



## The effect of planting media and seed soaking on the growth of true shallot (*Allium ascalonicum* L.) seeds

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### Abstract

The shallots are one of the commodities in high demand in Indonesia. One method for increasing shallot growth is to use good planting media and seed treatment. The purpose of this experiment was to see what effect planting media and seed soaking had on the growth of shallot TSS (*Allium ascalonicum* L.). A factorial randomized block design (RAK) with three replications was used in the experiment. The first factor is the planting media, which is divided into three levels: M1 (cocopeat), M2 (zeolite), and M3 (sand). The second factor is seed soaking, which has three levels: no soaking (P0), warm water 35°C 30 minutes (P1), and plain water 24 hours (P2). When compared to sand planting media, the treatment of cocopeat and zeolite planting media had a significant effect on germination capacity and fresh weight. Soaking shallot seeds in warm water at 35°C for 30 minutes and plain water for 24 hours had a significant effect on germination, plant height, number of leaves 7-21 DAT, fresh weight, and dry weight of shallots compared to not soaking. There is a significant interaction that affects the percentage of germination. However, there was no significant interaction between plant height, number of leaves, fresh weight, and dry weight of shallots and various planting media and seed soaking.

**Keywords:** cocopeat; germination; seed treatment

## 1. Introduction

Shallots are a commodity in high demand in Indonesia. The demand for shallots is currently increasing due to large imports and market fluctuations in shallot prices. In 2021, the amount of shallot produced will increase to 2 million tons (Badan Pusat Statistik, 2021). The productivity of True Shallot Seeds is higher than that of ordinary bulb seeds. There are three ways to make shallots from TSS seeds: sowing TSS seeds directly in the field (without planting), sowing TSS seeds first to produce seeds (seedlings), and planting mini bulbs (shallots sets), which are small tuber seeds (2-3 g/tuber) derived from TSS seeds (Sumarni et al., 2012b).

The Sanren variety of shallots has taller plants and more leaves. This is in accordance with the description of Sanren shallots because they have taller plants and more leaves than Lokananta shallots. Hybrid seed shallots are suitable in the lowlands - highlands are more resistant to Fusarium wilt and Anthracnose, 3 - 4 bulbs per plant, weight per bulb 15 - 25 g and have a strong aroma (Mughtar & Syafrudin, 2019). True shallot seed exhibited remarkable growth and yield performance in Central Sulawesi. This is a result of the cultivation technology used and the climate conditions being suitable. The percentage of flowering plants in the yield component was a high 78%, with several capsules/flower coming in at 80,67. Since tuber seeds may contain viruses and diseases, it is anticipated that successful shallot seed production will be able to replace the tuber seed supply (Wahyuni & Padang, 2020).

Nevertheless, the public's demand is not being met by this increase in production. One strategy for boosting shallot growth is to select high-quality planting media and seed treatment. One strategy to promote shallot growth is to select a high-quality planting medium. maximum growth in a setting with enough porosity for plants. One of cocopeat's many qualities is its capacity to hold enormous volumes of water and loosen the soil (Irawan & Hidayah, 2014). Aside from that, zeolite planting media is incredibly simple to cultivate. This kind of soil has good drainage and aeration (the availability of air pockets); however, because of its small cumulative surface area, it has a very low water-holding capacity and dries out more quickly (Dewi et al., 2020). According to the findings of Salsinha (2016), Fe zeolite alginate (3:1) promotes the growth and yield of shallots (*Allium cepa* L.). Then, for planting media, sand has good aeration (availability of air pockets) and drainage, but the cumulative surface area is relatively small, so the water holding capacity is very low or the soil dries out more quickly. The weight of the sand makes straightening the stem easier (Dewi et al., 2020).

Utilizing TSS presents a challenge because the seeds must be cultivated in a nursery for twenty to twenty-five days before being transplanted into the field. This means that the process of producing bulbs takes longer than with seed bulb crops, as they must be buried in the ground. Efforts must be made to extend seed germination and accelerate seedling establishment in order to shorten the time spent in the nursery (Agung & Diara, 2017). The hard structure of seeds prevents roots and spots from growing as well as oxygen and water from penetrating the seed coat. By soaking seeds in hot water, the hard, dry seed coats can be loosened and their pores opened, speeding up the process of seed absorption. For one to two days, soak the seeds in cold water. Mutia & Diyanti (2022), claim that it has the ability to soften seeds, allowing enzymes to be activated and germination to proceed more quickly. Thus, the purpose of this experiment was to ascertain how planting media and seed soaking affected the growth of shallot TSS (*Allium ascalonicum* L.), as well as how they interacted.

## 2. Methods

This study was conducted in the Leuwikopo greenhouse at Agriculture Faculty of IPB University from March to April of 2023. Seedling trays, digital scales, sprayers, ovens, and rulers were among the tools used in this study. TSS seeds of the Sanren variety, zeolite, cocopeat, sand, and water were the materials used in the study. Three replications of a factorial randomized block design (RAK) were used in the experiment. First and foremost, there are three levels of planting media: cocopeat (m1), zeolite (m2), and sand (m3). Each planting medium used has the following particle densities: sand is 1,263 gr/cm<sup>3</sup>, zeolite is 1,061 gr/cm<sup>3</sup>, and cocopeat is 0,021 gr/cm<sup>3</sup>. The second factor is seed soaking, which comes in three stages: control without soaking (p0), warm water at 35°C for 30 minutes (p1), and plain water for 24 hours (p2). Up to one TSS shallots seed per tray hole is planted, using the Sanren variety. Every replication has six seeds in it. NPK 16-16-16 fertilizer is applied at 14 Day After Treatment (DAT) to each tray hole at a rate of 50 gr/m<sup>3</sup> or 20 ppm. Volume of tray hole: 79,55 cm<sup>3</sup>.

Following that, factorial RAK variance (ANOVA) was used to evaluate the data from the observations at a significant level of 5%. Duncan was then used to test for the presence of an average difference between treatments at a significant level of 5%. The following formula was used to determine the parameters observed: the percentage of seed germination (%) at 7 DAT. Germination percentage (%) = (A/B) x 100. Where plant samples were taken, up to four plants per replication for each treatment, and in the oven at 60°C for three times in a row, plant samples were measured as follows: A: Number of seeds that germinated; B: Number of seeds planted; height (cm) at 7–35 DAT; number of leaves (strands) at 7–35 DAT; fresh weight (grams) at 35 DAT; and dry weight (grams) at 35 DAT.

## 3. Results and Discussion

### 3.1. The Germination Percentage

The results of the variance analysis (ANOVA) revealed that the planting medium and seed soaking had a real interaction on the percentage of shallot germination at 7 DAT. The

combination of treatments for each planting medium, including cocopeat, zeolite, and sand, with soaking the seeds in warm water at 35°C for 30 minutes and plain water for 24 hours, resulted in a higher average germination rate than when the seeds were not soaked. The cocopeat and zeolite planting media treatments had a significant effect on germination parameters when compared to sand planting media, and soaking in warm water at 35°C for 30 minutes and plain water for 24 hours had a significant effect on germination capacity when compared to no soaking (Table 1). According to SNI ([Badan Standarisasi Nasional, 1995](#)) standards, the percentage of good seed germination is greater than 85%. The treatment without soaking had the worst germination capacity of 37.04%, not even reaching 50%, let alone the SNI standard results (Table 1).

According to [Harist et al. \(2017\)](#), the higher the density, the smaller the total pore space, and the lower the density, the greater the total pore space. Low particle density implies low bulk density, but high porosity, and vice versa. Cocopeat has a particle density of 0,021 gr/cm<sup>3</sup>, zeolite has a particle density of 1,061 gr/cm<sup>3</sup>, and sand has a particle density of 1,263 gr/cm<sup>3</sup>. The lower the porosity of a planting medium, the denser the planting medium, which causes the structure of the planting medium to deteriorate and the amount of pore space in the planting medium to decrease ([Mubarok et al., 2012](#)). Sand media has a lower porosity than zeolite and cocopeat and a lower average percentage of germination (Table 1). However, earlier studies revealed that the loamy soil:sandy soil:vermiculite (2:1:1) combination produced the highest germination percentage, number of leaves/seedling, stem diameter, root length, fresh leaf weight, and dry weight ([Mahmoud et al., 2019](#)). The results of this investigation suggest that the combination of substantial air space and high moisture retention in agricultural media may have contributed to the observed increase in seedling growth. The outcomes correspond with the findings of [Al-Imama & Al-Jubury \(2011\)](#).

If the structure of the planting medium is poor, it can cause stunted growth due to difficulty in root movement into the planting medium ([Suyono et al., 2006](#)). Cocopeat has the ability to store a large amount of water while also loosening the soil ([Irawan & Hidayah, 2014](#)). According to [Istomo \(2012\)](#), cocopeat media also has micro pores that can inhibit greater water movement, resulting in increased water availability. Zeolite media, which is made of minerals, is resistant to decay, does not affect pH, and can absorb and store water well over time ([Khosiaris et al., 2002](#)). Previous studies have shown that the planting media combination of rice husk and cocopeat (4,9 days) exhibited the fastest germination rate. Many of the seeds died when they were planted straight from onion seeds in soil without a tidal swamp. Sanren (78%) germinated more readily on a combination of husk and cocopeat than Lokananta (65%), Trisula (60%) and Bima brebes (64%) ([Sopiana et al., 2023](#)).

The imbibition process, or the absorption of water by seeds, is accelerated by soaking them in warm water. This is because temperature creates pressure, which allows water to enter the seeds. According to [Schmidt \(2002\)](#), hot water causes tension in the outer cells to fracture, allowing oxygen and water to reach the seeds more quickly. This rupture of the macrosclereid layer is how hot water breaks the physical dormancy in legumes. If water is not absorbed by the seed, germination will not start. After soaking in hot water, the seed coat will allow oxygen and water to pass through. According to earlier study findings, germination percentage, germination rate, and vigor index of 100%, 4,51 days, and 27,02, respectively, were obtained from Sengon seeds immersed in hot water at 60°C for 4 minutes, and then submerged in cold water for 12 hours ([Marthen et al., 2018](#)). Seeds soaked in water at room temperature for 24, 48, or 72 hours after 20 minutes at 70°C had germination rates exceeding 97%, according to [Tadros et al. \(2011\)](#).

However, care should be taken since an embryo may be harmed by prolonged exposure to high heat, which would lower germination rates ([Koobonye et al., 2018](#)). *Allium cepa* seeds soaked in nitrogen demonstrated a high rate and energy of germination. The control treatment yielded the highest values for germination time GT, abnormal seedling percentage, and dead seed percentage. Therefore, the germination techniques had an impact on both the germination characteristics and the growth of onion seedlings ([Mostafa et al., 2019](#)).

Table 1. Average shallot germination percentage at 7 DAT (%)

Treatment	Germination (%)
Planting Media	
M1: Cocopeat	75.92 a
M2: Zeolite	66.67 ab
M3: Sand	61.11 b
<i>F test</i>	0.04*
Seed Soaking	
P0: Control without soaking	37.04 b
P1: Warm water at 35°C	87.04 a
P2: Plain water for 24 hours	79.63 a
<i>F test</i>	0.00*
Interaction	0.00*

Details:

\* : Significant effect on statistical tests ( $p < 5\%$ )

tn : No significant effect on statistical tests ( $p > 5\%$ )

### 3.2. Height of a Shallot Plant

The results of the variance analysis (ANOVA) revealed that there was a real interaction between planting media and seed soaking on shallot height at 7 DAT. There was no significant interaction between planting media and seed soaking on shallot plant height at 14 DAT - 35 DAT. Soaking in warm water at 35°C for 30 minutes and plain water for 24 hours had a significant effect on height of shallot when compared to no soaking, at 35 DAT, the treatment without soaking had the shortest height of only 6,16 cm (Table 2).

The planting media treatments of cocopeat, zeolite, and sand had no effect on shallot plant height at 7 DAT - 35 DAT. At 7 DAT - 35 DAT, the treatment of soaking seeds in warm water at 35°C for 30 minutes and plain water for 24 hours produced higher plant height than the treatment without soaking seeds (Table 2). This is thought to be because the nutrients in the planting medium cannot be optimally absorbed by the plant at the start of growth. Because shallot plants have their own food reserves to aid in growth at the start of the growth period (Tambunan et al., 2014). The porosity of zeolite media is superior to that of soil. This is due to the presence of empty spaces or pores in the crystal structure that can be filled with water, allowing it to store and retain a large amount of water. Vermicompost is required for zeolite media to help increase the supply of nutrients to plants and their ability to bind water (Mance et al., 2016).

The treatment with warm water at 35°C for 30 minutes and plain water for 24 hours produced higher plant height than the treatment without soaking the seeds (Table 2). This could be because the process of soaking shallot seeds in distilled water can stimulate the division and elongation of cells in the stem and accelerate the growth of root cells because the process of entering water and oxygen in the seeds wets the proteins and colloids in the seeds (hydration or imbibition), resulting in formation and activation. The enzyme causes increased metabolic activity, radical cell elongation, and subsequent growth (Fitri, 2015). It is suspected that soaking provides sufficient water for transport within the plant body, the results of research by Ghasemi et al. (2014) showed that soaking for 24 hours had a significant effect on shoot height compared to controls that were not soaked.

Table 2. Average height of shallot plants (cm)

Treatment	Height (cm)				
	7 DAT	14 DAT	21 DAT	28 DAT	35 DAT
<b>Planting Media</b>					
M1: Cocopeat	2.64 a	4.38 a	8.23 a	8.98 a	10.52 a
M2: Zeolite	2.91 a	4.30 a	8.39a	9.48 a	11.36 a
M3: Sand	2.22 a	3.22 a	7.18a	7.83 a	9.06 a
<i>F test</i>	tn	tn	tn	tn	tn
<b>Seed Soaking</b>					
P0: Control without soaking	0.67 b	2.08 b	4.61 b	5.10 b	6.16 b
P1: Warm water at 35°C	3.37 a	4.63 b	9.44 a	10.42 a	12.87 a
P2: Plain water for 24 hours	3.73 a	5.19 a	9.74 a	10.78 a	11.91 a
<i>F test</i>	0.00 *	0.01*	0.00 *	0.00 *	0.01 *
<b>Interaction</b>	0.03 *	tn	tn	tn	tn

Details:

DAT : Day After Treatment

\* : Significant effect on statistical tests ( $p < 5\%$ )

tn : No significant effect on statistical tests ( $p > 5\%$ )

### 3.3. The Number of Leaves on Shallots

Variance analysis (ANOVA) results demonstrated that the number of shallot leaves at 7 DAT was actually influenced by a real interaction between the planting medium and seed soaking. On the number of shallot leaves at 14 DAT - 35 DAT, there was no discernible difference in the interaction between the planting medium and seed soaking. When the seeds were soaked in warm water at 35°C for 30 minutes and in plain water for 24 hours for each planting medium—cocopeat, zeolite, and sand—the average number of shallot leaves at 7 days after transplanting was higher than when the seeds weren't soaked (Table 3). The parameters of shallot plant height at 14–35 DAT were not significantly affected by the planting media treatment (Table 3). Soaking in warm water at 35°C for 30 minutes and plain water for 24 hours had a significant effect on the quantity of shallot leaf when compared to no soaking, at 7 DAT-28 DAT, with the treatment without soaking having the smallest amount of leaf only 1,22 strands at 28 DAT (Table 3).

The average number of shallot leaves produced by the treatments of soaking the seeds in warm water at 35°C for 30 minutes and in plain water for 24 hours was higher than that of the treatment that did not soak the seeds at 7 DAT, and it did not differ significantly at 14 DAT to 35 DAT. Furthermore, the primary advantages of pre-soaking seeds are faster germination and increased seed viability. In this case, soaking water treatment for seed has no effect on increasing the number of leaves, therefore concentrate on providing conditions such as nutrient-rich soil in media planting.

The average number of shallot leaves at 7 DAT was then significantly affected by the treatment of cocopeat and zeolite planting media; specifically, the average number of leaves was 0,81 and 0,76 compared to the sand planting media's 0.61 (Table 3). These results align with the research conducted by Margiwiyatno (2007), where a mixture of sand and cocopeat at room temperature has the least impact on the quantity of shallot leaves. According to this study, zeolite by itself is insufficient to provide nutrition to shallot plants; however, zeolite can be added as a mixed medium in conjunction with soil that contains adequate nutrients for plant growth. Zeolite increased leaf number and area, shoot fresh weight (FW), edible root diameter and FW, fibrous root number, length, and FW, and harvest index. The addition of zeolite increased the concentrations of nitrogen (N) and potassium (K) in shoot tissues, as well as the medium's cation-exchange capacity. It was determined that zeolite had a significant positive effect on radish vegetative growth. To reduce nutrient leaching, zeolite may be recommended as a soil amendment for vegetable crops such as radishes. A 100 g kg-

1 soil zeolite level increased the average number of leaves by 77% to 8.53, compared to 4.81 in the controls (Baninasab, 2009).

Table 3. The average number of leaves

Treatment	Shallot Leaf (Strands)				
	7 DAT	14 DAT	21 DAT	28 DAT	35 DAT
<b>Planting Media</b>					
M1: Cocopeat	0.81 a	1.67 a	1.89 a	2.11 a	2.24 a
M2: Zeolite	0.76 a	1.39 a	1.61 a	2.17 a	2.52 a
M3: Sand	0.61 b	1.28 a	1.76 a	1.87 a	1.87 a
<i>F test</i>	0.00 *	tn	tn	tn	tn
<b>Seed Soaking</b>					
P0: Control without soaking	0.39 b	1.02 b	1.17 b	1.22 b	1.30 a
P1: Warm water at 35°C	0.89 a	1.74 a	2.09 a	2.45 a	2.78 a
P2: Plain water for 24 hours	0.92 a	1.57 a	2.00 a	2.48 a	2.56 a
<i>F test</i>	0.00 *	0.01 *	0.01 *	0.00 *	tn
<b>Interaction</b>	0.00 *	tn	tn	tn	tn

Details:

\* : Significant effect on statistical tests ( $p < 5\%$ )

tn : No significant effect on statistical tests ( $p > 5\%$ )

### 3.4. Fresh Weight of Shallots

The analysis revealed that cocopeat and zeolite planting media had a significant effect on the fresh weight of shallots, with an average of 0,56 grams and 0,41 grams compared to 0,35 grams for sand planting media. Soaking the seeds in warm water at 35°C for 30 minutes and plain water for 24 hours resulted in a higher fresh weight with an average of 0,59 grams and 0,48 grams compared to 0,25 grams without treatment. However, the results of the variance analysis (ANOVA) revealed that there was no real interaction between the planting medium and seed soaking on the fresh weight of shallots (Table 4).

The above results are in accordance with Margiwiyatno (2007) research, where cocopeat media produced the highest fresh weight formation. Fresh weight formation was highest in cocopeat growing media, followed by zeolite, and lowest in sand growing media. The fresh weight of shallots treated with zeolite planting media is not significantly different from that of cocopeat because they share the same properties, namely the ability to absorb a large amount of water and thus meet nutritional needs. As a result, the fresh weight of the cocopeat and zeolite treatments is greater than that of sand. This is due to the high water retention capacity of cocopeat, which causes the water content in the roots to increase. Cocopeat has a high porosity and aeration level. Aeration in the soil means that nutrient absorption will be efficient (Simbolon et al., 2018). Zeolite can automatically regulate the pH balance of the media due to the unique acidity-wetness properties of zeolite so it is very suitable for use as a planting media component (Mance et al., 2016). While 100% soil or 100% cocopeat is not advised, 80% cocopeat and 20% soil is the ideal mixture ratio. To supply nutrients that are absent from cocopeat, balanced fertilization should be applied after using it as planting media (Cahyo et al., 2019). Sand, on the other hand, is easily eroded by wind or water due to its low cohesiveness and consistency, which makes it resistant to the process of separation. In order to support plant growth, sand planting media needs higher irrigation and fertilization levels (Margiwiyatno, 2007). One of the factors that play an important role in plant metabolism is the water content of leaf cells (Anshar et al., 2014). Each variety differs in its ability to support life and growth in individuals from various climates.

Table 4. Average fresh weight of shallots (grams)

Treatment	Fresh Weight (gram)
Planting Media	
M1: Cocopeat	0.56 a
M2: Zeolite	0.41 a
M3: Sand	0.35 b
<i>F test</i>	0.01 *
Seed Soaking	
P0: Control without soaking	0.25 b
P1: Warm water at 35°C	0.59 a
P2: Plain water for 24 hours	0.48 a
<i>F test</i>	0.00 *
Interaction	tn

Details:

\* : Significant effect on statistical tests ( $p < 5\%$ )

tn : No significant effect on statistical tests ( $p > 5\%$ )

### 3.5. Dry Weight of Shallots

There was no interaction between the planting media and soaking seeds and the dry weight of shallots, as indicated by the analysis's results, which also revealed that the planting media of cocopeat, zeolite, and sand had an average dry weight of 0,04 grams, 0,04 grams, and 0,03 grams, respectively (Table 5).

Cocopeat, zeolite and sand planting media did not have a significant effect on the average dry weight of shallots. Cocopeat has a high carbon (C) to nitrogen (N) ratio (112:1) and a high lignin content, which can inhibit plant nutrient immobilization (Krishnapillai et al., 2020). However, zeolite have several flaws, including poor and optimal water absorption capacity, the presence of many impurities such as Na, K, Ca, Mg, and Fe, and poor crystallinity. The presence of these impurities can reduce the activity of the zeolite (Mance et al., 2016). Sand is also known for its difficulty in retaining water, which has an impact on growth on a daily basis (Rosalina et al., 2019)

According to his research, the pH of the soil+cocopeat and compost and cocopeat mixed media was found to be lower than that of the soil+compost mixed media group, which was found to be 6,3–7. To keep sand media from drying out, irrigation must occur on a regular basis or at a steady flow (Margiwiyatno, 2007). Planting shallots in well-drained, rich, loose soil is recommended. Rich soil enhances tuber growth. A suitable pH range for soil is 5,5 to 6,5, which is about neutral. Because they drain well and have good aeration, sandy or silt loam soils are ideal for planting. Acidic or alkaline soil isn't even good for growing onions; soil that is too acidic (pH less than 5,5) dissolves aluminum in the soil hazardously, which stunts plant growth. Plants cannot absorb manganese salts in soil that has a pH of more than 7 or 6,5, which leads to small tubers and low yields (Ashari, 1995).

Weight loss in shallot tubers is related to water content, which affects the quality of the bulbs, particularly their freshness. According to Azmi et al. (2018), shrinkage occurs because the water content remains high and the respiration rate remains high. Previous results showed that providing planting media in the form of 75% topsoil + 25% compost could respond to shallot production. Ecoenzyme and the interaction of ecoenzyme in the growing media had no effect on shallot production as measured by wet tuber weight per plot, dry bulb weight per plot, and tuber diameter (Luta et al., 2022).

An average of 0,05 grams and 0,04 grams were obtained from the seed soaking treatment using warm water at 35°C for 30 minutes and plain water for 24 hours, as opposed to 0,02 grams without treatment (Table 5). High dry weight is a sign of healthy plant growth, which is determined by how quickly roots absorb nutrients from the soil and

grow longer and more numerous roots. High vigor seeds have a faster rate of formation and translocation of raw materials to the embryonic axis, which increases the amount of dry matter that accumulates. Elevated dry weight may suggest effective use of seed food reserves (Nurussintani et al., 2012).

Table 5. Average dry weight of shallots (gram)

Treatment	Dry Weight (gram)
Planting Media	
M1: Cocopeat	0,04 a
M2: Zeolite	0,04 a
M3: Sand	0,03 a
<i>F test</i>	tn
Seed Soaking	
P0: Control without soaking	0,02 b
P1: Warm water at 35°C	0,05 a
P2: Plain water for 24 hours	0,04 a
<i>F test</i>	0,00*
Interaction	tn

Details:

\* : Significant effect on statistical tests ( $p < 5\%$ )

tn : No significant effect on statistical tests ( $p > 5\%$ )

#### 4. Conclusions

The treatment of cocopeat and zeolite planting media had a significant effect on germination percentage and fresh weight when compared to sand planting media. Soaking shallot seeds in warm water at 35°C for 30 minutes and plain water for 24 hours had a significant effect on germination, plant height, number of leaves 7-21 DAT, fresh weight, and dry weight of shallots compared to not soaking. There was a significant interaction that influences the percentage of germination percentage. There was no significant interaction between plant height, number of leaves, fresh weight, and dry weight of shallots and various planting media and seed soaking.

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#### Author Contribution

Conceptualization, K.D.P.; Methodology, K.D.P.; Software, K.D.P.; Validation, K.D.P.; Formal Analysis, K.D.P.; Investigation, K.D.P.; Resources, K.D.P.; Data Curation, K.D.P.; Writing – Original Draft Preparation, K.D.P.; Writing – Review & Editing, K.D.P.; Visualization, K.D.P.

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Not applicable.

#### Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.



## Data Availability Statement

Primary data is based on requested due to confidentiality or ethical constraints.

## Conflicts of Interest

The authors declare no conflict of interest.

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