

# Effect of Seed Extraction Methods of Tomato on Physiological Quality of Seeds and Seedlings

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## Research article

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## Abstract

## Background

*Tomato (Solanum lycopersicum L.) belongs to the family Solanaceae is one of the most popular and most processed vegetable crops worldwide with a great nutritional contribution to the human diet. Though the demand for tomato products is increasing, its production is constricted by shortage of quality seeds due mainly to lack of appropriate seed extraction method. Inadequate seed extraction method also causes extensive disease epidemics. Conversely, empirical evidences are scanty on instant and latent effects of seed extraction methods. This study was therefore initiated to evaluate the effects of extraction methods on the physiological quality of seeds and seedlings of tomato.*

## Result

*Results revealed that the highest germination percent (99.33 and 89.76% under laboratory and field conditions respectively) was obtained at 2% HCl for 60 minutes. Whereas, the maximum weight of 1000 seeds (4.277 g) was found at 1% of HCl for 30 minutes. Mycoflora load was maximum (36%) at fermentation for 72 hours. Yet, no mycoflora was detected from higher concentrations of HCl acid greater than 2% and time durations longer than 60 minutes.*

## Conclusion

the major seed quality parameters such as seed germination percent, seedling length, seedling fresh weight, seedling dry weight, and vigour indices were significantly higher at extraction method of 2% HCl for 60 minutes. Thus, it can be concluded that the maximum physiological seed quality and best performance of seedlings of tomato obtained from 2% HCl for 60 minutes seed extraction method of tomato.

## Background

Tomato (*Solanum lycopersicum* L.) belongs to the family *Solanaceae* is one of the most popular and worldwide consumed vegetable crops (Valenciano and Battistuzzi, 2012; Asfaw and Eshetu, 2015). It is also the most processed vegetable crop and ranks first in commercial and nutritional contribution to the human diet (Jayathunge et al., 2012; Yusufe et al., 2017).

Domestic production and export of tomato in Ethiopia is significantly increasing. It provides higher income for producers as well as a source of hard currency for the country (EIA, 2012; Asfaw and Eshetu, 2015; FAO, 2019). However, shortage of quality seeds constricted its production. Specifically, local-open pollinated varieties are being replaced by imported hybrids.

The success of germination, growth and final yield of crops largely depends on the quality of seeds. Quality of seed refers to the viability, freedom from damage, healthy, purity and vigour attributes of a seed that enables the emergence and establishment of normal seedlings under a wide range of environments (Kailappan and Karunanithy, 2006; Khan, 2013; Nemati et al., 2010; Finch-Savage and Bassel, 2016).

Multiple factors affect seed quality of tomato by such as fruit maturity, methods of seed extraction, fermentation period and temperature (Nemati et al., 2010). A mucilaginous gel substance in tomato seeds has germination inhibitors. Thus, seed extraction includes removal of pulp and the gelatinous substances surrounding the seed (Vishwanath et al., 2006).

The pulp and gel surrounding the seeds can be removed by different methods of extraction such as natural fermentation, mechanically or using chemicals. Sodium carbonate, sodium hydroxide, ammonium hydroxide, hydrochloric acid, acetic acid, calcium hypochlorite, pectinases and sulfuric acid are among commonly used chemicals for tomato seed extraction (Demir and Samit, 2001; França et al., 2013; Rival et al., 2016). A specific concentration of chemicals applied to the fleshy fruits together with the pulp and seeds for a specific time period.

Natural fermentation and manual seed extraction methods are commonly used methods but not effective for large scale production. Chemical methods mostly preferred as they are easier and faster for large scale production and obtain disease-free seeds (Kailappan and Karunanithy, 2006; Nemati et al., 2010; Vishwanath et al., 2016). However, it is evident that chemicals with higher concentration harm the embryo of seeds so as affect their nutritive value, germination percent and other seed quality parameters.

Acid methods of seed extraction mostly used to get rid of the gelatinous pulp from surrounding seeds (Sachan et al., 2009; Ankit et al., 2016; CAFT, 2017). Large scale seed producers often prefer HCl acid (1 to 3%) extraction method as it speedily separates the gel ensuring a clean and very bright seed coat (Eevera and Vanangamudi, 2006; Rival et al., 2016). HCl acid extraction method avoids temperature extremes during fermentation. It also efficiently breakdown and quickly clean the gelatinous substance from seeds; and enable to abolish bacterial canker (Desai, 2004). However, it can be deteriorative unless the duration of time and levels of concentrations are not optimized.

Seed mycoflora load also influenced by extraction methods. Unsatisfactory seed extraction methods cause extensive disease epidemics (Ankit et al., 2016). However, empirical evidences are scanty on the instant and latent effects of methods of tomato seed extraction on seed physiology and seedling performance. This study was therefore initiated to examine the effects of seed extraction methods of tomato on the physiological quality of seeds and seedlings of tomato.

## Results

Data on selected seed quality parameters and seedling characteristics were recorded during the course of the study. Results of the study are presented and discussed sequentially as follows.

### Weight of 1000 seeds

Results revealed that treatment means of weight of 1000 seeds was significantly ( $P < 0.01$ ) affected by seed extraction methods. Generally, weight of 1000 seeds decreased with increasing concentration of HCl and time length.

Table 1  
Effects of different seed extraction methods and time length on weight of 1000 seeds and seed mycoflora

Treatments	Weight of 1000 seeds (g)	Seed mycoflora (%)
Fermentation for 24 hrs	4.140 <sup>ab</sup>	22.67 <sup>c</sup>
Fermentation for 48 hrs	3.107 <sup>d</sup>	29.33 <sup>b</sup>
Fermentation for 72 hrs	1.727 <sup>e</sup>	36.00 <sup>a</sup>
1% HCl for 30 min	4.277 <sup>a</sup>	17.33 <sup>d</sup>
1% HCl for 60 min	4.037 <sup>ab</sup>	9.33 <sup>e</sup>
1% HCl for 90 min	3.940 <sup>ab</sup>	1.33 <sup>fg</sup>
2% HCl for 30 min	4.233 <sup>a</sup>	4.00 <sup>f</sup>
2% HCl for 60 min	4.007 <sup>ab</sup>	0.00 <sup>g</sup>
2% HCl for 90 min	3.350 <sup>cd</sup>	0.00 <sup>g</sup>
3% HCl for 30 min	3.653 <sup>bc</sup>	1.33 <sup>fg</sup>
3% HCl for 60 min	2.843 <sup>d</sup>	0.00 <sup>g</sup>
3% HCl for 90 min	1.440 <sup>e</sup>	0.00 <sup>g</sup>
Significance	**	***
LSD (5%)	0.514	3.370
CV (%)	4.0	19.8
Means within a column sharing common letter(s) are not significantly different at 5% level of significance; * = significant at $p < 0.05$ ; LSD = Least significant difference; CV = Coefficient of variation.		

### Seed mycoflora

The per cent mycoflora detection was significantly ( $P < 0.001$ ) affected by seed extraction methods. The highest percentage of mycoflora (36%) observed from fermentation method for 72 hours followed by fermentation for 48 hours (29.33) (Table 1). On the other hand,

mycoflora load drastically decreased as HCl concentration together with time length increased (Fig. 1. B).

Seeds extracted with (A = 3% HCl for 60 minutes, B = 2% HCl for 90 minutes, C and E = 3% HCl for 90 minutes; D = fermentation for 72 hours)

First count germination *and* Germination percentage

The analysis of variance showed that first count germination was significantly ( $P < 0.001$ ) affected by treatment effects. Seed extraction methods and time length also showed a significant ( $P < 0.001$ ) influence on germination per cent of seeds both in laboratory and open field pot trials (Table 2).

Table 2

Effects of different seed extraction methods and time length on first count germination, germination percentage and seed emergence

Treatments	First count germination (%)	Germination percentage (%)	Seed emergence (%) (open field in pot)
Fermentation for 24 hrs	75.00 <sup>bcd</sup>	85.00 <sup>cd</sup>	81.00 <sup>bc</sup>
Fermentation for 48 hrs	59.33 <sup>e</sup>	69.67 <sup>f</sup>	69.08 <sup>de</sup>
Fermentation for 72 hrs	23.00 <sup>f</sup>	30.67 <sup>g</sup>	33.71 <sup>f</sup>
1% HCl for 30 min	72.67 <sup>cd</sup>	76.33 <sup>e</sup>	68.79 <sup>de</sup>
1% HCl for 60 min	76.00 <sup>bcd</sup>	81.33 <sup>d</sup>	73.36 <sup>cd</sup>
1% HCl for 90 min	78.00 <sup>bcd</sup>	85.33 <sup>cd</sup>	76.46 <sup>bcd</sup>
2% HCl for 30 min	82.00 <sup>bc</sup>	86.67 <sup>c</sup>	78.72 <sup>bc</sup>
2% HCl for 60 min	93.33 <sup>a</sup>	99.33 <sup>a</sup>	89.76 <sup>a</sup>
2% HCl for 90 min	83.33 <sup>b</sup>	94.67 <sup>b</sup>	81.99 <sup>ab</sup>
3% HCl for 30 min	79.67 <sup>bc</sup>	88.67 <sup>c</sup>	79.48 <sup>bc</sup>
3% HCl for 60 min	75.33 <sup>bcd</sup>	81.67 <sup>d</sup>	73.33 <sup>cd</sup>
3% HCl for 90 min	69.33 <sup>d</sup>	73.33 <sup>ef</sup>	64.10 <sup>e</sup>
Significance	***	***	***
LSD (5%)	9.802	4.632	8.508
CV (%)	8.1	3.5	7.0
Means different letter within a column are significantly different at 5% level of significance; LSD = Least significant difference; CV = Coefficient of variation.			

Lengthening the time from 60 into 90 minutes or increasing the concentration of HCl from 2% into 3% also resulted significantly reduced germination percentage (94.67%) and (88.67%), respectively. This might also be due to corrosive effect of acid over prolonged period (Vishwanath et al., 2006; Nemati et al., 2010; França et al., 2013).

## Seeds emergence

Significantly maximum (89.76%) seeds emergence was obtained from 2% HCl for 60 minutes followed by 2% HCl for 90 minutes (81.99%).

## Seedling length

Among all extraction methods, significantly ( $P < 0.01$ ) maximum seedling length (14.00 cm) was recorded at 2% HCl for 60 minutes followed by 1% HCl for 90 minutes (13.50 cm).

## Seedling fresh weight and dry weight

Seedling fresh weight was significantly ( $P < 0.01$ ) affected by the treatment effects. Seedling dry weight was also significantly ( $P < 0.01$ ) influenced by seed extraction methods.

Table 3

Effects of different seed extraction methods and length of time on seedling length, seedling fresh weight and seedling dry weight

Treatments	Seedling length (cm)	Seedling fresh weight (g)	Seedling dry weight (g)
Fermentation for 24 hrs	13.13 <sup>abc</sup>	541.6 <sup>ab</sup>	24.16 <sup>b</sup>
Fermentation for 48 hrs	11.93 <sup>ef</sup>	490.6 <sup>d</sup>	19.06 <sup>d</sup>
Fermentation for 72 hrs	10.77 <sup>g</sup>	437.1 <sup>e</sup>	13.71 <sup>e</sup>
1% HCl for 30 min	12.15 <sup>def</sup>	518.2 <sup>c</sup>	21.82 <sup>c</sup>
1% HCl for 60 min	12.95 <sup>bcd</sup>	531.4 <sup>bc</sup>	23.14 <sup>bc</sup>
1% HCl for 90 min	13.50 <sup>ab</sup>	541.6 <sup>ab</sup>	24.16 <sup>b</sup>
2% HCl for 30 min	13.05 <sup>bc</sup>	558.1 <sup>a</sup>	24.85 <sup>b</sup>
2% HCl for 60 min	14.00 <sup>a</sup>	556.2 <sup>a</sup>	26.73 <sup>a</sup>
2% HCl for 90 min	12.60 <sup>cdef</sup>	544.8 <sup>ab</sup>	24.31 <sup>b</sup>
3% HCl for 30 min	12.73 <sup>bcde</sup>	538.5 <sup>abc</sup>	23.85 <sup>b</sup>
3% HCl for 60 min	11.73 <sup>f</sup>	484.2 <sup>d</sup>	19.09 <sup>d</sup>
3% HCl for 90 min	10.83 <sup>g</sup>	450.7 <sup>e</sup>	15.40 <sup>e</sup>
Significance	**	**	**
LSD (5%)	0.8913	22.45	1.821
CV (%)	4.2	2.6	5.0
Means different letter within a column are significantly different at 5% level of significance; LSD = Least significant difference; CV = Coefficient of variation.			

## Vigour indices

Both vigour index I and II were significantly affected by extraction method and time length at  $P < 0.001$  and  $P < 0.01$  respectively.

Table 4  
Effects of different seed extraction methods and length of time on seed vigour index I and vigour index II

Treatments	Seed vigour index I (unit)	Seed vigour index II (unit)
Fermentation for 24 hrs	1116 <sup>bc</sup>	2054 <sup>c</sup>
Fermentation for 48 hrs	832 <sup>fg</sup>	1329 <sup>f</sup>
Fermentation for 72 hrs	329 <sup>h</sup>	417 <sup>h</sup>
1% HCl for 30 min	929 <sup>ef</sup>	1666 <sup>e</sup>
1% HCl for 60 min	1054 <sup>cd</sup>	1883 <sup>d</sup>
1% HCl for 90 min	1153 <sup>bc</sup>	2062 <sup>c</sup>
2% HCl for 30 min	1132 <sup>bc</sup>	2154 <sup>bc</sup>
2% HCl for 60 min	1391 <sup>a</sup>	2655 <sup>a</sup>
2% HCl for 90 min	1194 <sup>b</sup>	2301 <sup>b</sup>
3% HCl for 30 min	1129 <sup>bc</sup>	2115 <sup>c</sup>
3% HCl for 60 min	959 <sup>de</sup>	1560 <sup>e</sup>
3% HCl for 90 min	794 <sup>g</sup>	1128 <sup>g</sup>
Significance	***	**
LSD (5%)	109.4	170.8
CV (%)	6.5	5.7
Means different letter within a column are significantly different at 5% level of significance; LSD = Least significant difference; CV = Coefficient of variation.		

## Discussion

### Weight of 1000 seeds

Among treatment means of fermentation, the seed weight was decreased by 58.28% as the duration of fermentation increased from 24 to 48 hours. Whereas, soaking of tomato fruits in 1% of HCl for 30 minutes resulted in maximum weight of 1000 seeds (4.277 g) across all treatments. This result was also statistically similar with 2% HCl for 30 minutes, fermentation for 24 hours, 1% HCl for 60 minutes, 2% HCl for 60 minutes and 1% HCl for 90 minutes (Table 1). Figure 3. (A). This might probably be due to the presence of gelatinous substance adhered to the seeds and partial removal of mucilage (Vishwanath et al., 2006). The minimum weight (1.440 g) was obtained from 3% HCl for 90 minutes.

### Seed mycoflora

No mycoflora was detected from higher concentrations (2% and more HCl) and time lengths of 60 minutes and longer. As par with this result Vishwanath et al., (2006) also reported the highest mycoflora load from fermentation extraction and the lowest load from higher concentration of (2.5% HCl) acid extraction methods. It has been evidenced that fusarium, root nematodes and verticillium pathogens resided deep within seed coats and fuzz could be disinfected by acid extraction method (Vishwanath et al., 2006; Dick and Dick, 2014; Ankit, 2016).

### First count germination

The highest first count germination (93.3%) was recorded at 2% HCl for 60 minutes whereas the lowest (23%) was recorded from seeds extracted with 72 hours fermentation (Table 2). On the contrary, Ankit et al. (2016) reported the highest first count germination (93.33%) from 1% HCl for 30 minutes over fermentation and NaCO<sub>3</sub> method of tomato seed extraction. In contrast, the current study showed that

lower concentration (less than 2% HCl) and shorter length of time (shorter than 60 minutes) resulted in lower first count germination percent. The difference might be due to difference in fruit mesocarp thickness of the varieties.

## Germination percentage

The highest germination per cent (99.33 and 89.76% in laboratory and open field pot experiments respectively) was obtained at 2% HCl for 60 minutes followed by 2% HCl for 90 minutes (94.67% and 81.99% in laboratory and open field pot experiments respectively). However, the lowest germination percent (30.67) was shown from fermentation process for 72 hours and lower concentration (less than 2% HCl) as well as 2% HCl for 30 minutes. This finding is supported by Demir and Samit (2001).

Germination per cent highly declined when fermentation period prolonged from 24 to 48 then to 72 hours (Fig. 3. B). Darken and swollen (imbibed) seed coat was also observed (Fig. 4. C). An extended period of fermentation likely imposed seeds to germinate during extraction process and protruded radicle killed during seed drying process. Nemati et al., (2010) also reported analogous findings. As shown in Fig. 3. (B), the lower germination in lower concentration (1% HCl) and shorter dipping time (less than 60 minutes) might probably be due to presence of inhibitors in the gelatinous substance adhered to the seeds (Vishwanath et al., 2006).

Lengthening the time from 60 into 90 minutes or increasing the concentration of HCl from 2% into 3% also resulted significantly reduced germination percentage (94.67%) and (88.67%), respectively. This might also be due to corrosive effect of acid over a prolonged period (Vishwanath et al., 2006; Nemati et al., 2010; França et al., 2013).

## Seeds emergence

The lowest seeds emergence (33.71%) was found at fermentation for 72 hours (Table 2). The poorest germination might be either the seeds were damaged by fungal pathogens or seeds were germinated during extended fermentation time thus failed to germinate during germination test. This finding is in conformity with the results of Evera and Vanangamudi (2006) and Nemati et al., (2010) who reported that decreased seed emergence due to fermentation longer than 48 hours. This might be due to premature sprouting and reduced germination from extended fermentation period.

## Seedling length

The minimum seedling length 10.77 cm and 10.83 cm recorded at fermentation for 72 hours and 3% HCl for 90 minutes respectively (Table 3 and Fig. 3. B). Quite the reverse, Ankit et al., (2016) testified a maximum seedling length (13.49 cm) from 24 hours fermentation. On the contrary, Nemati et al., (2010) reported no significant difference between short-term fermentation and severe plant height reduction with long-term fermentation. In the current study, the difference in seedling length might be due to difference in date of germination. Earlier germinated seedlings probably had longer periods for root and shoot growth.

## Seedling fresh weight and dry weight

Maximum fresh weight (558.1 g) found at 2% HCl for 30 minutes which is statistically at par with 2% HCl for 60 minutes (556.2 g) (Table 3).

The highest seedling dry weight (26.73 g) was observed from 2% HCl for 60 minutes followed 1% HCl for 30 minutes (24.85 g) time length. Minimum dry weight (13.71 g) and (15.4 g) was recorded from 72 hours fermentation and 3% HCl for 90 minutes (Table 3).

## Vigour indices

The highest vigour index I (1391) and Vigour Index II (2655) were obtained from dibbing of the crashed tomato fruits in 2% HCl for 60 minutes while the lowest vigour index I (329) and Vigour Index II (417) were recorded from fermentation for 72 hours (Table 4).

There was a severe increase in seed vigour indices with increasing HCl concentration and time length up to 2% HCl for 60 minutes then radically decreased beyond that point (Fig. 4. A and B). However, CAFT (2017) recommended 3% HCl for 30 minutes to get the best vigour and seed quality of tomato. But, in this study seed vigour was reduced over 2% HCl. This might be due to differences in pulp thickness. On the other hand, Demir and Samit (2001) reported that best seed vigour can be obtained from 2 and 3% HCl acid extraction.

## Conclusion

The aforementioned results showed that most important seed physiological quality and seedling characteristic parameters such as first count, germination per cent, seed emergence, seedling length, seedling fresh weight, seedling dry weight, vigour index I and vigour index II were significantly higher at extraction method of 2% HCl for 60 minutes. Furthermore, no mycoflora was detected from seeds extracted by

dipping into 2% HCl for 60 minutes. Thus, it can be concluded that the maximum physiological seed quality of tomato can be obtained from a seed extraction method of dipping within 2% HCl concentration for 60 minutes period of time.

## Methods

### Experimental Site Description

The experiment was conducted both in laboratory and field demonstration site of Horticulture department, College of Agriculture and Environmental Sciences, University of Gondar. It has been implemented in the year 2019/20. The experimental site is located at an altitude of 1906 m.a.s.l

### Experimental Materials

Tomato (*Solanum lycopersicum* L. Var. Galilea) fruits with uniform size, shape and fully ripened were collected from youth tomato producers' farm in Mecha district. The variety Galilea is produced by Hazera Seeds Ltd, South Africa (Pty) and commercially supplied by Greenlife Trading PLC in Ethiopia. Hydrochloric acid (HCl) was also used for seed extraction treatment.

### Treatments and Experimental Design

Natural fermentation and HCl acid seed extraction methods exposed for different time lengths were the treatments. Accordingly, seeds were extracted through fermentation periods of 24, 48 and 72 hours and dipping of tomato fruits in a 1%, 2%, and 3% (V/V) HCl acid concentration for 30, 60 and 90 minutes. Hence, there were 12 treatment combinations of replicated three times. Consequently, there were a total of  $12 * 3 = 36$  experimental units laid out in a completely randomized design.

### Experimental Procedures

Tomato seeds were extracted from about 1 kg of (about ten) fruits var. Galilea. Fruits with uniform size, shape and fully ripened were considered. These fruits were crushed and squeezed within clean plastic buckets. Then, seeds were extracted in both fermentation and HCl acid extraction methods. The physiological qualities of seeds and seedling were then evaluated.

### Fermentation extraction method

The tomato fruits were wrinkled manually. Then, the pulp along with gelatinous material and the seed were allowed to ferment for a period of 24, 48 and 72 hours at room temperature of 18 to 33 °C and relative humidity of 42%. The mixture was agitated daily to allow uniform rate of fermentation and elude discolouration. The seeds were repeatedly lapped with tap water. Good seeds and abnormal seeds together with other debris were separated by sink and floating method on the water surface. Finally, the good seeds were surface dried over rough papers for three days at room temperature and then weighed and packed in plastic bags (ISTA, 2007).

### HCl acid extraction method

Fully ripened tomato fruits with uniform maturity stage were lanced and broken. A 1%, 2%, and 3% HCl acid solution (V/V) was prepared for each kg of tomato fruits in a volume/weight basis. The juicy pulps with gelatinous substances were dipped for 30, 60 and 90 minutes. The seeds were then extracted, dried and packed with similar procedures followed during the fermentation process (Fig. 5).

A = Surface drying of seeds; B = Seeds extracted with HCl acid; C = Seeds extracted with fermentation for 72 hours; D = Weighed and packed of 500 seeds

### Data Collection

#### Weight of 1000 seeds (g)

once the seeds extracted, samples of 1000 seeds were randomly taken from each treatment combinations, dried according to Rao et al. (2006) and weighed with sensitive balance. The weight was recorded expressed in grams to three decimal places (ISTA, 2007; França et al., 2013; Debela et al., 2016).

#### Seed mycoflora count (%)

the presence of fungi on seeds was detected by blotter method as recommended by ISTA (2007). For each treatment combination, 100 seeds were planted on a double layer moistened blotters of petri plates. Afterwards, the petri plates were maintained at room temperature ranging 18–33 °C and relative humidity of 42%. About seven days later, the number of infected seeds (fungal colonies) were counted according to Vishwanath et al., (2006) and expressed as percentage.

#### First germination count (%)

a first-day germination count was taken on the fifth day after planting based on ISTA (2007).

## Germination (%)

Germination test was undertaken under laboratory and field conditions. For the laboratory trial, 100 seeds representing each treatment combination were planted in petri dishes, a moistened double-layer blotter paper inside. Petri dishes then placed in the laboratory at room temperature. Then again, the field trial was carried out. Six randomly selected seeds were planted on triplicated pots. Germination count was begun on the 5th day and lasts on the 14th day after planting. Germination test for normal seedlings was recorded and expressed in percentage based on ISTA (2007).

## Seedling emergence (%)

randomly selected six seeds were planted on triplicated pots representing each treatment combination. Adequate moisture was maintained to make as suitable as possible for seed emergence. Seedlings emerged 3 cm above the soil surface was recorded on 7th to 14th days after planting according to Debela et al., (2016), calculated and expressed as a percentage of seed emergence.

## Seedling length (cm)

Seedling length includes the length of shoot tips to root tips. Ten randomly selected normal seedlings were considered. On the day of the final count, the length from the collar region to the tip of the primary shoot was measured as shoot length (cm) and from the collar region to the tip of primary root were measured as root length (cm). Then, the seedling length was computed by using the following formula, Seedling length (cm) = Shoot length (cm) + Root length (cm).

## Seedling fresh weight (g)

On the final count day, four normal seedlings were uprooted from the growing pots of each treatment and the entire biomass weighed (Debela et al., 2016). The value was expressed in gram.

## Seedling dry weight (g)

the seedlings considered for fresh weight were dried in a hot-air oven at 80°C temperature for 24 hours. The weight of the dried seedlings was recorded and the average weight was calculated and expressed as seedling dry weight in grams (Debela et al., 2016).

## Vigour indices

Vigour index-I and vigour index-II were calculated using the procedure suggested by (Finch-Savage and Bassel, 2016; Abdul-Baki and Anderson, 1973 cited in Ankit et al., 2016) and expressed in whole number.

Vigour index-I = Germination (%) X Seedling length (cm)

Vigour index-II = Germination (%) X Seedling dry weight (g)

# Data Analysis

Data collected from laboratory and field (pot) experiments were subjected to analysis of variance (ANOVA) using GenStat statistical software version 15.1. All significant pairs of treatment means were compared using Fisher's LSD (Least Significant Difference Test) at 5% level of significance.

## Abbreviations

Not applicable

## Declarations

**Ethics approval and consent to participate:** Not applicable

**Consent for publication:** Not applicable

**Availability of data and materials:** The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Competing interests:** The authors declare that they have no competing interests

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**Authors' contributions:** AD contributed in data collection, analysis, interpretation of results, write up and coordination of the entire research activities. TT and BM involved in data collection, analysis and interpretation of results.

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## Figures

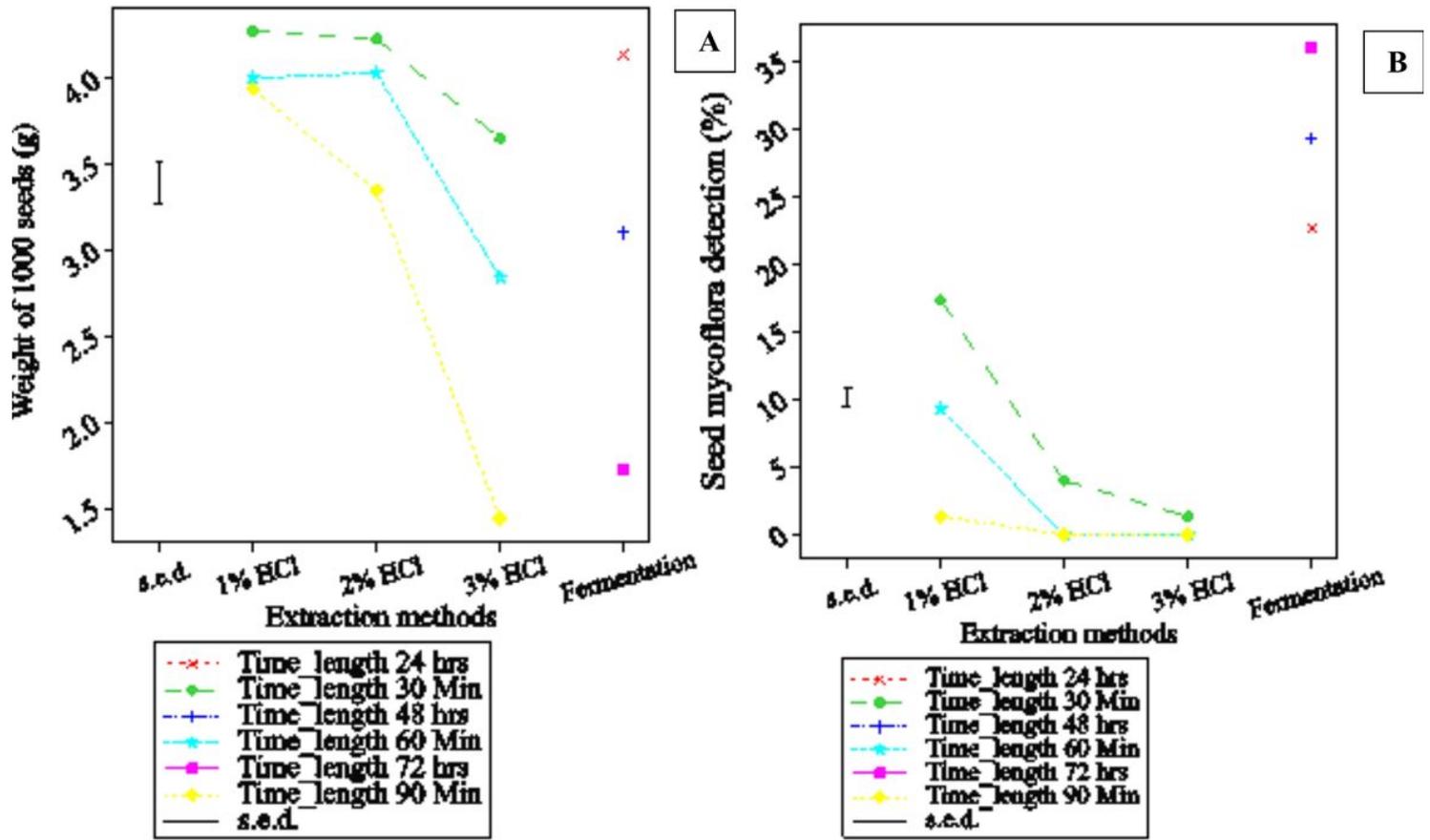


Figure 1

Seed extraction methods affected 1000 seeds weight (A) and seed mycoflora (B)

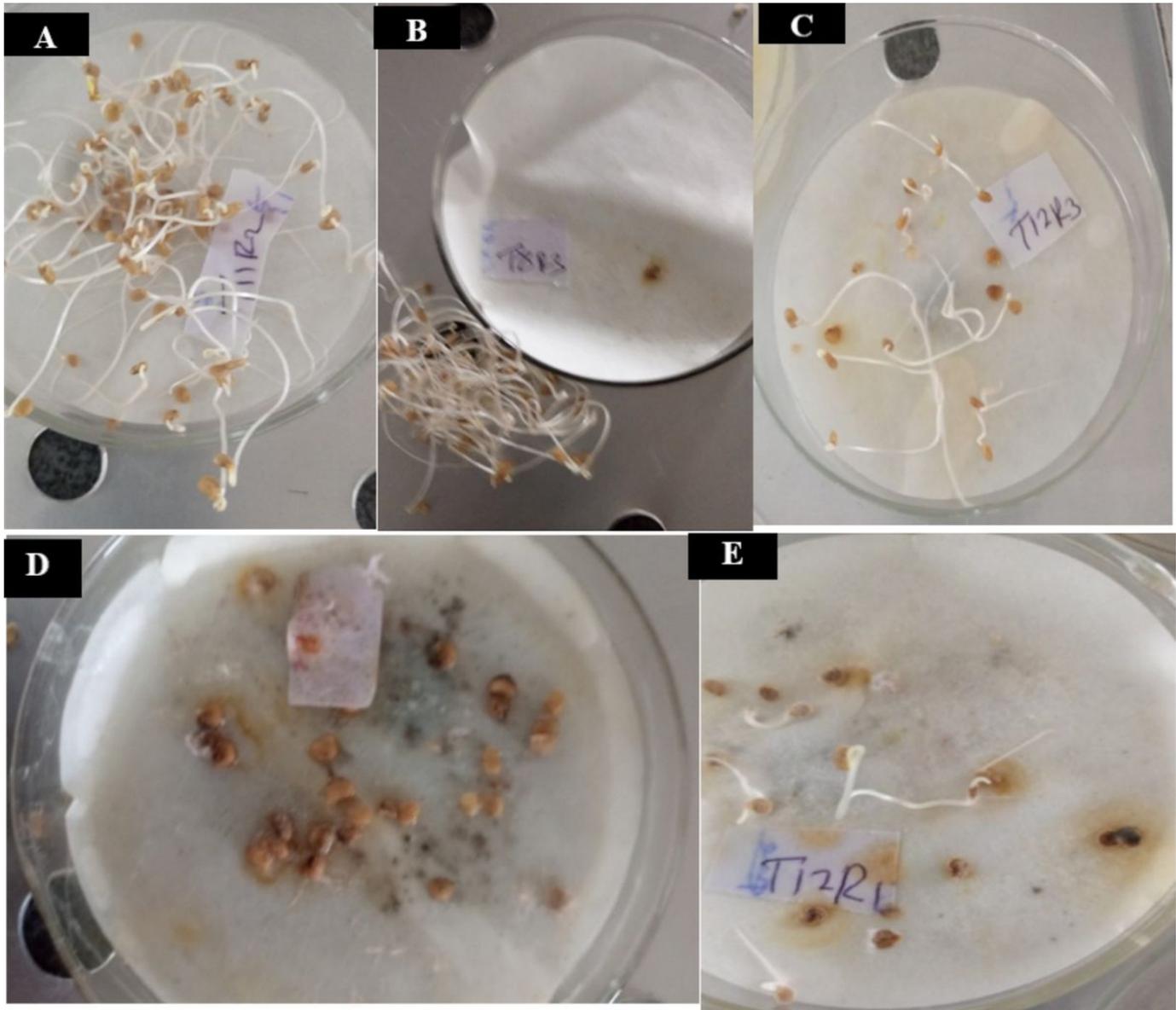


Figure 2

Effects of seed extraction methods on seed germination after 14 days of planting

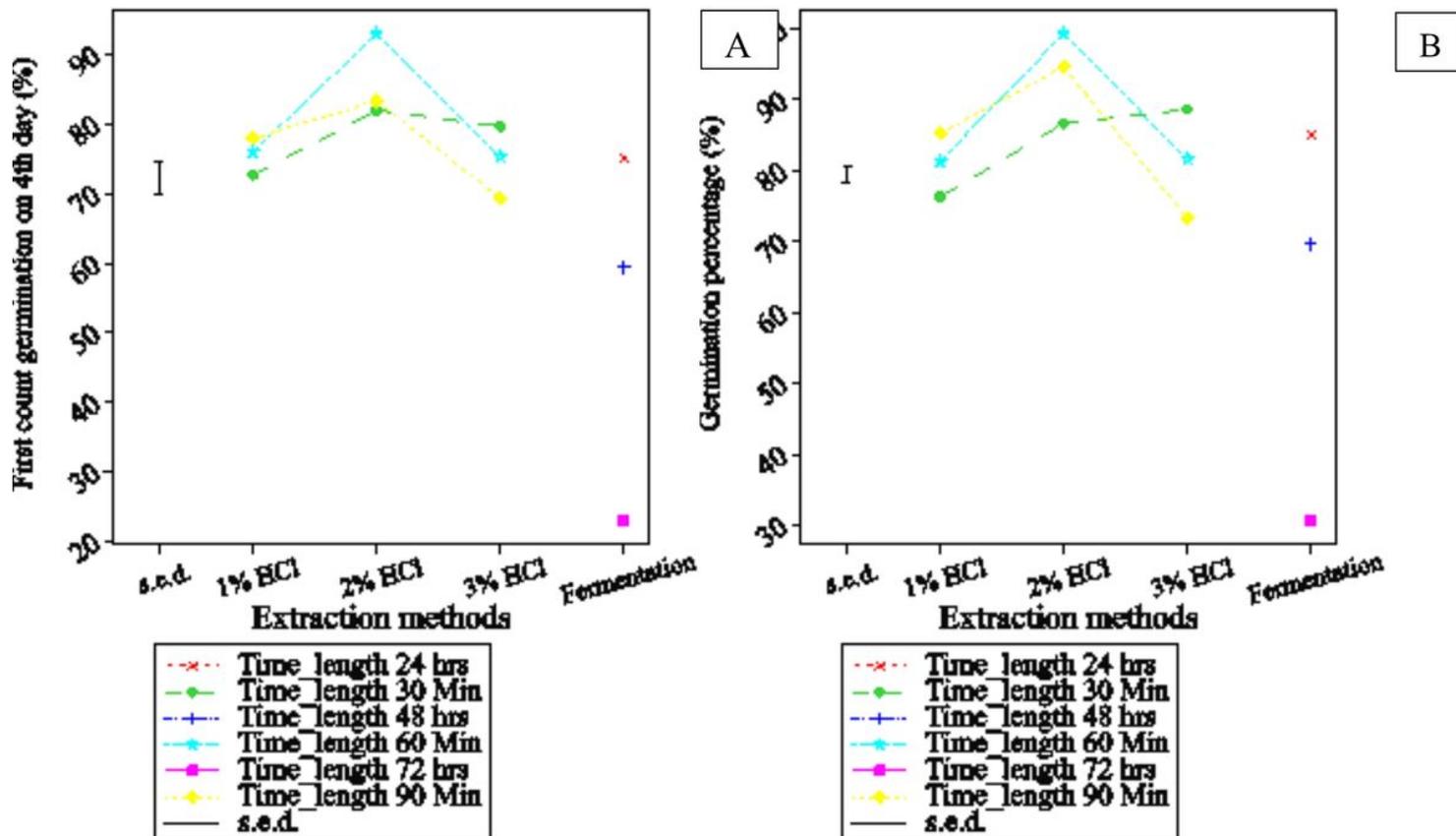
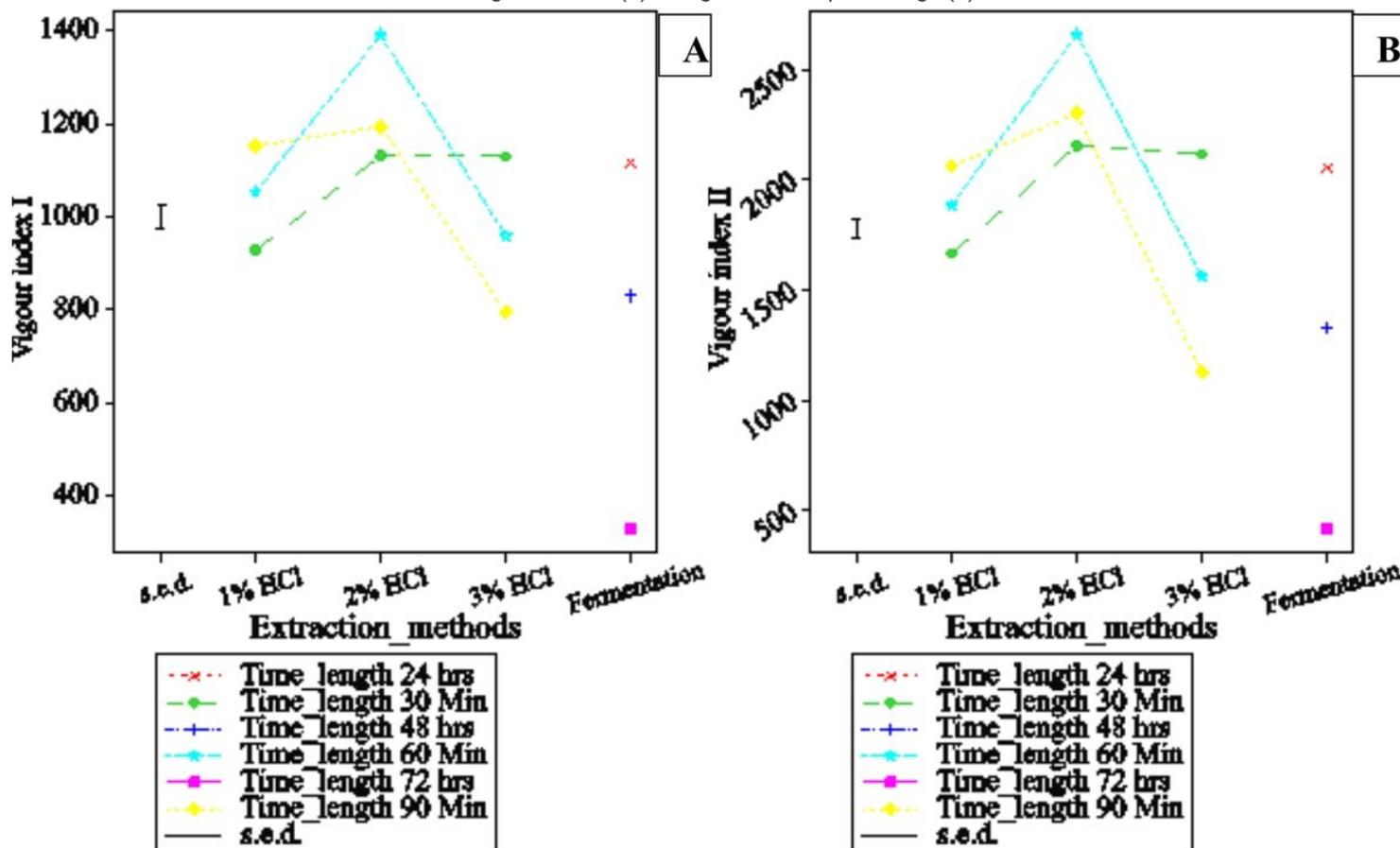


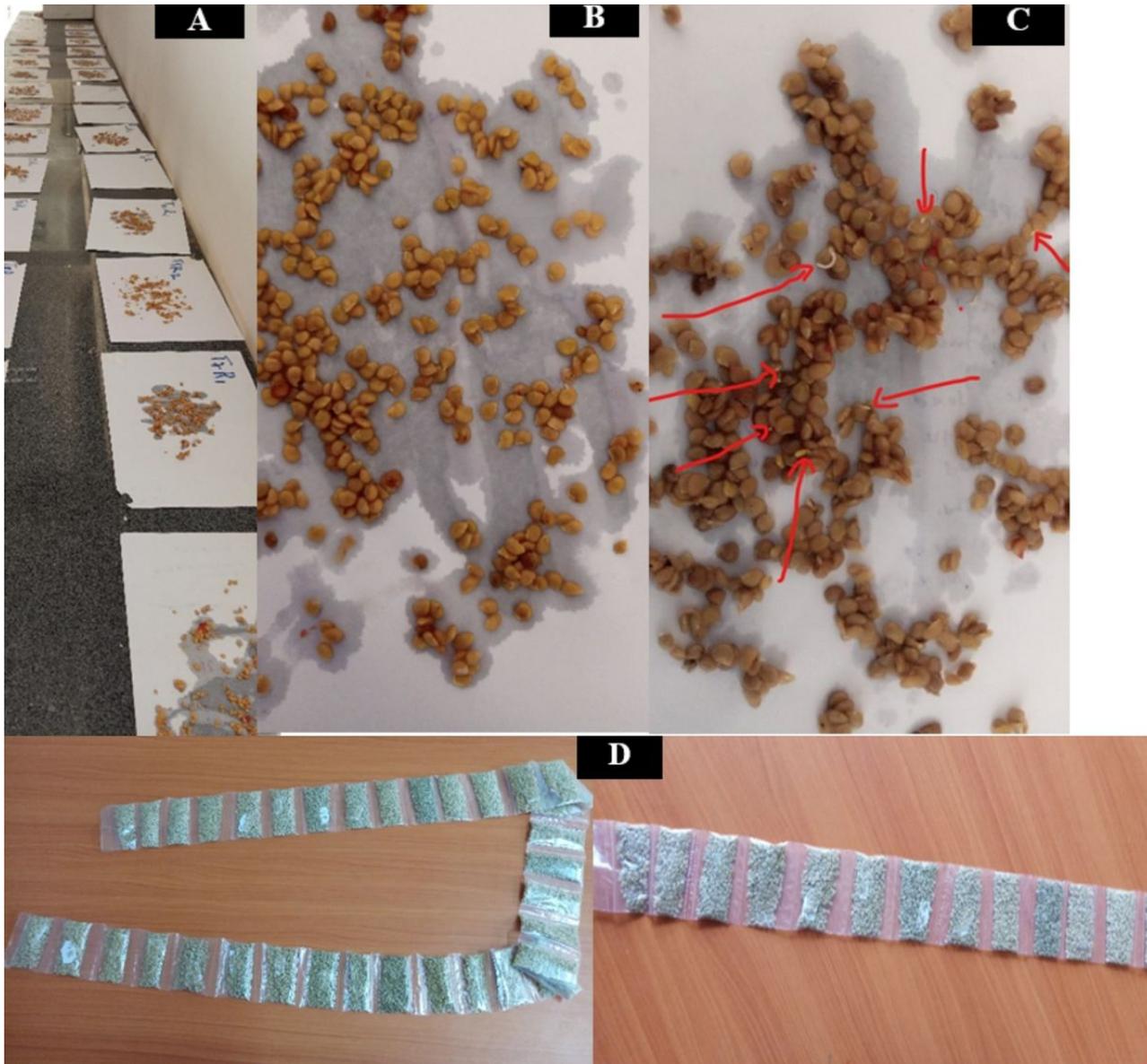
Figure 3

Seed extraction methods influenced first count germination (A) and germination percentage (B)



**Figure 4**

Changes in vigour indices I and II in response to seed extraction methods



**Figure 5**

Seeds extracted with different methods