at 4ºC/min to 300ºC and held for 1min. The injector and detector were held at 220 and 200ºC, respectively. Diluted samples (1:10 hexane, v/v) of 1 μL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. The separated components of the essential oil were identified by matching with the National Institute of Standards and Technology (NIST) published.

3. Results and Discussions

3.1 Quantity parameters

Data presented in Table (1) and illustrated in Fig. (1) show the effects of storage periods, packaging materials and their interaction on weight of stored dry leaves in both seasons.

Regarding the effect of storage periods, there was a significant decline in leaves weight in parallel with extending of storage periods from 0 to 8 months while this decline was insignificant within 8 and 12 month. As regards to the effect of packaging materials, there were significant variations among the different packaging types and the lowest loss in weight was obtained by using of polyethylene bags followed by kraft paper bags, polypropylene bags and then linen bags. These variations within materials may be owing to its dissimilar air permeability. As for the effect of treatments interaction, results revealed that samples stored for only 4 months and packed in polyethylene bags followed by kraft paper bags, polypropylene bags and then linen bags recorded significantly the minimum loss in weight in comparison to other treatments.

These findings were in harmony with those reported by [9] on *Rosmarinus officinalis* who found that increasing of storage period increased weight loss of herb as well as the different packaging materials had significant effects on weight loss during storage.

Table 1: Effect of storage periods, packaging materials and their interaction on weight (g) of stored dry leaves in both seasons of 2015/2016 and 2016/2017

Packaging materials / Storage periods	Polyethylene bags	Kraft paper bags	Polypropylene bags	Linen bags	Mean
0 months	100.00	100.00	100.00	100.00	100.00
4 months	99.05	98.79	96.69	95.19	97.43
8 months	98.29	96.98	94.85	93.17	95.82
12 months	98.18	96.78	94.74	92.97	95.67
Mean	98.88	98.14	96.57	95.33	

LSD at 0.05
Storage periods = 0.40
Packaging materials = 0.40
Interaction = 0.57

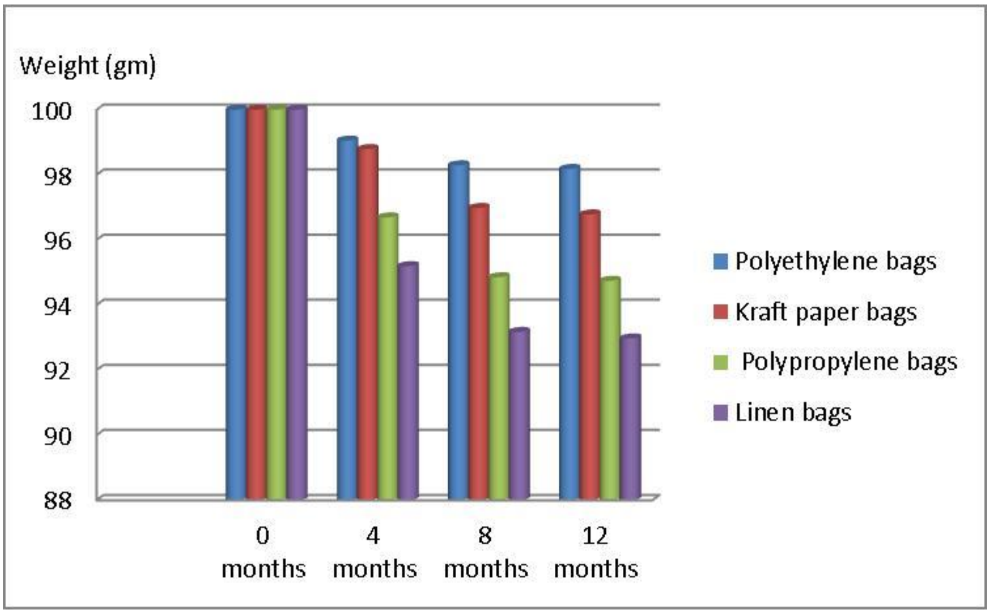

Fig 1: Effect of the interaction among treatments on weight of dry leaves

3.2 Quality parameters

3.2.1 Essential oil percentage

Data presented in Table (2) and illustrated in Fig. (2) show the effects of storage periods, packaging materials and their interaction on essential oil percentage of stored dry leaves in both seasons.

Dealing with the effect of storage periods, there was a significant reduction in volatile oil percentage with prolonging of storage periods and the lowest percent was detected after storage for 12 months. Concerning the influence of packing materials, results showed significant variations among the different materials and the least loss in oil percent was recorded by using of polyethylene bags followed by kraft paper bags, polypropylene bags and then linen bags. The combination between treatments proved that, samples stored for only 4 months and packed in polyethylene bags followed by kraft paper bags, polypropylene bags and then linen bags significantly recorded the lowest loss in oil percent as compared to other treatments.

These results were in good line with other researches on stored family Lamiaceae plants i.e. on *Ocimum basilicum*, [10] reported that the losses of total essential oil were 19, 62 and 66% at three, six and seven months storage, respectively; [11] found a decrease in essential oil percentage in the dried leaves stored for 6 and 12 months in either cotton or polypropylene packages comparing to paper packages which recorded the highest values and [9] who observed that increasing of storage period caused decreasing in the volatile oil % which should not exceed 2 months and the relative small quality degradation that occurred during storage was in jute sacks. Also, [12] stored spearmint and peppermint herbs for 12 months and the herb was sampled each 3 months, He indicated that extended storage period resulted in the reduction of essential oil percent of the two herbs. Packaging containing polyethylene showed superiority in their keeping capacities to their contents among all the tested ones.

Table 2: Effect of storage periods, packaging materials and their interaction on essential oil percentage of stored dry leaves in both seasons of 2015/2016 and 2016/2017.

Packaging materials / Storage periods	Polyethylene bags	Kraft paper bags	Polypropylene bags	Linen bags	Mean
0 months	3.63	3.63	3.63	3.63	3.63
4 months	3.44	3.33	3.24	3.18	3.30
8 months	3.33	2.99	2.95	2.90	3.04
12 months	3.03	2.83	2.74	2.68	2.82
Mean	3.36	3.20	3.14	3.10	

LSD at 0.05
Storage periods = 0.0
Packaging materials = 0.03
Interaction = 0.04

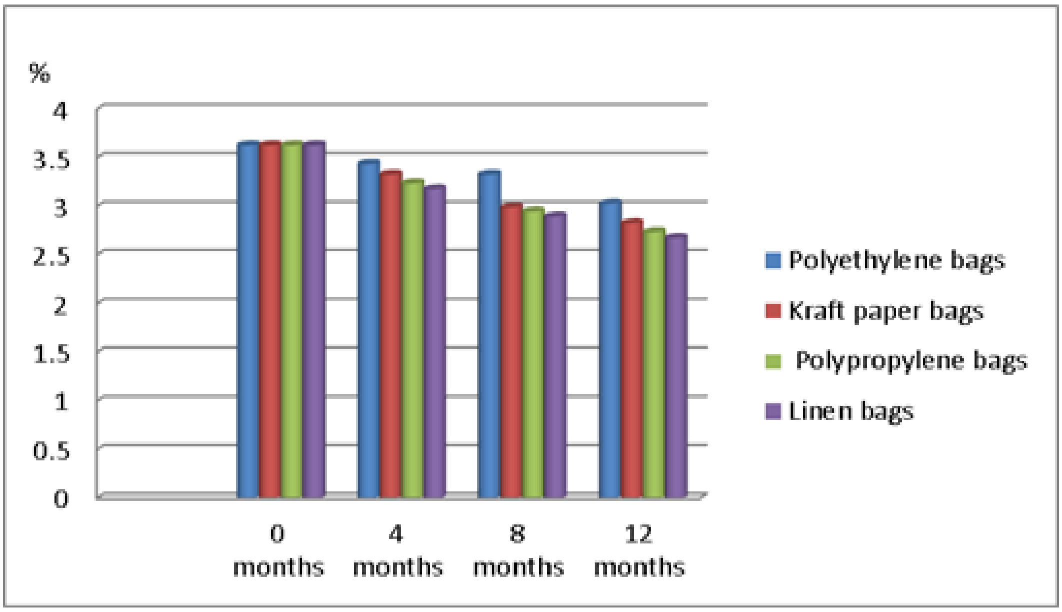

Fig 2: Effect of the interaction among treatments on essential oil percentage of dry leaves.

3.2.2 Essential oil chemical composition

Data presented in Tables (3, 4 and 5) verified the effect of interaction between treatments on essential oil chemical constituents.

The conducted GC-MS analysis before storage proved that oilchemical composition comprises mainly of carvone (53.10 %)and d-limonene (30.28 %) as two principal components.

During storage periods a decrease in d-limonene content andan increase in carvone content were observed then the concentration of carvone was decreased after four months of storage. This is probably attributed to the conversion of d-limonene to carvone during storage duration. The values of carvone constituent were 67.94, 70.18, 71.60 and 61.15% after four months of storage; 64.89, 62.85, 62.89 and 59.99% after eight months of storage and 65.02, 61.15, 61.50 and 61.57% after twelve months of storage for polyethylene, kraft paper, polypropylene and linen bags, respectively. Whereas, the values of d-limonene constituent were 19.56, 18.89, 18.11 and 24.12% after four months; 20.25, 22.74, 22.91 and 24.14% after eight months and 19.96, 22.59, 22.50 and 22.55% after twelve months for polyethylene, kraft paper, polypropylene and linen bags, respectively.

Table 3: Constituents of Siwa mint volatile oil before and after storage of dry leaves for 4 months in different packing materials.

Compound	Before storage	After storage for 4 months			
		Polyethylene bags	Kraft paper bags	Polypropylene bags	Linen bags
α-Pinene	2.42	1.04	0.77	0.62	1.27
Camphene	0.18	0.10	0.08	-	0.11
Sabinene	1.25	0.48	0.40	0.36	0.64
2-α-Pinene	2.73	1.18	0.94	0.80	1.46
α-Myrcene	-	0.35	0.29	0.30	0.51
1,3,8-p-Menthatriene	-	0.24	0.20	0.18	0.14
α- Humulene	-	0.15	0.13	0.11	0.11
D-limonene	30.28	19.56	18.89	18.11	24.12
1,8-Cineole	3.80	4.99	4.44	4.28	5.45
ç-Terpinene	-	0.20	0.17	0.16	0.17
4-Terpineol	-	-	-	0.18	0.29
Trans-ocimene	0.12	-	-	-	-
p-Mentha-1,4(8)-diene	-	-	-	0.07	-
Cis-sabinene hydrate	0.40	0.21	0.20	0.21	0.29
Isomenthone	-	0.18	0.16	-	0.19
Trans-ρ-mentha-2,8-dienol	0.15	-	-	-	-
Cis-limonene oxide	0.12	-	-	-	-
L-Menthone	0.33	-	-	0.16	-
Isoborneol	0.25	0.22	0.19	-	0.26
p-Menth-1-en-4-ol	0.31	0.27	0.20	-	-
p-Menth-1-en-8-ol	0.47	0.14	0.14	0.28	0.18
Cis-dihydrocarvone	0.50	0.34	0.25	0.26	0.32
Trans carveol	0.53	0.33	0.23	0.24	0.47
Cis carveol	0.31	0.12	0.10	0.10	0.19
Pulegone	1.01	0.59	0.57	0.66	0.73
Carvone	53.10	67.94	70.18	71.60	61.15
α-Bourbonene	0.50	0.33	0.35	0.28	0.46
α-elemene	0.18	0.10	0.10	0.08	0.23

Trans caryophyllene	0.46	0.32	0.35	0.29	0.46
α-Cubebene	0.16	-	-	-	-
Germacreden-D	0.19	0.23	0.26	0.29	0.40
Bicyclogermacrene	0.12	0.08	0.09	-	-
Cadina-1,3,5-triene	-	0.09	0.09	0.09	0.13
Cubenol	0.13	0.09	0.08	0.14	0.11
Total identified compounds	100	100	100	100	100
Total hydrocarbon compounds	38.59	24.45	23.11	21.74	30.21
Total oxygenated compounds	61.41	75.55	76.89	78.26	69.79

Table 4: Constituents of Siwa mint volatile oil after storage of dry leaves for 8 months in different packing materials.

Compound	After storage for 8 months			
	Polyethylene bags	Kraft paper bags	Polypropylene bags	Linen bags
α-Pinene	1.05	1.17	1.21	1.34
Camphene	0.10	0.11	0.11	0.12
Sabinene	0.54	0.58	0.55	0.65
Trans sabinene hydrate	0.27	0.17	0.17	0.30
2-α-Pinene	1.29	1.42	1.41	1.55
α-Myrcene	0.38	0.42	0.44	0.47
1,3,8-p-Menthatriene	0.26	0.20	0.25	0.24
α- Humulene	0.15	0.14	0.15	0.16
D-limonene	20.25	22.74	22.91	24.14
1,8-Cineole	5.49	5.72	5.57	5.93
ç-Terpinene	0.21	0.22	0.22	0.22
4-Terpineol	0.32	0.38	0.38	0.26
Trans-ocimene	-	-	-	-
p-Mentha-1,4(8)-diene	-	-	-	-
Cis-sabinene hydrate	-	-	-	-
Isomenthone	0.20	0.19	0.19	0.20
Trans-ρ-mentha-2,8-dienol	-	-	-	-
Cis-limonene oxide	-	-	-	-
L-Menthone	-	-	-	-
Isoborneol	0.28	0.27	0.26	0.28
p-Menth-1-en-4-ol	-	-	-	-
p-Menth-1-en-8-ol	0.18	0.16	0.17	0.17
Cis-dihydrocarvone	0.40	0.33	0.33	0.32
Trans carveol	0.53	0.43	0.38	-
Cis carveol	0.18	0.14	0.14	0.62
Pulegone	0.66	0.64	0.63	0.67
Carvone	64.89	62.85	62.89	59.99
Carvone oxide	0.18	0.17	0.15	0.18
α-Bourbonene	0.59	0.43	0.41	0.61
α-elemene	0.18	0.14	0.12	0.17
Trans caryophyllene	0.56	0.39	0.39	0.56
α-Cubebene	-	-	-	-
Germacreden-D	0.43	0.27	0.26	0.44
Bicyclogermacrene	0.13	-	0.09	0.12
Cadina-1,3,5-triene	0.18	0.11	0.12	0.17
Cubenol	0.12	0.12	0.10	0.12
α-Cadinol	-	0.09	-	-
Total identified compounds	100	100	100	100
Total hydrocarbon compounds	26.30	28.34	28.64	30.96
Total oxygenated compounds	73.70	71.66	71.36	69.04

Table 5: Constituents of Siwa mint volatile oil after storage of dry leaves for 12 months in different packing materials.

Compound	After storage for 12 months			
	Polyethylene bags	Kraft paper bags	Polypropylene bags	Linen bags
2-Thujene	0.08	-	-	-
α-Pinene	1.27	1.38	1.39	1.51
Camphene	0.11	0.12	0.13	0.14
Sabinene	0.55	0.62	0.59	0.62
Trans sabinene hydrate	-	-	0.21	-
2-α-Pinene	1.44	1.60	1.60	1.69
α-Myrcene	0.46	0.45	0.45	0.39

1,3,8-p-Menthatriene	0.26	0.21	0.25	0.24
α- Humulene	0.18	0.16	0.18	0.17
D-limonene	19.96	22.59	22.50	22.55
1,8-Cineole	5.76	6.29	6.29	6.35
ç-Terpinene	0.27	0.23	0.25	0.24
4-Terpineol	0.47	0.36	0.40	0.35
Trans-ocimene	-	-	-	-
p-Mentha-1,4(8)-diene	-	-	-	-
Cis-sabinene hydrate	0.18	0.25	-	0.21
Isomenthone	-	-	0.28	0.20
Trans-ρ-mentha-2,8-dienol	-	-	-	-
Cis-limonene oxide	-	-	-	-
L-Menthone	0.21	0.20	-	-
Isoborneol	-	0.31	0.33	0.27
p-Menth-1-en-4-ol	-	-	-	-
p-Menth-1-en-8-ol	0.45	0.19	0.19	0.17
Cis-dihydrocarvone	0.39	0.33	0.35	0.29
Trans carveol	0.56	0.52	0.48	0.37
Cis carveol	0.18	0.18	0.16	0.12
Pulegone	0.72	0.64	0.65	0.62
Carvone	65.02	61.15	61.50	61.57
Carvone oxide	0.19	0.18	0.19	0.16
α-Bourbonene	0.37	0.55	0.44	0.49
α-elemene	0.11	0.18	0.15	0.15
Trans caryophyllene	0.35	0.51	0.43	0.45
α-Cubebene	-	-	-	-
Germacreden-D	0.26	0.41	0.16	0.32
Bicyclogermacrene	-	-	-	0.10
Cadina-1,3,5-triene	0.10	0.16	0.19	0.14
Cubenol	0.10	0.12	0.17	0.12
α-Cadinol	-	0.11	0.09	-
Total identified compounds	100	100	100	100
Total hydrocarbon compounds	25.77	29.17	28.71	29.20
Total oxygenated compounds	74.23	70.83	71.29	70.80

The obtained results were in harmony with [13] who cultivated spearmint in Sudan and stored dry herb at ambient room temperature for 12 months. They found that carvone content was slightly increased during the first four months and progressively decreased at the end of the storage period. Also, several investigators found that most essential oils are thermolabile or heat sensitive and are subjected to oxidative changes in the presence of air and light, and during storage. These changes usually have adverse effects on flavor quality as most flavoring materials are to some extent thermolabile or heat sensitive. Changes in essential oil components during storage could occur by rearrangement, hydrogenation or dehydrogenation of other components [14, 15].

Based on the previous results, the loss of properties after storage for four months was the lowest in all packing materials as follows:-

1. In polyethylene bags the drug properties were: dry weight 99.05 g, essential oil percent 3.44% and carvone percent 67.94%.
2. In kraft paper bags the drug properties were: dry weight 98.79 g, essential oil percent 3.33% and carvone percent 70.18%.
3. In polypropylene bags the drug properties were: dry weight 96.69 g, essential oil percent 3.24% and carvone percent 71.60%.
4. In linen bags the drug properties were: dry weight 95.19 g, essential oil percent 3.18% and carvone percent 61.15%.

Although polyethylene material recorded superior preserve properties all the time as well as polypropylene material had higher preserve properties than linen, both of them are not recommended today as packing materials due to environmental and health commitments. There are possible problems caused by plastic bags. First, plastic bags are mostly made from oil, natural gas or coal, and these are all limited natural resources that must be conserved. Second, the manufacture of plastic produces large quantities of harmful pollutants. Third, old and unwanted plastic bags being composed of toxic chemicals and are not always easy to dispose of. The major chemicals that go into the making of plastic bags are highly toxic, these chemicals can cause an array of maladies ranging from birth defects, cancer, and nerve and immune disorders, to blood and kidney ailments [16].

On the other side, kraft paper bags and linen bags are environmentally friendly and healthy. They are made of materials that are easily regrown, highly bio-degradable, easy to recycle, toxic free and no negative chemical reactions could happen within herb and packet. So, both of kraft paper bags and linen bags could be preferred as safe packaging materials especially for storing of medicinal and aromatic plants.

4. Conclusion

According to the results of this investigation, it can be concluded that under room temperature conditions, the dry leaves of Siwa Oasis mint (*Mentha spicata* L. cv. Siwa) should be stored for only 4 months in kraft paper bags followed by linen bags to reach the minimum loss in quantity and quality characters.

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