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Evaluation of ultrasound technology to break seed dormancy of common lambsquarters (*Chenopodium album*)

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Abstract

Although seed dormancy is advantageous for annual plants in the wild, unsynchronized germination in the laboratory leads to increased error in measurements. Therefore, techniques to promote and synchronize germination are routinely used. Ultrasound is one of the newest methods for breaking dormancy in weed seeds. We have investigated whether ultrasonic waves can be used to break seed dormancy of common lambsquarters (*Chenopodium album*), a highly competitive annual weed that leads to significant reduction of yields of corn, soybeans, and sugar beets. Ultrasonic waves with frequency of 35 kHz were applied for 0 (control), 5, 10, 15, and 30 min using a completely randomized design. The results showed that the use of ultrasound waves generally enhanced the traits under investigation in the treated samples compared with the control sample. The maximum enhancement of germination percentage (180%), seedling dry weight (78%), and seedling vigor index I (271%) and II (392%) was seen in the common lambsquarters samples treated with ultrasound for 15 min and seedling length (40%) at 30 min compared with the control samples. Radical lengths were not statistically different from controls under any treatment and plumule length only increased marginally. These changes are reflected in seedling vigor index I and II measurements. For some of these traits, increasing the length of ultrasound treatment to 30 min had negative effects. These results demonstrate that ultrasound technology can be used as a quick, and efficient nondestructive method to break seed dormancy in common lambsquarters.

KEYWORDS

common lambsquarters, germination percentage, seed dormancy, ultrasound technology

1 | INTRODUCTION

To produce consistent high yields from agricultural systems, weed control is essential. Common lambsquarters (*Chenopodium album*) is one of the world's most problematic weeds (Moechnig, Stoltenberg,

Boerboom, & Binning, 2003). Characteristics such as high growth rate, effective competition for resources and nutrients, high seed production, and seed germination under a wide range of environmental conditions means that common lambsquarters prevents crop growth and results in reduced crop yield (Schuster, Shoup, &

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Al-Khatib, 2007). An important step to designing effective weed control is to understand the weed's biology and life cycle, especially the ecophysiological characteristics of its seeds (Long, Tan, Baskin, & Baskin, 2012).

The transition from seed to seedling is a key phenological transition and is under tight genetic and environmental control (Finch-Savage & Leubner-Metzger, 2006; Graeber, Nakabayashi, Miatton, Leubner-Metzger, & Soppe, 2012). Seeds of annual plants like weeds frequently exhibit dormancy at maturity. This key adaptive trait is established during mid-maturation of the seed within the mother plant (Alboresi et al., 2005; Karssen, Brinkhorst-van der Swan, Breckland, & Koornneef, 1983), and its aim is to block seed germination other conditions that would otherwise be favorable for germination. Without seed dormancy, soil seed banks could not be established or maintained and is therefore essential for annual weeds like lambsquarters. Seed dormancy plays a role in weed survival, and the time of germination is an important phenomenon in the plant life cycle because the establishment of weeds depends on their seed germination ability. Seed dormancy in annual plants enables a large portion of the seed bank of weeds to correctly time germination with the seasons and/or to remain in the soil for a long time.

Although seed dormancy is advantageous for plants in the wild, it is a hindrance in the laboratory as varied germination rates and timings can alter seedling counts and sizes at a given time. Therefore, laboratory methods to uniformly break dormancy and promote germination are frequently used. Environmental conditions such as time since seed shed, light quality (sensed primarily by phytochromes), temperature, nutrient availability, humidity, and some gases break seed dormancy (Kępczyński & Sznigir, 2013; Penfield & King, 2009). In laboratory conditions, cold stratification, scarification, light and temperature alternations, or incubation with water and/or germination-promoting solutions are typically used to break seed dormancy in weeds (Alebrahim, Rashedmihasel, Meighani, & Baghestani, 2011; Alshallash, 2018; Chu, Jusaitis, Aspinall, & Paleg, 1978; Fallahi, Babaei Ghaghelestany, Asadi Gakieh, & Hatami Gharah Ghovini, 2016; Humphries, Chauhan, & Florentine, 2018; Majd, Aghaie, Monfared, & Alebrahim, 2013; Tang et al., 2008). Of these, dry after-ripening, and application of nitrate, cold, thiourea, water or nutrient solutions have shown to be effective in breaking dormancy in common lambsquarters (Chu et al., 1978; Cumming, 1963; Henson, 1970; Herron, 1953; Holm & Miller, 1972a, 1972b; Tang et al., 2008; Williams, 1962, 1963). However, these protocols are not all uniformly effective, and some require considerable time or resources. Therefore, inexpensive, nondestructive, and effective alternatives are required.

Ultrasound is a new physical method that involves the use of sound frequencies in the inaudible range (20–100 kHz) for interaction with materials. Ultrasound can change the state of the substance and even accelerate reactions. The presence of hard cell walls in some plants prevents the penetration of water and oxygen into the cell and reduces their germination potential. One of the reasons for the improvement in germination rate is the stimulation of the

cellular wall of the seed by ultrasound. The advantages of the ultrasound technique are that it is simple, inexpensive, and environmentally friendly (Abbaspour-Gilandeh, Kaveh, & Jahanbakhshi, 2019; Goussous, Samarah, Alqudah, & Othman, 2010; Kaveh, Jahanbakhshi, Abbaspour-Gilandeh, Taghinezhad, & Moghimi, 2018). Ultrasound technology can also be used in the solid-phase microextraction and the detection of bioactive compounds in plants (Asfaram, Sadeghi, Goudarzi, Kokhdan, & Salehpour, 2019). Researchers have discussed the use of ultrasound technology to stimulate germination of various seeds such as maize, barley, rice, and sunflower seeds and emphasized its importance (Ding et al., 2018; Rifna, Ramanan, & Mahendran, 2019; Sadeghianfar, Nazari, & Backes, 2019; Yaldagard, Mortazavi, & Tabatabaie, 2008). Liu et al. (2016) demonstrate that the use of ultrasound waves increased and improved the growth of tall fescue (*Festuca arundinacea*) and Russian wild rye (*Psathyrostaehys juncea* Nevski) seedlings. Likewise, germination of sesame (*Sesamum indicum* L.) seeds treated with ultrasound waves showed increased germination and growth (Shekari, Mustafavi, & Abbasi, 2015). Therefore, we set out to determine whether the application of ultrasound would be effective in lambsquarters. Based on a review of past research, it was found that no information is available evaluating the ability of ultrasound technology to break seed dormancy of common lambsquarters. Therefore, the purpose of this study was to use ultrasound waves as a nondestructive, simple, and low-cost test to break dormancy in common lambsquarters.

2 | MATERIALS AND METHODS

2.1 | Collecting seeds

In this research, common lambsquarters seeds were collected from Moghan Agricultural and Natural Resources Research Field, Iran.

2.2 | Pretreatment of seeds

Samples were pretreated with produced waves using ultrasound bath (a Bandelin DT 255 H model with internal dimensions of 325 × 175 × 305 mm and volume of 5.5 L). This device is capable of producing ultrasonic waves at the frequency of 35 kHz and the power of 230 W. The ultrasound bath tank was first filled with two liters of distilled water. Then, four replicates of samples were exposed to ultrasound waves with at five time lengths of 0 (control sample), 5, 10, 15, and 30 min.

2.3 | Measuring germination indicators

After each ultrasound treatment, 50 common lambsquarters seeds were sterilized and placed in a Petri Dish. Then, 10 ml of distilled water was added to the samples and they were put in a germinator

at the temperature of 20°C (ISTA, 1996). Germinated seeds were counted every 24 hr for 2 weeks, and germination percentage of the samples was measured. Plumule and radicle were measured using a digital caliper. After 2 weeks (14 days) dry weight of common lambsquarters seedlings was measured by removing 10 samples from each petri dish to an oven at 70°C for 24 hr. Moisture and dry matter in the common lambsquarters seeds were measured using Equations 1 and 2 (Jahanbakhshi, Abbaspour-Gilandeh, Ghamari, & Heidarbeigi, 2019; Jahanbakhshi, Abbaspour-Gilandeh, & Gundoshmian, 2018; Jahanbakhshi, Rasooli Sharabiani, Heidarbeigi, Kaveh, & Taghinezhad, 2019; Jahanbakhshi, Yeganeh, & Shahgoli, 2019).

$$MC = \frac{M_w - M_d}{M_w} \times 100 \quad (1)$$

$$DM = \frac{M_d}{M_w} \times 100 \quad (2)$$

where MC is the moisture content of seed (%), M_w is the initial mass of seed (mg), M_d is the mass of dried seed (mg), and DM is the dry matter seed (%).

The Seedling vigor index I (SVI) and II (SVII) of common lambsquarters seed were calculated through Equations 3 and 4 (Bajji, Kinet, & Lutts, 2002).

$$SVI = GP \times SL \quad (3)$$

$$SVII = GP \times SDW \quad (4)$$

where SL is the seedling length, SDW is the seedling dry weight and GP is the germination percentage.

2.4 | Statistical analysis

In this experiment with a randomized complete design was used to analyze the effect of ultrasound at five levels (0 or control, 5, 10, 15, and 30 min) with four replications (20 treatments). The effect of ultrasound on parameters (GP, SL, SDW, SVI, and SVII) was analyzed. SAS 9.4 software was used for analysis and to perform statistical operations. Means of the treatments were compared based on LSD test ($p \leq .05$; Jahanbakhshi & Kheiralipour, 2019).

3 | RESULTS AND DISCUSSION

The results of analysis of variance for the effect of ultrasound waves on weed germination indices of common lambsquarters are reported in Table 1, and the data are presented in Figure 1 and further analyzed in Table 2.

3.1 | Germination percentage (GP)

Pretreating common lambsquarters seed with ultrasound increases their germination percentage (GP; Tables 1 and 2, Figure 1a). The percentage of germination in the control sample (without ultrasound, 29.75%) was the lowest. The highest germination percentage was observed in pretreatment with ultrasound for 15 min (83.25%). Pretreatment with 5 min is not statistically different from control. Thirty minutes of ultrasound treatment promoted germination more than the control but to a lesser extent than 10 or 15 min. Because of the decrease in percent germination at 30 min compared with 15 min, there was no linear relationship between increasing the ultrasound application time and percent germination. These results are similar to those reported by Sharififar, Nazari, and Asghari (2015). The germination of barley (*Hordeum vulgare*) and fennel (*Foeniculum vulgare*) can also be improved after ultrasound and increasing the duration of ultrasound application reduced the percentage of germination (Fateh, Noroozi, Farbod, & Gerami, 2012; Yaldagard et al., 2008). Other researchers reported that increasing the ultrasound application time from 10 to 20 min to break dormancy in the wild yam (*Dioscorea* spp.) seed yielded unfavorable results and led to decrease in germination percentage (Andriamparany & Buerkert, 2019).

3.2 | Seedling dry weight (SDW), plumule length (PL), seedling length (SL), and radicle length (RL)

Once the seed has germinated, it can then extend beyond the confines of the seed coat and transition into a seedling. We therefore measured whether pretreatment with ultrasound would have effects on other commonly measured early seedling traits. We found that pretreating common lambsquarters seed ultrasound alters the seedling dry weight, and the length of the plumule and seedling (Tables 1 and 2, Figure 1).

TABLE 1 Analysis of variance of ultrasound waves effects on the germination indices of common lambsquarters

Source of variations	df	Germination percentage (%)	Seedling dry weight (mg)	Plumule length (mm)	Seedling length (mm)	Radicle length (mm)	SVI	SVII
Treatment	4	0.658**	0.187**	0.155 ^{ns}	0.072*	0.069 ^{ns}	1.011**	1.417**
Error	15	0.076	0.008	0.064	0.019	0.050	0.093	0.097
CV	—	7.02	3.57	8.40	3.99	8.68	4.09	4.84

Abbreviation: ns, not-significant.

*Significant at 5% probability level.

**Significant at 1% probability level.

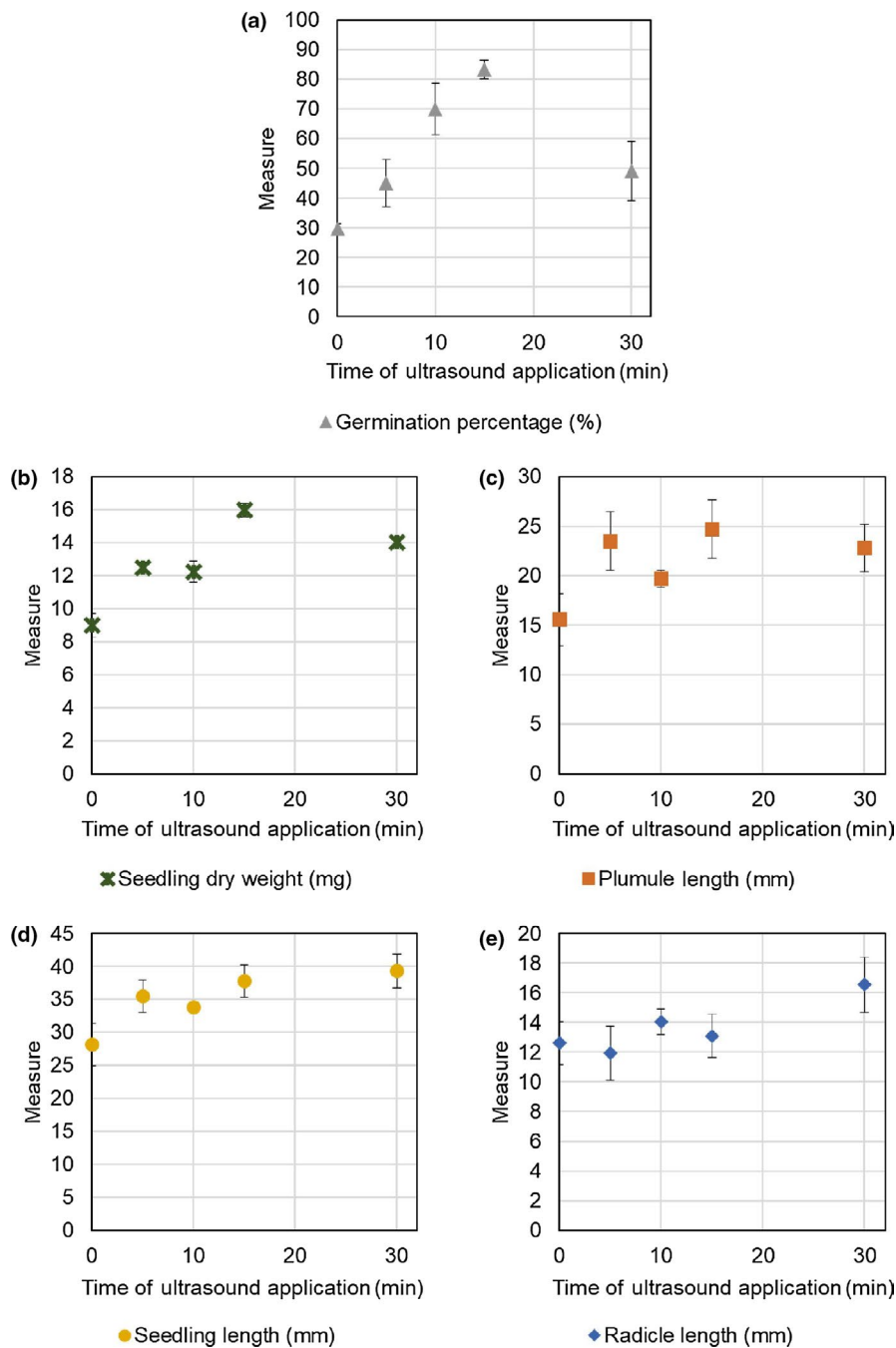


FIGURE 1 Effect of pretreatment with ultrasound on common lambsquarters (*Chenopodium album*) germination percentage and other commonly measured seedling traits. Data are averages \pm standard error of four treatments. Each treatment was composed of 50 common lambsquarters seeds measured 2 weeks after ultrasound treatment applied for the time indicated. Germination indices are (a) Germination Percentage, (b) Seedling Dry Weight (mg), (c) Plumule Length (mm), (d) Seedling Length (mm), and (e) Radicle Length (mm)

Seedling dry weight common lambsquarters showed a significant difference compared with untreated seeds after the ultrasound pretreatments (Tables 1 and 2, Figure 1b). The smallest seedling dry weight belonged to the control sample (9.00 mg). The largest seedling dry weight (16.00 mg) was observed in 15 min of ultrasound treatment where dry weight of the seedlings increased by 36.1% compared with the control sample. Similar to germination percentage and plumule length, increasing the time of ultrasound application (30 min) reduced seedling dry weight compared with 15 min (Table 2, Figure 1). In similar studies conducted on myrtle (*Myrtus communis*), researchers reported that ultrasound application, caused an increase in radicle and plumule dry weight in addition to breaking dormancy (Alvandian, Vahedi, & Taghizadeh, 2013).

Plumule length of common lambsquarters showed a significant difference compared with untreated seeds after the ultrasound pretreatments (Tables 1 and 2, Figure 1d). The longest plumule length was observed in the 15 min of ultrasound treatment (24.70 mm), and the shortest was seen in the control sample (15.50 mm). In 15- and 30-min ultrasound treatments, the seedling length increased 58.8% and 46.6%, respectively, over the control. The pattern for promotion of plumule length is similar to seedling length (Table 2, Figure 1) where the measure at 10 min of ultrasound pretreatment is less than that at 5 or 15 min (Table 2, Figure 1c). Pretreatment with ultrasound lead to a 27%–59% increase in plumule length. Researchers have reported that the use of ultrasound technology breaks down the cell wall in the seeds. Breaking the cell wall in the seeds increases the

TABLE 2 Comparison of the ultrasound mean effects on the germination indices of common lambsquarters

Ultrasonic pretreatments (min)	Germination percentage (%)	Seedling dry weight (mg)	Plumule length (mm)	Seedling length (mm)	Radicle length (mm)	SVI	SVII
0	29.75 c	9.00 d	15.55 b	28.15 b	12.60 a	843.2 c	270.0 c
5	45.00 c	12.50 bc	23.50 a	35.45 ab	11.95 a	1,598.4 bc	562.0 bc
10	70.00 ab	12.25 c	19.70 ab	33.75 ab	14.05 a	2,352.0 ab	861.0 b
15	83.25 a	16.00 a	24.70 a	37.10 a	13.10 a	3,125.6 a	1,328.8 a
30	49.00 bc	14.00 b	22.80 ab	39.35 a	16.55 a	1,921.8 b	697.0 b

Note: Means followed by the same letters in the same column do not have a significant difference based LSD multiple range test at 5% level.

water uptake in the cell and ultimately increases the germination and its indices (such as plumule length; Baker, Robertson, & Duck, 2001; Patero & Augusto, 2015).

Seedling length of common lambsquarters showed a significant difference compared with untreated seeds when ultrasound pretreatments were used (Tables 1 and 2, Figure 1c). The longest seedling length of common lambsquarters was seen after 30 min of ultrasound treatment (39.35 mm), and the shortest length was for the control sample (28.18 mm). The trend shows a roughly linear increase in length with increasing time with a 20%–40% increase over the control samples. In similar studies, researchers reported that using ultrasound increased the radicle of barley for 13%–16% compared with the control sample (Yaldagard et al., 2008).

Pretreating common lambsquarters seed with ultrasound does not result in a statistical difference for radicle length. (Tables 1 and 2, Figure 1e). Although pretreatment with 30 min of ultrasound does result in a 1.4-fold increase in radicle length, the differences are not statistically different. This is because of the high degree of variability seen between the replicated samples.

3.3 | Seedling vigor index I (SVI) and II (SVII)

The results of analysis of variance showed that seedling vigor index I (SVI) and II (SVII) of common lambsquarters showed significant differences at 1% probability level (Table 1). The highest vigor longitudinal and vigor weighted indices were obtained at 15 min of ultrasound treatment. By increasing ultrasound application time to 30 min, the SVI and SVII decreased and the lowest amount of them was observed in the control sample (Table 2). In 15 min of ultrasound treatment, the SVI and SVII of common lambsquarters increased by 64% and 80%, respectively, in comparison with the control sample. The results presented here are consistent with the findings of other researchers (Machikowa, Kulrattanarak, & Wonprasaid, 2013; Risca, Fartais, & Stiuca, 2007; Yaldagard et al., 2008).

3.4 | Potential mechanism for ultrasound effect and usefulness of ultrasound applications

Many plants regulate dormancy by imposing physical dormancy. Physical dormancy occurs when the seed coat is impermeable to

water and/or gasses that are required for germination. Ultrasound may be promoting germination in common lambsquarters, because it is breaking the physical dormancy. One of the mechanisms through which ultrasound is said to exert its effects is the change that it creates in the plasma membrane to facilitates the entrance and exit of water and mineral elements into cells (Miano et al., 2015). Increasing the activity of alpha-amylase enzyme and, as a result, increasing the rate of starch hydrolysis, is another effect of ultrasound waves (Kratovalieva et al., 2012). Ultrasound brings about microscopic cracks in the seeds' skin, which facilitates water absorption, and accelerates the germination process (Qin, Xu, Zhong, & Alfred, 2012).

Another type of dormancy is physiological dormancy. Physiological dormancy prevents germination from occurring unless certain chemical changes occur and is the most common type of dormancy (Finch-Savage & Leubner-Metzger, 2006). Physical and physiological dormancy do occur together resulting in combinational dormancy; this happens in *Geranium robertianum* (Vandelook & van Assche, 2010). It is known that *Chenopodium* species exhibit physiological dormancy, and the depth of dormancy depends on the seed coat's thickness, which in turn is determined by the environment experienced by the mother plant during seed maturation (Penfield & MacGregor, 2017 and references therein). Breaking physiological dormancy in nature happens through many routes including elevated or fluctuating temperatures, which could be taken to extreme with fire or freezing/thawing events, drying, or passage through the digestive tracts of animals. Breaking physiological dormancy in the laboratory is often accomplished through scarification, after-ripening in dry storage, and cold or warm stratification (Finch-Savage & Leubner-Metzger, 2006). Therefore, it may be that the ultrasound is sufficiently altering the chemistry of the seeds to promote germination and the other traits measured.

As this method requires soaking seeds in water and immersing them in the ultrasound bath, it is unlikely that it can be used in the field to promote germination from the weed seed bank to control plants in the field. Therefore, instead we propose that this method will be useful for researchers who are trying to understand the eco-physiological characteristics of weed seeds. A better understanding of the behaviors and characteristics of weed seeds will allow for better design of effective weed control programs. Therefore, when researchers are looking for differences between treatments, or wild populations, it is essential to be able to experimentally determine the maximum value that can be obtained, particularly with regards

to germination percentage as all of the other traits measured herein, or on any seedling, depend on whether or not the seed has germinated. Pretreatment with ultrasound promotes germination, as well as seedling dry weight and the length of the plumule and seedling; if researchers want to study the germination indicators other than germination percentage, further work will be required to determine if the effects are separable.

Although common lambsquarters is a problematic weed, it also has some medicinal uses and is eaten as a leafy vegetable (Choudhary, 2020) and has other potential applications in range-land/pasture rehabilitation, soil conservation, and animal husbandry. Therefore, when studying lambsquarters for its good as well as its bad properties, it will be important to promote synchronous germination to a high percentage. Ultrasound may play a role in accomplishing this goal.

4 | CONCLUSION

In this study, we investigated whether ultrasound pretreatment could induce dormancy break in common lambsquarters. Germination percentage, seedling length, seedling dry weight, and seedling vigor index I and II were measured. Different lengths of the ultrasound pretreatment were provided to determine when the maximum dormancy break could be obtained. The results showed that ultrasound pretreatment of common lambsquarters can increase most of the germination indices measured. The highest and lowest germination percentages were equal to 83.25% and 29.75%, respectively, which belonged to the 15 min of ultrasound treatment and the control sample, respectively. As a consequence of the increased germination or independently of it, other seedling measures were also altered in response to the ultrasound treatment. Maximum seedling length (39.35 mm) was observed after 30 min of ultrasound treatment which showed a 40% increase compared with the control sample. Seedling dry weight and the seedling vigor index I and II of common lambsquarters were promoted most by the 15-min ultrasound treatment, and there was a significant difference between the treated samples and the control sample for all other timepoints. The results of this study showed that by using ultrasound technology as a non-destructive method, the percentage of germination can be improved in common lambsquarters seeds. Ultrasound is therefore an effective way to increase germination percentage in common lambsquarters and to induce the maximum increase, a pretreatment time of 15 min is recommended.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

ETHICAL APPROVAL


This study does not involve any human or animal testing.

INFORMED CONSENT

Written informed consent was obtained from all study participants.

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