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Growth, yield and anatomy of alpinia calcarata (*Heen Araththa*) as affected by irrigation frequency

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Abstract

Alpinia calcarata, often known as Heen Araththa, is a precious medicinal plant owing to its chemical properties in herbal treatments. The growth, yield and anatomical features related to the synthesis and accumulation of secondary metabolites of this plant are highly affected by drought stress. Therefore, this work investigated the influence of irrigation frequency on growth, yield and selected anatomical features of *A. calcarata.* Five levels of irrigation frequencies including daily watering (T_1), 7-day (T_2), 14-day (T_3), 21-day (T_4) and 28-day (T_5) intervals were investigated in a protected plant house under controlled environmental conditions. *A. calcarata* rhizomes with a similar number of sprouting buds planted in common potting media (topsoil: sand: compost- 1:1:1) were arranged in Completely Randomized Design in triplicates. After three weeks of establishment, five distinct irrigation frequencies were implemented, with pots being watered daily up until that point. Growth and yield parameters (shoot height (cm), number of roots, number of shoots,

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number of leaves per shoot, leaf area (mm^2), rhizome dry weight (g)) and the anatomical parameters (root epidermis thickness (μm), rhizome epidermis thickness (μm) and oil cell density ($μm^2$)) were collected 10 weeks after establishment. Data were analyzed using Analysis of Variance (ANOVA) employing the SAS analysis system. Results revealed that all selected growth, yield and anatomical parameters of *A. calcarata* were significantly ($p<0.05$) affected by irrigation frequency. Significantly ($p<0.05$) the highest mean shoot height (20.8cm), mean number of roots (11.3), mean number of shoots (6.8), mean rhizome dry weight (9.3g) and significantly ($p<0.05$) higher mean leaf area (24.9mm^2) and mean number of leaves per shoot (4) of *A. calcarata* weregiven by 14-day irrigation frequency. It was also observed that the mean root epidermis and rhizome thickness of *A. calcarata* gradually increased in response to escalating drought stress. In contrast, the highest oil cell density was observed under the lowest drought stress. Based on the results, it can be concluded that the most promising irrigation frequency for higher growth and yield of *A. calcarata* is watering with 14-day intervals. Daily watering appeared to be the most suitable treatment to enhance oil cell density associated with secondary metabolite production of *A. calcarata*.

Key words: *Alpinia calcarata*, Anatomy, Drought stress, Growth and yield, Oil cell density

Introduction

Alpinia calcarata, a rhizomatous plant belonging to the family *Zingiberaceae,* is frequently used as a systemic medicinal source in Sri Lanka. *Alpinia Roxb*. is the largest and most extensively distributed genus in the *Zingiberaceae* family, with over 230 species and it can be found in the Pacific and all of Southeast Asia (Larsen *et al.*, 1998; Smith,1990).

Due to its medicinal properties, *A. calcarata* is often used in Sri Lankan traditional systems of medicine. The therapeutic advantages of *A. calcarata* rhizomes are numerous and it is widely recognized that the rhizomes have antibacterial anthelmintic, antifungal (Arambewela *et al.*, 2004), antioxidant (Arambewela & Arawwawala, 2005), gastroprotective (Arambewela *et al.*, 2009), anticancer and antidiabetic qualities. Similarly, Asha *et.al* (2012) state that, treatments for rheumatism, bronchial asthma, and asthma include medications made from *A. calcarata.* A study conducted by Chelladurai *et al.* (2021) to check for phytochemicals, revealed that *A.calcarata* rhizome extract shows the presence of terpenoids, tannins, flavonoids, saponins and cardiac glycosides. Essential rhizome oil of *A. calcarata* grown in Sri Lanka is reported to contain chemical constituents such as a-pinene, pincne, cymcne, cineol, limoncnc, camphcnc, camphor, terpineol, bomeol-terpeneol, muurolcnc, cara tol, fenchy, acetate,ct-eudesmol and trace amount of unalool, fcnchol, fcnchone, a-cadinene and p-caryophyllene (Arambewela & Alagiyawanna, 2006).

The biosynthesis and variations in plant secondary metabolites are greatly influenced by environmental variables such as drought or flooding, extremes of light and temperature, poor soil, or harmful substances (Verma & Shukla, 2015). Among these variables, "drought stress" is a significant environmental element that influences both the growth and content of secondary metabolites in plants (Li *et al.*, 2020). Drought stress reduces photosynthetic rates since there is less water available for roots to absorb, which increases the formation of phenolic compounds in plants (Ncube *et al.*, 2012). Therefore, a decrease in plant growth during a drought is to be expected. In addition, the generation of specific secondary metabolites is influenced by the growth and development of medicinal plants (Li *et al.*, 2020). Some studies have shown that plants subjected to drought stress have higher concentrations of secondary metabolites than plants grown in well-irrigated environments (Kleinwächter & Selmar, 2015). For instance, under drought stress, secondary metabolites like tannins, terpenoids, and alkaloids are increased (Liu *et al.*, 2017). On the other hand, several secondary metabolites such as phenols, flavonoids, and saponin might be reduced by drought (Alhaithloul *et al.*, 2019).

However, the analysis of secondary metabolites is an extremely skill, capital, and technologically-intensive operation. One affordable way to determine a medicinal plant's secondary metabolites is by assessing its anatomical features. However, an understanding of the relationship between morphogenesis and the synthesis and accumulation of specific medicinal components in medicinal plants is essential when examining anatomical features to gain insight into the production of secondary metabolites in organs and tissues. For example, Belkheir *et al*. (2016) reported that the mesophyll and palisade cells contained flavonoids, although the majority of phytochemicals were found in the epidermal cells of the roots, leaves, and rhizomes. Similarly, evaluating the anatomical characteristics helps in authenticating plant materials used in medicine (Uma & Muthukumar, 2014) and evaluating the quality of medicinal products (Li *et al.*, 2020).

Thus, the current research aimed to assess the effect of drought stress on the growth, yield and anatomical characteristics of *A. calcarata,* an economically important medicinal plant in Sri Lanka.

Materials and methods

Evaluation of growth and yield parameters of *Alpinia calcarata*

The present study was conducted in a plant house under controlled environmental conditions at Faculty of Agriculture, University of Ruhuna from June to September 2022. During the study period, the average monthly temperature, relative humidity (RH) and the light intensity of the protected plant house were 33°C, 70% and 25000 Lux respectively. *A. calcarata* rhizomes with a similar number of sprouting buds were planted in plastic pots of 10 inches x10 inches, filled with potting media (topsoil: sand: compost in 1:1:1 ratio). To ensure better establishment of rhizomes and the roots, pots were irrigated daily for 3 weeks and closely monitored to avoid fungal attacks. After 3 weeks of establishment, five different irrigation frequencies (daily watering-T_1, 7-day interval-T_2, 14-day interval-T_3, 21-day interval-T_4 and 28-day interval-T_5) were practised. Experimental units were arranged in Completely Randomized Design (CRD) with 3 replicates. Irrigation was accomplished to the field capacity level and the field capacity was determined using the gravimetric method. The water requirement to reach field capacity was determined using the equation below.

Water needs for the plants to reach field capacity = (Moisture amount in field capacity level) - (Initial moisture in the soil)

Data on the shoot height (cm), number of roots, number of shoots, number of leaves per shoot, leaf area (mm^2) and rhizome dry weight (g) were collected 10 weeks after establishment. Data were analyzed using Analysis of Variance (ANOVA) employing the statistical analysis system (SAS 9.1 statistical software) and Statistix 10 (non-parametric analysis). The means were separated using Duncan Multiple Range Test (DMRT).

Evaluation of anatomical parameters of *A. Calcarata*

As anatomical parameters, data on root epidermis thickness (μm), rhizome epidermis thickness (μm) and oil cell density (μm^2) were measured under different irrigation frequencies. For this, thin cross sections (one cell layer) of rhizome and root were obtained using a sharp blade and these were stained with safranin. For the staining procedure, the sections were transferred to 25% alcohol for 2 minutes, 50% alcohol for 2 minutes and 1% safranin solution in 50% alcohol for 30 seconds respectively. Then the sections were washed in 50% alcohol and 70% alcohol separately (over-stained sections were de-stained in acid alcohol and transferred again to 75% alcohol for 3 minutes). Thereafter, stained sections were mounted on a drop of glycerin. Finally, the cross sections were observed under a Trinocular microscope and the measurements and photographs were taken.

Results and Discussion

Evaluation of growth and yield parameters of *A. Calcarata*

Different irrigation frequencies significantly ($P<0.05$) affected the shoot height, mean number of roots, mean number of shoots, mean leaf area, mean number of leaves per shoot and the rhizome dry weight of *A. calcarata* (Figure 1).

As revealed by the experimental results, significantly ($p<0.01$) the highest shoot height (20.8cm: Figure 1A), the highest root number (11.3: Figure 1B), the highest number of shoots (6.8: Figure 1C) and the highest rhizome dry weight (9.3 g: Figure 1F) were recorded from the $T_{3.}$ Although significantly ($p<0.01$) the highest mean leaf area (29.8 mm^2: Figure 1D) and the highest number of leaves per shoot (4.7: Figure 1E) were recorded from $T_{1,}$ T_3 also recorded a higher leaf area (24.9cm^2: Figure 1D) and a higher number of leaves per shoot (4: Figure 1E). However, as shown in the results, the variation pattern for the given growth and yield parameters of *A. calcarata*

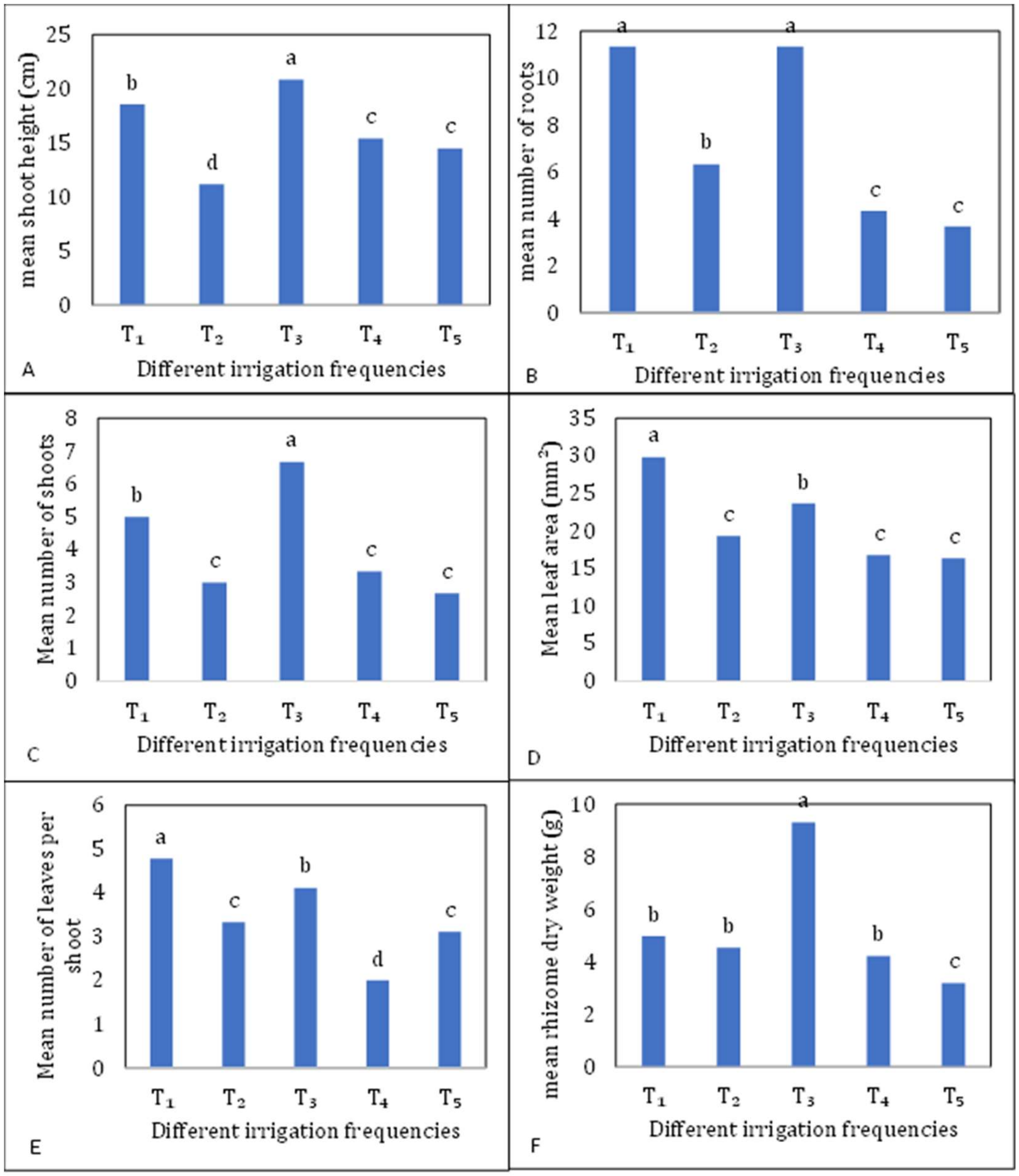

Figure 1. Effect of different irrigation frequencies on growth parameters of *A. calcarata* (A-mean shoot height(cm), B-mean number of roots, C-mean number of shoots, D-mean leaf area (mm^2), F-mean number of leaves per shoot, F- mean rhizome dry weight (g) of *A. calcarata*)

Note: Means with similar letters in a column are not significantly different at $p<0.05$ T_1-daily watering, T_2-7 days interval, T_3-14 days interval, T_4-21 days interval- and T_5-28 days interval across the five different irrigation frequencies is mostly similar, with the 14-day irrigation frequency emerging as the most appropriate treatment for *A. calcarata.*

In the majority of the cases, the overall trend suggests that increasing irrigation frequency is associated with a greater tendency to enhance growth and yield parameters. For example, an experiment by Khorasaninejad *et al,* (2011), to find the effect of drought stress on growth parameters, essential oil yield and constituent of *Mentha piperita* L.: a medicinal plant, discovered that the highest values of growth parameters and essential oil percent and yield were observed under 100% field capacity. Similarly, Shekofteh *et al.* (2015) observed that simultaneous application of 1 mM salicylic acid and 100 % field capacity produced the highest yield and yield components of *P. ovata* Forssk. The most frequent irrigation regime, which consisted of two-day watering intervals over a range of irrigation regimes evaluated, produced the greatest plant height, fresh and dry flower weight, and flower count of *Matricaria chamomila*, a significant essential oil-bearing plant (Razmjoo *et al*.,2008). However, it is worthy to note that, in this instance, *A. calcarata* has functioned remarkably at the medium frequent irrigation treatment, such as 14-day of irrigation frequency effectively under the given experimental settings.

Additionally, a thorough examination of the variation pattern of the graphs in Figure 1 reveals that, the growth and yield parameters of *A. calcarata* decline significantly after T_3, as irrigation frequency decreases. Likewise, Khalil *et al.* 's (2010) research showed that water stress has a major impact on the morphological and biochemical characteristics of basil plants while the plants' height, number of branches, number of leaves, leaf area, and fresh and dry weights of the first cut all exhibited substantial increases under 50% soil moisture; however, these metrics also considerably dropped as the water stress level increased. The yield of fresh and dry herbs in pots, plant height, stem diameter, number and length of auxiliary shoots, and essential oil yield all reduced as the soil water content dropped (Hssanai, 2006). Additionally, the findings of a different study on *Ocimum basilicum* revealed that plant height, stem diameter, leaf area index, number and area of leaves, herb yield, and leaf chlorophyll concentrations all dropped as soil water content fell (Alishah *et al.,* 2006). The findings showed that extended drought conditions significantly reduced the growth and yields of *Tulipa edulis*: an important medicinal plant, compared to the well-watered control (Miao et al., 2015).

The water requirements of plants have long been a critical focus for researchers in the agricultural sector, as understanding these needs is essential for improving crop productivity, sustainability, and resource management. Numerous research initiatives, including those by Sawadogo *et al*., (2023), Bao *et al*. (2022) and Bülbül *et al*. (2022) to evaluate the various elements influencing irrigation performance and requirements have grown popular in this subject. However, based on the current experiment's results, where *A. calcarata* performed well at a moderate irrigation frequency, we can hypothesize that other factors, such as the physical and chemical properties of the growth media, container size/volume, and external factors like evapotranspiration and temperature, may directly or indirectly influence the determination of the optimal water amount for maximizing its growth and yield characteristics. Therefore, we recommend conducting further comprehensive studies to investigate the effects of these internal and external factors on determining the moisture requirements and irrigation schedule for optimal growth of *A.calcarata* under these conditions.

Evaluation of anatomical parameters of *A. Calcarata*

Based on the results of the study, different irrigation frequencies significantly ($P < 0.05$) affected the given anatomical features such as the root epidermis thickness (Figure 2A) and rhizome epidermis thickness (Figure 2B) of *A. calcarata.* The results of the anatomical features indicate that, significantly ($p<0.05$) the highest root epidermis thickness (46.9 µm: Figure 2A) and the highest rhizome epidermis thickness (47.8 µm: Figure 2B) were recorded by T_5 while, T_1 recorded the lowest root epidermis thickness and the lowest rhizome epidermis thickness as, 28.5 µm (Figure 2A) and 21.8 µm (Figure 2B) respectively.

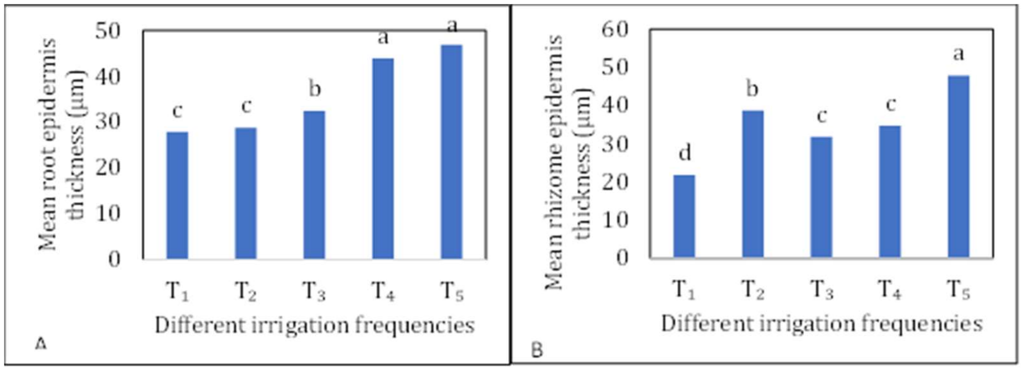

Figure 2. Effect of different irrigation frequencies on anatomical features of *A. calcarata.* (A- mean root epidermis thickness, B- mean rhizome epidermis thickness) of *A. calcarata.*

According to Figures 2A and 2B, the epidermis of roots and rhizomes appears to thicken in response to greater drought stress. This may be to defend against excessive water loss through transpiration during drought stress. Ghafoor *et.al* (2019) also recorded that water stress caused an increase in root epidermis thickness as well as the stem epidermis thickness. Reduced root growth rate and a higher percentage of roots with developed endodermis and exodermis are common responses to deficient soil water conditions in the rhizosphere (Enstone *et. al*, 2002).

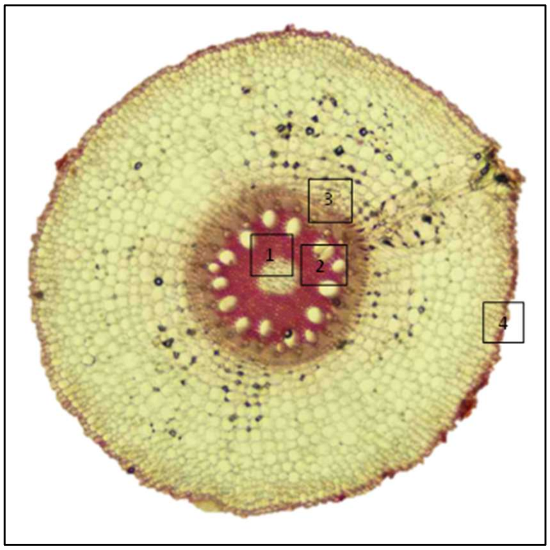

Figure 3. Cross section of primary growth in *Alpinia calcarata* root (stained with safranine) (10x10)

As revealed by the experimental results, different irrigation frequencies significantly ($P < 0.05$) affected the oil cell density of *A. calcarata* (Figure 4). In contrast to the variation of root and rhizome epidermis thickness under five different irrigation frequencies, T_1 recorded the highest oil cell density (98 μm^{-2}: Figure 4) while T_5 recorded the lowest oil cell density (52 μm^{-2}: Figure 4) of *A. calcarata*,

Oil cells are the fundamental sources that produce beneficial chemical compounds that have potential applications in medicine, food, cosmetics, and the agriculture industry. Based on the results of the current study, it appears that optimal irrigation conditions could encourage increased oil cell density and drought stress motivated a significant reduction in oil cell density of *A. calcarata* (Figure 4). Similar results were obtained by Khorasaninejad *et al.* (2011) for essential oil yield (ml/plant) and essential oil percentage of *Mentha piperita L.* under four drought stress conditions. Water deficit stress during flower bud formation had the most significant negative impact on seed oil content, with the study concluding that severe water deficit reduced both oil content and oil yield of Brassica napus L (Shekari et al., 2016).

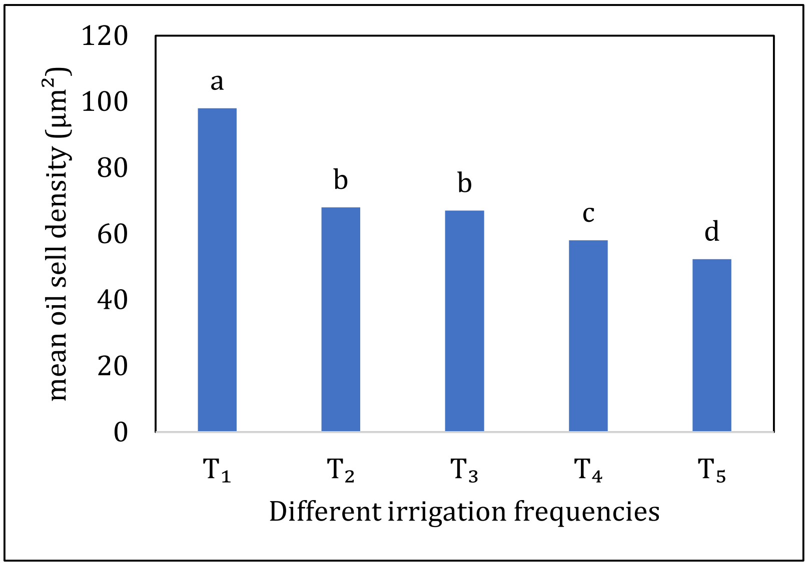

Figure 4. Effect of different drought stress on oil cell density of *A. calcarata*

Alhaithloul *et al.* (2019) also reported that drought may cause a decrease in phenols, flavonoids, and saponin, among other secondary metabolites. In contrast, Khalid (2006) reported that water stress treatments increased essential oil percentage, main constituents of essential oil, proline, and total carbohydrate content of *Ocimum sp.* According to a study on three species of ocimum (*O. basilicum* Genovese, *O.* x *africanum*, and *O. americanu),* the yield of essential oils grew dramatically in one species during a drought, whereas it continuously declined in two other species (Mulugeta & Radácsi, 2022). Similar to the previous experiment, another study involving six different Lamiaceae species (*Lavandula latifolia*, *Mentha piperita*, *Salvia sclarea*, *Salvia lavandulifolia*, *Thymus capitatus*, and *Thymus mastichina*) found that only *S. sclarea* plants exhibited a decrease in essential oil content under drought conditions, while the other species under investigation showed no changes in essential oil content. This illustrates that the effect of moisture on the essential oil content is species-dependent. In addition to species, plant density appears to be a significant factor in influencing the oil content along with irrigation frequency (Tarighi *et al.*, 2012). Thus, we recommend assessing the impact of these variables such as plant density, species variability, and other highly linked environmental factors on oil cell density or oil production of *A. calcarata* together with the drought stress. Furthermore, from a pharmacological perspective, it is imperative to investigate how the quality of essential oils varied under different drought stresses to get a high-quality output from this plant.

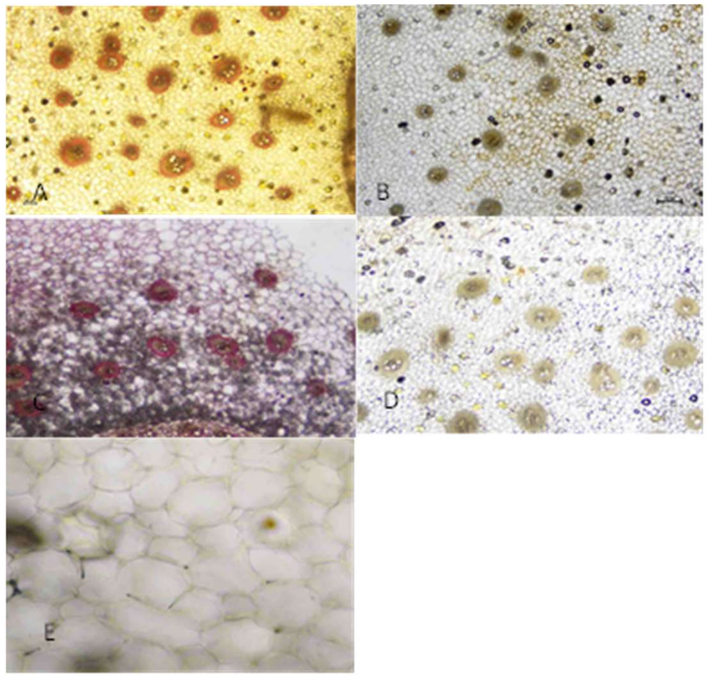

Figure 5. Difference in oil cell distribution in the rhizome of *A. calcarata* at different irrigation frequencies (stained with safranine) (10x10) (A: Daily watering, B: 7-day watering, C:14-day interval watering, D: 21-Day interval watering, E: 28-Day interval watering)

Conclusion

In the present experiment, the growth and yield parameters and anatomical parameters of *A. calcarata* ewre evaluated under five different drought stress treatments. The experimental findings revealed that, among the investigated irrigation frequencies, the 14-day interval irrigation frequency significantly ($p<0.05$) increased the growth and yield parameters (shoot height (cm), number of roots, number of shoots, number of leaves per shoot, leaf area (cm^2), rhizome dry weight (g)) of *A. calcarata*. It was also observed that the mean root epidermis and rhizome thickness of *A. calcarata* gradually increased in response to escalating drought stress. In contrast, the highest oil cell density was observed under the lowest drought stress. Hence, the general conclusion suggests that a 14-day irrigation frequency is the most appropriate for optimal growth and yield performance, while daily watering resulted in the highest oil cell density, leading to the greatest accumulation of secondary compounds in *A. calcarata.*

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