# Ultrasonic Influence on Germination and Early Growth of Bell Pepper Seeds

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# Ultrasonic Influence on Germination and Early Growth of Bell Pepper Seeds

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**Abstract:** *This study investigates the impact of varying durations of 40 kHz ultrasound frequency on seed germination parameters, including germination rate, seedling growth, and seedling vigor index (SVI). The results of the germination test revealed no significant effects (p>0.05) on the germination rate of bell pepper seeds in sand, while under ideal conditions, significant changes (P<0.05) were observed, indicating an accelerated germination rate due to ultrasound treatment. Specifically, T2 (10 mins exposure) showed the highest germination rate under ideal conditions, suggesting a positive influence of ultrasound on germination. Further exploration of seedling growth parameters demonstrated a distinct response to varying ultrasound durations. Ultrasound treatment significantly influenced (p>0.05) growth parameters. Longer ultrasound exposure enhanced root development, shoot elongation, and an overall increase in total length. The root-shoot length ratio also increased, indicating a favorable impact on seedling vigor. Significant changes (p<0.05) in SVI across all media were observed, emphasizing the role of ultrasound treatment in influencing seedling vigor.*

**Keywords:** Ultrasound technology, ultrasound treatment, seed germination, germination enhancement, seed vigor.

## 1. INTRODUCTION

Seed germination is a complex process that involves the interplay of biochemical and physiological mechanisms, impacted by a range of internal and external factors, these elements include hormones, temperature, light, water, nutrition, and oxygen [1], [2], [3]. Water imbibition starts the germination process by reactivating physiological processes and metabolic pathways that promote embryo development, radicle emergence, and seedling growth [4]. According to Finch-Savage & Footitt, seeds possess the capacity to control their dormancy through the perception of many environmental factors, including as light, nitrate, temperature, and water availability which function as signals that indicate ideal circumstances for germination [5]. In previous years, diverse techniques were employed mainly to improve the germination or overcoming the dormancy. Some examples of these techniques are seed priming, which involves bio-priming [6], osmo- and hydro-priming [7], hormonal priming [8], stratification [9], genetic engineering [10], soil fertilization [11], and regulating hormones to improve the germination (Sharma et al., 2004). Rifna et al. also summarized the emerging technologies in seed germination enhancement [1]. In their work, some techniques being highlighted involves high-pressure processing, pulse electric field processing, ultrasound technology, ozone processing, UV light treatment, magnetic field, microwave radiation, non-thermal plasma, and plasma activated water as pretreatment on seeds. Although these techniques were able to showcase a reliable result in terms of improving the germination of some selected seeds, most of these techniques were time consuming and relatively expensive in nature [12], making it more attractive to seek for other alternatives that overcomes these drawbacks.

Among these technologies, ultrasound treatment is one of the promising techniques that could present diverse advantages in the seed industry, particularly in improving the germination and vigor of seeds. Alfalahi et al. claim that this physical treatment, which uses heat-generating ultrasonic waves, quickly affects seeds mechanically and chemically, compared to previous seed priming techniques, it is also simpler to apply[12]. According to Goussous et al., ultrasonic treatment facilitates moisture absorption into plant cells, which raises the moisture content of seeds over time[13]. Chen et al. also emphasized that the energy generated by ultrasonic vibration changed the cell wall structure, and it increased the permeability of seed cells' inner and outer membranes, which accelerated the biochemical reaction in cells and promoted seed sprouting [14]. The application of ultrasonic stimulation has the potential to enhance seed coat cavitation and promote a high rate of permeability, hence elevating the rate and/or speed of germination in seeds [15]. In general, ultrasonic mechanism mainly included two aspects: (a) the formation of bubbles and voids induced by acoustic wave vibration and (b) the turbulence of solid induced by acoustic wave which can result in expansion and compression of solid medium (sponge effect) [16].

A variety of crops, including alfalfa and broccoli [17], black beans [18], corn [19], barley [20], chickpea, wheat, watermelon, and pepper [13], watermelon, melon, leek, pepper, carrot, tomato, and aubergine [21], have been shown to benefit from ultrasonic treatments in terms of germination. While this technology is said to be capable of operating to the pressure waves of frequency over the range of 20 kHz [1], Ampofo et al. reported that the sprouting time of common beans with ultrasound treatment (40 kHz, 360 W) was reduced by 60 h while accumulating large amounts of active substances (polyphenols, anthocyanins) [22]. Chiu found that both 40 kHz and 80 kHz could improve seeds' hydration and sprouting rate, which might be due to the changes in seed coat structure induced by mechanical vibration caused by ultrasound [23]. Due to the limited capacity of the equipment, which operates only at a 40 kHz frequency, and previous studies suggesting that this frequency positively affects the early growth of some seeds, this study investigated the effects of 40 kHz ultrasound frequency on bell pepper (*Capsicum annuum*) seeds, considering varying exposure times. The paper also aims to evaluate the responses of seed germination, seed vigor, and seedling shoot and root lengths to different exposure times at the 40 kHz frequency.

## 2. MATERIALS AND METHODS

*2.1 Seed Materials*

The untreated seeds (from year 2013 accession) were collected from the National Plant Genetic Resources Laboratory (NPGRL) at the University of the Philippines-Los Baños. The collected seeds were allowed to rest at room temperature for three (3) days before the treatment.

*2.2. Seed Ultrasonic Treatments*

Before subjecting the seeds to sonication treatment, they underwent surface sterilization in a 30-ppm hypochlorite solution for 10 minutes. Subsequently, the seeds were thoroughly washed with flowing water, followed by a final rinse with distilled water. The seeds were then categorized into four (4) groups: (a) controlled samples, soaked in distilled water for 10 minutes without ultrasound treatment [T0]; (b) samples exposed to 40 kHz for 5 minutes [T1]; (c) samples exposed to 40 kHz for 10 minutes [T2]; and (d) samples exposed to 40 kHz for 15 minutes [T3]. The categorized samples were placed in a bath-type ultrasound machine at a fixed frequency of 40 kHz for their specified durations. Following the ultrasound treatment, the samples were allowed to sit at room temperature for 30 minutes to facilitate the removal of unbound water.

*2.3. Germination Experiments*

After the ultrasonic treatment, germination tests were conducted by sowing the seeds in three (3) replications, each comprising 3 subsamples with 25 seeds per subsample. The seeds were placed in a petri dish lined with tissue paper (ideal condition), and a separate batch was sown in a container filled with sand (standard condition). Both the petri dishes and containers were positioned outside the SeedTech Laboratory, subject to observed ambient temperature conditions (27°C). Germination counts were performed every two (2) days following the sowing period. Seeds were considered to germinate when radicles were visibly protruding, reaching an approximate length of 2mm. The percentage germination (PG) was calculated using Equation 1.

$$\mathrm{PG} = \frac{\text{germinated seeds}}{\text{total seeds sown}} \mathrm{x}100\% \qquad \text{Equation 1.}$$

*2.4. Seedling root length, shoot length, total length, and root and shoot length ratio measurements.*

After the 12th day of germination, all germinated seedlings were meticulously extracted from the sand, ensuring complete removal without leaving any roots on the ground. Subsequently, the seedlings were washed thoroughly using flowing water to eliminate any attached sand and other foreign matter. After the cleaning process, a ruler was used to measure the lengths of the roots and shoots of the seedlings. The total length was also obtained by adding the seedling root and shoot length. The root-shoot ratio was also calculated using Equation 2.

$$\text{Root} - \text{shoot ratio} = \frac{\text{root length}}{\text{shoot length}} \qquad \text{Equation 2}$$

*2.5. Seedling Vigor Index*

The seedling vigor index (SVI) was also calculated by the Equation 3 (Shams et al., 2018).

$$\text{SVI} = (\text{root length} + \text{shoot length})\ (\text{GP}) \qquad \text{Equation 3}$$

*2.6. Statistical Analysis*

The data gathered in this study that was laid out in a completely randomized design were analyzed using one-way analysis of variance and least significant difference (LSD) through the Statistical Tool for Agricultural Research (STAR) 2.0 software developed by the International Rice Research Institute to determine the differences between treatment means at a 5% level of significance by LSD.

## 3. RESULTS AND DISCUSSIONS

*3.1. Seed Germination*

The effects of varying durations of ultrasound treatment on the seed germination of bell pepper seeds sown in two different planting media, sand, and filter paper, were included in this report. From the results in Figure 1, for the standard condition, the highest germination rate on day 12 was observed in T1, and T2, while the lowest germination rate was noted in T3, and T0 (untreated) samples, with the corresponding germination rate of 73.33%, 66.67%, 65.78%, and 65.78%, respectively. However, the LSD test revealed that no significant effects ($p>0.05$) were observed in the germination rate of bell pepper seeds in this planting medium. For samples under ideal condition, T2 exhibited the highest germination rate, next to T3 and T1 which is 19.11%, 16.89%, and 13.78% higher than untreated seeds (T0). As supported by the LSD test, significant changes ($p<0.05$) were noticed in the germination rate of seeds in tissue paper which means that the ultrasound treatment has an impact on the accelerated germination rate. These results could be due to the different properties of the planting media used for the ultrasound-treated seeds. The sand medium may have different moisture retention and aeration properties compared to tissue paper, which could affect the germination process. Sand, being denser and having less consistent moisture distribution, might not be able to provide significant effects on the germination effects of ultrasound as effectively as tissue paper. Tissue paper provides a more uniform and well-aerated environment, which could enhance the beneficial effects of ultrasound treatment on seed germination.

Many seeds regulate dormancy by creating physical dormancy. This type of dormancy occurs when the seed coat is impermeable to water and/or gasses that are required for the germination process [24]. The ultrasound effect in this case may be promoting germination in bell pepper seeds because it is breaking the physical dormancy within the seeds. According to Mariano et al., one of the ways that ultrasound works is by modulating the plasma membrane, which makes it easier for water and mineral elements to enter and exit cells [25]. Another impact of ultrasonic waves is that they increase the activity of the alpha-amylase enzyme, which in turn increases the rate of starch hydrolysis [26]. According to Qin et al., ultrasound creates a microscopic crack in the seed coat, which improves water absorption and accelerates the germination process [27]. Babaei - Ghaghelestany et al. also emphasized that ultrasound treatment can possibly break the physiological dormancy in seeds by altering the chemistry of the seeds to promote seed germination [24]. Furthermore, endosperm modification—such as ultrasonic starch degradation—may cause an increase in the rate of enzyme-catalyzed hydrolysis processes in the seeds. Consequently, higher hydrolysis enzyme activity may result in faster sonication-induced germination and faster embryo development [28]. However, only the appropriate duration and frequency would stimulate the enzyme activities and promote cell growth by increasing cell physiological activity, while at higher frequency and duration may lead to damage to the cell and its structures [28][29][30].

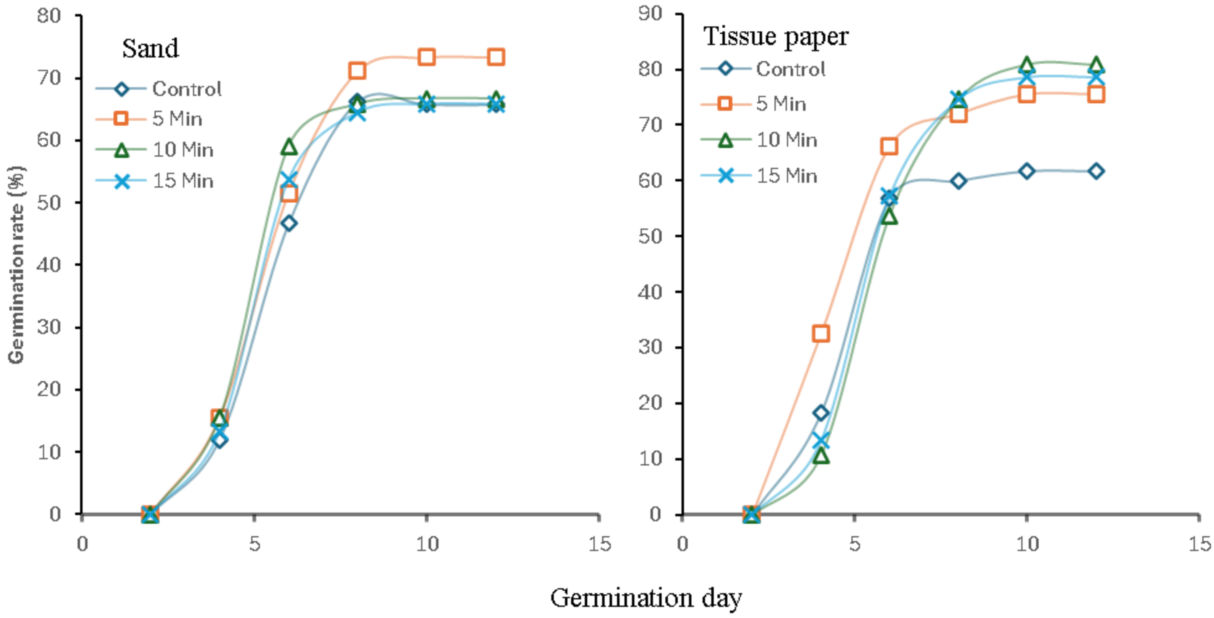


Fig. 1. Germination test (%) using sand and tissue paper as seed mediums under varying durations of 40 kHz ultrasound exposure. Values are means (n=9). No significant differences were observed for the germination test in sand. However, significant differences were observed in the germination test using tissue paper as the medium (not significant at day 2, *** at day 4, * at days 6 and 8, *** at days 10 and 12). * F test significant at $p \leq 0.05$; ** F test significant at $p \leq 0.01$; *** F test significant at $p \leq 0.001$; ns - not significant.

From these observations, it is evident that the application of 40 kHz frequency on pepper seeds was able to increase the seedling emergence of the pepper seeds. Likewise, the application of ultrasound waves resulted in enhanced and accelerated germination in barley seeds [30], higher germination percentages of compromised *Arabidopsis thaliana* seeds, increased the germination process, and germination rates in rice seeds [15], as well as triggered dormancy break in *Chenopodium album* [24]. Miano et al. also reported that ultrasound treatment has also a good effect on seed germination and other parameters [25]. This result contrasts with the findings of Goussous et al. [13] and Miano & Agusto [31] where the ultrasound treatment has a negative effect on the germination rate of pepper and beans, which could be due to the possible excessive breakdown of cell walls or even damage in embryo of the seeds.

*3.2. Root length, shoot length, total length, and root and shoot length ratio*

The investigation into the effects of different durations of 40 kHz ultrasound treatment on pepper seedlings has yielded notable results in terms of seedling growth parameters, as shown in Figure 2. The seedling root length, shoot length, total length, and root-shoot length ratio were assessed across T0, T1, T2 and T3 where the measured values, as presented in Figure 3, demonstrated a distinct response to varying ultrasound durations. The data reveals that the application of ultrasound had a significant impact on the growth parameters of pepper seedlings, as supported by the LSD test at *$p<0.05$*.

Examining the specific growth parameters, it is evident that seedlings subjected to ultrasound treatments exhibited variations in root length, shoot length, and total length compared to the control group. Notably, the increase in seedling root length from 3.07cm (T0) to 4.97cm (T3) suggests a positive correlation between longer ultrasound exposure and enhanced root development. The same trend is observed on the shoot length and the total length, indicating an overall positive influence of ultrasound on the growth of both root and shoot components. This an evident that ultrasound waves stimulate root and shoot elongation leading to a higher total length as compared to controlled samples (T3, 4.14cm longer than controlled). Similarly, Liu et al. (2016) found that after five years of storage, aged fescue seeds treated with ultrasonic waves had longer roots and shoots [32] . Although it is evident that ultrasound technology was able enhance the seedling root and shoot length. However, the increase in the seedling root and shoot length as compared to untreated samples varied depending on the intensity and duration of the treatment [12].

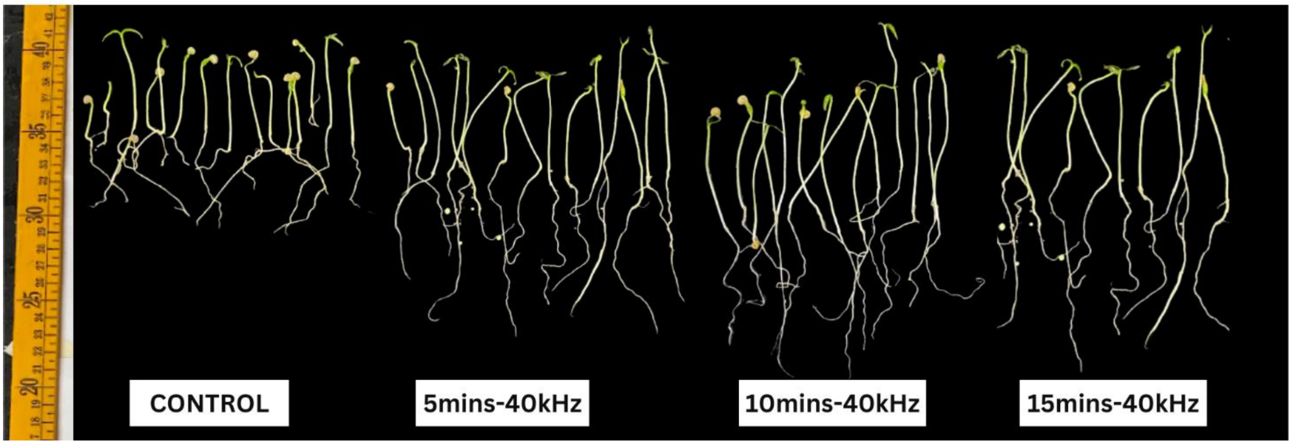


Fig. 2. Comparison of seedlings under control (untreated), 5mins, 10 mins, and 15 mins exposure to 40 kHz ultrasound frequency.

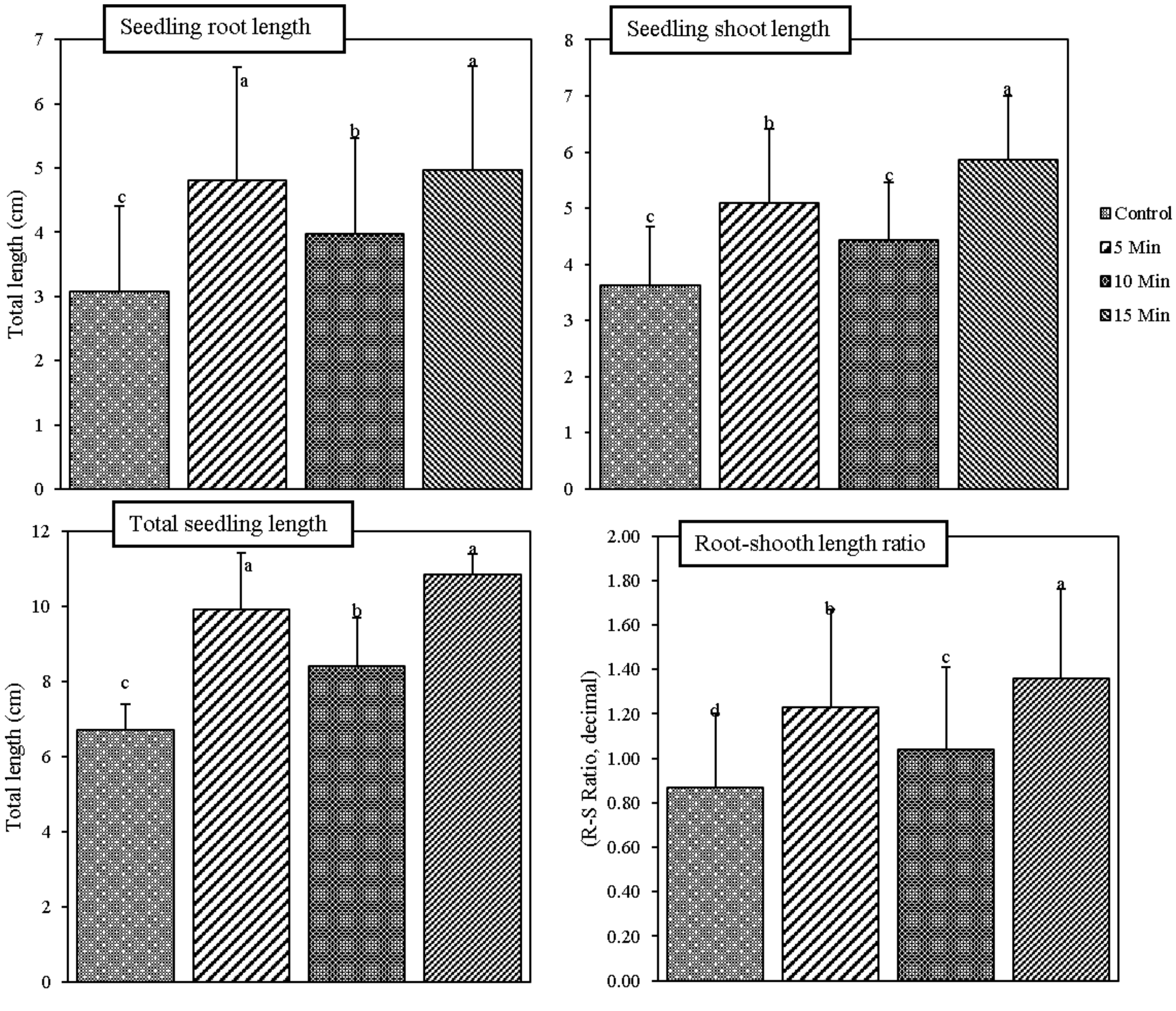


Fig. 3. Seedling root and shoot lengths, total length, and root-shoot ratio of under varying durations of 40 kHz ultrasound exposure. Values are means (n=125). Different letters (a, b, and c) indicate significant differences among treatments * F test significant at ($p \leq 0.05$).

The root-shoot length ratio also revealed a significant increase, where T1, T3, and T2 demonstrates 0.11, 0.07, and 0.03 increase as compared to T0. High R/S ratio indicates that seedling root is longer than its shoot, while low R/S ratio suggests that seedling shoot is longer than its root. Plants with a higher proportion of roots can compete more effectively for soil nutrients, while those with a higher proportion of shoots can collect more light energy [33].

*3.3. Seedling Vigor Index*

The Seedling Vigor Index, alternatively referred to as seed power or seed viability, quantifies a seed's ability to germinate in challenging environments. Seeds exhibiting a higher Seed Vigor Index are characterized by greater vigor [34]. The effects of varying durations of ultrasound treatment on the SVI of bell pepper seeds sown in standard media and ideal condition, were investigated in this report. The results in Figure 4 revealed that after 12 days in sand, the SVI values for T0, T1, T2, and T3 were 435.83, 725.93, 563.94, and 599.31, respectively. While for the seeds in filter paper is 435.83, 725.93, 563.94, and 599.32, sequentially. As supported by the LSD test, significant changes ($p<0.05$) were observed in the SVI across all planting media. This finding suggests that the ultrasonic treatment duration played a significant role in influencing seedling vigor.

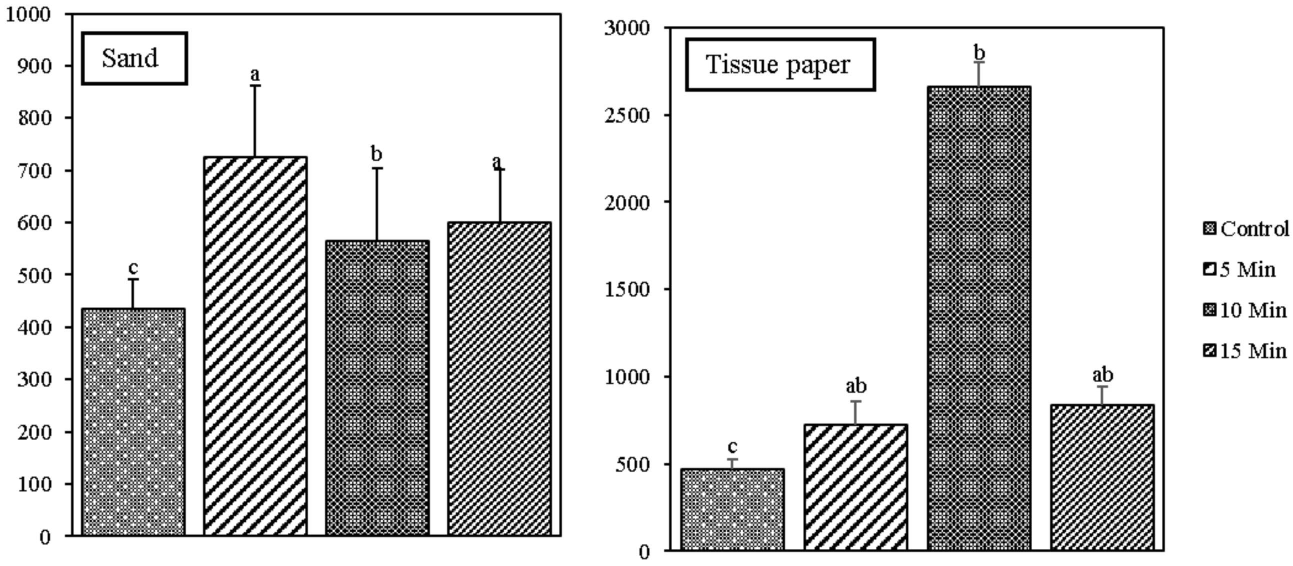


Fig. 4. Seedling vigor index from seeds planted in sand and tissue paper under varying durations of 40 kHz ultrasound exposure. Different letters (a, b, and c) indicate significant differences among treatments * F test significant at ($p<0.05$).

For seeds planted in sand, T1 exhibited the highest vigor, which is then flowed by T3, and T2, while untreated samples (T0) demonstrate the lowest vigor seedlings. For seedlings under ideal conditions, a different trend was observed, in which T2 has the highest seedling vigor observed, more than two-fold than the other treatments, and lowest vigor was still observed in T0. The findings of this report suggest that regardless of the treatment time, the ultrasound technology has a good effect on the SVI as compared to untreated seeds. The action of acoustic waves may be responsible in the changes in the microstructure of the bell pepper seed coat, which accelerates the water imbibition and promotes hydration. Therefore, the dormant seeds gradually regain the respiration and become more vigorous after imbibing sufficient water, thereby increasing the activity of metabolism-related enzymes [14].

## 4. CONCLUSIONS

This study has highlighted the beneficial effects of ultrasound technology on the germination rate, seedling vigor index, and seedling characteristics on the 2013 accession of bell pepper. The results of the experiment revealed that regardless of the duration to 40 kHz frequency, ultrasound treated seeds demonstrated a good effect in all parameters being examined in this report. A significant increase ($p<0.05$) in seed germination were observed in samples under tissue paper. Furthermore, a significant increase also was noted in the SVI of ultrasound treated seeds where 166.56% (T1-sand) and 565.08% (T2-ideal condition) increase in the seed vigor as compared to controlled samples. The same trend was observed in the seedling characteristics where an improvement in seedling root length, shoot length, total length, and root-shoot length ration were noticed. In brief, ultrasound treatment has a beneficial effect on aged bell pepper seeds with good effects on seed quality.

## 5. RECOMMENDATIONS

While this study suggests that ultrasound technology is a promising and effective method for improving the early growth of bell pepper seeds, the authors recommend that future studies investigate not only the influence of duration but also the combined effects of duration and frequency of ultrasound treatment on the seeds. Additionally, it is recommended that future research explore the optimized combination of these factors to achieve the best effects on the early growth of bell pepper seeds. This includes the use of statistical techniques such as Response Surface Methodology (RSM) and other methods like factorial design and regression analysis to identify the optimal treatment conditions.

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## 7. DECLARATION OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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