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

Alemu Degwale (alemudegwalle@gmail.com)
Wolaita Sodo University  https://orcid.org/0000-0002-3494-1285

Tiru Tesfa
University of Gondar, College of Agriculture and Environmental Sciences

Belete Meseret
University of Gondar, College of Agriculture and Environmental Sciences

Research

Keywords:

DOI: https://doi.org/10.21203/rs.3.rs-33074/v1

License: © This work is licensed under a Creative Commons Attribution 4.0 International License.  Read Full License
Abstract

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. Even though its demand is increasing with increasing profitability, the production constricted by lack of quality seeds due mainly to lack of proper seed extraction method particularly for large scale seed production. Extensive disease epidemics might also be due to unsatisfactory seed extraction method. Empirical evidences are scanty on instant and latent effects of seed extraction methods of tomato seeds. Thus, this study was initiated to investigate the effect of seed extraction methods on physiological quality of seeds and seedlings of tomato. The experiment was carried under laboratory and field conditions in a completely randomized design. 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, maximum weight of 1000 seeds (4.277g) was found at 1% of HCl for 30 minutes whereas maximum mycoflora load (36%) was observed from 72 hours fermentation. Yet, no mycoflora was detected from higher concentration (2% and more HCl) and time length (60 minutes and longer time). Seed quality parameters like 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 maximum physiological seed quality and best performance of seedlings of tomato can be obtained from 2% HCl for 60 minutes.

Background

Tomato (Solanum lycopersicum L.) belongs to the family Solanaceae is one of the most popular and worldwide consumed vegetable crops. It is also the most processed vegetable crop and ranks first in commercial and nutritional contribution to the human diet (Dandena et al., 2013; Isack and Monica, 2013; Asfaw and Eshetu, 2015; Ankit et al., 2016; Mawardii et al., 2017). Domestic production and export of tomato in Ethiopia is significantly increasing with increasing profitability providing higher income for producers (EIA, 2012 and Debela et al., 2016) and making a significant contribution to the national economy (Baredo et al., 2014). However, the production is constrained by lack of quality seeds especially of local open pollinated varieties which are being replaced by imported hybrids. The success of germination, growth and final yield of crops largely depend on quality of seeds. Quality of seed refers to the viability, free 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; and Savageand Bassel, 2016).

Tomato seed quality is affected by factors such as fruit maturity, methods of seed extraction, time length of fermentation, and fermentation 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 (Jeffrey, 2004; and Vishwanath et al., 2006).

The pulp and gel surrounding the seeds can be removed by different extraction methods such as natural fermentation, using chemicals or by mechanical means. 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; and Rival et al., 2016). A specific concentration of chemicals applied to the fleshy fruits together with the pulp and seeds for 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; Ankit et al., 2016; and Vishwanath et al., 2016). However, it is evident that chemical with higher concentration harm the embryo of seeds so as affect their nutritive value, germination percentage and other seed quality parameters.

Acid extraction methods especially 1–3% hydrochloric acid largely used for separating tomato pulp and seeds (Sachan et al., 2009; Nemati et al., 2010; Ankit et al., 2016; and CAFT, 2017). This method is often favored by large commercial producers as it rapidly degrades the gelatinous seed coating resulting in the production of a very bright clean seed sample (Eevera and Vanangamudi, 2006; and Rival et al., 2016). Efficient breakdown of the gel surrounding the seed and quick cleaning, avoiding of the low and high temperature problem, eradication of bacterial canker and producing bright looking seed coat are important features of acid extraction method (Desai, 2004). However, it can be deteriorative on seed quality if application period and concentration are not appropriate.

Seed mycoflora load also influenced by extraction methods. Unsatisfactory method of tomato seed extraction is one of the reasons of extensive disease epidemics (Ankit et al., 2016). Empirical evidences are scanty on the instant and latent effects of seed extraction methods and time length of fermentation on seed physiology and seedling quality of tomato. This study was therefore intended to investigate the effects of seed extraction methods and time length of fermentation on physiological quality of seeds and seedlings of tomato.

Materials And Methodologies

The experiment was carried out in the year 2019/20 at Horticulture Department laboratory of College of Agriculture and Environmental Sciences, University of Gondar, Ethiopia located at an altitude of 1906 m.a.s.l.

Treatments and Experimental Design

Fermentation time for 24, 48 and 72 hours and dipping of tomato fruits with 1%, 2%, and 3% HCl acid for 30, 60 and 90 minutes were the treatment combinations. Thus, there were about 12 treatment combinations replicated thrice; $3^3 = 36$ experimental units laid out in a CRD.
Experimental Procedures

For each treatment, about 1 kg of uniform size, shape and fully ripened tomato fruits var. Gelila were taken directly from production field. The fruits crashed and squeezed with in a clean plastic buckets. Then, seeds were extracted with both methods following specific procedures for each. After extraction process, the quality of seeds was evaluated by seed physiology and seedling characteristics. Seedlings were planted in petri dishes and pots in laboratory and on field conditions, respectively.

Fermentation extraction method: The fruits were wrinkled manually then pulp along with gelatinous material and the seed was allowed to ferment for a period of 24, 48 and 72 hours at room temperature of 18 – 33 °C and relative humidity of 42%. The mixture was agitated daily to allow uniform rate of fermentation and elude discoloration. The seeds were repeatedly lapped with tap water. Good seeds and abnormal seeds and other debris were separated by sink and floating 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, 2020; Nemati et al., 2010).

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

Data Collection

Weight of 1000 seeds (g): once the seeds extracted, samples of 1000 seeds were randomly collected from each treatment combinations and weighed with sensitive balance to three decimal places expressed in grams (ISTA, 2007; França et al., 2013; Debela et al., 2016).

Seed mycoflora: the presence of fungi on seeds was detected by blotter method as recommended by ISTA (2020). 100 seeds were planted on a double layer moistened blotters of petri plates maintained at room temperature in three replications. After 7 days, the number of infected seeds (fungal colonies) were counted according to Vishwanath et al., (2006) and expressed as percentage.

First count (%): a first count data was taken on the 4th day after planting based on ISTA (2007).

Germination (%): 100 seeds representing each treatment were planted in petri dish in which moistened blotter paper was inside and the process triplicated. It will be better to convert it in to Average seedling fresh weightPetri dishes then placed in laboratory at room temperature. The final count was on 14th day of germination test for normal seedlings and expressed in percentage based on ISTA (2020).

Seedling emergence (%): randomly selected six seeds were planted on triplicated pots representing each treatment. 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 percentage of seed emergence.

Seedling length (cm): Seedling length includes the length of shoot tips to root tips. Ten random selected normal seedlings were considered. On the day of final count, the length from the collar region to the tip of the primary shoot were 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.

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.

Vigour indices: The vigour indices were calculated using the procedure suggested by (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 in laboratory and on 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. Correlation analysis was also performed to detect the linear relationship among seed physiological quality attributes and seedling characteristics.

Results And Discussion

Data on selected seed quality parameters and seedling characteristics were recorded during the course of the study. The results of the study are presented and discussed sequentially as follows.
The study results revealed that all seed physiological characteristics were significantly influenced by the extraction methods along with duration of time (Table 1).

Weight of 1000 seeds

Results revealed that means of weight of 1000 seeds extracted with fermentation and HCl acid methods with different time duration were significantly (P<0.01) different. Maximum weight of 1000 seeds (4.277g) was recorded at 1% of HCl dipping for 30 minutes even if 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). As shown in Fig.3. (A) dominantly higher seed weight in 1% HCl across all time lengths as compared with higher concentrations of HCl. The minimum weight (1.440 g) was obtained from 3% HCl for 90 minutes. Generally, weight of 1000 seeds decreased with increasing concentration of HCl. Maximum weight of 1000 seeds at lower HCl concentrations might probably be due to partial removal of mucilage and presence of gelatinous substance adhered to the seeds (Vishwanath et al., 2006).

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^{ab}               | 22.67^{c}          |
| Fermentation for 48 hrs | 3.107^{d}               | 29.33^{b}          |
| Fermentation for 72 hrs | 1.727^{a}               | 36.00^{a}          |
| 1% HCl for 30 min      | 4.277^{a}               | 17.33^{d}          |
| 1% HCl for 60 min      | 4.037^{ab}              | 9.33^{e}           |
| 1% HCl for 90 min      | 3.940^{ab}              | 1.33^{f}           |
| 2% HCl for 30 min      | 4.233^{a}               | 4.00^{f}           |
| 2% HCl for 60 min      | 4.007^{ab}              | 0.00^{g}           |
| 2% HCl for 90 min      | 3.350^{cd}              | 0.00^{g}           |
| 3% HCl for 30 min      | 3.653^{ab}              | 1.33^{g}           |
| 3% HCl for 60 min      | 2.843^{d}               | 0.00^{g}           |
| 3% HCl for 90 min      | 1.440^{f}               | 0.00^{g}           |

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 percent mycoflora detection was significantly (P< 0.001) affected by seed extraction methods and time length. 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 as well as time length increased (Fig. 2. B). No mycoflora was detected from higher concentration (2% and more HCl) and time length (60 minutes and longer time). As par with this result Vishwanath et al., (2006) also reported highest mycoflora load from fermentation extraction and lowest load from higher concentration of (2.5% HCl) acid extraction methods. It has been evidenced that fusarium, root nematodes and verticillum pathogens resided deep within seed coats and fuzzes usually emerge during germination could be disinfected by acid extraction method (Vishwanath et al., 2006; Dick and Dick, 2014; and Alabi, 2019).

First count germination

The analysis of variance showed that first count germination was significantly (P< 0.001) affected by treatment effects. 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 highest first count germination (93.33%) from 1% HCl for 30 minutes over fermentation and NaCO$_3$ 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 lower first count germination percent. The difference might be due to difference in fruit mesocarp thickness of the varieties.

Germination percentage

Seed extraction methods and time length showed a significant (P < 0.001) influence on germination percent of seeds both in laboratory and open field pot trials (Table 2). The highest germination percent (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 lab and open field pot experiments respectively). However, the lowest germination percent (30.67) was shown at 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 percent highly declined when fermentation period prolonged from 24 to 48 then to 72 hours (Fig. 4. B). Darken and swollen (imbibed) seed coat was also observed (Fig. 1. C). 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 ndings. As shown in Fig. 4. (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).

### 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 bcd                  | 85.00 cd                   | 81.00 bc                               |
| Fermentation for 48 hrs  | 59.33 e                    | 69.67 f                    | 69.08 de                               |
| Fermentation for 72 hrs  | 23.00 f                    | 30.67 g                    | 33.71 f                                |
| 1% HCl for 30 min        | 72.67 cd                   | 76.33 e                    | 68.79 d                                |
| 1% HCl for 60 min        | 76.00 bcd                  | 81.33 d                    | 73.36 cd                               |
| 1% HCl for 90 min        | 78.00 bcd                  | 85.33 cd                   | 76.46 bcd                              |
| 2% HCl for 30 min        | 82.06 bc                   | 86.67 c                    | 78.72 bc                               |
| 2% HCl for 60 min        | 93.33 a                    | 99.33 a                    | 89.76 a                                |
| 2% HCl for 90 min        | 83.33 b                    | 94.67 b                    | 81.99 ab                               |
| 3% HCl for 30 min        | 79.67 bc                   | 88.67 c                    | 79.48 bc                               |
| 3% HCl for 60 min        | 75.33 bcd                  | 81.67 d                    | 73.33 cd                               |
| 3% HCl for 90 min        | 69.33 d                    | 73.33 ef                   | 64.10 e                                |

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; and Ankit et al., 2016).

**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%). The lowest seeds emergence (33.71%) was found at fermentation for 72 hours (Table 2). The lowest 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**

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). 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. 4. B). Quite the reverse, Ankit et al., (2016) tested 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, 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**

Seedling fresh weight was significantly (P < 0.01) affected by the treatment effects. Maximum fresh weight (558.1g) found at 2% HCl for 30 minutes which is statistically at par with 2% HCl for 60 minutes (556.2g) (Table 3).

**Seedling dry weight**

Seedling dry weight was significantly (P< 0.01) influenced by seed extraction methods. 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 (13.71g) and (15.4 g) was recorded from 72 hours fermentation and 3% HCl for 90 minutes (Table 3).
### 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>bc</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>bcd</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>    |

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. 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. 5. 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.

### 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>c</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>          |

Means different letter within a column are significantly different at 5% level of significance; LSD=Least significant difference; CV=Coefficient of variation.
Conclusion
The aforementioned results showed that most important seed physiological quality and seedling characteristic parameters such as first count germination, germination percent, 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 dibbing in to 2% HCl for 60 minutes. Thus, it can be concluded that 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.

Declarations

Ethics approval and Consent to participate – Not applicable

Consent for Publication
This piece of manuscript entitled as “Effect of Seed Extraction Methods on Physiological Quality of Seeds and Seedlings of Tomato” is our own original research work and agreed to submit for your journal to be published. Please consider it

Availability of data and Materials
We confirm that all data and materials for regarding this manuscript are available.

Competing interests
We, the first Author as well as the Co-Authors confirm that the attached manuscript hereunder entitled as “Effect of Seed Extraction Methods on Physiological Quality of Seeds and Seedlings of Tomato” is our own original work.

Funding
We want to thank University of Gondar, College of Agriculture and Environmental Sciences for funding the research and providing all the necessary facilities required for the research.

Author’s contribution
The contribution of the first Author was as principal investigator of the research and the Co-Authors were co-investigators during the research implementation.

Acknowledgement – Not applicable

References
Abdul-Baki A.A. and Anderson J.D., 1973. Vigour determination in soybean seeds by multiple criteria. Crop Science1:3:630-633.
doi.org/10.2135/cropsci1973.0011183X001300060013x

Alabi O.A., 2019. Good quality seed production guide for smallholder farmers. A Field Guide for Extension Workers in South Sudan. Agriculture Extension Specialist at the Technical Assistance (TA) for Increased Agriculture Production of Smallholders in South Sudan implemented by AESA with funding from the European Union. https://fcluster.org/sites/default/files/documents/good_quality_seed_production_guide_aesa-fao_19_06_2019.pdf

Ankit R., Sasidharan N. and Kalyan R., 2016. Effect of seed extraction procedures on seed quality parameters in tomato. Advances in Life Sciences5(20).
doi=pjbs.2010.814.820

Asfaw Z. and Eshetu D., 2015. Production and management of major tomato crops in Ethiopia. Volume I. December 2015, Addis Ababa, Ethiopia. Ethiopian Institute of Agricultural Research, Addis Ababa, Ethiopia; KOPIA, Ethiopia Centre, Addis Ababa, Ethiopia. Printed at Eth-Cana printing press 149 pages, Addis Ababa, Ethiopia.

Baredo Y., 2012. Gamo Gofa zone diagnosis and planning document, Livestock and Irrigation Value Chains for Ethiopian Smallholders (LIVES) project. Ethiopia: Addis Ababa. http://prime-ethiopia.org/wp-content/uploads/2015/03/Gamo%20Gofa%20zone%20report%20-%20final.pdf

CAFT [Centre of Advanced Faculty Training in Horticulture (Vegetables)], 2017. Advances in quality seed production of vegetable crops. Department of Vegetable Science; YSP University of Horticulture and Forestry Nauni - 173 230 Solan, Himachal Pradesh.
http://www.yspuniversity.ac.in/vgc/caft/Compendium2017-18.pdf

Dandena G., Bekele A. and Lemma D., 2013. Effects of Gibberellic acid and 2, 4- Dichlorophenoxy Acetic Acid spray on vegetative growth, fruit anatomy and seed setting of tomato (Solanum lycopersicum esculentum Mill.). Sci. Technol. Arts Res. J., 2(3):25-34.
http://www.academicjournals.org/app/webroot/article/article1379675813_Gelmesa%20et%20al.pdf
Debela B.K., Belew D. and Nego J., 2016. Evaluation of tomato (*Lycopersicon esculentum* Mill.) varieties for growth and seed quality under Jimma condition, South Western Ethiopia. *International Journal of Crop Science and Technology*, Volume 2, Issue 2, 2016, Page: 69-77. DOI: 10.5897/JPBCS2015.0543.

Demir I. and Samit Y., 2001. Quality of tomato seeds as affected by fruit maturity at harvest and seed extraction method. *Gartenbauwissenschaft*, 66 (4). S. 199–202. ISSN 0016–478X. http://docsdrive.com/pdfs/ansinet/pjbs/2010/814-820.pdf.

Desai B.B., 2004. Seed handbook, biology, production, processing and storage. 2nd Edn. *Marcel Decker Inc., New York*, pp: 233-359. doi: 10.1093/aob/mcj036.

Dick J. A. and Dick A. A., 2014. Tomato seed disinfection with chlorine. *Tomato solutions.* http://www.tomatosolutions.ca/sitebuildercontent/sitebuilderfiles/tomdis.pdf.

EIA (Ethiopian Investment Agency), 2012. Investment opportunity profile for production of fruits and tomatoes in Ethiopia; 2012. http://ethemb.se/wp-content/uploads/2013/07/Production-of-Fruits-and-vegetables-in-Ethiopia.pdf.

Finch-Savage F.W.E. and Bassel G.W., 2016. Seed vigour and crop establishment: extending performance beyond adaptation. *Journal of Experimental Botany*, Vol. 67, No. 3 pp. 567–591. doi:10.1093/jxb/erv490.

França L.V., Croda M.D., Nascimento W.M. and Freitas R.A., 2013. Physiological quality of eggplant seeds with different extraction and drying methods. *Journal of Seed Science*, v.35, n.1, p.51-55. https://www.scielo.br/pdf/jss/v35n1/07.pdf.

Isack M.E. and Monica L., 2013. Effect of post-harvest handling practices on physico-chemical composition of tomato. *Journal of Agricultural Technology* Vol. 9(6):1655-1664. doi.org/10.1080/19315260.2013.837134.

ISTA (International Seed Testing Association), 2020. International rules for seed testing. Edition 2020.https://www.seedtest.org/en/international-rules-for-seed-testing—content—1–1083.html.

Jeffrey H. M., 2004. Tomato seed production. An organic seed production manual for seed growers in the Mid-Atlantic and Southern U.S. https://www.researchgate.net/publication/336083525_TOMATO_SEED_PRODUCTION_An_organic_seed_production_manual_for_seed_growers_in_the_Mid-Atlantic_and_Southern_US/link/5d8d5a96a6fdcc25549e75a2/download.

Kailappan P. and Karunanithy C., 2006. Seed processing equipments: advances in science and technology. *Agrobios Publisher, India.* 804-826. https://www.researchgate.net/publication/235747673_Seed_Processing_Equipment.

Khan N., 2013. Genetic and Physiological Quality of Tomato Seed and Seedlings. Thesis submitted in fulfillment of the requirements for the degree of doctor at Wageningen University. http://www.wageningenseedlab.nl/thesis/nkhan/Thesis%20Noorullah%20Khan.pdf.

Nemati H., Nazdar T., Azizi M. and Arouiee H., 2010. The effect of seed extraction methods on seed quality of two cultivars tomato (*Solanum lycopersicum* L.). DOI: 10.3923/pjbs.2010.814.820.

Vishwanath K., Rajashekhar B.S., Kalappa V.P., Muniyappa V. and Nagarajappa A., 2006. Influence of seed extraction methods on seed quality in leaf curl resistant tomato varieties. *Journal of Asian Horticulture*, 2:250-254 https://www.academia.edu/3882528/Influence_of_Seed_Extraction_Methods_on_Seed_Quality_in_Leaf_Curl_Resistant_Tomato_Varieties.

Figures

Page 8/11
Figure 1

Seeds extracted with different methods: 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

Figure 2

Effects in 1000 seeds weight (A) and seed mycoflora detection (B) due to HCl concentration.
Figure 3

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

Figure 4

Changes in first count germination (A) and germination percentage in response to extraction method and time length (B)
Figure 5

Changes in vigour index I and II in response to extraction method and time length