Palynological evaluation of endemic species, *Corynandra elegans* Chandore *et al*. from konkan region of Maharashtra, India.

[1]Shweta S. Jadhav (SRF)

[1]Vikram P. Masal (Associate Professor)

[1]Dapoli Urban Bank Senior Science College, P.G. Research Center, Department of Botany, Dapoli 415712, Maharashtra, India.

Corresponding Author: masalvikram@gmail.com

Abstract:

Corynandra elegans Chandore *et al.* (Cleomaceae) is a perennial, endemic species of the South Konkan region of Maharashtra, India, within the Western Ghats biodiversity hotspot. *C. elegans* is geographically restricted species and its limited distribution makes pollen studies essential for understanding reproductive biology and informing conservation efforts. In this study, the plant collected from study area used as research material. The flowering process was documented through field observations and from plants cultivated in pots at the research site. The morphology of pollen observed and examined with a compound microscope. The study utilized in vitro germination and three staining methods to characterize its germination rate. The results showed that despite being a short floral longevity, *C. elegans* flowers are characterized by a high number of stamens and abundant pollen production. Pollen grains of *C. elegans* are medium sized, oval shaped, with three germinating pores and has monad dispersion. The Modified Alexander staining method could most suitable to determine pollen viability of *C. elegans*. The highest pollen germination rate was 64.98% ± 3.79% at 24 hrs incubation at room temperature and the length of pollen tube was 0.14 mm after incubation for 4 hrs at room temperature after cultured in 20% of sucrose. In conclusion, the analysis of *C. elegans* pollen morphology and vitality offers a theoretical foundation that can guide future breeding and conservation strategies.

Keywords: Alexander staining**,** Corynandra, Flower, Germination, Pollen etc.

1. Introduction:

Pollen is the essential male gametophyte in seed plants, carrying the genetic material necessary for sexual reproduction. Because its viability directly dictates the success of pollination, fertilization, and seed quality, it remains a primary focus of research ranging from structural studies to the molecular regulation of pollen tube growth. All of these investigations are essential for bridging practical plant cross-breeding techniques with underlying theoretical knowledge (Liu *et al*., 2023). *Corynandra elegans* Chandore *et al*. belongs to genus *Corynandra* in family cleomaceae, which is an endemic species with restricted distribution in Ratnagiri region of Maharashtra, India (Chandore *et al*., 2016). It is a perennial plant with a restricted growth in different environment and geographic range (Chandore *et al*., 2016). This necessitates the conservation of pollen resources. Previous work on *C. elegans* has concentrated on its morphological features, with only limited attention to reproductive aspects such as pollen morphology and flowering characteristics. This research-employed microscopy to study pollen morphology, tracked the flowering dynamics of flowers, optimized in-vitro germination protocols. The findings aim to inform reproductive biology and hybridization breeding of *C. elegans.*

2. Material and Methods:

2.1. Study site**:**

Experiments were conducted at Lanja, central facility lab and Dapoli, PG research lab from October to November of 2025. Our test plants were collected from its habitat and had grown and flowered in the plantation.

2.2. Observation of single flower flowering dynamic process

A study of the flowering dynamics of a flower of *C. elegans* was conducted in October to November 2025. Number of mature bud as well as fresh flowers were used for observations. We observed and photographed the flowers several times over the past three years on from its natural habitat and the pollen viability determined by collecting pollens from fresh flowers at study site.

2.3. Pollen morphology observation

2.3.1. Microscopic studies

Fresh flowers were collected and used for study by dusting pollens over the cavity slide containing glycerin. In compound microscope, 3 slides were prepared to study. The measurement of pollen characters was made from number of grains taken at random. Microphotographic work was carried out using Photomicroscope fixed in central facility lab at Lanja, Ratnagiri. The terminology used was in accordance with the studies by Halbritter (2018).

2.4. Study on Methods for Determining Pollen Viability

2.4.1 In Vitro Germination of *Corynandra elegans* Chandore *et al*.

The Effects of Different Medium Components on the In Vitro Germination of *C. elegans*

The effect of different medium components on *in vitro* germination of pollens were investigated using optimal concentrations of sucrose, boric acid (H_3BO_3), and calcium chloride ($CaCl_2$). Freshly collected flowers between 7:30 and 8:00 a.m. were used for the study. Cavity slides were used in triplicates for each medium. Pollens were dusted over the different media and incubated for 4 hours at room temperature, maintaining humidity. Germination was assessed microscopically after 4 hours, with five random fields per slide and three repeats per treatment. Pollen germination was defined as a tube length exceeding the pollen grain diameter. The following formula was used to calculate the in vitro germination rate of *C. elegans*: pollen germination rate (%) = number of germinated pollen grains/total number of pollen grains on slide × 100.

Table 1: Experimental Design for Media component optimization

Group	Media component type	Concentrations			
Control (CK)	Distilled water	100 ml			
Treatment 1	Sucrose (%)	5	10	15	20
Treatment 2	Boric acid (mg/L)	50	100	150	200
Treatment 3	Calcium chloride (mg/L)	50	100	150	200

The Effects of Different Medium Components on the In Vitro pollen tube growth of *C. elegans*

Pollen tube growth was investigated using the same method as in vitro germination. Further, the pollen tube length was measured in micrometers using a calibrated ocular micrometer at 400X magnification.

Effect of Incubation Time on the In Vitro Germination Rate

A study was conducted using selected medium concentration to determine the germination rate and pollen tube length after different incubation times (2 h, 4 h, 8 h, 12 h, 24 h) at room temperature.

2.4.2. Pollen Viability of *C. elegans* by Staining Methods

The pollen viability was assessed through three distinct histochemical assays: TTC (2.3.5-Triphenyl Tetrazolium Chloride) staining method, Modified Alexander staining method and KI-I_2 staining method (Rathod *et al*., 2018).

Observation and Results:

3. Study on methods for determining pollen viability

3.1. Flowering dynamics observation of flower of *C. elegans* Chandore *et al.*

Flowers of *C. elegans* are arranged in recemes, typically bearing about 15- 20 flowering buds that undergo anthesis in an upward progression from the base. Individual flowers exhibit a short flowering period, completing anthesis within a few hours. Development of bud and the period of anthesis, depends on the photoperiod and temperature.

A three-stage dynamic process of the opening of single flower was artificially categorized: Mature bud stage (stage I), the pre-anthesis phase is marked by a shift in floral pigmentation. A day before, corollas in matured bud eventually starts changing the colour from greenish with some pink to its characteristic pink colour (Figure 1c). Before opening of a flowers corollas slightly dilate, indicating the flowers were about to open. Stage II, the flowers open during the morning hours, reaching their diameter at its maximum and all anthers disperses with a visible coting of yellow pollens at the apex (Figure 1d). Stage III, a drop in afternoon sunlight causes rapid corolla wilting and depending on the wind intensity, corolla falls off on the same day or within 2- 3 days. Anthers are also affected by winds and eventually they fell off leaving stigma behind. In brief, the floral longevity of *C. elegans* flowers is very short that is about few hours to days.

Figure 1. Flowers at different stages: (a) Flower inflorescence, (b) Early bud stage, (c) Mature bud stage, (e) End dispersed pollen stage.

3.2. Pollen grain morphological characteristics

The results showed that the shape of pollen is oval with monad as a dispersal unit and with three pollen apertures. Pollen is medium sized with 20 µm wide and 40-45 in length. Size of hydrated pollen is about 35-

38 µm. According to the observations about polar axis, 28-30 µm is the shortest in equatorial view; 35-38 µm is longest in equatorial view whereas 26-28 µm is shortest polar view and 30-32 µm is longest polar view.

3.3. Studies on the method of pollen viability

3.3.1. In vitro germination method to determine the pollen viability

The Effects of Different Medium Components on the In Vitro Germination of *C. elegans*

The addition of sucrose, H_3BO_3 and $Cacl_2$ in the liquid medium notably influenced germination rates in vitro (Figure). Studies show 20% sucrose is optimal for promoting pollen germination. Pollen germination rates increased significantly as sucrose concentrations were increase from 5%, 10%, 15% to 20%. However, further increases in sucrose concentration to 25% or higher (30%) resulted in decreased germination rates (unpublished data). The pollen germination rate in H_3BO_3 differs with the increased concentrations. While 100mg/L of H_3BO_3 effectively promoted pollen germination, both lower (50mg/L) and higher (200mg/L) concentrations failed to support growth, indicating variable range of efficacy for boric acid. Following the optimal germination at 100mg/L H_3BO_3, the medium with 50mg/L $Cacl_2$ showed decent pollen germination rate. In summary, the most effective concentrations for pollen growth in *C. elegans* were 20% sucrose (yielding a 58.62% germination rate), 100mg/L H_3BO_3 (44.81%) and 50mg/L $Cacl_2$ (15.74%). Each of these treatments performed significantly better than the control group (CK), which showed no germination.

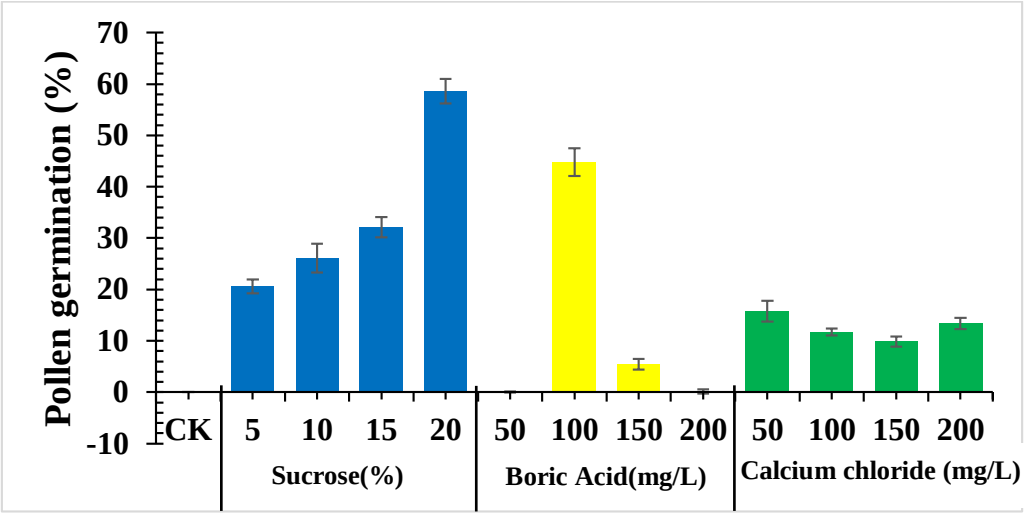

Figure 2(a): Effect of different concentration of Sucrose, H_3BO_3, $Cacl_2$ on in vitro germination of *C. elegans*

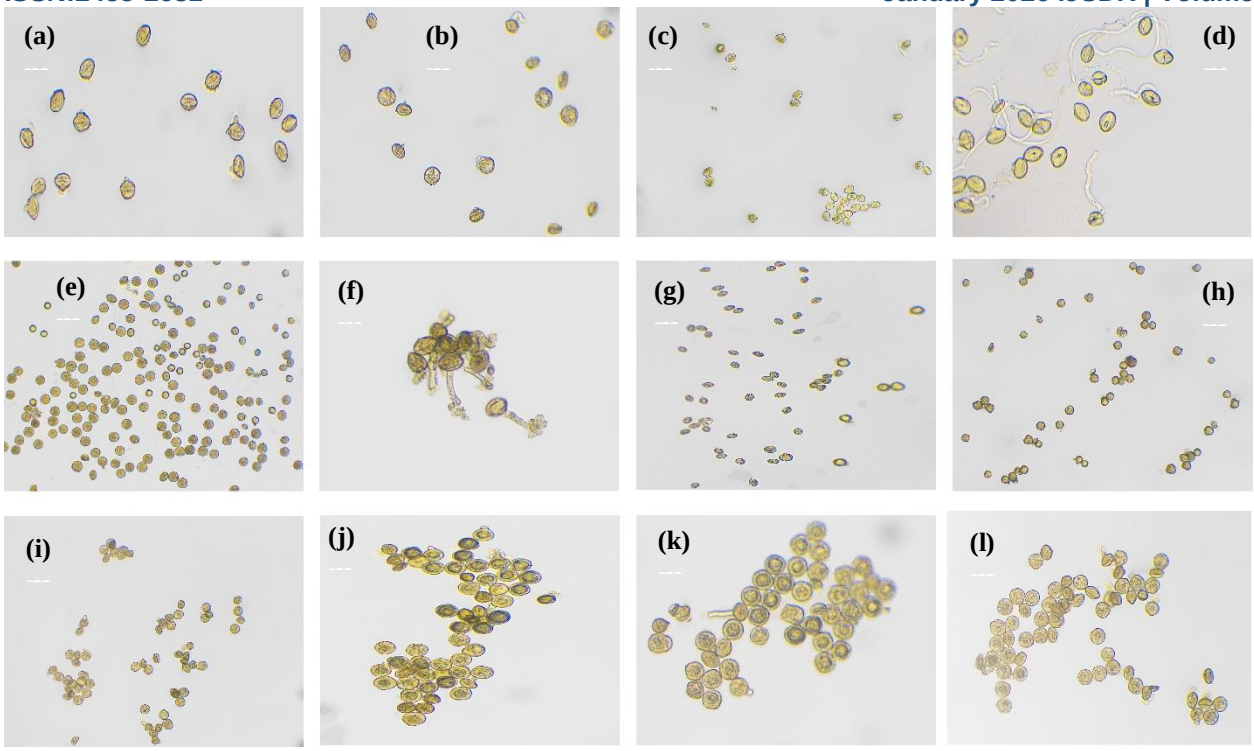

Figure 2(b): The Effects of Different Medium Components on the In Vitro Germination of *C. elegans* : (a)5%,Sucrose, (b)10% Sucrose, (c)15% Sucrose, (d)20% Sucrose, (e)50mg/L H_3BO_3, (f)100mg/L H_3BO_3, (g)150mg/L H_3BO_3, (h)200mg/L H_3BO_3, (i)50mg/L $Cacl_2$, (j)100mg/L $Cacl_2$, (k)150mg/L $Cacl_2$, (l)50mg/L $Cacl_2$

The Effects of Different Medium Components on the In Vitro pollen tube growth of *C. elegans*

In vitro pollen tube growth was significantly impacted by presence of sucrose, H_3BO_3 and $Cacl_2$ in the liquid medium. Research indicates that a 20% sucrose concentration is most effective for enhancing pollen tube growth. Pollen germination rates increased significantly as sucrose concentrations were increase from 5%, 10%, 15% to 20%. However, further increases in sucrose concentration to 25% or higher (30%) resulted in decreased germination rates (unpublished data). The pollen germination rate in H_3BO_3 differs with the increased concentrations. While 100mg/L of H_3BO_3 effectively promoted pollen germination, both lower (50mg/L) and higher (200mg/L) concentrations failed to support growth, indicating variable range of efficacy for boric acid. Following the optimal germination at 100mg/L H_3BO_3, the medium with 50mg/L $Cacl_2$ showed decent pollen germination rate. In summary, the most effective concentrations for pollen growth in *C. elegans* were 20% sucrose (yielding a 58.62% germination rate), 100mg/L (44.81%) and 50mg/L (15.74%). Each of these treatments performed significantly better than the control group (CK), which showed no germination.

The Effects of Different Medium Components on the In Vitro pollen tube growth of *C. elegans*

Following the optimal germination rate at 20% sucrose medium, pollen tube growth showed highest pollen tube length, average about 0.14 mm after incubation for 4 hrs at room temperature.

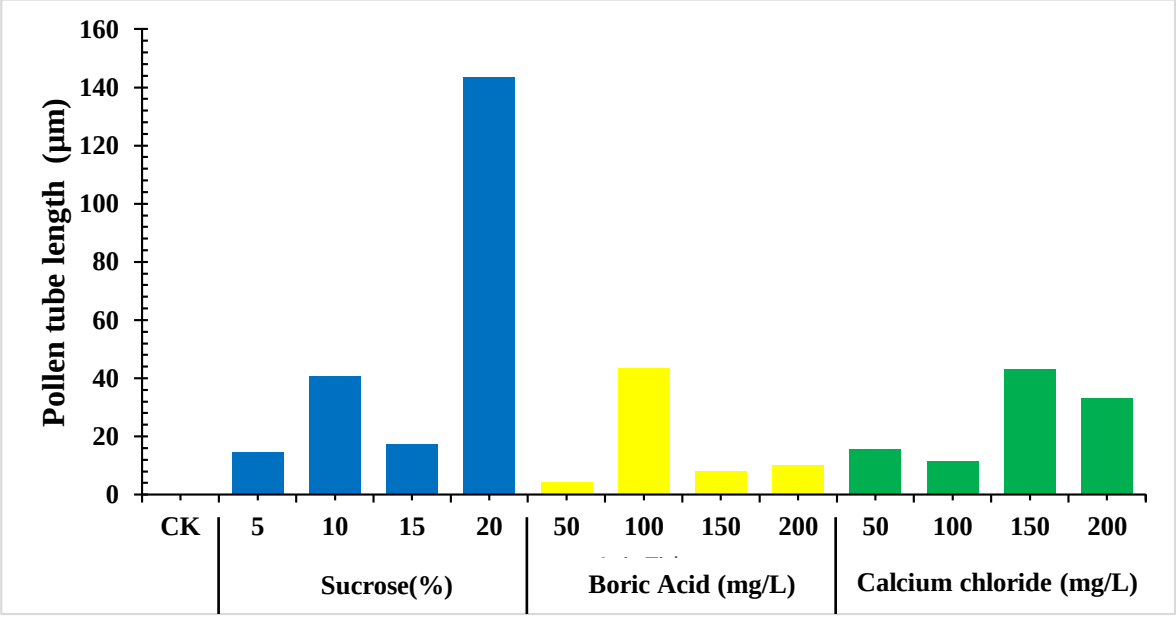

Figure 2(a): Effect of different concentration of Sucrose, H_3BO_3, $Cacl_2$ on in vitro pollen tube growth of *C. elegans*

Effect of Incubation Time on the In Vitro Germination Rate

A significant increase in pollen germination rate and pollen tube length was observed with prolonged incubation (Figure 2(a) & 2(b)) when pollen grains were cultured in the liquid medium 20% sucrose at room temperature. As a result, the highest germination was 64.98% ± 3.79% and the pollen tube reached at 0.069 ± 0.005mm in 24 hrs. A positive correlation was observed between incubation duration and the germination frequency of *C. elegans* pollen.

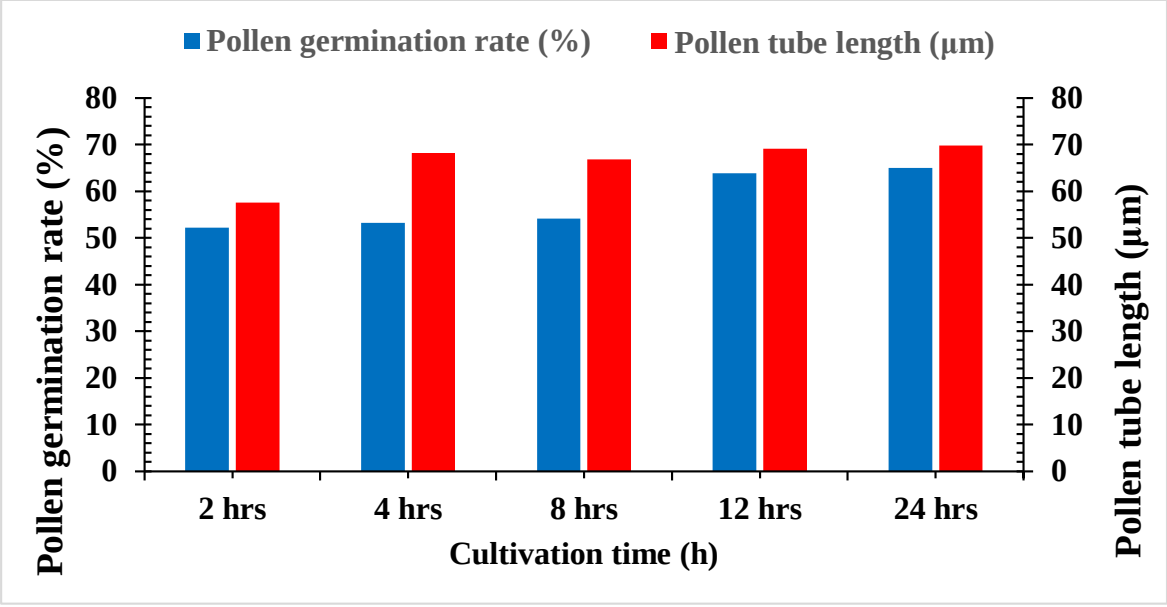

Figure 3(a): Effect of incubation time on in vitro pollen germination and tube growth

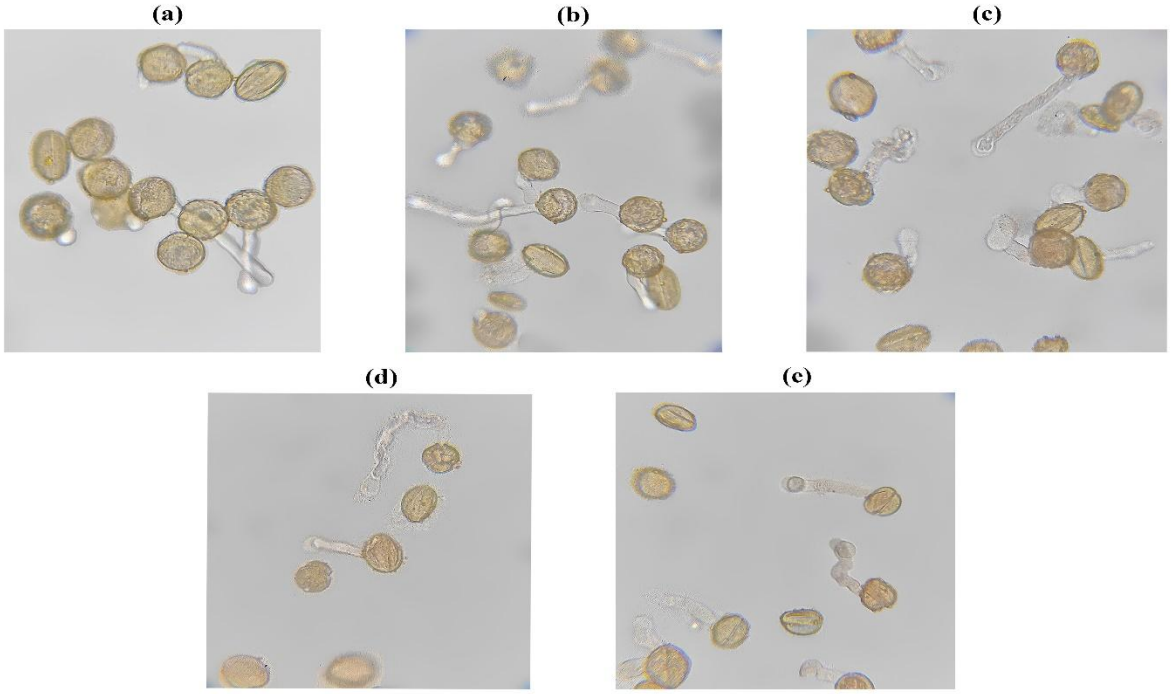

Figure 3(b): Effect of incubation time on in vitro pollen germination and tube growth at different time of incubation (a) 2 h, (b) 4 h, (c) 8 h, (d) 12 h, (c) 24 h.

3.3.2. Pollen Viability of *C. elegance* Measured by Three Different Staining Methods

To assess the pollen viability of *C. elegans*, three staining techniques were evaluated, each producing varying results. A comparative analysis determined that TTC (triphenyl tetrazolium chloride) was unreliable, as pollen grains frequently germinated despite remaining unstained.

In contrast, both KI-I2 and the modified Alexander's staining method effectively distinguished between viable and non-viable grains. Ultimately, the modified Alexander's method was identified as the most precise and dependable technique for measuring pollen viability in this species.

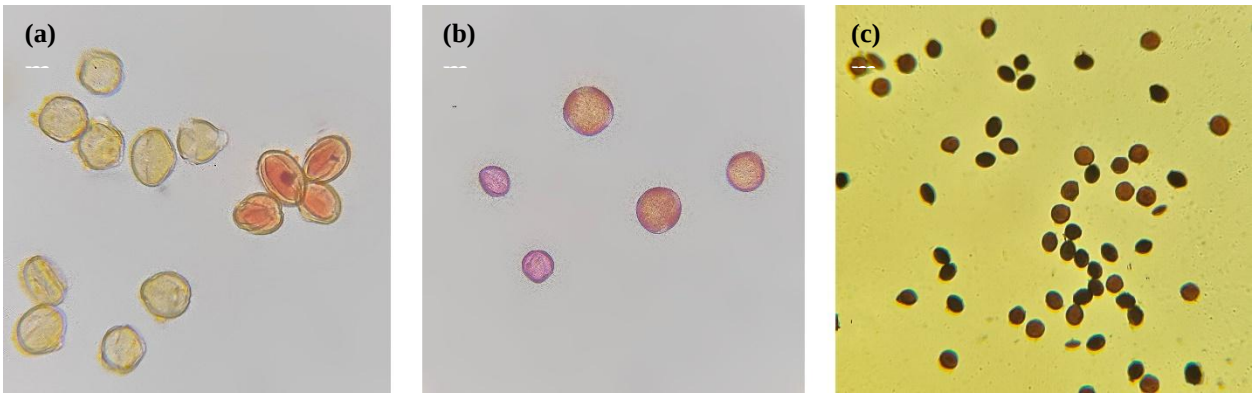

Figure 4: Pollen grain staining: (a)TTC staining method (b)Modified Alexander staining method (c) KI-I2 Staining method

4. Discussion

4.1. Pollen morphological characteristics of *C. elegans* Chandore *et al.*

Palynological data probably helpful at every levels of systematics, especially in angiosperms (Liu *et al*., 2023). The morphological of pollen are often characteristic and represents family, families, or genus. The characteristics may or may not be similar among species and pollen can vary among others in size, aperture, and in exine stratification. The study of pollen should encompass all structural and ornamental aspects like the size of grains according to the appearance and shape of pollen, pollen outer wall ornamentation and number and location of germination pores (Halbritter *et al*., 2018). Microscopic analysis of pollen morphology revealed that *C. elegans* produces oval-shaped, monad pollen grains that are dispersed as single units. The pollen of the species is classified as medium-sized, with a width of 20–25 µm and a length of 40–45 µm; hydrated grains measure approximately 35–38 µm in diameter.

Observations of the microscopic morphology of pollen revealed that pollen grains of *C. elegans* were oval in shape, monad pollen unit, monad dispersal unit and peculiarities. The pollen size is medium with width and length ranges from 20- 25(µm) and 40- 45(µm) respectively. Size of hydrated pollen is about 35- 38(µm). Three apertures have been observed and found to be positively correlated with pollen germination. In brief, the study of pollen morphology can provide diagnostic data towards systematic classification of this species.

4.2. Methods for the Determination of Pollen Viability

In vitro germination assays are regarded as a reliable method for accurately predicting pollen viability. Due to the lack of specificity for this plant species, the most important step of in vitro germination was selecting the appropriate medium for pollen germination angiosperms (Liu *et al*., 2023). Pollen germination was significantly enhanced by the supplementation of a basal medium with 15% sucrose, 100 mg/L boric acid, and 20 mg/L calcium chloride. Sugars like sucrose provide energy for pollen tube growth and the stigmatic surface exudes essential nutrients, such as boron and calcium that are absent in pollen and are required for germination. Essential micronutrients, specifically calcium (Ca) and boron (B), play a fundamental role in regulating pollen development and germination, pollen tube growth, sugar synthesis, and accumulation. To assess pollen viability, the staining method is widely regarded as the simplest and fastest approach. The pollen viability of *C. elegans* was assessed using three staining methods (Rathod *et al*., 2018); and it yielded differential results with different methods. Comparative analysis revealed TTC's pollen viability was not reliable because germination in unstained pollen was clearly visible. Modified Alexander's staining method and KI-I2 showed differentiation in non-viable and viable pollen grains. Modified Alexander's staining method was found as a most suitable method for accurate determination of pollen viability of *C. elegans*.

5. Conclusion

The study results showed that the flowers of *Corynandra elegans* Chandore *et al.* lasted for short duration and individual flower bears high number of stamens each covered with countless pollens. The temporal pattern of anthesis and senescence depends on photoperiod, temperature and environment. Pollen grains are morphologically oval-shaped, monad in dispersion and with tree apertures. The germination rate *for C. elegans* reached its maximum value (64.98% ± 3.79%) at 24 hrs incubation at room temperature and the length of pollen tube was 0.14 mm after incubation for 4 hrs at room temperature based on the selected liquid medium that 20% sucrose. The Modified Alexander staining method proved to be the most reliable approach for

distinguishing between viable and non-viable pollen grains, outperforming the three alternative staining protocols tested.

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