Impact of fast and slow desiccation on *Garcinia imberti* seed and seedling vigour

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Abstract

*Garcinia imberti* Bourd. endemic to the southern Western Ghats is classified as endangered by the IUCN (2018). The seeds as the sole means of propagation with initial moisture content (MC) of 62.8% are sensitive to desiccation. Studies on the responses of the seed to drying require ascertaining of the critical moisture content (CMC) as a basic requisite for germplasm conservation. Responses of *G. imberti* seeds to fast drying with activated silica gel (25 ± 2 °C, 6 ± 1% RH) and to slow drying under laboratory conditions (28 ± 2 °C, 60 ± 2% RH) were evaluated for seed and seedling vigour. When the MC was reduced to 56% by 48 hours of slow drying or 6 hours of fast drying, 75 to 90% normal seedlings were produced respectively. In the case of fast dried seed (6 hours), seed associated parameters except mean germination time showed peak values with maximum germination and enhanced root length. Below the CMCs of 16.4 and 26.3% (fast and slow drying respectively), half of the tested seeds become non viable. Thus for germplasm conservation the present study proposes 6 hour's of fast drying to retain viability and normal seedling development of *G. imberti*.

Keywords

Agasthyamala Biosphere Reserve; Desiccation; Moisture content; Recalcitrant seed; Seed storage practises

Introduction

The Genus *Garcinia* L. of the family Clusiaceae comprising 400-600 tropical species (1) is an important component of Western Ghats flora. This genus has 9 species and 2 varieties indigenous to the Western Ghats and in particular 7 of the species and 2 varieties are endemic to the Agasthyamala Biosphere Reserve (ABR) (2). *G. imberti* is one of the endangered and endemic species to ABR (3) confined to an altitudinal range of 600-1200 msl (4). The niche specificity of the species is related to this narrow distribution, recruitment failure due to high fruit/seed predation rate and an extended period of seed dormancy (5).

In general, *Garcinia* seed propagation is characterised by the peculiar ‘Garcinia type’ hypogeal germination (6) along with dormancy. Similar to the reported dormancy of *G. gummi-gutta* (7), *G. imberti* seeds germinate only after a period of 5-6 months due to the physical barrier of the seed coat (8). According to Normah et al.
(9) all *Garcinia* species show dormancy, except *G. mangostana*. Most species of the genus *Garcinia* also shed their seeds at high moisture contents with low viability and desiccation sensitivity (10) and are thus considered to be recalcitrant. Recalcitrant seeds lose viability at relatively high moisture contents and their desiccation sensitivity imposes a severe problem for the long term conservation of many tropical plants' genetic resources (11, 12). Consequently the main challenge for the *ex situ* conservation of *G. imberti* is its sensitivity to drying of the seeds below a critical moisture content. This leads to quick loss of viability though the reduction of moisture content to a minimum safe level that reduce seed vigour and seed storability. The germination performance of *G. imberti* seeds subjected to desiccation has been studied in detail along with features of low flower to fruit ratio, poor natural regeneration and crucial fruit/seed predation. *Ex situ* conservation of plant species via seed is the most convenient and reliable method for gene banks and cryobanks (13-15). Studies on sensitivity to desiccation have been undertaken on many tropical and sub- tropical species to achieve germplasm storage and the present study has followed the responses of the seed of *G. imberti* to such an approach.

**Materials and Methods**

*G. imberti* fruits were harvested between September and November of 2015-2017 with 20 accessions each in the evergreen forests at Chemunji (1168 m, N 8° 41' 28.0", E 77° 11' 4.8"), Bonacaud (943 m, N 8° 45' 25", E 77° 11' 20") and Ponmudi (1003 m, N 8° 45' 50.2", E 77° 6' 48.5") located at altitudes ranging from 600 to 1200 msl of southern Western Ghats. Fully ripened fruits were identified by their brilliant yellowish-green and fruits were directly collected from the mother trees. After harvesting, the fruits were brought to the laboratory and the outer coat removed by manual pressing. These seeds were thoroughly washed under running water and thereafter allowed to surface dry in laboratory conditions.

Seed desiccation effect on the loss of viability was studied by subjecting the seed to two different treatments (16): (a) seeds in open plastic trays were exposed to laboratory conditions (slow desiccation) (28 ± 2°C, 60 ± 2% RH) for 240 hours and at each 24 hour interval the water content was determined; (b) seeds in 4L capacity desiccators containing 3 kg of activated silica gel (fast desiccation) (25 ± 2°C, 6 ± 1% RH) were dried for 63 hours. The moisture content was determined every three hours. The silica gel was replaced as soon as it lost its indicative blue colour.

The following aspects were studied in order to assess impact of seed drying on physiological potentials:

**Moisture content**

For each drying method seed moisture content was determined using the high constant temperature oven method (130 ± 2°C for two hours). Ten replicates of two seeds each were made and calculated on a fresh weight basis.

**Seed germination**

Germination tests were carried out with ten replicates of 10 desiccated and non-desiccated de-coated seeds each, rolled in an acid free moist germination paper kept in a seed germinator without light (30 ± 2°C, 80% RH). The viability was checked on the basis of the germinated seed percentage. Seeds were scored as germinated when the radicle reach 5 mm. Observations on germination were noted daily. The seed germination associated parameters of speed of germination (SPG), mean germination time (MGT) (17), mean daily germination (MDG), peak value (PV) and germination value (GV) (18) were calculated.

**Seedling length**

Seedling length was obtained by measuring the lengths of the root and shoots with five replicates of 10 seedlings (100 days old) each, using a millimetre ruler. The results were expressed in centimetres.

**Normal and abnormal seedlings**

Evaluations were performed 60 days after sowing by computing the percentages of normal seedlings, using the presence of well-developed shoots and root system as the criteria.

**Seed Vigour Index (SVI)**

After 100 days of drying SVI was calculated by multiplying germination percentage and seedling length (19) before planting in the field.

**Statistical analysis**

The data were statistically analysed by one-way ANOVA and values expressed as mean ± standard error. Significance of differences
between mean values was tested by least significant difference (LSD) (P < 0.05). Means followed by the same letter in the column do not differ significantly by Tukey's post hoc test of Honest Significant Difference.

Results

G. imberti seeds are the sole propagation agent with an initial moisture content of 62.8 ± 0.15%. Seeds showed a type of hypogeal germination and possess 1.2 ± 0.2 years of dormancy with only 38 ± 1.3 % of germination. During desiccation, both seed moisture content (MC) and germination percentage (GP) concurrently declined irrespective of the drying method indicating the recalcitrant nature of the seed. The drying method and duration significantly affected the level of seed moisture content and the final seed moisture content was higher when slowly dried at room temperature compared with fast drying with silica gel. In G. imberti, the fast drying method improved germination along with other seedling physiological characters in comparison with the slow drying. It took more than 240 hour to reach a moisture content of 17.68% with the slow drying method. Whereas when fast dried, it took only 48 hours to attain a moisture content of 17.75% (Fig. 1-a).

Fast dried seeds germinated earlier than those that had been slow dried implying that drying speed influenced physiological potential and level of damage sustained by the seeds. During fast drying, the MC of the seeds reduced quickly along with significant reductions in GP. In this regard at 51-54th hours of fast drying the germination percentage rapidly declined from 50.5 to 25.1 % and consequently it is suggested that the MC at the 51th hour is the critical moisture content of the species i.e., 16.4 %. For the slow dried seeds the significant germination drop from 43.0 to 25.8 % was observed between 192-216th hours indicating that the critical moisture content i.e., 26.33 % (Fig. 1-h.) was reached at 192 hours of drying. The 6 hours of fast desiccated and 48 hours of slow desiccated seeds registered cent percent of germination (Fig. 1-c). All seed associated parameters except MGT showed peak values at 6 hour of fast desiccation while only PV and GV registered peak values at 48 hours of slow desiccation (Fig. 1-d).

The Pearson correlation coefficient of fast (r= 0.94**) and slow (r= 0.98**) desiccated seed associated parameters indicated that the MC and GP were positively correlated even though the MGT and GP were negatively correlated and significant at both 5 and 1 % levels. MDG as the average number of seeds germinating per day of the definite test period showed positive correlation with GV which indexed combined speed and total germination. MGT of desiccated seeds displayed negative correlation with MC, GP, MDG, SPC, PV and GV in both drying methods (Fig.1b-g). The minimum safe MGT and maximum PV and GV were noted in the 24th hour of slow desiccation and the MGT and GP were negatively correlated (r = -0.802**) and significant at both levels. The fast (r = 0.915**) and slow (r = 0.923**) desiccated seeds showed positive correlation between PV and GV as an index that combines speed and total germination.

The root and shoot length were affected by slow and fast drying. With fast drying, the longest root value was 2.4 cm and shoot value was 3.46 cm attained after 6 hours of drying to reach a moisture content of 56.2%. Whilst after slow drying, the seeds produced roots of variable length and it was observed that seeds dried for 192 hour produced longer roots (2.9 cm). The shoot length exhibited maximum i.e., 3.1 cm at 144 hours of slow desiccation, however it produced very low percentage of normal seedlings (Table 1). The physiological potential of seeds subjected to slow drying is more affected at lower levels of moisture.

Normal seedling production of 67.6 % was enhanced to 75.0 and 92.5 % upon to 48 hours of slow and 6 hours of fast desiccation respectively (Table 1). The employed desiccation methods exposed different levels of sensitivity of seeds to desiccation and the production of normal seedlings at the observed moisture content values. More than 50% normal seedlings were produced at 50.6 % MC of slow drying and 40.2% MC of fast drying respectively. Monitoring the rate of seed vigour index showed a decline in the vigour of the seeds during desiccation. The seeds subjected to fast drying had higher seed vigour index than those of seeds subjected to slow drying, indicating the damage caused by slow desiccation. This was proved with the comparative 6 hours fast and 48 hours of slow desiccation with seed vigour index i.e., 590 and 400 respectively (Table 1).

Discussion

G. imberti seeds, which are recalcitrant with 62.8 % initial moisture content, are the sole medium of propagation. The seeds of most Garcinia species are reported (20) as not true seeds which are devoid of endosperm/cotyledons and embryonic axis or any structure remotely similar to it. The recalcitrant seeds in general remain sensitive to desiccation throughout their development and have limited longevity, even in an environment with elevated moisture content (21). It was reported that, desiccation sensitive seeds are most common in tropical rainforests (22). The G. imberti seeds with hypogeal type of germination (38±1.3%) by nature possess 1.2±0.2 years of dormancy. Seed decoating was reported as the best method for
alleviating its physical dormancy in *G. imberti* (8). When desiccated, both moisture content and germination percentage became concurrently and significantly reduced irrespective of their

Fig. 1 a-h. Seed germination associated parameters of both fast and slow desiccated seeds
Table 1. Assessment of adventitious root length, shoot length, seed vigour index and percentage of normal seedling of both fast and slow desiccated seeds (n=10)

<table>
<thead>
<tr>
<th>Period</th>
<th>Adventitious root length (cm)</th>
<th>Shoot length (cm)</th>
<th>Seed vigour index</th>
<th>Normal seedling (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fast</td>
<td>Slow</td>
<td>Fast</td>
<td>Slow</td>
</tr>
<tr>
<td>(Hours)</td>
<td>0</td>
<td>1.58 ± 0.23</td>
<td>1.58 ± 0.23</td>
<td>398.3 ± 43.0</td>
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<tr>
<td></td>
<td>1</td>
<td>1.68 ± 0.29</td>
<td>2.40 ± 0.22</td>
<td>406.7 ± 46.7</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.43 ± 0.16</td>
<td>3.46 ± 0.18</td>
<td>369.9 ± 14.5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.23 ± 0.00</td>
<td>2.88 ± 0.15</td>
<td>205.00 ± 14.4</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.18 ± 0.01</td>
<td>1.20 ± 0.02</td>
<td>163.5 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.09 ± 0.01</td>
<td>1.18 ± 0.01</td>
<td>150.7 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1.07 ± 0.01</td>
<td>1.52 ± 0.36</td>
<td>145.7 ± 1.8</td>
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<td></td>
<td>7</td>
<td>1.04 ± 0.10</td>
<td>1.14 ± 0.03</td>
<td>139.3 ± 2.4</td>
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<tr>
<td></td>
<td>8</td>
<td>0.99 ± 0.02</td>
<td>1.05 ± 0.03</td>
<td>124.3 ± 2.8</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>0.94 ± 0.04</td>
<td>1.04 ± 0.02</td>
<td>120.8 ± 3.4</td>
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<td>10</td>
<td>0.87 ± 0.01</td>
<td>0.99 ± 0.04</td>
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<td></td>
<td>11</td>
<td>0.76 ± 0.05</td>
<td>0.96 ± 0.03</td>
<td>96.8 ± 4.1</td>
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<tr>
<td></td>
<td>12</td>
<td>0.59 ± 0.06</td>
<td>0.90 ± 0.05</td>
<td>82.7 ± 4.9</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>0.51 ± 0.06</td>
<td>0.84 ± 0.02</td>
<td>78.5 ± 1.6</td>
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<tr>
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<td>14</td>
<td>0.45 ± 0.02</td>
<td>0.58 ± 0.02</td>
<td>75.33 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>0.29 ± 0.02</td>
<td>0.51 ± 0.02</td>
<td>62.00 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>0.19 ± 0.06</td>
<td>0.49 ± 0.02</td>
<td>53.2 ± 2.9</td>
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<tr>
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<td>17</td>
<td>0.09 ± 0.01</td>
<td>0.40 ± 0.04</td>
<td>25.3 ± 0.8</td>
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<tr>
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<td>18</td>
<td>0.42 ± 0.26</td>
<td>2.28 ± 0.36</td>
<td>370.0 ± 44.9</td>
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<td>19</td>
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<td>2.32 ± 0.40</td>
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<td>20</td>
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<td>2.60 ± 0.13</td>
<td>358.8 ± 34.5</td>
</tr>
<tr>
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<td>2.66 ± 0.24</td>
<td>3.08 ± 0.41</td>
<td>160.1 ± 23.4</td>
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<tr>
<td></td>
<td>22</td>
<td>1.20 ± 0.38</td>
<td>1.82 ± 0.50</td>
<td>216.0 ± 18.3</td>
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<tr>
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<td>23</td>
<td>2.86 ± 0.14</td>
<td>2.14 ± 0.13</td>
<td>75.33 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>0.34 ± 0.11</td>
<td>1.54 ± 0.39</td>
<td>62.00 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>1.06 ± 0.26</td>
<td>1.00 ± 0.1</td>
<td>59.6 ± 6.1</td>
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</tbody>
</table>

The seed is directly correlated with the tolerable moisture content. In *G. imberti*, the critical moisture content significantly varies with respective germination percentage in accordance with the fast and slow drying methods adopted. It was stated that differential responses to desiccation at different drying rates denote difficulty to define a single critical moisture content value for recalcitrant seeds (27). Hence, desiccation to 16.4 and 26.3% moisture content levels at fast and slow drying respectively may be considered as two different critical moisture contents of *G. imberti*. Effect of fast and slow desiccation on *Saraca asoca* seeds with respect to different critical moisture contents *i.e.*, 36.3 and 46.9% values were reported earlier (28). During fast desiccation, the peripheral *G. imberti* seed cells may dry fast unlike the embryo-like structure that may provide safe water physiology favouring extended seed viability. On continued desiccation, the moisture content of the *G. imberti* seeds was reduced further along with significant reduction of germination percentage. The reduction of water strongly affects the nature of physical and biochemical reactions (29). Alterations in cell membranes were the main structural damage associated with seed desiccation (30) and due to these types of irreversible damages, seeds suffer from reduced germination and vigour or even loss of viability (31). The *G. imberti* seeds registered significant loss of viability below 16 % moisture content.
Seed germination associated parameters are important factors in seed biology and it varied with methods of seed drying. In *G. imberti*, mean germination time of the both fast and slow desiccated seeds displayed negative correlation with moisture content, germination percentage, mean daily germination, speed of germination, peak value and germination value. A similar observation was noted in desiccated *S. asoca* seeds in which both leachate conductivity and MGT displayed negative correlation with MC, GP, MDG, SPG, PV and GV (28). Mean germination time of *G. imberti* seeds gradually increased during both methods of desiccation which is linked to seed vigour loss. It was pointed out that irrespective of the desiccation method adopted, the inverse proportionality between MDG and MGT values signifies the adverse effect of deterioration on S. asoca seeds during desiccation (28). Slower water absorption capacity and smaller surface area to mass ratio (34) were the possible factors of longer MGT in large seeded species (35). All seed associated parameters except MGT showed the peak value significant at both 5 and 1 % levels at 6 hour in fast desiccated *G. imberti* seeds as an indication of seed vigour. The maximum MGT of slow desiccated seeds noted in the 240 hour of desiccation indicated damage due to drying of seeds. One of the first indication of seed ageing was an enhanced MGT as reported earlier (36).

In dried *G. imberti* seeds, though MDG was decreased, increased MGT showed the adverse effect of vigour deterioration during desiccation. Hence, the Pearson correlation coefficient of seed associated parameters indicated that the MGT and GP are negatively correlated and significant with both of the desiccation methods in *G. imberti*. It was reported that the drying rate affected not only the *Genipaa mericana* seed germination percentage, but also the germination associated parameters especially speed of germination (37). Germination aspects like SPG, PV, MDG and GV were high in seeds with high MC, while they were dropped considerably during desiccation. The 24 hours of fast and slow desiccated seeds showed that, the GP, SPG, MDG, PV, GV and percentage of normal seedlings were highest in fast desiccated seeds; however the seed vigour index was higher in slow desiccated seeds. Nevertheless with respect to seed desiccation and germplasm storage practices, the 24 hours of slow desiccation in seeds with more water content may not support extended seed storage. The slow drying contributed homogeneous dehydration and seed tissues spend a prolonged period at intermediary water contents which leads to extensive membrane damages (29, 30). It was also observed that *G. imberti* when subjected to 72 hours of slow drying beyond the proposed optimum period of 48 hours slow drying with 56% moisture content, the germination percentage remained unaltered, but seedling vigour parameters were observed to be deteriorated. It was pointed out the fact that, membranes are particularly susceptible to damage during slow desiccation, because the degradative process emerges to be aqueous based and oxidative in nature (27).

The GV and PV were correlated, because the parameter of PV influenced the value of GV. Most of desiccation treatments in *G. imberti* followed the correlation between PV and GV and were linked. GV was an index of combining seed germination and its speed (18) which was closely linked to *Pinus ponderosa* seedling survival (39).

The root and shoot length of *G. imberti* seedlings were negatively affected by both desiccation methods. Corroborating the statement by Martins et al. (38) the seedling growth rate as expressed by length of plumule and radicle was significantly reduced for Archonto phoenix alexandrae seeds with lower moisture content (below 28.2%) than seeds at optimum levels (47.5–34.6%). At 6th hour of fast desiccated *G. imberti* seeds, the highest root length of 2.43 and shoot length of 3.46 cm were attained at higher moisture content, and it gradually decreased to lower moisture content because it ruptured the viable cells during dehydration. In *G. imberti* seeds 6 hours of fast desiccation lowered MC from 62.8 to 56.2 % with 100 % germination and an enhanced root length compared to non desiccated seeds (5). Dresch et al. (40) pointed out that the shoot length was negatively affected by fast desiccation at low moisture content in *Campomanesia adamantium* seeds.

After slow drying, the highest root length i.e., 2.9 cm and shoot length i.e., 3.1 cm were attained at reduced MC, however it produce only very less percentage of normal seedlings. A reduction in the root length was noted in seeds of Hancornia speciosa (41), and *Eugenia pyriformis* (42) after desiccation. The maximum seed vigour index i.e., 590 (6th hour) and 402 (24th hour) of both fast and slow desiccated seeds respectively and below the critical moisture content, seeds exhibited less *G. imberti* germination percentage and seed vigour index. The seedling length and seed vigour index of *G. imberti* were also reduced during continuous desiccation. It was reported that, the whole plant length was negatively affected by the rate of dehydration of the *Campomanesia xanthocarpa* seeds that delayed seedling growth (43).

The percentage of abnormal seedling of *G. imberti* as higher in the intermediary moisture levels (17.6% to 45.02%). Many authors (44-46) stated that, seeds dried at intermediate moisture content could endure harmful aqueous based reactions, leading to reduced germination percentage and increased abnormal seedlings. The employed desiccation methods exhibited different levels of sensitivity of *G. imberti* seeds to desiccation and the production of normal seedlings at the observed moisture content values. Similar observation revealed the occurrence
of normal seedlings of *Campomanesia adamanantium* favoured by the seed drying methods and decreased linearly with the MC (40). More than 50% normal seedlings of *G. imberti* were produced at 40.2% moisture content of fast and 50.6 % moisture content of slow desiccation respectively. According to a previous study (40), the seeds of *C. adamanantium* produced 56 % of normal seedlings at 21.1 % of moisture content after fast and 17.2 % of moisture content after slow desiccation respectively. The highest percentage of normal seedling i.e., 92.5 (6th hour) and 75 % (48th hour) were produced both fast and slow desiccated seeds respectively indicated the vigour of the *G. imberti* seeds. It was point out that, fast dried *Citrus reshni* seeds were more advantageous and it provided more root protrusion along with lower percentage of abnormal seedlings (47). In *G. imberti*, seeds at less moisture content registered more seed vigour index and produced a higher percentage of normal seedlings. The study reported a similar observation that, *C. reshni* seeds dried from 46% (fresh) to 25% moisture content had a higher percentage of normal seedling compared to the fresh seeds (47). To conclude with, 6 hours of fast desiccation was found to be optimal for cent percentage *Garcinia imberti* seed germination and seedlings with maximum vigour.

**Acknowledgements**

The authors greatly acknowledge the Director, JNTBGRI for facilities provided and SERB, Department of Science and Technology (DST), Government of India for financial support. The Authors express sincere thanks to the Kerala University for approved Ph.D. programme on *Garcinia imberti* Bourd.

**Conflict of Interest**

There is no conflict of interest among the authors.

**Author’s contributions**

Both authors contributed equally to carry out the research and prepare the article.

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