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Original Article

Study of Essential Oil and Effect of Temperature and Seed Appendages (pappus) on Germination Characteristics in Coltsfoot

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ABSTRACT

Coltsfoot (*Tussilago farfara* L.) is a perennial plant of the Asteraceae family that has been used as an ancient folk remedy to treat coughs, bronchitis, and asthmatic disorders. Ripe seeds were collected from *T. farfara* plants growing in Pol-e Zangholeh, Iran, in this study. Samples were gathered by the Tarbiat Modares University (TMU) Department of Horticultural Sciences laboratory in late April 2016. The extracted seed essential oil constituents identified and quantified using GC/MS and GC, respectively. Phy,tol, n-Nonadecane, n-Tetradecane, and 4,4-Dimethyltetracyclo [5.2.1.02,6.03,5] decane, with amounts of 30.5%, 11.4%, 9.8%, and 6.2%, respectively, were the main components in the seeds oil of *T. farfara*. The germination variables were studied using a factorial experiment with 5 replicates × 50 seeds in a totally randomized design. Treatments include: cultivating non-pappus-bearing seeds in a Petri dish and placing them at 4 °C (c1t1) and 25 °C (c1t2), Cultivating pappus-bearing seeds in a Petri dish and keeping them at 4 °C (c2t1) and 25 °C (c2t2) and cultivating of non-pappus-bearing seeds treated with sodium hypochlorite 1.5% for 20 minutes and keep them at 4 °C (c3t1) and 25 °C (c3t2). The interaction between culture type and temperature was significant ($p \leq 0.01$) for the germination percentage of seeds, germination rate, and seed vigor of *T. farfara*, according to analysis of variance. The rising seed germination rates of C3t2 and C3t1 were reported at 76.4% and 74.8%, respectively. The results revealed the closest positive correlation between germination rate, vigor index, and seed germination percentage ($p \leq 0.001$).

INTRODUCTION

Tussilago farfara L., often known as Coltsfoot, is a perennial Asteraceae plant found in East Asia, North Africa, Siberia, and Europe [1]. In Iran's damp mountainous areas, including Tehran, Azerbaijan, and the Northern Provinces, *T. farfara* is widespread [2]. Above ground, the plants grow to a height of 5-15 cm, although up to 30 cm during fruit dispersal. *T. farfara*'s yellow blooms appear before the leaves in early spring (February-April) [3]. They generate a lot of wind-dispersed achenes that may spread up to 4 kilometers [1]. Its delicate leaves are used as a vegetable in China and Europe, and the flower buds are used in China to make a nutritious tea and as a dietary supplement [4]. Cough, bronchitis, and asthmatic diseases have all been treated with this conventional folk remedy [4]. In previous investigations, a few bioactive compounds have been examined, including sesquiterpenoids, triterpenoids, chlorogenic, and flavonoids [5], phenylpropanoids, chromones, and pyrrolizidine alkaloids [4]. Above ground, the plants reach 5–15 cm in height, although up to 30 cm during fruit dispersal, and the yellow flowers grow on stems with no leaves in early spring, then the large leaves appear later [3]. The chemical makeup of *T. farfara* essential oils, predominantly from buds and flowers, has only been the subject of a few investigations [6]. 14-Hydroxy-Z-caryophyllene, *α*-Cadinol, 4,4-Dimethyl tetracyclo (5.2.1.02,6.03,5] decane, Humulene epoxide II and (*E*)-Nerolidol. While there hasn't been a single investigation on the volatile oils of *T. farfara* seeds, the primary compounds found in the essential oil of *T. farfara* organs from Iran were n-Undecane, n-Tetracosane, Phytol, Hexacosane, n-Tetradecanol, n-Nonadecane, and Caryophyllene oxide [2]. *T. farfara* spreads by seeds and rhizomes and produces large numbers of achenes capable of dispersal up to 4 km in the wind

[2]. Seed germination is a crucial developmental step that impacts population renewal in many plant species [7]. Seed vigor as the key quality factor affects establishment ability and performance [8]. The fluffy pappus heads of the tiny *T. farfara* seeds link to seed-bearing achenes. The pappus is a hairy structure in the germinal aperture of Coltsfoot seeds that plays an essential role in wind dispersal [9]. The effects of decelerating enzyme activity were revealed by improving the mobilization of total lipids and protein reserves in the previous studies [10]. Fluctuating temperatures play a vital role in the seed germination of various species, and it has been found that many grassland species show improved germination when treated with fluctuating temperatures [11]. It is crucial to note, however, that some herbal substances may prevent the growth and germination of other plants [12]. Many different physical (e.g., seed soaking, manual scarification, cold stratification, heat shocks, light irradiation, and magnetic fields) and chemical (e.g., scarification with acidic or basic chemicals, plant growth regulators, and osmotic treatments) treatments are used to improve germination [13].

The sodium hypochlorite was efficient as physical removal of parchment to promote and hasten the emergence of seedlings in papaya and coffee seeds [14, 15]. Even though there are many reports on the advantages of sodium hypochlorite for seeds of various species, it is crucial to emphasize that understanding the ratio of sodium hypochlorite solution for a given seed quantity, as well as the concentration of the active chlorine solution and the soaking time, is crucial to defining the methodology to be used [15]. A great piece of research included numerous treatments and evaluated their direct impact on seed germination to increase germination, but these efforts yielded no discernible improvement in germination [11]. In this study, the *T. farfara* seeds' essential oil composition, along with the germination percentage, germination rate, and seed vigor index by various methods, including pappus-bearing seeds, non-pappus-bearing seeds, and non-pappus-bearing seeds treated with sodium hypochlorite are investigated. The effect of temperature and seed appendages (pappus) on germination parameters is studied.

MATERIALS AND METHODS

Seed Collection Site

In late April 2016, the *T. farfara* mature seeds were collected in Pol-e Zangholeh of Iran (51º 20′ 17″ N, 36º 48′ 11″ E). The geographic coordinates and site collected information of *T. farfara* are shown in Table 1.

Fig. 1 Hydrodistillation of essential oil of *T. farfara* L. seeds using a Clevenger type apparatus.

Essenti al oil Extraction (EO)

In the five grams of *T. farfara* collected seeds and in the flowering stage, the EO was extracted by hydrodistillation that long three hours using a Clevenger-type apparatus [16] in the lab of the Department of Horticultural Sciences at Tarbiat Modares University (Fig. 1). The essential oils separated from the water were dried over anhydrous sodium sulfate and kept in the dark at 4 °C until analysis.

Essential Oil Analysis

Gas chromatography (GC) analysis was performed using Agilent Technologies 7890B (Santa Clara, CA, USA) with a flame ionization detector. The instrument was equipped with an HP-5 fused silica column (length 30 m, inner diameter 0.32 mm, and film thickness 0.25 µm), and helium was used as the carrier gas at a flow rate of 1.0 mL/minute.

Table 1 Geographical coordinates and collection site Information of *T. farfara*

Sampling location	Province	Average rainfall (mm)	Mean annual temperature (ºC)	humidity	Latitude	Longitude	Altitude (m a.s.l.)
Pol-e Zangholeh	Mazandaran	1081	10.7	32	51º20′17″	36º48′11″	2010

The individual peaks qualification was carried out by injecting the oil into a Thermoquest–Finnigan gas chromatography, coupled with a trace mass spectrometer (GC/MS) with the same parameter for fused silica column (except for the inner diameter of 0.25 mm), oven temperature, injector temperature, carrier gas and flow rate. The ionization voltage was 70 eV. Ion source and interface temperatures were 200° and 250 °C, respectively. Identification was confirmed by comparison of each component's mass spectra with those of the internal mass spectra library of the main library, Wiley 7.0 and Adams, and further identification was based on a comparison of peak retention indices by using a homologous series (C8 to C24) noted under the same operating conditions and the published data (Adams, 2007). Identification was confirmed by comparison of each component's mass spectra with those of the internal mass spectra library of the main library, Wiley 7.0 and Adams, and further identification was based on a comparison of peak retention indices by using a homologous series (C8 to C24) recorded under the same operating conditions and the published data [17].

Germination Experiment

T. farfara seeds were dried and stored at room temperature (25 °C) after being rinsed three times with water. The study was conducted in a factorial experiment arranged in a completely randomized design with 5 replicates × 50 seeds. Treatments include: 1. cultivating non-pappus-bearing seeds in a Petri dish and placing them at 4 °C (c1t1) and 25 °C (c1t2), 2. cultivating of pappus-bearing seeds in a Petri dish and placing them at 4 °C (c2t1) and 25 °C (c2t2), 3. cultivating of non-pappus-bearing seeds treated with sodium hypochlorite 1.5% for 20 minutes and placing them at 4 °C (c3t1) and 25 °C (c3t2) (Fig. 2). For each step, Petri dishes, distilled water, and other Equipment were autoclaved at 180 °C for 2 hours (Relative humidity 50%). The first germination count was performed on the third day, and the last counting was performed 20 days after applying treatments. Seeds with a root length of about 2 mm were called germinated seeds [18].

Germination percentage = number of viable seeds initiated / number of germinating seeds × 100 [18].

The germination rate (GR) was calculated according to the formula proposed by Maguire [19]. GR=E1/N1+E2/N2+... En/Nn, which GR, germination rate, E1, E2… En, number of normal plants computed at the first count, second count until the last count, N1, N2…; Nn, number of days of sowing the first count, the second count until the last count.

The vigor index value was computed by the following formula suggested by Abdul-Baki and Anderson [20]. Vigor index = Germination (%) × total seedling length (cm)

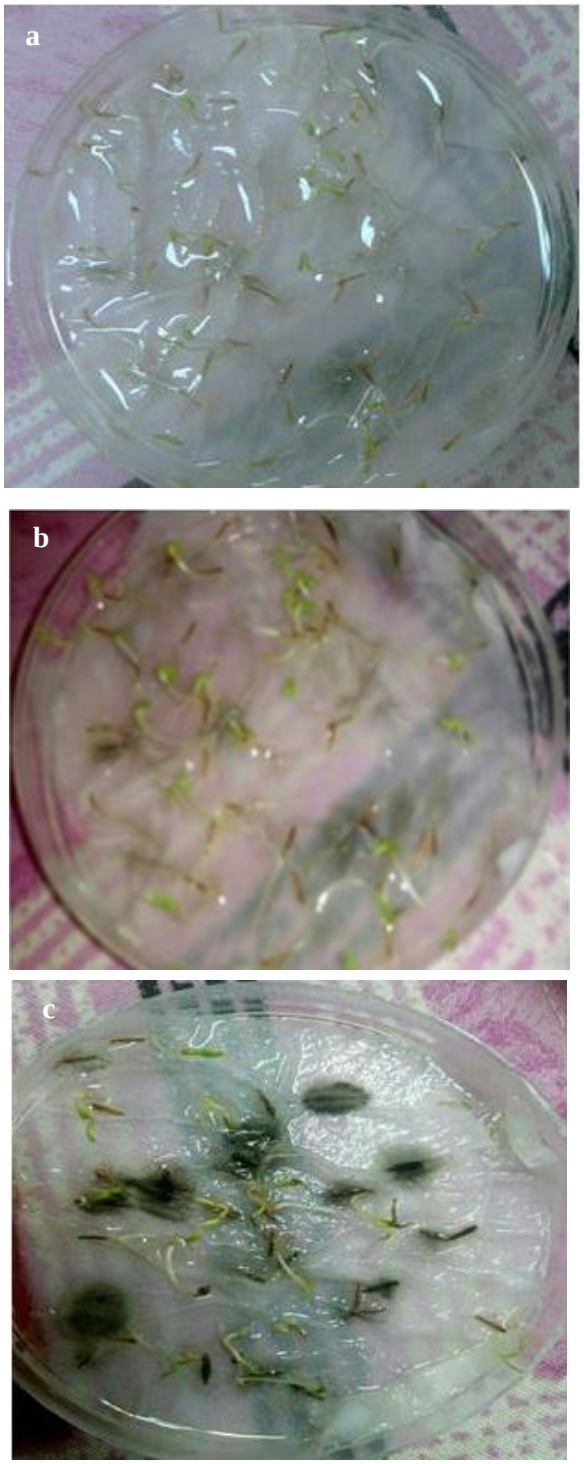

Fig. 2 Treatments of germination test of *T. farfara* L.; A:cultivating of non-pappus bearing treated with sodium hypochlorite 1.5% for 20 minutes and placing them at 4 °C (c3t1) and 25 °C (c3t2), B: cultivating of non-pappus bearing in a Petri dish and placing them at 4 °C (c1t1) and

25 °C (c1t2), C: cultivating of pappus bearing in a Petri dish and placing them at 4 °C (c2t1) and 25 °C (c2t2).

Statistical Analyses

All results were analyzed according to an analysis of variance based on a completely randomized design with three replications, using SAS Statistical Package Program version 9.0 and SPSS software version 20. The significant differences between the group means were separated using the Least Significant Difference (LSD) test at a 5% probability level [21]

RESULTS AND DISCUSSION

Seed Essential Oil Composition

The essential oil yield (v/w % relative to dry weight of plant) from the leaves of *T. farfara* was 0.02. Compared to other research, Ferrer and Venskutonis [22] reported the essential oil content of flowers of *T. farfara* from Lithuania and France were 0.07% and 0.09% (w/w). Norani, Ebadi [2] showed that the essential oil yield of the leaf and flower of *T. farfara* from different regions of Iran was 0.02- 0.09. Overall, 29 compounds were identified in seed oil, representing 94.2 % (Fig. 3). Table 2 shows the major components of *T. farfara* seeds. The major compounds in the seeds oil of *T. farfara* were phytol, *n-nonadecane*, *n-tetradecane*, and 4,4-Dimethyltetracyclo [5.2.1.02,6.03,5] decane with the amount of 30.5 %, 11.4 %, 9.8 % and 6.2 %, respectively. In previous phytochemical studies on the essential oils of *T. farfara*, researchers have identified a series of sesquiterpenoids, such as Tussilagone, *ß*-Bisabolene, and Bisabolene epoxide derivatives [23], 14-Hydroxy-Z-caryophyllene, *α*-Cadinol, 4,4-Dimethyl tetracyclo[5.2.1.02,6.03,5] decane, Humulene epoxide II and (*E*)-Nerolidol in leaves and *n*-Undecane, *n*-Tetracosane, Phytol, Hexacosane, *n*-Tetradecanol, *n*-Nonadecane and Caryophyllene oxide in flowers [2]. Investigation and comparison of the essential oil compounds of *T. farfara* seeds showed that they have similar compounds with the leaf and flower of this plant [2].

Germination Characteristics

The interaction between culture type and temperature was significant (p0.01) for the germination percentage of seeds, germination rate, and seed vigor of *T. farfara* (Table 3). Furthermore, the kind of culture had significant impacts on all evaluated attributes (p0.01). Figure 4-A depicts the highest seed germination percentages in c3t2 and c3t1. The non-pappus-bearing seeds treated with sodium hypochlorite at temperatures of 25 °C and 4 °C, with 76.4% (c3t2) and 74.8% (c3t1), respectively, showed the highest seed germination percentage. The lowest germination percentage of seeds was related to c1t1 and c1t2, with 27.6 % and 28.4 %, respectively. In other words, the non-pappus-bearing seeds were used at temperatures of 4 °C and 25 °C. The highest germination rate, 3.16, was obtained in c3t2 when non-pappus-bearing seeds were treated with sodium hypochlorite at a temperature of 25 °C (Fig. 4-B). According to Khatibzadeh, Azizi [24] reports a study on *levisticum officinale* germination. The lowest seed germination rate was recorded for C1t2, at 2.36. The highest vigor indexes are 1.14 and 1.12 for c3t2 and c3t1, respectively (Fig. 4-C). Furthermore, c1t1 and c1t2 had the lowest vigor index values of 0.41 and 0.42, respectively. The results show that the germination factors increased by sodium hypochlorite when removed pappus and seed treatment. A temperature of 25 °C has a beneficial influence on the percentage of seeds, germination rate, and seed vigor compared to 4 °C. Lan, Li [9] reported that the removal of pappus significantly ($P < 0.05$) affected the germination index of A. venetum (12.8% increase) and P. pictum (10.1% increase) seeds. Pappus might act as a mechanical barrier at the seed's germinal aperture and limit oxygen absorption, thereby decreasing the seed germination. *Centaurea iberica* germination was accomplished at temperatures between 15 and 25 °C, in another study [25]. Gorai and Gasmi [26] suggested the highest and lowest germination percentages of *Salvia aegyptiaca* L. were obtained at 30 °C and 4 °C, respectively. Rathinavel and Dharmalingam [8] reported that seed disinfection increased the germination factors and seed quality of Cotton. Average seed germination following all of the sodium hypochlorite treatments on seed germination of *Brassica oleracea* was significantly higher than other treatments [27]. In general, seeds lacking pappus treated with sodium hypochlorite had the highest number of germination factors. This result is probably related to the inhibitory effect of sodium hypochlorite contamination. Plant species seeds taken in various years or from different places exhibit higher variation in germination quantity and quality [28]. Therefore, it is necessary to study the ecological behavior of each specific population for species conservation and cultivation.

Table 2 Chemical composition of volatile components of *T. farfara* L. seeds obtained by hydrodistilation and analyzed by GC-MS and GC-FID

No	RT	Components	%	RI *
1	4.9	*α*-Pinene	0.4	968
2	5.2	*a*-Phellandrene	2.3	1006
3	7.0	*p*-Cymene	0.7	1025
4	7.2	n-Undecane	2.0	1090
5	8.4	4,4- Dimethyltetracyclo [5.2.1.02,6.03,5] decane	6.2	1155
6	11.9	n-Decanol	0.7	1270
7	12.7	1-Tridecene	1.3	1289
8	14.5	*α*-Copaene	1.2	1378
9	14.8	*n*-Tetradecane	9.8	1392
10	15.3	(*E*)-Caryophyllene	0.4	1422
11	16.5	*α*-Humulene	0.7	1452
12	17.1	Germacrene D	1.3	1484
13	17.2	*β*-Selinene	2.3	1490
14	17.7	*β*-Bisabolene	0.3	1517
15	18.1	*δ*-Cadinene	3.4	1526
16	19.4	Spathulenol	0.2	1578
17	19.5	Caryophyllene oxide	2.3	1594
18	20.1	Humulene epoxide II	3.1	1612
19	21.2	*α*-Cadinol	0.2	1658
20	21.6	*n*-Tetradecanol	3.2	1671
21	21.8	*n*-Pentadecanol	0.7	1772
22	24.6	Caffeine	0.5	1849
23	24.9	*n*-Nonadecane	11.4	1870
24	26.1	Phytol	30.5	1925
25	27.1	Isophytol	0.9	1946
26	31.7	n-Docosene	0.4	2196
27	33.5	Tricosane	2.8	2300
28	35.1	Tetracosane	2.4	2400
29	39.9	Hexacosane	2.6	2600
Total compounds			94.2 %	

* RI: retention indices according to the normal alkanes between C8-C24

Table 3 Analysis of Variance of Percentage of Germination, germination rate and seed vigor of *T. farfara* L.

		Mean Square		
Dependent Variable	DF	Vigor index	Germination rate	Percentage of Germination
Type of culture	2	0.27 **	0.73 **	1212.4 **
Temperature	1	0.00003 ns	0.002 ns	0.013 ns
Type of culture × Temperature	2	1.03 **	0.29 **	4585.73 **
Error	24	0.33	0.34	6.24
CV	-	4.6	4.2	4.6

** and ns significant at 0.01 level of probability and non-significant, respectively.

Table 4 Simple correlation among germination rate, vigor index and percentage of germination of *T. farfara* L.

	Percentage of Germination	Germination rate	Vigor index
Percentage of Germination	1.000	0.678 ***	1.000 ***
Germination rate	-	1.000	0.678 ***
Vigor index	-	-	1.000

*** Significant at 0.001 level of probability.

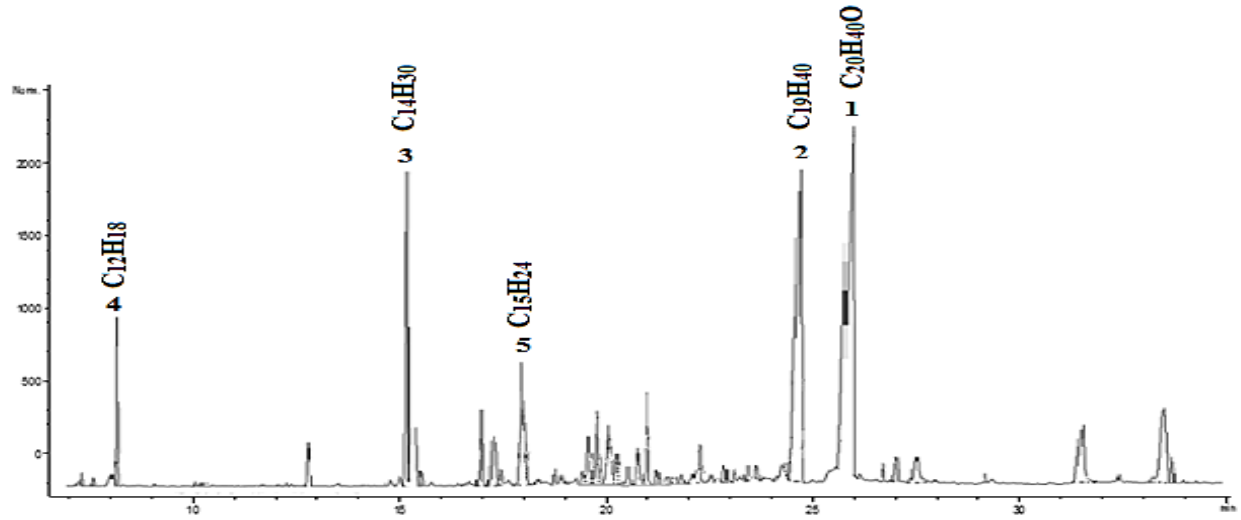

Fig. 3 GC profile of essential oil of *T. farfara* L. seeds
1: Phytol, 2: *n*-Nonadecane, 3: *n*-Tetradecane, 4: 4,4- Dimethyltetracyclo [5.2.1.02,6.03,5] decane, 5: *δ*-Cadinene

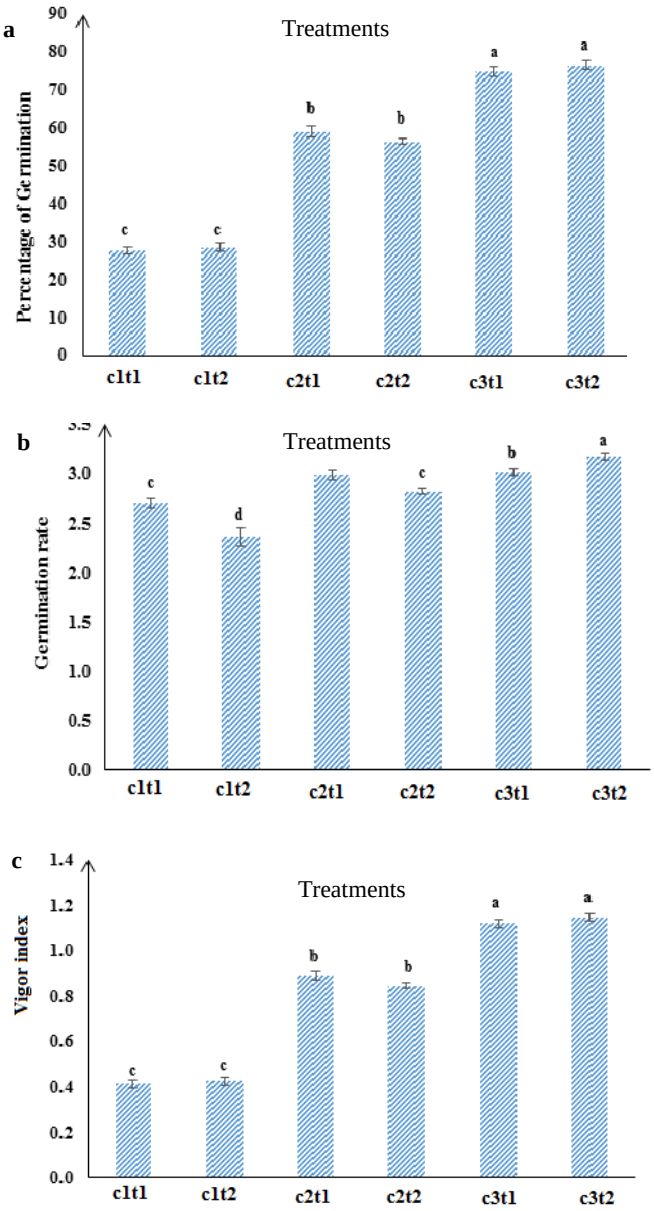

Fig. 4 Germination percentage (A), germination rate (B), and vigor index (C) of *T. farfara* L. seeds under different treatments.

Correlation between Germination Rate, Vigor Index and Germination Percentage of Seeds

The correlation between germination rate, vigor index, and germination percentage of seeds from *T. farfara* is presented in Table 4. The results showed the strongest positive correlation between the vigor index and germination percentage ($r= 1.00$, $p\leq0.001$). The correlation between germination rate and percentage was significant ($r= 0.678$, $p\leq0.001$). These results were in agreement with which high correlation was observed between germination rate and germination percentage of *Dorema ammoniacum* D. Don.

CONCLUSION

In conclusion, the essential oils of *T. farfara* seeds were studied for the first time in this study. *T. farfara* seeds have a relatively low yield of essential oil. This study discovered that removing pappus from *T. farfara* seeds improves germination. The vigor index was favorably associated with germination percentage in all treatments. The findings of this study also revealed that low temperature and pappus might be the causes of decreased germination percentages in *T. farfara* seeds in natural settings, which could contribute to *T. farfara* spreading via rhizomes. As a result, the rhizome propagation strategy is recommended for domesticating *T. farfara*.

Conflict of Interests

The authors have not declared any conflict of interest.

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