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Studies on changes in chemical parameters of *Zanthoxylum rhetsa* during storage

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Abstract
Zanthoxylum rhetsa (Triphal) is an underutilized spice tree widely used by tribal healers for many years. To study the effect of different packaging materials on physico-chemical parameters of *Zanthoxylum rhetsa* during storage at ambient tem- perature, different packaging *viz*. Gunny bag (Control), LDPE bags, HDPE bags, PET (polyethylene terephthalate) jars, Aluminium pouches lined with polyethylene, shrink packaging, CFB boxes, Woven bags and brown paper bags were used. After packing the samples were stored at ambient temperature and the samples were analysed for both physico-chemical qualities at regular intervals of 2 months for a year. The decrease in essential oil, sabinene and terpenen-4-ol content was observed during storage along with a decrease in alcohol soluble extractive and water-soluble extractive. Other factors such as moisture, PLW, and size remained the same. PET bottles could be used to store *Zanthoxylum rhetsa* upto 12 months at ambient condition. The moisture content of the *Z. rhetsa* at the end of storage period was 12.236%. The physiological loss in weight was maintained through the storage period with an average of 2.22%. The physico-chemical factors like essential oil was 1.301%, sabinene was 14.260%, and terpenen-4-ol was 31.056% at 12 months. The ash percent 7.410%, acid insoluble ash 0.156%, alcohol soluble extractive 11.460% and the water soluble extractive 12.893%. The fruit size (diameter in mm) was 7.596 mm and colour value L* (16.461) a* (11.243), b* (15.424) was observed. Yeast and mould growth was observed 0.183 cfu/g−1 at 12 months in PET.

Keywords: *Essential oil*, packaging, sabinene, storage, terpenen-4-ol, *Zanthoxylum rhetsa*

Introduction
Since the dawn of human civilization, the Indian Traditional System of Medicine has played a crucial role in providing healthcare, with records showing that it has successfully treated a wide range of diseases. Considering that 80% of the world's population relies on it as their first line of defence, it also shows promise for protecting human health. (Mallya *et al*., 2019) [41] Traditional medicines are becoming increasingly important due to their therapeutic properties, with 80,000 plant species known to have them. (Karunamoorthi *et al*., 2013) [32] In the Rutaceae family, which is made up of 150 genera, 12 tribes, 7 subfamilies, and potentially 1600 species, *Zanthoxylum* is the largest genus. It can also be found in arid areas. It grows in warm tropical to subtropical or warm temperate zones. (Dassanayake and Fosberg, 1985) [19]. *Zanthoxylum rhetsa* is widely distributed in India, Southeast Asia, Malaysia, and north Queensland. It can be found in India's evergreen woods in Assam, Meghalaya, and the Western Peninsula from the Coromandel and Konkan southward, (Kirtikar and Basu, 1993) [34-35] *Zanthoxylum rhetsa* is a spiny deciduous tree with a height range of 25-30 m. Its leaves are spirally organised, compound, imparipinnate, alternating, and grouped at the extremities of twigs. It has tiny, greenish-yellow, polygamous blooms and small, nearly spherical fruits with black seeds. (Aziz *et al*., 2022) [35] The woody pericarp of the tiny fruits is used as a spice by the inhabitants of Goa, Konkan and Kanara beaches, and Coorg, known locally as "tirphal" (Indian) or "batangberduri" (Malay). It acts as a topical anaesthetic and gives the tongue a tingling feeling. (Shaari *et al*., 2013) [77].

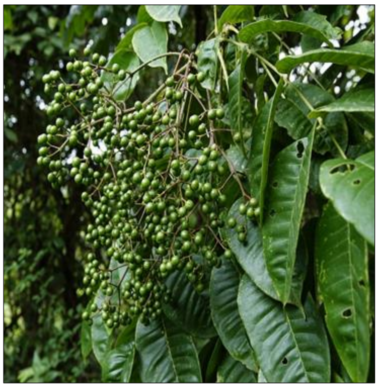

Fig 1: *Zanthoxylum rhetsa* fruits on Tree

Fig 2: Dried fruits of *Zanthoxylum rhetsa*

Table 1: Vernacular Names of Z. rhetsa

Region/Language	Name
English	Indian Prickly Ash, Cape Yellowwood, Indian Ivy-rue (Thejashwini *et al*.2020) [87]
India	Mullilavu, Mullilam, Kothumurikku, Triphala, Teppal, Tirphal (Joy *et al*. 1986; Jirovetz *et al*. 1998; Poornima and Krishnakumar, 2018) [31, 30, 58]
Bangladesh	Bazna, Bazinali, Kantahorina (Alam and Hassan, 2006; Hossain *et al*. 2015) [5, 25]
Thailand	Makhwaen (Bubpawan *et al*. 2015) [15]
Nepal	Rukhboke Timur, Gaai Simal Rukh (Rajbhandari and Amatya, 2019) [60]
Malaysia	Hantu duri, Chenkring (Wong, 2002) [91]

Z. rhetsa has been used by tribal healers for many years and has many pharmacological properties. Its fruit and stem bark are aromatic, energising, astringent, digestive, and stomachic, and its fruits are tasty, good for cholera, asthma, bronchitis, heart prob- lems, piles, and toothaches. An essential oil obtained from the carpels is administered to treat cholera and inflammatory dermatosis, and dandruff and dry eczema are treated with seed oil. The root bark has cholinergic, hypoglycaemic, and spasmolytic properties. (Mallya *et al*., 2019) [40] Z. rhetsa is a plant that contains alkaloids, alkamides, lig- nans, coumarins, terpenes, flavonoids, and phenolic chemicals. Spices absorb moisture from the air due to their hygroscopic nature, which causes powdered goods to soften and clump. Spices lose their flavour and perfume when they are preserved because some aromatic components that are easily volatile oxidise or the volatile oil concentration decreases. Infestation with insects makes spices more likely to degrade during storage, while high humidity, heat, and oxygen hasten the process. (Chaliha *et al*., 2013) [16].

The essential oil concentration of the fruits of *Zanthoxylum rhetsa* is dependent on the amount of sabinene, which is one of the primary ingredients. Standardised packaging materials are used to preserve flavour and enhance shelf life. Indian prickly ash is traditionally packaged in gunny or jute bags with or without a polyethylene liner. As packaging affects the factors that cause or encourage food deterioration, it is crucial for extending the shelf life of goods. The suitable material must have certain properties, such as mechanical and barrier properties, for packaging. (Sangroniz *et al*., 2019) [72].

From ancient times, *Zanthoxylum rhetsa* has been used to treat a wide range of ailments, and it still has an important place in modern Ayurvedic therapies. Although it has a variety of significant pharmacological features, challenges must yet be over- come before it can be used in clinical trials and medication development. Establishing a unified global evaluation system and coming up with strategies to improve the calibre of grown plants and the efficiency of extraction techniques are required to make identification easier.

Materials and Methods

Experimental materials and sample preperation

Samples were taken from local farmers from coastal Konkan region of Ratnagiri district. In July 2020, the *Z. rhetsa* fruits that were gathered for storage after harvesting were utilised. 350 g of *Z. rhetsa* were individually stored for storage in a variety of packaging materials, including a gunny bag (Control), a low-density polyethylene (LDPE) bag, a high-density polyethylene (HDPE) bag, a PET (polyethylene terephthalate) jar, an aluminium pouch lined with polyethylene, shrink packaging, a CFB box, a woven bag, and a brown paper bag.

Plate 3: Different packaging materials used for storage of *Z. rhetsa*

Experimental design and treatments

Using a Factorial completely randomised design (FCRD), a three-factor experiment was set up and replicated three times. The type of packaging, and the length of storage were the two variables used in this experiment.

Table 2: Treatment details

Main treatment	**09 Treatments**
	T1: Gunny bag (Control)
	T2: Low Density Polyethylene bag (LDPE)
	T3: High Density Polyethylene bag (HDPE
	T4: PET (polyethylene terephthalate) jar
	T5: Aluminium pouches lined with polyethylene
	T6: Shrink packaging
	T7: CFB Box
	T8: Woven Bag
	T9: Brown Paper Bag
Sub Treatment	**07 Treatments**
	T1: 0 days
	T2: 2 Month
	T3: 4 Month
	T4: 6 Month
	T5: 8 Month
	T6: 10 Month
	T7: 12 Month
Treatment combination	9 × 7 = 63
Replication	3 (Three)
Statistical design	FCRD

Measurements

The samples were evaluated for physical properties like Physiological loss in weight, Size and Shape of fruit, Colour, etc as well for chemical parameters such as Moisture percent, Essential oil content, sabinene and terpenen-4-ol content, total ash, acid insol- uble ash, alcohol soluble extractive and water soluble extractive. Data were collected every two months for a year.

To calculate physiological loss in weight (%) used the method described by (Sahoo *et al*., 2014) [69-70]. In *Z. rhetsa* fruit size is determined by taking measurement of diameter using a vernier calliper. Typically, a fruit's sphericity, aspect ratio, ellipsoid ratio, and slenderness ratio are used to describe its shape. It is possible to measure shape characteristics of fruit separately or in combination with size measurements (Mohsenin, 1986) [44] The colour of the dried samples was assessed using a colourimeter (Konica Minolta Holdings Inc., model CR-400/410 chromameter, Tokyo, Japan), and the results were expressed in accordance with the CIE Lab system (Hutchings) with reference to illuminant D65 and a viewing angle of 10°. Both the fresh powder and the stored powder's L*, a*, and b*values were calculated.

The moisture content in the sample was determined by using the moisture analyser. The moisture content of the given sample was determined by using the following formula (Ranganna, 1986) [64]. The essential oil content of *Z. rhetsa* was estimated by hydro distillation as given by (Bubpawan *et al*., 2015) [15] Fifty grammes of *Z. rhetsa* fruits were weighed and then put in a flask with a circular bottom. Distilled water was then added until the sample was completely covered. The Clevenger arm, condenser, and round bottom flask are all connected. The temperature is then reduced to 60°C and the combination is allowed to continue boiling for an additional hour after the water has simmered for a while at 100°C. After an hour, the tube is let to settle and cool. In the collection tube where it is collected, the oil develops a layer.

The essential oil with the colour of golden yellow was obtained. Sabinene and terpenen-4-ol content of *Z. rhetsa* was isolated by using Gas chromatography/ mass spectrometry method describe by (Rana and Blazquez, 2010) [63] The oil was analysed using a GC/MS (Agilent 19091S-433) fitted with an HP5MS capillary column. The column temperature was held at 60 °C for 6 minutes, then programmed at 3°C/min to 180°C, and finally at a rate of 10%/min to 280°C. It was used as carrier gas at a flow rate of 2 mL/min, injection volume was 0.5 mL in the split mode, and injector temperature was 250 °C. The various compounds were identified using mass spectra and verified by comparing their retention indices to C8-C32 n-alkanes and matching their spectral data with NIST05 Library and literature data.

Acid Insoluble Ash, Alcohol soluble and water soluble extractives were determined by the method described in the ayurvedic pharmacopoeia part 1 volume IX. The microbial analysis of stored *Z. rhetsa* was done at the interval of 0 Days, 2 month, 4 month, 6 month, 8 month, 10 month and 12 months by the method described by A. O. A. C procedures. The sample under study was powdered and 1 gm of the same was added to 9 ml of distilled water to create dilution of 10^{-1} the serial dilution was continued till 10^{-5}. Potato dextrose agar was prepared with 39 gm of PDA powder in a litre of distilled water. Media was then autoclaved and poured to set in Petri dish is sterilized laminar air flow. One mL of sample is placed in the centre of the plate and the sample is distributed evenly using gentle downward pressure. Place the plate in incubator in a horizontal position for 5 days.

Statistical Analysis

As a part of this experiment, the storage treatment and storage time was estimated by use of Factorial completely randomized design (FCRD) to improve experimental and statistical accuracy. The observations were recorded in replicates of three and critical differences was calculated to compare the results of analysis of different treatments using mean value and ANOVA. Analysis and interpretation of data was carried out in accordance with Panse and Sukhatme (1985) [54] and Ambedkar (2014) [8] using Factorial Completely Randomized Design and valid conclusions were drawn only on significant differences between treatment mean at 5 percent level of significance.

Results and Discussion
Physical parameters
Physiological loss in weight (%)
The data on the Physiological loss in weight percent value for *Z. rhetsa* fruits during storage in different packaging materials are presented in Table no. 3 and graphically depicted in Fig. 1

The physiological weight loss was measured in order to calculate the amount of moisture lost by the fruits of *Z. rhetsa* through various packaging materials. The change in PLW (%) of the stored fruits was significantly influenced by various packaging materials. At the treatment T4 (PET bottle) (1.804%), the lowest mean for PLW was noted while the highest mean for PLW was recorded in treatment T1 (gunny bag) (2.886%). The data showed a slight rise in PLW (%) *Z. rhetsa*, which may be due to moisture loss in the surrounding environment *i.e.*, the packaging material. At 12 month of storage the minimum physiological loss in weight was found in treatment T4 (1.902%).

The interaction between different packaging materials with respect to the storage duration was also found to be significant.

The physiological loss in weight occurs mainly through the respiration and transpiration losses along with some metabolic processes. The physiological loss in weight of fruits was found to increase with advancement of storage period irrespective of treatments. It was observed that the PLW increased during storage as a result of the ambient temperature and relative humidity. This low PLW of fruits may be attributed to diminished biological activities (respiration, inactivation of enzymes and restricted movement of free water) the above findings confirmed with the work done by Pimpalpalle *et al*. 2018 [57], Jeong *et al*. 2002 [29] and Prange and Delong, 2002 [59]. During the 12 month of storage period, it was found that storage had a substantial impact on the change in physiological weight reduction.

The data revealed that there is a slight increase in PLW (%) of *Z. rhetsa* which can be resultant in moisture loss in the surrounding *i.e.*, the packaging material. The PLW was found to increase in storage resultant of the environmental temperature and relative humidity. The effect of moisture was studied by Packiyasothy *et al*. 1983 [52] in black pepper.

Table 3: Effect of different packaging materials and storage period on PLW (%) of *Zanthoxylum rhetsa*

Treatments	Physiological loss in weight (%) Storage Period						Mean
	2 Month	4 Month	6 Month	8 Month	10 Month	12 Month	
T1	2.724	2.779	2.845	2.913	2.989	3.062	2.886
T2	2.714	2.766	2.829	2.884	2.947	3.001	2.857
T3	2.690	2.750	2.793	2.856	2.925	2.994	2.835
T4	1.702	1.750	1.783	1.826	1.862	1.902	1.804
T5	2.490	2.551	2.593	2.652	2.721	2.793	2.633
T6	2.112	2.164	2.214	2.273	2.335	2.402	2.250
T7	2.390	2.451	2.493	2.553	2.623	2.694	2.534
T8	2.624	2.679	2.745	2.813	2.890	2.958	2.785
T9	2.614	2.666	2.729	2.784	2.846	2.900	2.756
Mean	2.451	2.506	2.559	2.617	2.682	2.745	2.593

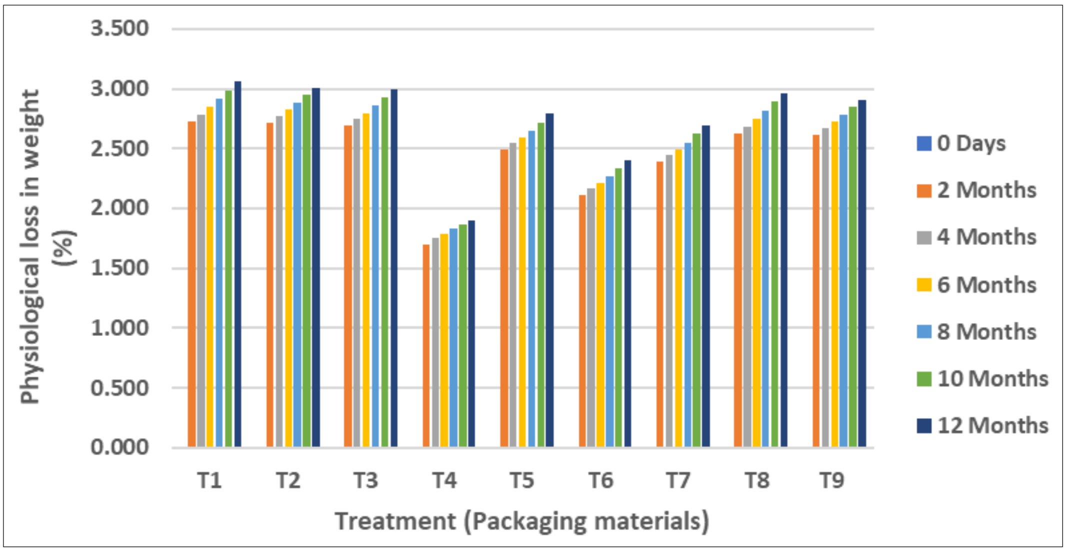

Fig 1: Effect of different packaging materials and storage period on PLW (%) of Z. rhetsa

Size of fruits (diameter in mm)
The data on the size (diameter in mm) of *Z. rhetsa* during storage in different packaging materials are presented in Table no. 4 and graphically depicted in Fig. 2

The size of dried stored fruits was significantly influenced by various packaging materials. The size of fruit was decreased with storage period and has a significant effect on the same. The significantly maximum mean size of fruit recorded in T5 (7.851 mm) followed by T4 (7.596 mm), while minimum size of fruits was recorded in T2 (6.949 mm). The results are significant interpret accordingly.

The interaction between different packaging materials with respect to the storage duration was also found to be significant. The decreasing trend in size of fruits was recorded throughout the storage period.

In this experiment, size of fruit was measured in order to calculate the amount of weight loss by the fruits of *Z. rhetsa* through various packaging materials. During the storage from 0 days to 12 months the fruit size was decreased. Due to loss of moisture, volatile content, size was decreased and respiration increases. The diameter of fruits decreased due to adsorption of moisture that resulted in shrinking of capillaries and expressing contraction in major, medium and minor axes along with the geometric mean diameter. The measurement of size of fruit is necessary for grading to get optimum market value. There is no research related to fruit sizes in *Zanthoxylum rhetsa* during storage. Detailed morphological study was done by Rajbhandari and Amatya (2019) [60] in which they described the average size of fruit 5-8 mm.

Shape of fruit

According to the study, the shape of all the fruits observed were oblong in a shape during the entire storage period. No change was seen in the shape as far as packaging material and storage period is concerned. The shape of all the fruits remained unchanged during the entire storage period. Variation in fruit size and shape is quantitatively inherited. No change was seen in the shape respective of packaging material and storage period. The oblong shaped fruits of *Zanthoxylum rhetsa* was observed. The shape of fruits of *Z. rhetsa* was described by Duagyod *et al*. (2020) [20].

Table 4: Effect of different packaging materials and storage period on Size of fruits of *Zanthoxylum rhetsa*

Treatments	Size (Diameter in mm) Storage Period							Mean
	0 Days	2 Month	4 Month	6 Month	8 Month	10 Month	12 Month	
T1	7.750	7.627	7.533	7.417	7.213	7.027	6.860	7.347
T2	7.303	7.210	7.147	7.017	6.867	6.663	6.433	6.949
T3	7.837	7.750	7.610	7.520	7.320	7.107	6.960	7.443
T4	7.810	7.773	7.713	7.623	7.507	7.410	7.333	7.596
T5	8.247	8.167	8.030	7.933	7.730	7.540	7.310	7.851
T6	7.330	7.243	7.163	7.073	6.993	6.893	6.817	7.073
T7	7.657	7.520	7.453	7.357	7.127	6.960	6.800	7.268
T8	7.990	7.823	7.733	7.630	7.457	7.270	7.090	7.570
T9	7.637	7.520	7.450	7.327	7.110	6.940	6.730	7.245
Mean	7.729	7.626	7.537	7.433	7.258	7.090	6.926	7.371

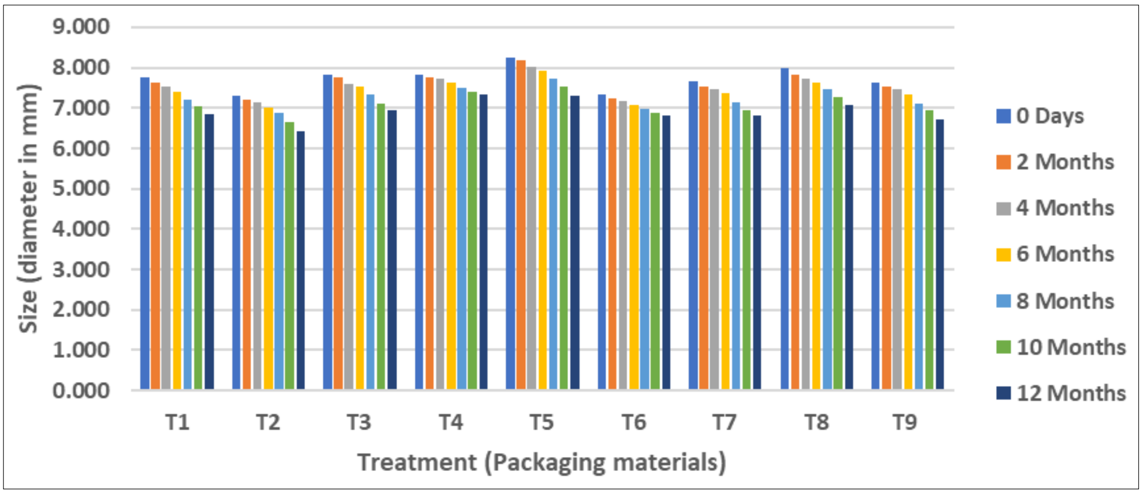

Fig 2: Effect of different packaging materials and storage period on Size of fruit (diameter in mm) of *Z. rhetsa*

Colour of fruit

Colour and appearance of food product helps in judging the acceptability of the product. The sensory quality of the food or its item may affect the decision-making process of purchasing food materials by the consumers. The colour of fruit was estimated by colorimeter which gives the L*, a* and b* values which stands for perceptual lightness, red and green, yellow and blue respectively.

L* value

The data for L* value for colour of fruits of *Z. rhetsa* during storage period are presented in Table no. 5 and graphically depicted in fig 3.

L* colour value was recorded to determine lightness of fruits of *Z. rhetsa* which got decreased with corresponding increase in storage period. The decrease in L* value of colour was increased in darkening of fruits of *Z. rhetsa* during storage of 12 months period.

The treatment T1 and T2 recorded the highest mean (16.462) L* value of colour followed by T4 (16.461), T9 (16.458) and T3 (16.455) while the lowest mean (16.441) for L* value was recorded in treatment T5 and T8. Lightness of the colour of fruit decreased significantly with increase in storage periods from 16.782 to 16.131 during 12 months storage period. Thus, it can be concluded that Lightness of the colour of fruits of *Z. rhetsa* decreased with increase in storage period. Interaction effect between storage period and different treatments was found to be statistically significant.

a* value

The data for a* value for colour of fruits of *Z. rhetsa* during storage period are presented in Table no. 6 and graphically depicted in fig 4.

As far as a* value is concerned, a* value was recorded to determine redness of *Zanthoxylum rhetsa* which increased with corresponding increase in storage period. The treatment T3 (11.257) recorded the significantly highest mean a* value for colour which was followed by T5 (11.250) while the lowest mean a* value of colour was recorded in treatment T1 and T6 (11.224) during the storage period of one year. The a* value of colour during storage of 0 days to 12 Months also increased significantly.

Redness of the colour in fruits of *Z. rhetsa* increased with increase in storage period from 10.901 to 11.616 upto 12 months of storage. Interaction effect between storage period and different treatments was found to be statistically significant.

b* value for colour

The data for b* value for colour during storage period are presented in Table no. 7 and graphically depicted in fig 5.

b* value was recorded to determine yellowness of *Zanthoxylum rhetsa* which decreases with corresponding increase in storage period. The treatment T2 (15.508) recorded the significantly highest mean b* value for colour followed by treatment T3 (15.506). The treatment T6 (15.420) significantly recorded the lowest mean b* value of colour which was followed by treatment T4 and T8 (15.424). The b* value of colour during storage of 0 days to 12 months also decreased significantly. Yellowness of the colour in *Z. rhetsa* decreased with increase in storage period from 15.779 to 15.118 upto 12 months storage. Interaction effect between storage period was studied among different treatments found to be statistically significant.

The value of colour shows that packaging material type and storage period have a significant effect on the colour of *Z. rhetsa* during storage. The similar line of work done by Meghwal and Goswami (2014) [42] who studied the changes in the colour of black pepper during storage in ambient condition. Also, the change in colour was recorded in *Zanthoxylum armatum* during different storage condition by Cheng *et al*. (2021) [17]. They observe impact of different storage condition on colour of *Zanthoxylum armatum.*

In storage studies minute changes in colour value indicates that the storage period doesn't have any significant change and the pepper retains its brownish black hue. Similar line of work was done by Rhim and Hong (2011) [67] on *Capsicum annum* where the colour was one of the quality parameters to be tested in terms of storage and observed a decreasing colour value range. The effect of the storage duration with respect to the colour change was found to be significant.

Table 5: Effect of different packaging materials and storage period on L* Colour of fruits of Zanthoxylum rhetsa

Treatments	L* Value of colour – Storage Period							Mean
	0 Days	2 Month	4 Month	6 Month	8 Month	10 Month	12 Month	
T1	16.807	16.690	16.553	16.450	16.360	16.233	16.143	16.462
T2	16.798	16.710	16.567	16.483	16.323	16.213	16.143	16.462
T3	16.816	16.700	16.530	16.417	16.353	16.250	16.140	16.455
T4	16.793	16.710	16.567	16.483	16.323	16.210	16.143	16.461
T5	16.793	16.640	16.523	16.473	16.320	16.267	16.113	16.441
T6	16.797	16.700	16.553	16.483	16.310	16.210	16.123	16.451
T7	16.800	16.713	16.540	16.470	16.317	16.227	16.110	16.452
T8	16.813	16.640	16.523	16.473	16.320	16.267	16.113	16.441
T9	16.807	16.647	16.527	16.457	16.380	16.273	16.147	16.458
Mean	16.802	16.683	16.543	16.466	16.334	16.239	16.131	16.454

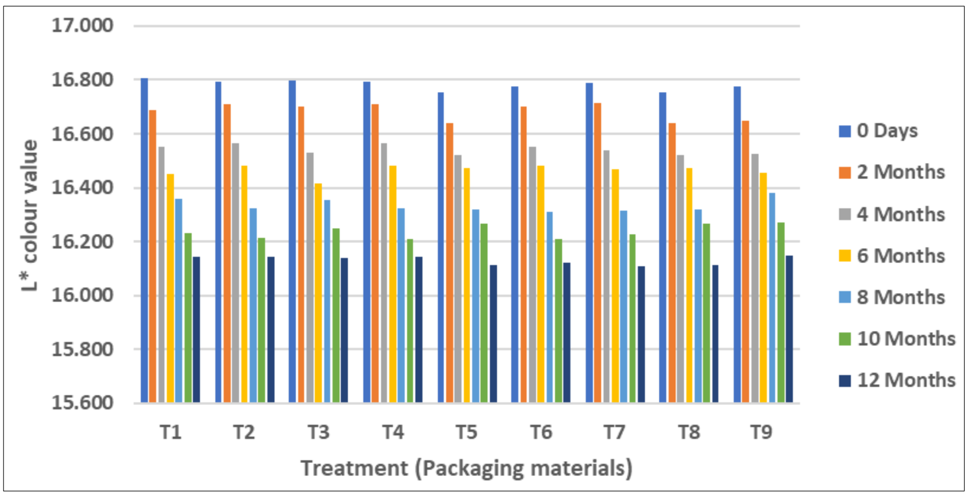

Fig 3: Effect of different packaging materials and storage period on L* colour value of *Z. rhetsa*

Table 6: Effect of different packaging materials and storage period on a* Colour of fruits of *Zanthoxylum rhetsa*

Treatments	a* Value of colour							Mean
	Storage Period							
	0 Days	2 Month	4 Month	6 Month	8 Month	10 Month	12 Month	
T1	10.900	11.010	11.090	11.190	11.310	11.480	11.590	11.224
T2	10.900	11.020	11.110	11.220	11.310	11.460	11.630	11.236
T3	10.900	11.040	11.120	11.260	11.370	11.460	11.650	**11.257**
T4	10.900	11.040	11.110	11.220	11.360	11.450	11.620	11.243
T5	10.900	11.010	11.100	11.260	11.370	11.460	11.650	11.250
T6	10.900	10.990	11.100	11.200	11.310	11.450	11.620	11.224
T7	10.900	11.000	11.100	11.210	11.330	11.480	11.590	11.230
T8	10.900	11.000	11.100	11.210	11.330	11.480	11.590	11.230
T9	10.910	11.020	11.110	11.220	11.310	11.460	11.600	11.233
Mean	10.901	11.014	11.104	11.221	11.333	11.464	11.616	11.236

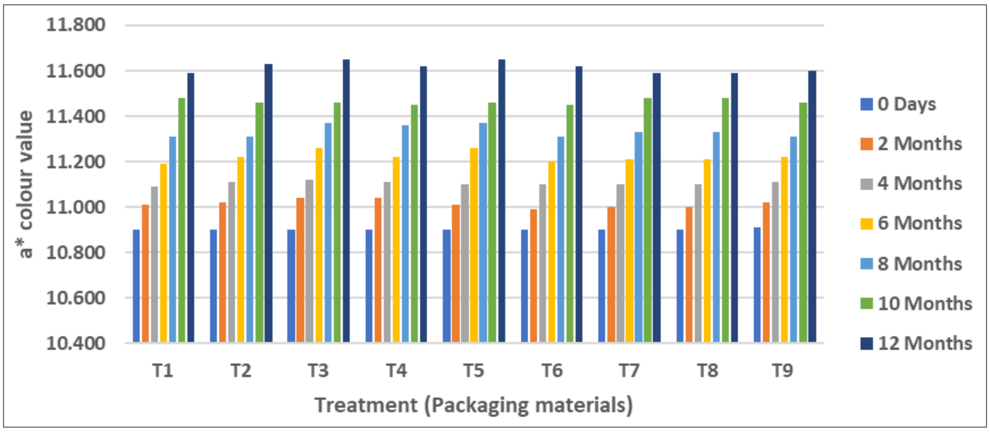

Fig 4: Effect of different packaging materials and storage period on a* colour value of Z. rhetsa

Table 7: Effect of different packaging materials and storage period on b* Colour of fruits of *Zanthoxylum rhetsa*

Treatments	b* Value of colour							Mean
	Storage Period							
	0 Days	2 Month	4 Month	6 Month	8 Month	10 Month	12 Month	
T1	15.778	15.720	15.630	15.510	15.400	15.320	15.110	15.499
T2	15.780	15.717	15.617	15.530	15.410	15.330	15.120	**15.508**
T3	15.779	15.717	15.630	15.507	15.400	15.320	15.170	15.506
T4	15.781	15.620	15.557	15.420	15.330	15.193	15.100	15.424
T5	15.778	15.620	15.560	15.420	15.330	15.220	15.100	15.436
T6	15.778	15.620	15.510	15.420	15.330	15.220	15.100	15.420
T7	15.776	15.650	15.580	15.450	15.370	15.290	15.150	15.463
T8	15.778	15.650	15.510	15.420	15.330	15.220	15.100	15.424
T9	15.780	15.710	15.630	15.510	15.400	15.320	15.110	15.497
Mean	15.779	15.669	15.580	15.465	15.367	15.270	15.118	15.464

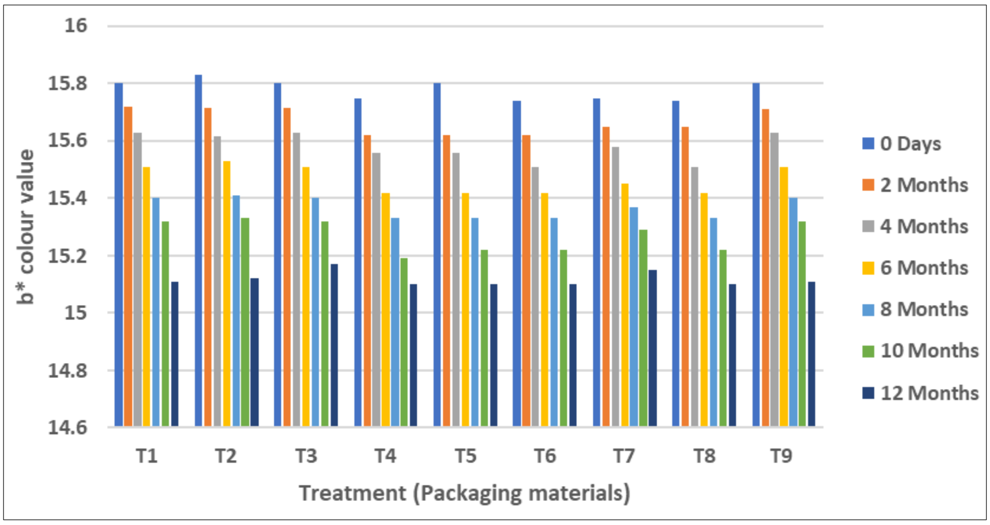

Fig 5: Effect of different packaging materials and storage period on b* colour value of *Z. rhetsa*

2. Chemical parameters

2.1 Moisture content (%)

The data on the moisture content for *Z. rhetsa* during storage in different packaging material are presented in Table no. 8 and graphically depicted in Fig 6.

Moisture content is an indication of changes in the water level present in the *Z. rhetsa*. A low moisture content is desirable for higher drug stability. The effect of different packaging materials has a significant effect on moisture. The significantly maximum mean moisture content was in T4 (12.236%) followed by T6 (12.151%), T3 (12.120%), T2 (12.111%), T5 (12.108), T7 (12.105%), T8 (12.061%), T9 (12.038%) and T1(12.010%) respectively. Minimum mean moisture content was found in T1 (12.010%).

The moisture was observed to decrease with storage period duration and has a significant effect on the same. The maximum (12.366%) total mean was found initially. The interaction between the packaging materials and the storage duration on the moisture (%) was significant. The significantly maximum moisture (12.080%) was recorded in T4 at 12 months of storage.

The values showed that the ability of the packaging materials to preserve moisture and maintain integrity of the spices. The results observed were in accordance with work done by Packiyasothy *et al*. (1983) [52]. The moisture was observed to decrease with storage period and has a significant effect on the same. The maximum total mean moisture was found initially (12.36%). Smitha *et al*, (2020) [83] in Kalmegh as well as Babarinde and Fabunmi, (2009) [14] in okra performed the similar line of work.

2.2 Essential oil (%)

The data on the essential oil (%) value for *Z. rhetsa* during storage in different packaging materials are presented in Table no. 9 and depicted graphically in Fig 7.

Essential oil is a concentrated hydrophobic liquid containing volatile chemical compounds from plants. It is what gives the plant its essence and is highly valued. The effect of the different packaging materials on the essential oil was found to be significant. The significantly maximum value of oil extracted from T4 (1.301%).

The storage duration was found to be significant effect on the essential oil content of *Z. rhetsa*. The maximum mean was found initially (1.535%) followed by 2 months (1.430%), 4 months (1.337%), 6 months (1.026%), 8 months (0.856%), 10 months (0.594%) and 12 months (0.357%) which shows minimum mean value.

The interaction between various packaging materials and storage period of *Z. rhetsa* on essential oil was found to be statistically significant. The maximum essential oil was recorded in T4 (0.870%) at 12 months of storage.

Table 8: Effect of different packaging materials and storage period on Moisture content (%) of Zanthoxylum rhetsa

Treatments	Moisture content (%)							Mean
	Storage Period							
	0 Days	2 Month	4 Month	6 Month	8 Month	10 Month	12 Month	
T1	12.366	12.210	12.123	12.013	11.917	11.810	11.640	12.010
T2	12.362	12.280	12.203	12.113	12.037	11.947	11.827	12.111
T3	12.368	12.300	12.247	12.120	12.043	11.960	11.810	12.120
T4	12.360	12.330	12.280	12.240	12.200	12.140	12.080	12.236
T5	12.368	12.250	12.190	12.140	12.067	11.950	11.800	12.108
T6	12.366	12.300	12.220	12.180	12.100	12.040	11.850	12.151
T7	12.368	12.277	12.223	12.140	12.037	11.947	11.750	12.105
T8	12.364	12.273	12.183	12.060	11.953	11.843	11.753	12.061
T9	12.370	12.253	12.133	12.013	11.943	11.833	11.720	12.038
Mean	12.366	12.275	12.200	12.113	12.033	11.941	11.803	12.104

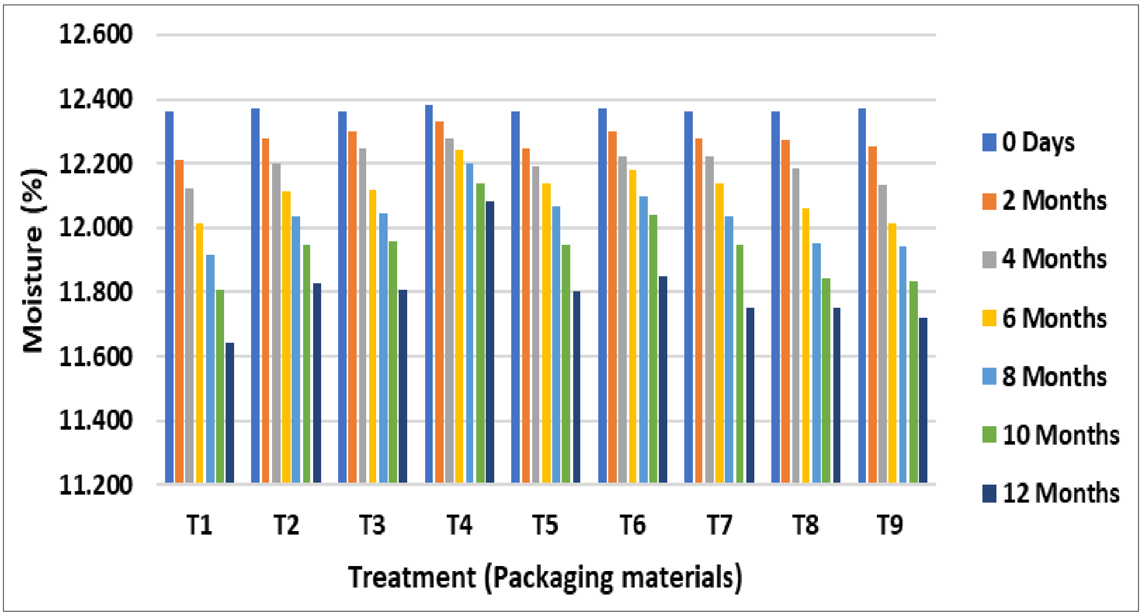

Fig 6: Effect of different packaging materials and storage period on moisture content (%) of *Z. rhetsa*

Table 9: Effect of different packaging materials and storage period on Essential oil (%) of Zanthoxylum rhetsa

Treatments	Essential oil (%) Storage Period							Mean
	0 Days	2 Month	4 Month	6 Month	8 Month	10 Month	12 Month	
T1	1.538	1.400	1.310	0.913	0.710	0.500	0.250	0.943
T2	1.535	1.410	1.300	0.913	0.820	0.500	0.280	0.968
T3	1.536	1.430	1.320	0.953	0.823	0.500	0.310	0.984
T4	1.531	1.520	1.450	1.390	1.240	1.107	0.870	**1.301**
T5	1.532	1.420	1.357	0.950	0.810	0.520	0.330	0.985
T6	1.537	1.440	1.390	1.130	0.920	0.680	0.400	1.071
T7	1.535	1.400	1.320	1.090	0.800	0.510	0.290	0.989
T8	1.536	1.420	1.280	0.923	0.790	0.500	0.230	0.958
T9	1.538	1.430	1.310	0.967	0.790	0.530	0.250	0.973
Mean	1.535	1.430	1.337	1.026	0.856	0.594	0.357	1.019
		S.Em ±				CD at 5%		
Treatments (T)		0.002				0.006		
Storage (S)		0.002				0.005		
Interaction (T×S)		0.006				0.016		

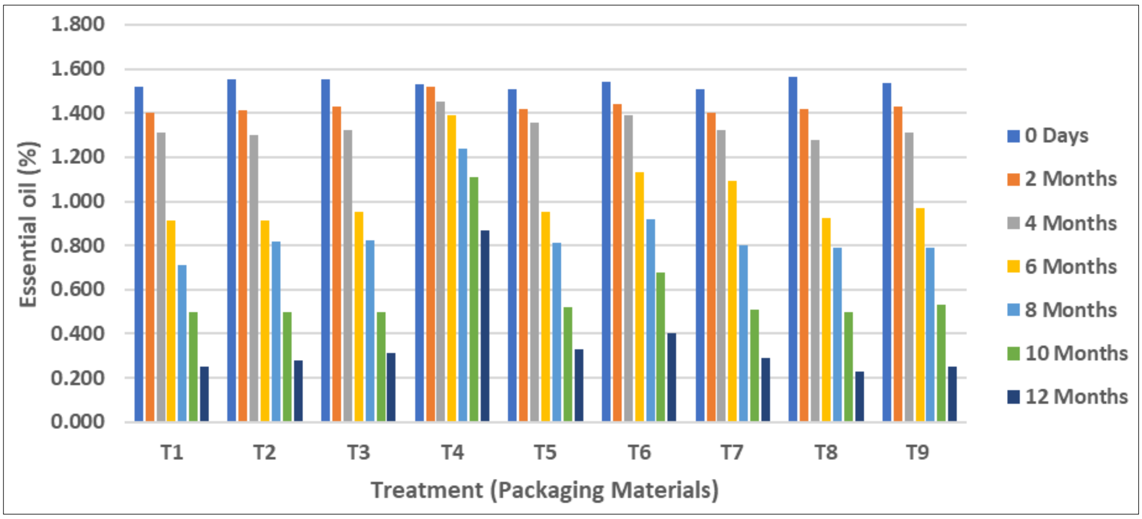

Fig 7: Effect of different packaging materials and storage period on essential oil (%) of *Z. rhetsa*

Essential oil is a concentrated hydrophobic liquid containing volatile chemical compounds from plants. It gives the plant its essence and is highly valued. The high yield even with a slight dip in oil content shows that packaging material acts as an environmental preserving barrier owing to its small porosity. These results are in accordance with work done on black pepper by Zachariah *et al*. (2019) [93]. The extreme reduction of the oil content is due to high porosity of the respective packaging materials which was presented by Thomas and Mani, (2017) [88] in the composition of oil found in *Hedychium matthewii*. In accordance to storage duration, the amount of essential oil content decreases. The same results were noted by Arabhosseini *et al*. (2006) on French Tarragon and by Ebadi *et al*. (2016) [21] on lemon verbena leaves.

2.3 Sabinene content (%)

The data on sabinene content (%) value for *Z. rhetsa* during storage in different packaging materials are presented in Table no. 10 and graphically depicted in Fig.8.

Sabinene is one of the key constituent to be found in the *Zanthoxylum* species. It provides citrus aroma and peppery flavor to *Zanthoxylum rhetsa* fruits. Sabinene content of *Z. rhetsa* was calculated by using Gas chromatography/ mass spectrometry method.

The significantly maximum 14.260% mean was found in treatment T4 followed by T6 (14.232%), T3 (14.221%), T2 (14.220%) and T5 (14.207%). While the minimum mean value was found in treatment T8 (14.164%). Sabinene showed a constant dip in yield during the time of storage and has a significant effect. The maximum sabinene content was found initially (14.929%). Interaction between the various packaging materials and storage period of *Z. rhetsa* on sabinene (%) was statistically significant. The maximum sabinene was recorded in T4 (13.210%) at 12 months of storage.

2.4 Terpenen-4-ol content (%)

The data on Terpenen-4-ol content (%) value for *Z. rhetsa* during storage in different packaging materials are presented in Table no. 11 and graphically depicted in Fig.9.

Terpenen-4-ol is one of the most prevalent and dominating constituent present in *Z. rhetsa.* It has antibacterial and antioxidant properties. With respect to the storage period the amount of terpnen-4-ol was decreased. There is slight change upto 6 months but after that there was rapid loss.

The maximum Terpenen-4-ol content was found initially (39.254%). Interaction between the various packaging materials and storage period of *Z. rhetsa* on Terpenen-4-ol (%) was statistically significant. The significantly maximum terpinen-4-ol was recorded in treatment T4 (31.056%) followed by T6 (30.554%), T5 (30.483%), T7 (30.458%), T3 (30.447%), T2 (30.433%), T9 (30.426%) and T1 (30.417%). The minimum mean terpenen-4-ol was recorded in T8 (30.401%). Terpenen-4-ol showed a significant effect on storage period.

Terpenen-4-ol was the major constituent present in the essential oil of *Z. rhetsa*. The findings of the present investigation showed that the concentration of constituents with a lower molecular weight decreased by prolonging storage time especially at room temperature. This phenomenon might be due to evaporation, oxidation and other unwanted changes in essential oils component during storage period. The similar line of work performed by Rowshan *et al*. (2013) [68] they studied the influence of storage conditions on the essential oil composition of *Thymus daenensis* Celak.

Table 10: Effect of different packaging materials and storage period on Sabinene content (%) of *Zanthoxylum rhetsa*

Treatments	Sabinene content (%) Storage Period							Mean
	0 Days	2 Month	4 Month	6 Month	8 Month	10 Month	12 Month	
T1	14.929	14.830	14.713	14.400	13.920	13.560	13.010	14.190
T2	14.926	14.822	14.700	14.440	13.950	13.600	13.103	14.220
T3	14.930	14.825	14.740	14.417	13.930	13.580	13.103	14.221
T4	14.927	14.829	14.703	14.457	13.990	13.643	13.210	**14.260**
T5	14.928	14.825	14.703	14.427	13.940	13.550	13.090	14.207
T6	14.929	14.827	14.740	14.490	13.950	13.527	13.140	14.232
T7	14.930	14.825	14.700	14.410	13.903	13.510	13.000	14.181
T8	14.928	14.829	14.720	14.400	13.880	13.400	13.020	14.164
T9	14.930	14.827	14.717	14.427	13.897	13.477	13.093	14.194
Mean	14.929	14.827	14.715	14.430	13.929	13.539	13.085	14.208

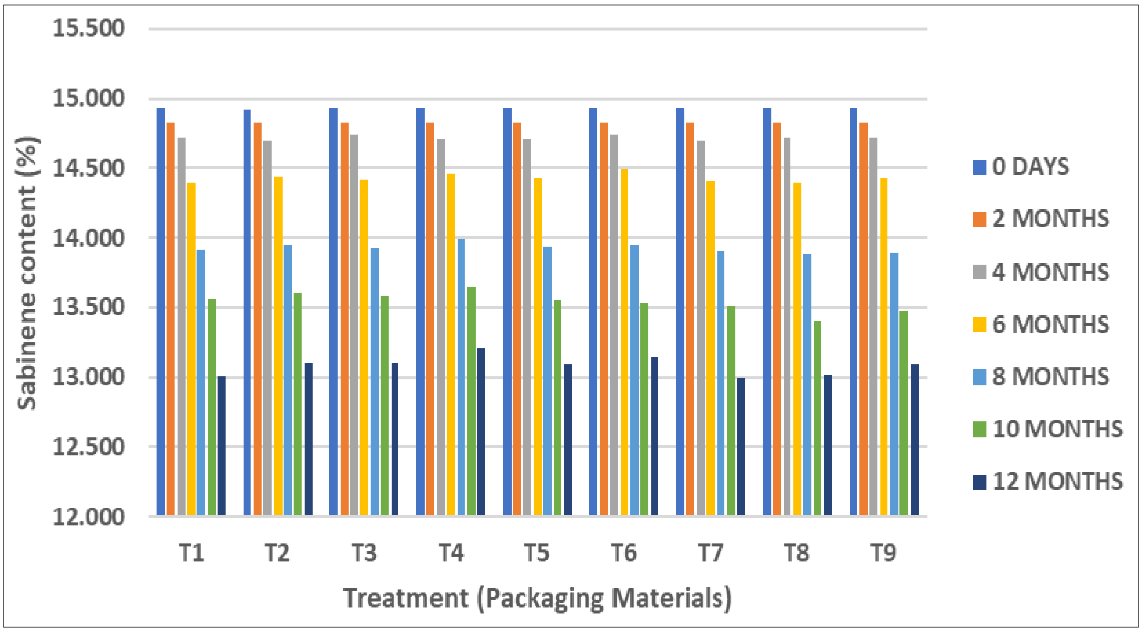

Fig 8: Effect of different packaging materials and storage period on sabinene (%) of *Z. rhetsa*

Table 11: Effect of different packaging materials and storage period on Terpenen-4-ol content (%) of *Z. rhetsa*

Treatments	Terpenen-4-ol content (%) Storage Period							Mean
	0 Days	2 Month	4 Month	6 Month	8 Month	10 Month	12 Month	
T1	39.260	38.480	36.200	33.870	26.267	21.600	17.243	30.417
T2	39.250	38.490	36.250	33.800	26.307	21.650	17.237	30.433
T3	39.258	38.450	36.290	33.870	26.280	21.680	17.230	30.447
T4	39.252	38.800	36.890	33.810	26.840	22.950	18.833	31.056
T5	39.251	38.460	36.230	33.800	26.660	21.800	17.233	30.483
T6	39.250	38.480	36.410	33.760	26.353	21.700	17.927	30.554
T7	39.260	38.480	36.307	33.760	26.510	21.730	17.157	30.458
T8	39.251	38.450	36.213	33.700	26.400	21.610	17.230	30.401
T9	39.254	38.470	36.333	33.753	26.283	21.690	17.243	30.426
Mean	39.254	38.507	36.347	33.791	26.433	21.823	17.481	30.520

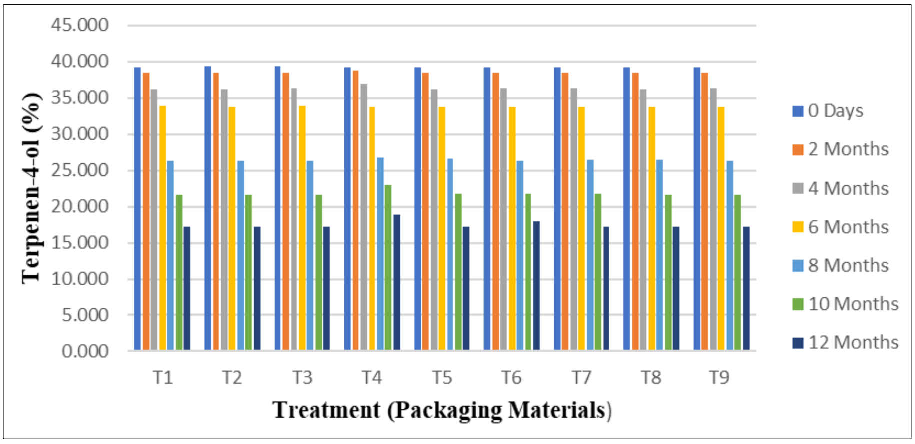

Fig 9: Effect of different packaging materials and storage period on Terpenen-4-ol (%) of *Z. rhetsa*

Total ash (%)

The data on total ash for *Z. rhetsa* during storage in different packaging materials are presented in Table 12 and graphically depicted in Fig.10.

Ash refers to the inorganic residue remaining after either ignition or complete oxidation of organic matter in a food sample. It helps to determine the amount and type of minerals in food and is considered important because the number of minerals can determine the physicochemical properties of foods and microorganisms' activity. The packaging materials were found to have a non-significant effect with the ash content. The maximum mean was found in treatment T3 (7.410%) while the minimum mean (7.407%) was recorded in T1, T4, T5 and T7.

Initially the maximum (7.409%) mean value of ash was recorded while lowest (7.400%) was recorded at 2nd and 12th month storage. Interaction between the various packaging materials and storage period of *Z. rhetsa* on ash content (%) was found to be statistically non-significant for ash content (%).

Ash refers to the inorganic residue remaining after either ignition or complete oxidation of organic matter in a food sample. It helps to determine the amount and type of minerals in food and is considered important because the number of minerals can determine the physicochemical properties of foods and microorganisms' activity. The packaging materials were found to have a significant effect with the ash content. It was found that the ash content decreased very little with respect to their individual storage in different packaging material. But when the comparison of different packaging material is considered, it was observed that the difference in value is due to the fact that ash content only gives an idea about non-volatile material present in food and undergoes very minimum change resulting in disintegration. The change in ash is also due to the microbial growth that converts organic component to inorganic form for their function. Same line of work is by Akusu and Emelike, (2019) [3] on black pepper where they observed the decrease in ash with storage of time on black pepper in different polythene bag of different micron size.

Acid insoluble ash (%)

The data on acid insoluble ash (%) value for *Z. rhetsa* during storage in different packaging materials are presented in Table no. 13 and graphically depicted in Fig.11.

The minimum mean value for acid insoluble ash (0.156%) is observed in treatment T4 followed by T7 and T6 (0.157%) while the maximum AIA mean value was recorded in treatment T1. The interaction effect of the packaging materials with AIA was significant. It was observed that mean value of acid insoluble ash for storage remain unchanged thereby it shows slight change in silica impurities throughout the storage period has significant effect. In interaction between the various packaging materials and storage period of *Z. rhetsa* on acid insoluble ash (%) was found to be statistically significant.

Total acid insoluble ash shows the impurity of silica from external silica (as contamination from soil at harvesting time or internal silica (biogenic silica). The following results are conformity with Selvarajan *et al*. (2019) [76] they revealed that all packaging material act as a successful barrier against dust and soil contamination with woven bag showing a minute contamination. It was observed that mean value of acid insoluble ash for storage slightly increases thereby it shows increase in silica impurities throughout the storage period.

Table 12: Effect of different packaging materials and storage period on Total ash (%) of Z. *rhetsa*

Treatments	Total ash (%)							Mean
	Storage Period							
	0 Days	2 Month	4 Month	6 Month	8 Month	10 Month	12 Month	
T1	7.413	7.400	7.413	7.400	7.410	7.413	7.400	7.407
T2	7.408	7.401	7.420	7.407	7.410	7.413	7.401	7.409
T3	7.410	7.400	7.420	7.407	7.410	7.420	7.400	7.410
T4	7.410	7.403	7.407	7.400	7.410	7.420	7.400	7.407
T5	7.408	7.400	7.420	7.400	7.410	7.420	7.403	7.407
T6	7.410	7.401	7.420	7.401	7.410	7.420	7.400	7.409
T7	7.409	7.400	7.420	7.400	7.408	7.407	7.401	7.407
T8	7.410	7.402	7.420	7.400	7.410	7.420	7.401	7.409
T9	7.408	7.400	7.417	7.400	7.409	7.420	7.400	7.408
Mean	7.410	7.401	7.417	7.402	7.410	7.417	7.401	7.408

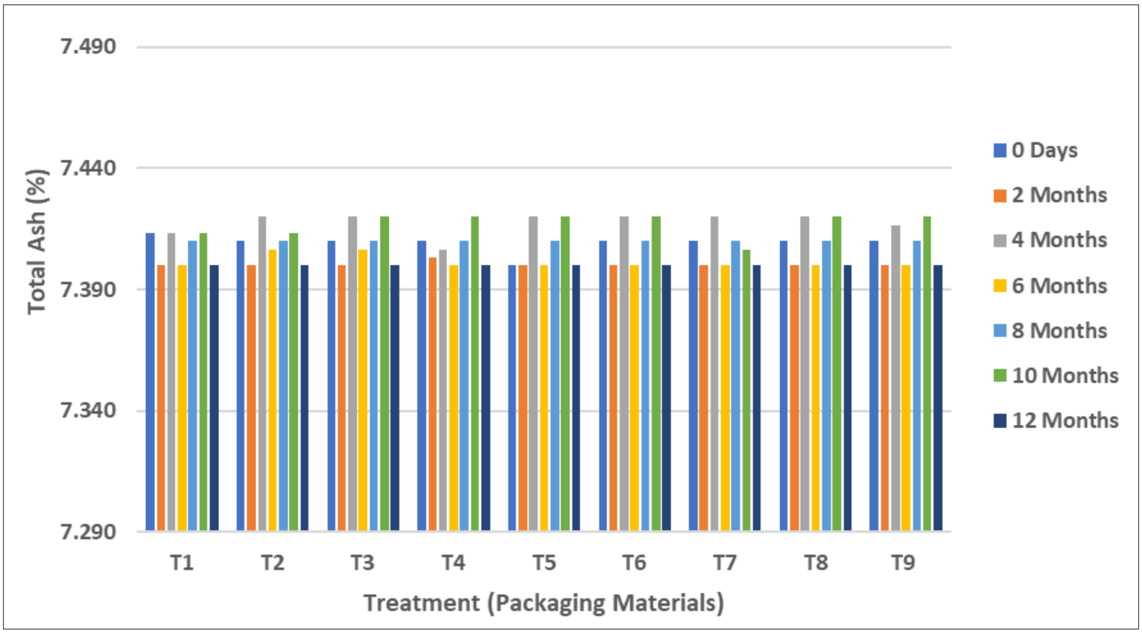

Fig 10. Effect of different packaging materials and storage period on Total ash (%) of Z. *rhetsa*

Table 13: Effect of different packaging materials and storage period on Acid insoluble ash (%) of *Z. rhetsa*

Treatments	**Acid insoluble ash (%)**							**Mean**
	Storage Period							
	0 Days	**2 Month**	**4 Month**	**6 Month**	**8 Month**	**10 Month**	**12 Month**	
T1	0.139	0.154	0.159	0.161	0.168	0.174	0.179	0.163
T2	0.134	0.152	0.159	0.162	0.168	0.172	0.177	0.162
T3	0.138	0.142	0.155	0.162	0.169	0.175	0.183	0.160
T4	0.134	0.147	0.152	0.156	0.163	0.168	0.172	0.156
T5	0.135	0.147	0.155	0.161	0.168	0.172	0.180	0.159
T6	0.139	0.142	0.151	0.159	0.165	0.170	0.178	0.157
T7	0.136	0.145	0.153	0.159	0.163	0.169	0.175	0.157
T8	0.135	0.149	0.158	0.164	0.170	0.178	0.183	0.162
T9	0.140	0.149	0.158	0.164	0.170	0.178	0.183	0.162
Mean	0.137	0.147	0.156	0.161	0.167	0.173	0.179	0.160
		S.Em ±			CD at 5%			
Treatments (T)		0.0002			0.0006			
Storage (S)		0.0002			0.0006			
Interaction (T×S)		0.0006			0.0017			

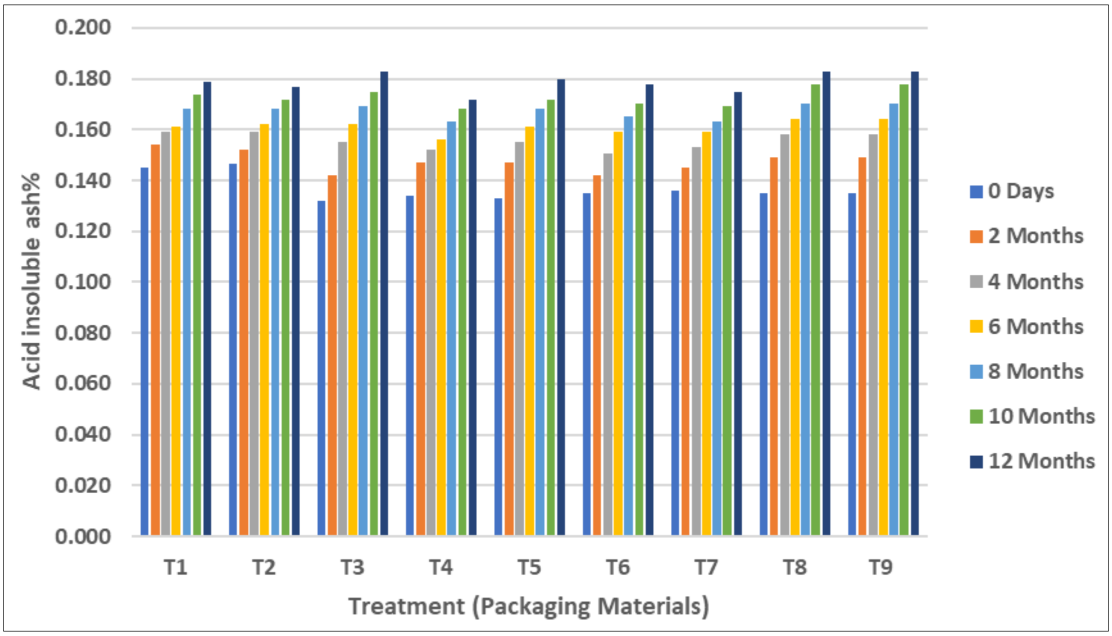

Fig 11: Effect of different packaging materials and storage period on Acid insoluble ash (%) of Z. rhetsa

Alcohol soluble extractive (%)

The data on alcohol soluble extractive value for *Z. rhetsa* during storage in different packaging materials are presented in Table no. 14 and graphically depicted in Fig.12.

Alcohol soluble extractive is an indication of existence of exhausted material or addition of adulterant or can be due to incorrect processing during drying. The significantly maximum mean value for the alcohol extractive value (%) was found in treatment T4 (11.460%) followed by T3 and T7 (11.450%). The effect on the ASE (%) with respect to various packaging materials was found to be statistically significant.

It was noticed that ASE for storage reduced with time. The effect of the storage duration on the ASE was found to be significant. The maximum mean value was found initially (11.630%) indicating that over the time the material is exhausted in the method of storage. Interaction between the various packaging materials and storage periods of *Z. rhetsa* on alcohol soluble extractive (%) was found to be statistically significant for mean of alcohol soluble extractive (%) for *Z. rhetsa*. The maximum ASE was reported in treatment T4 (11.230%) at 12 months of storage.

Alcohol soluble extractive is an indication of existence of exhausted material or addition of adulterant or can be due to incorrect processing during drying. Similar line of work was done by Shweta *et al*. (2020) [81] where the mixture of *Laghu sutashekhara Rasa* was stored at ambient temperature. The ASW (%) was found to be reduced in 6 months of storage where the range of reduction is from 6.62% to 3.37%. It was noticeable that ASE for storage reduced with time. Indicating that over time the material is either exhausted or the incorrectness in the method of storage.

Water soluble extractive (%)

The data on water soluble extractive value for *Z. rhetsa* during storage in different packaging materials is presented in Table no. 15 and graphically depicted in Fig.13.

The maximum mean water soluble extractive was recorded by T4 (12.893%) which was at par with T6 (12.880%), T7 (12.872%), T5 (12.860%), T3 (12.856%), T2 (12.855%) and T9 (12.852%), while lowest mean WSE recorded in T1 (12.813%).

The interaction between the different packaging materials was in significance to that of the WSE value. The storage of *Z. rhetsa* with respect to the water-soluble extractive (%) showed a similar result to that of alcohol extractive value i.e., the mean value decreased with passage of time where initially the maximum mean WSE (13.410%) was noticed. The values of the storage duration were found to be significant. Interaction between the various packaging materials and storage period of *Z. rhetsa* on water soluble extractive (%) was found to be statistically significant for mean of water-soluble extractive (%). The maximum WSE (%) was found in T6 (12.347%) at 12 months of storage.

Table 14: Effect of different packaging materials and storage period on Alcohol soluble extractives (%) of Z. *rhetsa*

Treatments	Alcohol Soluble Extractive (%)							Mean
	Storage Period							
	0 Days	2 Month	4 Month	6 Month	8 Month	10 Month	12 Month	
T1	11.630	11.580	11.493	11.443	11.333	11.290	11.117	11.412
T2	11.630	11.583	11.517	11.460	11.407	11.353	11.117	11.438
T3	11.630	11.600	11.543	11.513	11.417	11.330	11.117	11.450
T4	11.630	11.607	11.533	11.480	11.420	11.320	11.230	11.460
T5	11.630	11.590	11.540	11.480	11.430	11.330	11.117	11.445
T6	11.630	11.607	11.530	11.470	11.357	11.273	11.190	11.437
T7	11.630	11.600	11.543	11.513	11.417	11.330	11.117	11.450
T8	11.630	11.607	11.543	11.470	11.337	11.277	11.117	11.426
T9	11.630	11.580	11.527	11.470	11.403	11.307	11.117	11.433
Mean	11.630	11.595	11.530	11.478	11.391	11.312	11.138	11.439

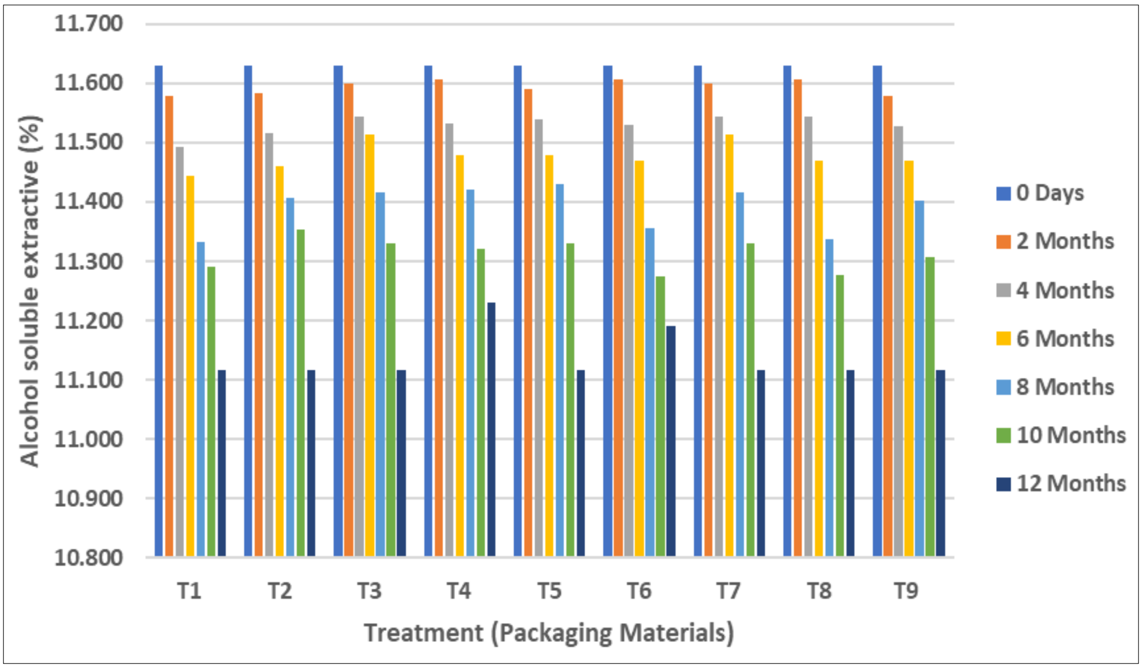

Fig 12: Effect of different packaging materials and storage period on Alcohol soluble extractive (%) of *Z. rhetsa*

Table 15: Effect of different packaging materials and storage period on Water soluble extractives (%) of Z. *rhetsa*

Treatments	Water soluble extractive (%)							Mean
	Storage Period							
	0 Days	2 Month	4 Month	6 Month	8 Month	10 Month	12 Month	
T1	13.407	13.270	13.033	12.827	12.640	12.207	12.307	12.813
T2	13.417	13.250	13.020	12.850	12.637	12.513	12.300	12.855
T3	13.410	13.250	13.033	12.830	12.657	12.510	12.300	12.856
T4	13.410	13.280	13.170	12.847	12.663	12.553	12.330	12.893
T5	13.403	13.257	13.033	12.880	12.660	12.493	12.293	12.860
T6	13.413	13.287	13.070	12.860	12.673	12.510	12.347	12.880
T7	13.407	13.277	13.093	12.830	12.670	12.510	12.317	12.872
T8	13.410	13.210	12.990	12.810	12.633	12.510	12.340	12.843
T9	13.410	13.233	13.010	12.860	12.630	12.510	12.310	12.852
Mean	13.410	13.257	13.050	12.844	12.651	12.480	12.316	12.858

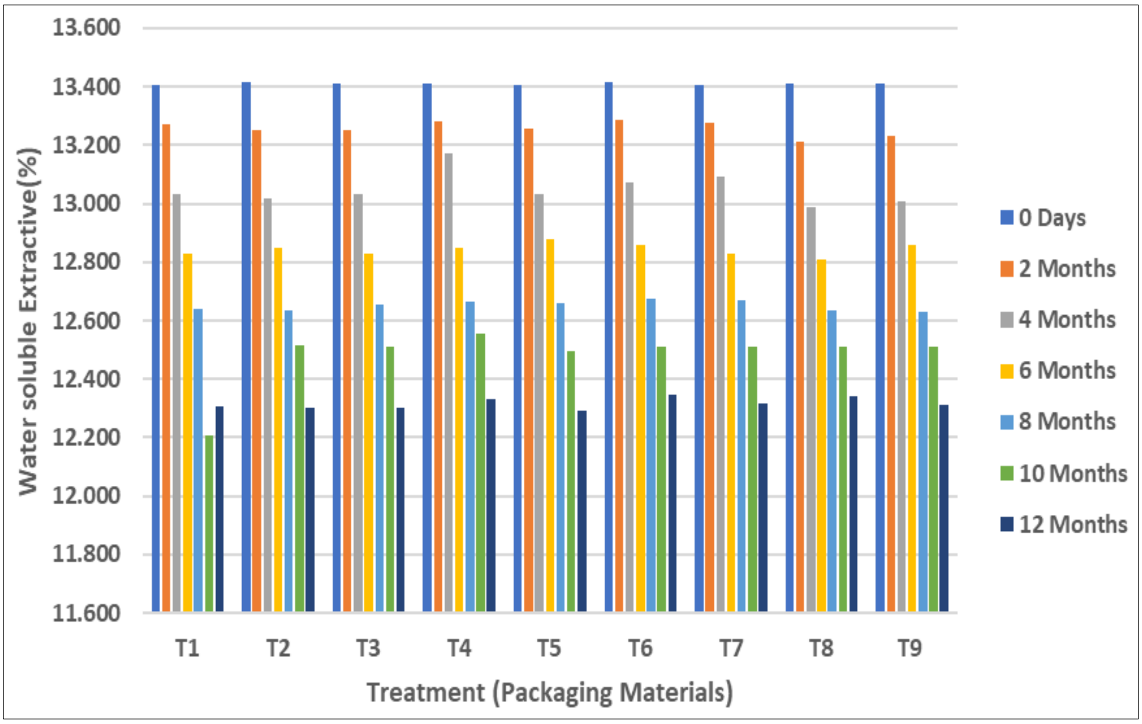

Fig 13: Effect of different packaging materials and storage period on Water soluble extractive (%) of *Z. rhetsa*

Water-soluble extractive is an indication of existence of exhausted material or addition of adulterant or can be found due to incorrect processing during drying. The following results may be due to the effect packaging materials on the *Z. rhetsa* either by exhausting or adulterating them naturally due to mixing of foreign object due to large porosity of the material. The same trend was observed by Jaluthriya *et al.* (2020) [7] in black pepper. The storage of pepper with respect to the water-soluble extractive showed a similar result to that of alcohol extractive value i.e., the mean value decreased with passage of time where initially has the maximum mean of 13.41%.

Microbial Analysis

Yeast and mould (cfu g-1)

The data on mould and yeast for *Z. rhetsa* during storage in different packaging materials is presented in Table no. 16 and graphically depicted in Fig. 14.

The minimum mean for microbial growth was for T4 (0.183× 10^4 cfu g-1). Mould and yeast infestation was found minimum initially (0.100 × 10^4 cfu g- 1). The interaction of infestation with respect to the storage time shows significant interaction.

Interaction between the various packaging materials and storage period of *Z. rhetsa* on microbial and yeast growth was found to be statistically significant for *Z. rhetsa*. The minimum value for growth in *Z. rhetsa* was observed at T4 (0.270 × 10^4 cfu g-1) followed by T6 (0.587× 10^4 cfu g-1) at 12 months of storage.

Mould and yeast are both decomposers of nature and converts the complex organic compound to simpler inorganic compounds. These organisms are responsible for the disintegration of spices. The yeast gives yellow white colored colony which are small in number and mould may appear black, yellow and green on PDA. These material shows less porosity and thereby prevents microbial infestation.

These result shows similar trend in black pepper stored in different density polyethylene bag as reported by Akusu and Emelike (2019) [3].

Table 16: Effect of different packaging materials and storage period on microbial growth (Yeast and mould) of *Z. rhetsa*

Treatments	Yeast and mould (cfu g-1) Storage Period							Mean
	0 Days	2 Month	4 Month	6 Month	8 Month	10 Month	12 Month	
T1	0.100	0.220	0.313	0.410	0.530	0.620	0.713	0.415
T2	0.100	0.210	0.290	0.433	0.510	0.600	0.710	0.408
T3	0.100	0.227	0.310	0.430	0.520	0.590	0.703	0.411
T4	0.100	0.130	0.150	0.180	0.210	0.240	0.270	**0.183**
T5	0.100	0.200	0.290	0.413	0.500	0.580	0.667	0.393
T6	0.100	0.180	0.230	0.300	0.380	0.510	0.587	0.327
T7	0.100	0.190	0.300	0.400	0.500	0.570	0.670	0.390
T8	0.100	0.227	0.310	0.420	0.530	0.600	0.710	0.414
T9	0.100	0.220	0.287	0.427	0.500	0.590	0.693	0.402
Mean	0.100	0.200	0.276	0.379	0.464	0.544	0.636	0.371

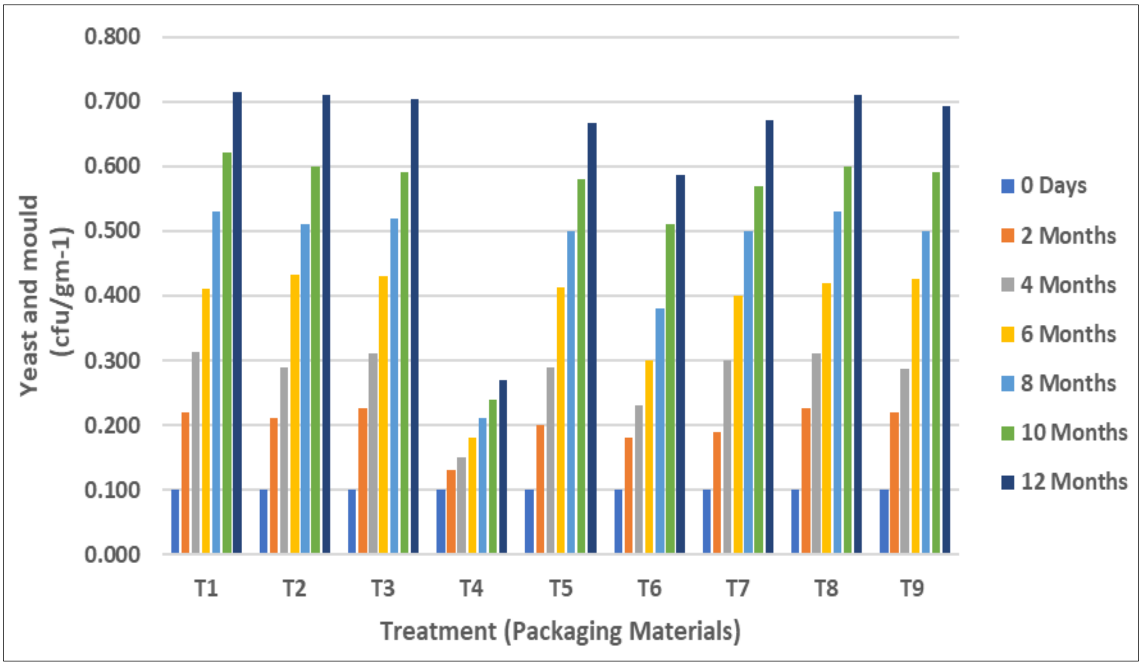

Fig 14: Effect of different packaging materials and storage period on Yeast and mould growth of Z. rhetsa

Conclusion

Based on the findings of the current studies, it can be concluded that *Z. rhetsa* stored in PET bottle (T4) exhibits the best outcomes when compared to other results. It has demonstrated good essential oil retention, sabinene, terpenen-4-ol content which is the key characteristic of *Z. rhetsa*. The PET bottle shows nearly constant amounts of alcohol soluble extractive and water soluble extractive, proving that it has not been contaminated by any external causes. Throughout the whole 12 months of storage, the PET bottle has also inhibited moisture loss, microbial growth. Due to their recyclable and reusable nature, they are also a cost-effective, superior, and environmental friendly solution when compared to traditional packaging material.

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