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Study of pollen germination in selected plant species

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Abstract

In flowering plants, pollen germination is an essential step in the reproductive cycle that has a direct impact on fertilization and seed development. In this work, the effects of different sucrose concentrations and culture conditions are examined in relation to the *in-vitro* germination viability of pollen from specific plant species. A variety of media, including Brewbaker's and Robert's media, sucrose solutions (2%, 4%, 6% and 8%) and natural nectars from Ixora and Periwinkle, were used to analyze pollen grains from ten plant species. For each species, the ideal circumstances were identified by monitoring the pollen tube development and germination rate at various intervals. The study improves knowledge of the reproductive biology of the plants and offers guidance for conservation and breeding initiatives. Subsequent investigations need to examine the genetic and environmental elements that impact pollen viability in a variety of plant species.

Keywords: Pollen germination; Pollen viability; Sucrose concentration; Germination media; Nectar; Pollen tube growth

1. Introduction

Pollen is the microgametophyte of seed plants that develops from the microspore (Punt *et al*., 2007.). For male gametes, the pollen serves as both a source and a means of transport. The male gametophytic generation is represented by the multicellular pollen grain, while the unicellular pollen grain is the microspore of seed plants (Halbritter *et al*., 2018.). It grows inside the anther, also known as pollen sac, where the tapetum- a transient, sporophytic, apoptotic tissue that acts as a bridge between the gametophytic generation and the mother plant tissues- nourishes the grains. Each grain is made up of several cells, which include exine that has been accumulated with the help of the sporophyte, intine that is of gametophytic origin and common walls (Pacini and Franchi., 2020.).

These include symmetric and asymmetric spores. Round, elliptical, triangular, rectangular, quadrangular and other geometric shapes are possible for the pollen. Based on the ratio of polar axis to equatorial diameter, the pollen grains were divided into eight form groups (prolate, spheroidal, sub-prolate, perprolate, oblate, oblate-spheroidal, sub-oblate and peroblate) (Erdtman., 1952.). The pollen grains can be classified based on the apertures i.e. Monocolpate, Tricolpate, Porate, Stephano Colpate (Wang and Dobritsa., 2018.). It can be classified based on the symmetry i.e. Radiosymmetric, Zonocolpate, Heteropolar (Viertel and Konig., 2022). It can be also classified based on the surface ornamentation i.e. Reticulate, Echinate, Psilate, Verrucate (Williams and Mazer., 2016.).

The anther is the site of pollen formation (Bedinger., 1992.). The structure of the pollen wall is a complicated composite. Sporopollenin, a stiff, water-impermeable, chemically resistant biomaterial, is present in the exine, the outermost layer. The cellulose intine attaches firmly to the exine and forms a compliant, water permeable layer just inside (Katifori *et al*., 2010.). The central cytoplasmic region is the source of the fertilization nuclei. The pollen tube is started by the intine

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emerging from one of the pores on pollen grains where the exine is thinner or absent. The process of pollen tube formation entails fast actin cytoskeleton remodeling as well as significant endocytosis and exocytosis (McCormick., 2013.).

The transfer of pollen from its production location to the female landing point is known as pollination. It is followed by fertilization and seed development if it is effective (Pacini., 2008.). There are two kinds of pollination: self-pollination and cross-pollination. Pollen grains are moved from the anther of one plant to the stigma of another plant is called cross-pollination. On the other hand, self-pollination occurs when pollen grains proceed from an anther to the stigma of the same plant or flower (Mangena and Mokwala., 2018.). There are two types of pollinating agents: abiotic pollinating agents and biotic pollinating agents. Abiotic pollinating agents are non-living agents like wind (Anemophily), water (Hydrophily) (Faegri and Van der Pijl., 1979.). Biotic pollinating agents are living agents like insects (Entomophily), birds (Ornithophily), bats (Chiropterophily), etc (Proctor et al., 1996.).

The process by which a pollen grain hydrates and starts to develop a pollen tube after landing on a suitable stigma is known as pollen germination. This tube helps carry sperm cells for fertilization by extending through the style and toward the ovule (Taylor and Helper., 1997.). In a basic, sucrose and agar media, increasing the concentrations of boric acid, gibberellic acid, and IAA (0.5-1.0 ppm) enhanced pollen germination and tube growth without having any negative effects (Patel and Mankad., 2014.). The following, otherwise fatal., high temperature and quick tube elongation produced the best pollen germination plants at temperatures between 28°C and 31°C under 80%. Thermotolerance is a complicated relative humidity acquisition. According to physiological phenomena, pollen germination was reduced at temperature over 37°C, and tube elongation was reduced at temperature above 32°C that involved at least some heat shock proteins (Burke *et al*., 2004.).

The ability of pollen grains to survive, develop, germinate or flourish is known as pollen viability, and it is crucial for seed plants to successfully reproduce sexually. With the help of a suitable stigma, viable pollen can successfully germinate, create a pollen tube and transfer sperm cells to ovule, resulting in fertilization (Dafni and Firmage., 2000.). On a nutrient medium, pollen is cultivated in order to track pollen tube growth and germination rates. The type of sugars, boric acid, calcium chloride, pH, and other medium composition elements can all have a big impact on germination success. A raffinose-based medium that facilitated high in vitro germination rates was discovered, for example, in a study on wheat pollen (Impe *et al*., 2020.).

2. Material and methods

The practical work for the dissertation was completed at Gujarat University's Ahmedabad General Laboratory of Botany.

2.1. Plant Material

Table 1 List of selected plants

Sr. No.	Botanical name of plants	Family	Common Name
1.	*Adhatoda vasica* Nees	Acanthaceae	Ardusi, Malabar nut
2.	*Bauhinia purpurea* L.	Fabaceae	Orchid tree, butterfly tree, Kanchnar
3.	*Calliandra haematocephala* Hassk.	Mimosaceae	Powder puff
4.	*Cascabela thevetia* (L.) Lippod.	Apocynaceae	Pili karen, yellow oleander
5.	*Hymenocallis caribaea* (L.) Herb.	Amaryllidaceae	Garden lily
6.	*Jatropha integerrima* Jacq.	Euphorbiaceae	Peregrina, spicy jatropha
7.	*Senna occidentalis* (L.) Link.	Caesalpiniaceae	Sundaro, kasundaro,coffee weed
8.	*Tabernaemontana divaricata* (L.) R.Br. ex Roem. & Schult.	Apocynaceae	Chandni, Crape jasmine, Tagar
9.	*Tecoma stans* (L.) Juss. ex Kunth.	Bignoniaceae	Yellow bells, yellow elder
10.	*Tridax procumbens* L.	Asteraceae	Pardesi bhangaro, tridax daisy

2.2. Requirements

The practical work was completed in a conventional laboratory using a variety of tools, chemicals, glassware, and other supplies.

2.3. Chemicals

- Sucrose (C6H12O6)
- Boric acid (H3BO3)
- Triphenyl tetrazolium chloride
- Calcium nitrate [Ca(NO3)2]
- Magnesium sulphate (MgSO4)
- Potassium nitrate (KNO3)

2.4. Glass-wares

- Watch glass
- Glass road
- Beaker
- Slide
- Dropper
- Capillary
- Measuring cylinder
- Measuring flask
- Petri plates
- Test tubes

2.5. Sample Collection

The fresh flowers were collected from Gujarat University's campus. Pollen is the first prerequisite for experimentation. Early in the morning, the flowers of a couple of selected plants were gathered. Because pollen germination is stronger during the developmental stages, pollen grains gathered from freshly dehisced anther. Using a needle, remove the pollen grains from the blooms, then gather the majority of the grains in a watch glass-containing medium. Typically, pollen is used shortly after flower dehiscence to maximize germination.

2.6. Methods

The hanging drop and the seated drop are the two most commonly used pollen germination techniques for small-scale experiments. An insignificant amount of pollen grain and culture media were used in these two techniques. These techniques were inapplicable when comparing how certain chemicals affected tube growth and pollen germination.

In this *in-vitro* experiment, a significant number of pollen grains were cultivated in media using suspension culture method. 2-10 ml of culture media were used each time for the experiment.

2.7. Pollen Viability Test

A viability test is required to verify the viability of pollen before the experiment starts. Iodine Potassium Iodide (IKI) and 2,3,5 Triphenyl tetrazolium Chloride (TTC) were used to assess pollen viability. The staining approach is the first technique. To prepare the IKI solution, 1 gm of KI and 0.5 gm of iodine were dissolved in 100 ml of distilled water. After five minutes, the viable pollen from the watch glass or slide was counted after the pollen was placed in the IKI solution. In the second method, 20 ml of distilled water were used to dissolve 0.2 gm of TTC and 12 gm of sucrose. Once the 1% TTC solution was ready, a drop was placed on the slide, pollen was mixed with the solution, and a cover slip was placed over the slide. The viable pollen is checked and counted under a microscope from the slide after a 2 hr incubation period.

2.8. Media Preparation

These techniques used a variety of mediums for the pollen germination experiments. This experiment uses a natural substance, such as nectar to promote pollen germination.

2.9. Sucrose Solution

Standardized the optimal sucrose concentration for in vitro plant germination, attempting to offer a broad range of concentrations. A solution of sucrose ranging from 2-10% was made and used for the practical germination process.

2.10. Preparation of sucrose solution

- To make a solution of 2% sucrose: 2 gm of sucrose is dissolved in a small amount of distilled water, then additional distilled water is added to reach a volume of 100 ml.
- Concurrently, distilled water was used to prepare a 2-10% sucrose solution.
- Several concentrations of sucrose solutions, containing 3-4 ml were kept in watch glass.
- A needle was used to directly sprinkle pollen grains into the watch glass.
- To maintain humidity, the watch glass is set on a petri dish with damp filter paper.
- The watch glass was examined under a microscope after an incubation period of 15 minutes.
- For 2 hr, further reading was done every 15 minutes.

2.11. Brewbaker's media

According to the species, pollen grains were cultivated with certain concentrations of sucrose, boric acid, magnesium sulphate, and calcium nitrate, which together created a dependable culture medium. For *in-vitro* pollen germination, Brewbaker and Kwack suggested a standard medium in 1963. As a result, the material used for this investigation is standardized.

Brewbaker and Kwack (1963) *in-vitro* germination media are composed of the following:

Table 2 List of Component Use in Media Preparation

Constituents	**Amount (mg/l)**
Sucrose (C6H12O6)	1,00,000
Boric acid (H3BO3)	100
Calcium nitrate (Ca (NO3)2)	300
Magnesium sulphate (MgSO4)	200
pH	7.3

- The media used by Brewbaker and Kwack (1963) were stored in 10 distinct 3-4 ml media containers with watch glass.
- Using a needle, pollen grains from specific plants were directly sprinkled into a watch glass.
- To maintain humidity, the watch glass is set on a petri dish with damp filter paper.
- The watch glass was examined under a microscope after an incubation period of 15 minutes.
- For 2 hr, further reading was done every 15 minutes.

2.12. Calculations

$$\% \text{ of germinated pollen grains } = \frac{\text{Number of germinated pollen grains } * \ 100}{\text{Total number of pollen grains}}$$

$$\% \text{ of pollen showing tube growth } = \frac{\text{Number of pollen showing tube growth } * \ 100}{\text{Total number of pollen grains}}$$

$$\% \text{ of bursting pollen grains } = \frac{\text{Number of bursted pollen grains } * \ 100}{\text{Total number of pollen grains}}$$

2.13. Robert's Media

Roberts and other researchers proposed a uniform medium for pollen germination *in-vitro* in 1983. The following is the makeup of Roberts *et al.*'s *in-vitro* germination media:

Table 3 List of Component Use in Media Preparation

Constituents	Amounts (mg/l)
Sucrose (C6H12O6)	1,00,000
Boric acid (H3BO3)	100
Calcium chloride (CaCl2)	362
Potassium nitrate (KNO3)	100
pH	8

- The media used by Roberts et al., 1983 were stored in 10 distinct 3-4 ml media containers with watch glass.
- Using a needle, pollen grains from specific plants were directly sprinkled into a watch glass.
- To maintain humidity, the watch glass is set on a petri dish with damp filter paper.
- The watch glass was examined under a microscope after an incubation period of 15 minutes.
- For 2 hr, further reading was done every 15 minutes.

2.14. Nectar

The nectar from *Ixora coccinea* L. and *Catharanthus roseus* (L.) G. Don flowers was collected and stored in separate watch glasses. Nectar was gathered with capillary assistance.

- Using a needle, pollen grains from specific plants were directly sprinkled into a watch glass.
- To maintain humidity, the watch glass is set on a petri dish with damp filter paper.
- The watch glass was examined under a microscope after an incubation period of 15 minutes.
- For 2 hr, further reading was done every 15 minutes.

3. Result and discussion

These *In-vitro* pollen germination experiments used a variety of media types, varied element concentrations, and attempted to identify the most effective media for pollen germination. The following tables show the effects of each component on germination and tube growth. Each component is crucial to the growth of the pollen tube and plays a significant role in many chemical reactions.

The outcomes of the experiments are as follows:

3.1. Pollen Germination and Bursting of selected plants in 2% sucrose solution

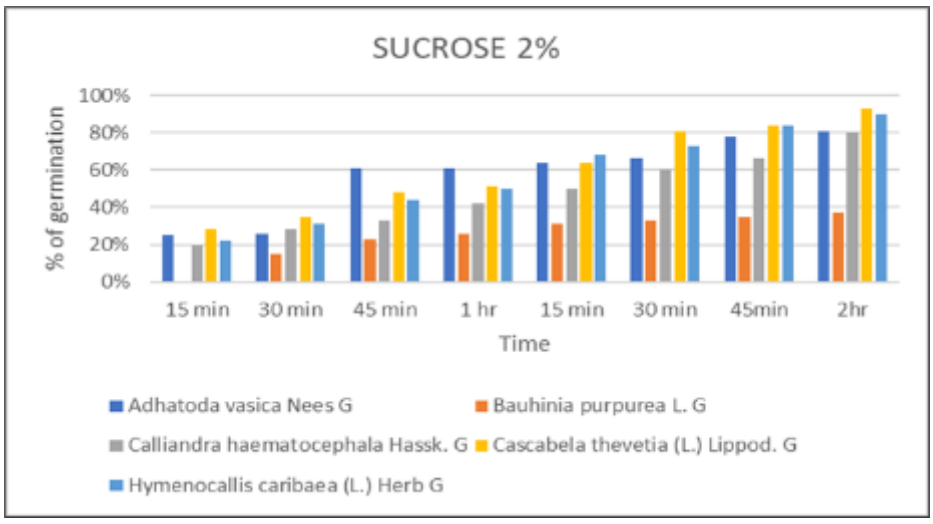

Figure 1 % of germination in 2% sucrose

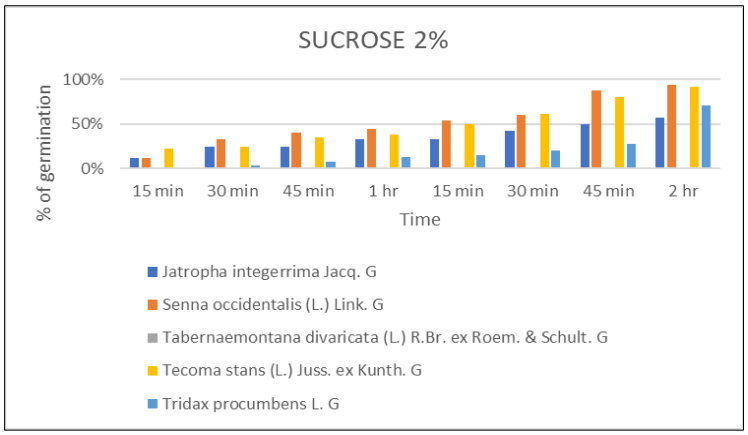

Figure 2 % of germination in 2% sucrose

Above graph reveals that the maximum germination (94%) in *Senna occidentalis* (L.) Link. and minimum (0%) in *Tabernaemontana divaricata* (L.) R.Br. ex Roem. & Schult.

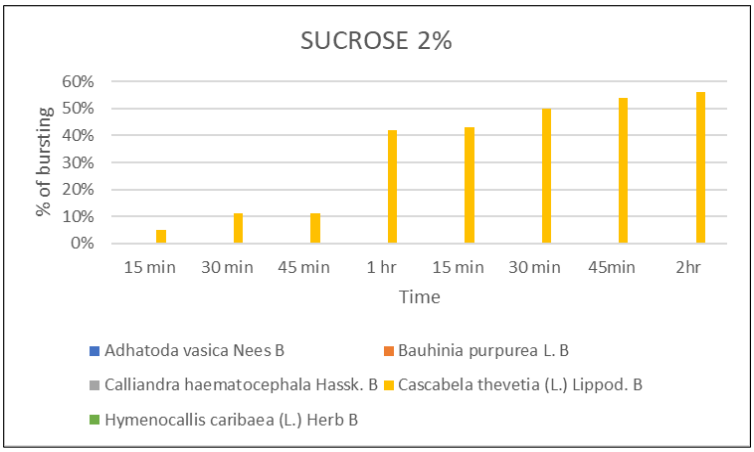

Figure 3 % of bursting in 2% sucrose

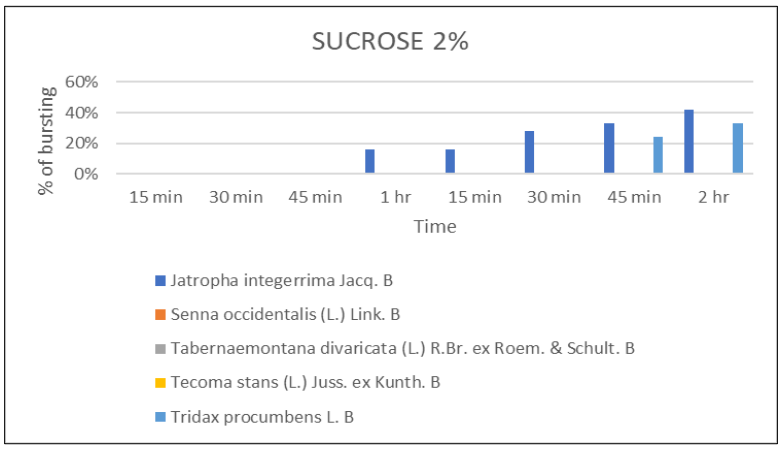

Figure 4 % of bursting in 2% sucrose

Above graph reveals the maximum bursting shown in *Cascabela thevetia* (L.) Lippod. (56%).

3.2. Pollen Germination and Bursting of selected plants in 4% sucrose solution:

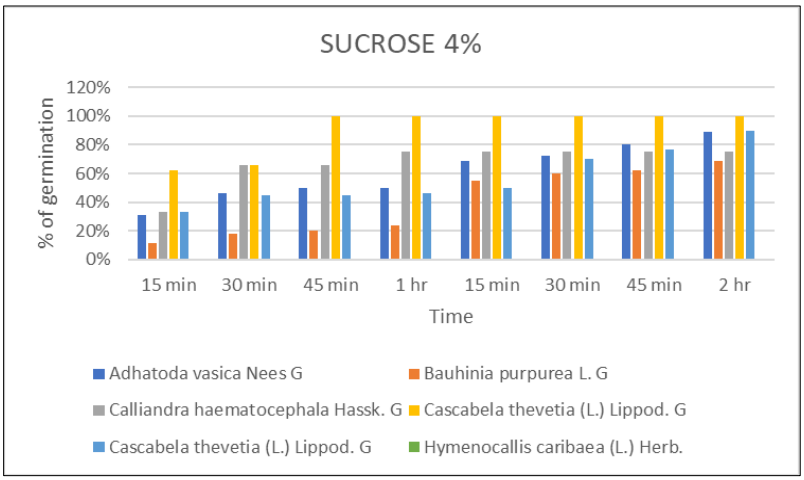

Figure 5 % of germination in 4% sucrose

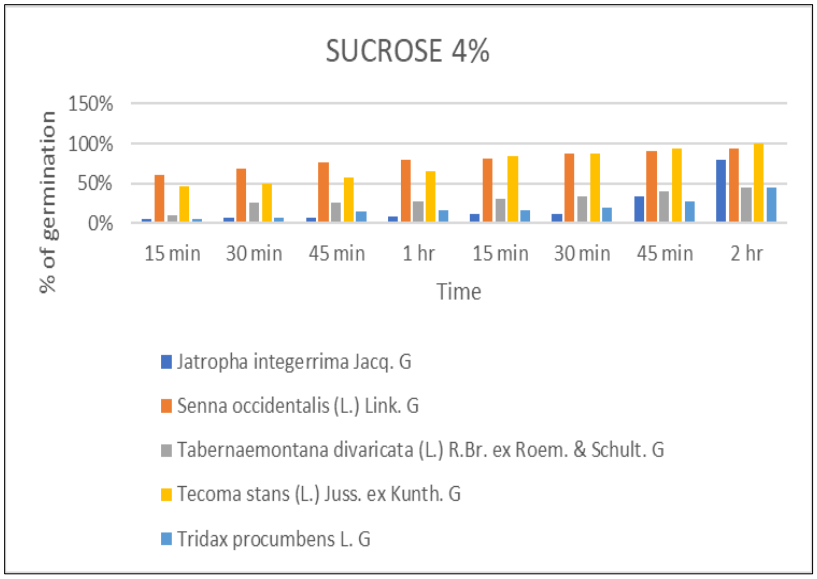

Figure 6 % of germination in 4% sucrose

Above graph reveals that the maximum germination (100%) in *Tecoma stans* (L.) Juss. ex Kunth. & *Cascabela thevetia* (L.) Lippod. and minimum (45%) in *Tabernaemontana divaricata* (L.) R.Br. ex Roem. & Schult. & *Tridax procumbens* L.

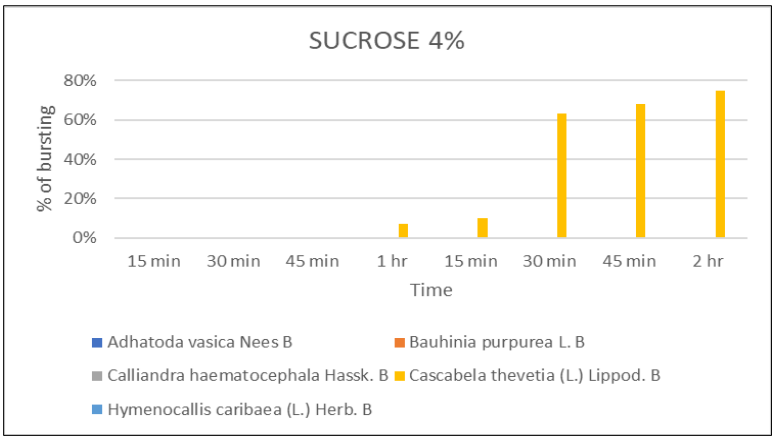

Figure 7 % of bursting in 4% sucrose

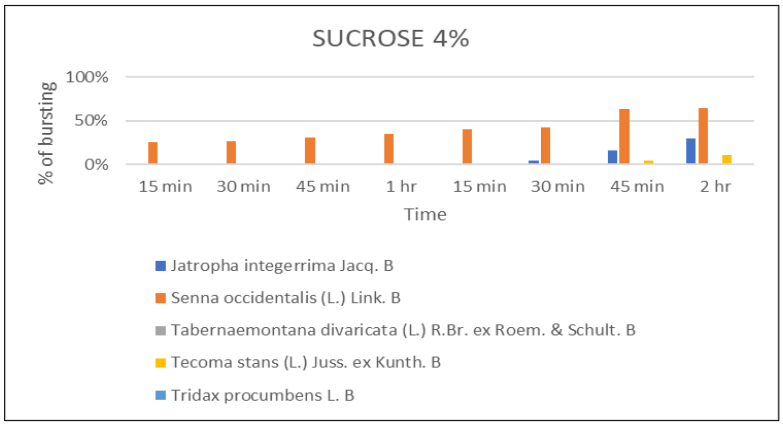

Figure 8 % of bursting in 4% sucrose

Above graph reveals the maximum bursting shown in *Cascabela thevetia* (L.) Lippod. (75%).

3.3. Pollen Germination and Bursting of selected plants in 6% sucrose solution

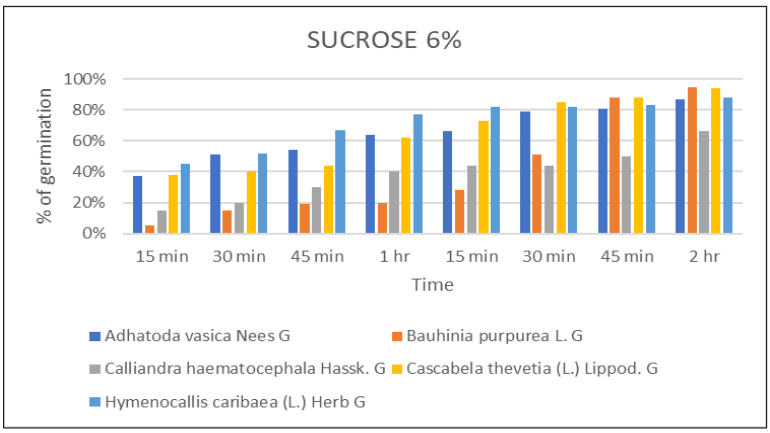

Figure 9 % of germination in 6% sucrose

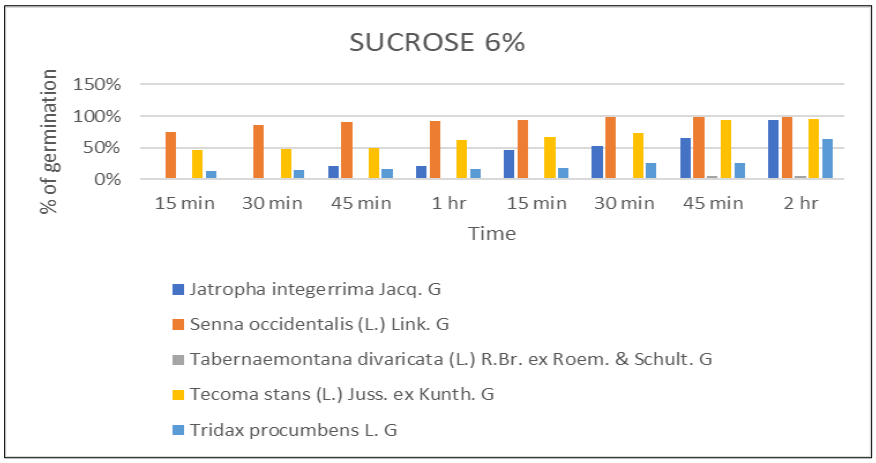

Figure 10 % of germination in 6% sucrose

Above graph reveals that the maximum germination (98%) in *Senna occidentalis* (L.) Link.and minimum (6%) in *Tabernaemontana divaricata* (L.) R.Br. ex Roem. & Schult. & *Tridax procumbens* L.

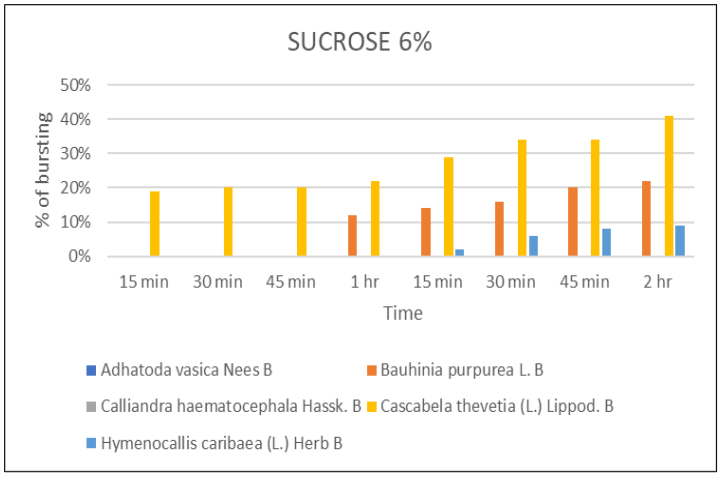

Figure 11 % of bursting in 6% sucrose

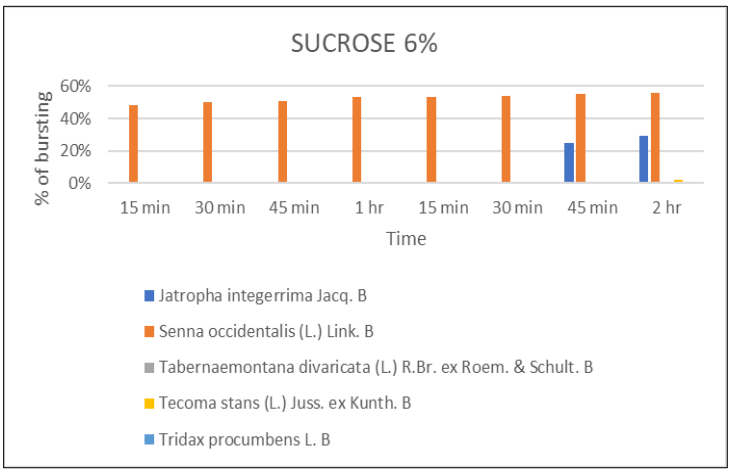

Figure 12 % of bursting in 6% sucrose

Above graph reveals that the maximum bursting shown in *Senna occidentalis* (L.) Link. (56%).

3.4. Pollen Germination and Bursting of selected plants in 8% sucrose solution

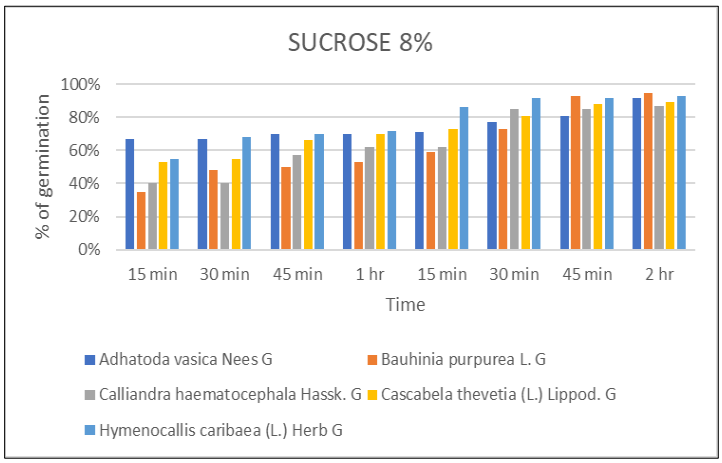

Figure 13 % of germination in 8% sucrose

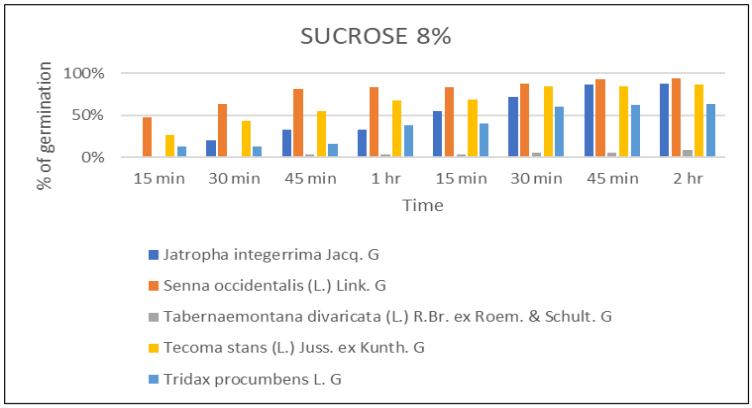

Figure 14 % of germination in 8% sucrose

Above graph reveals that the maximum germination (95%) in *Bauhinia purpurea* L. and minimum (9%) in *Tabernaemontana divaricata* (L.) R.Br. ex Roem. & Schult. & *Tridax procumbens* L.

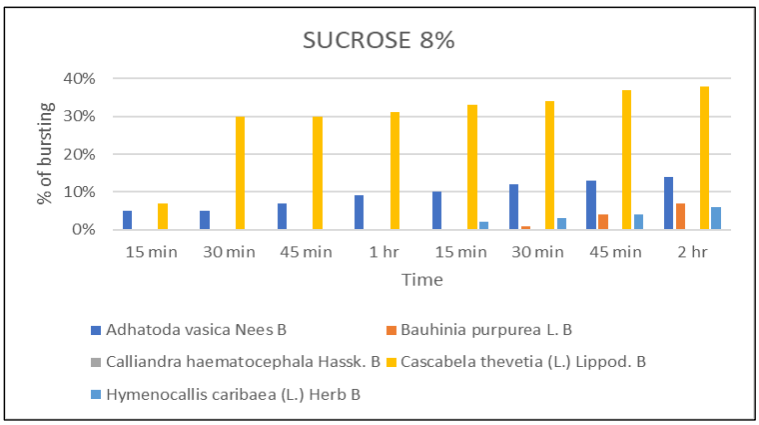

Figure 15 % of bursting in 8% sucrose

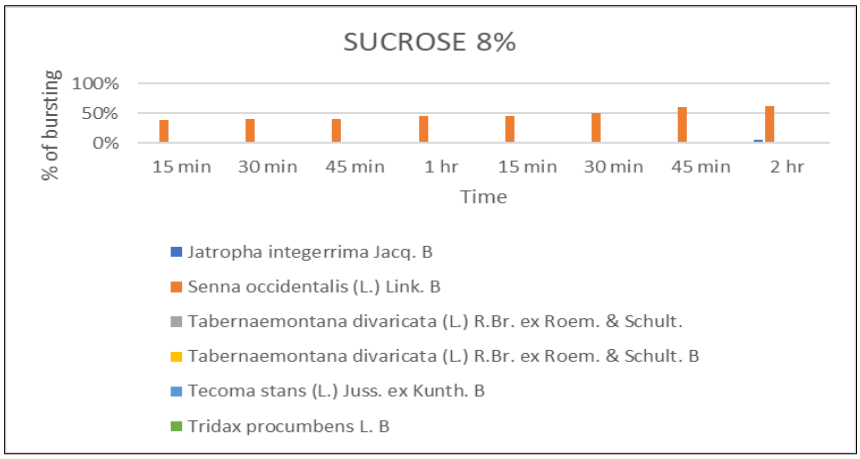

Figure 16 % of bursting in 8% sucrose

Above graph reveals that the maximum bursting shown in *Senna occidentalis* (L.) Link. (62%).

3.5. Pollen Germination and Bursting of selected plants in Brewbaker's media:

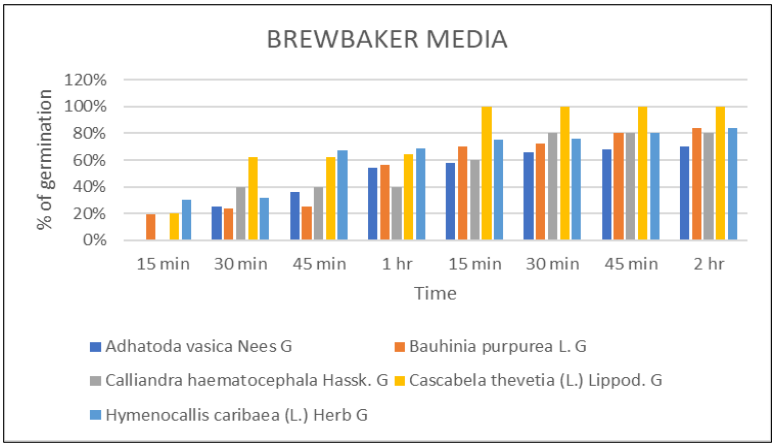

Figure 17 % of germination in Brewbaker's media

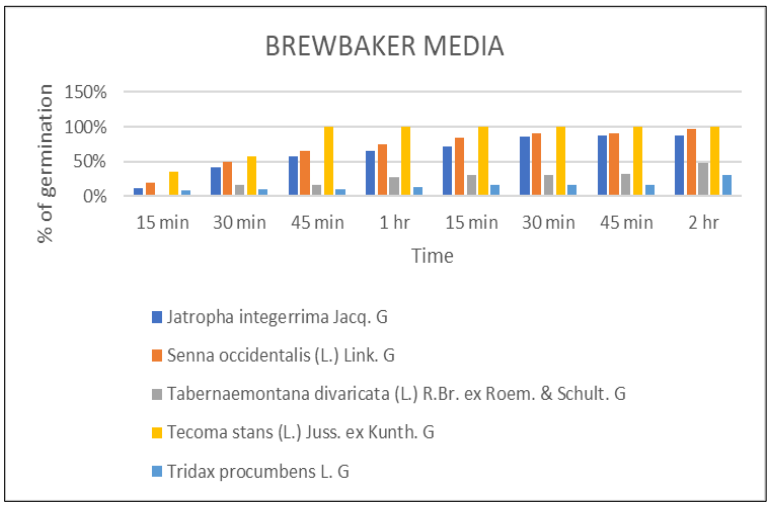

Figure 18 % of germination in Brewbaker's media

Above graph reveals the maximum germination (100%) in *Cascabela thevetia* (L.) Lippod. & *Tecoma stans* (L.) Juss. ex Kunth. and minimum (30%) in *Tridax procumbens* L.

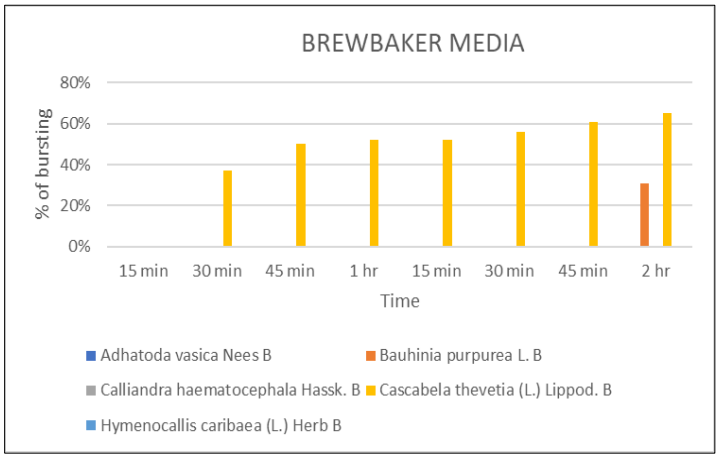

Figure 19 % of bursting in Brewbaker's media

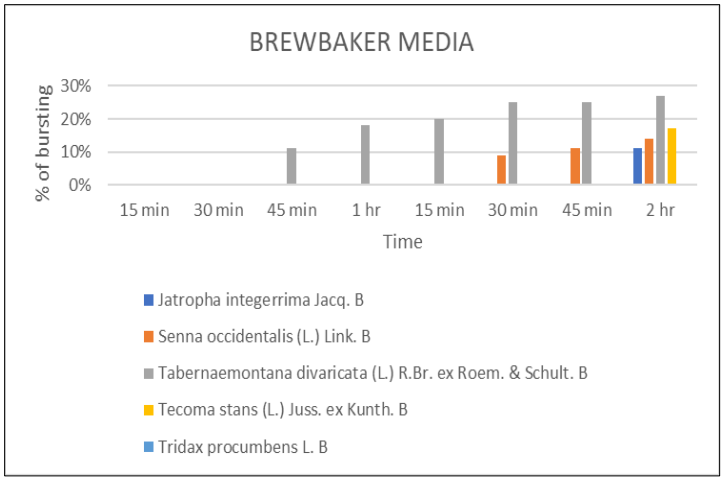

Figure 20 % of bursting in Brewbaker's media

Above graph reveals the maximum bursting shown in *Cascabela thevetia* (L.) Lippod. (65%).

3.6. Pollen Germination and Bursting of selected plants in Robert's media

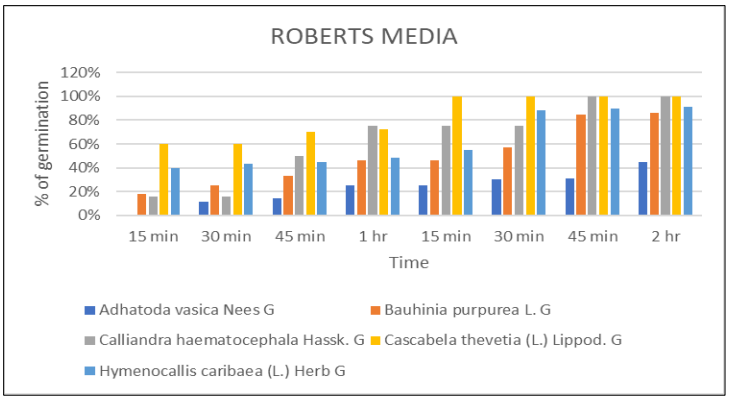

Figure 21 % of germination in Robert's media

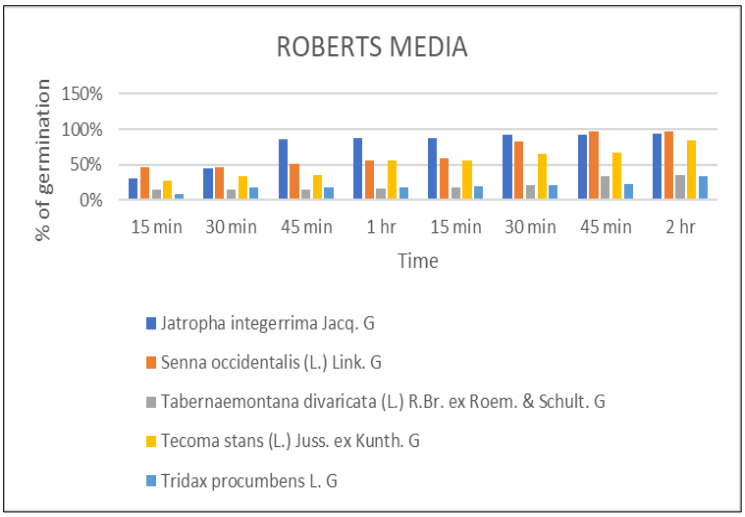

Figure 22 % of germination in Robert's media

Above graph reveals the maximum germination (100%) in *Cascabela thevetia* (L.) Lippod. & *Calliandra haematocephala* Hassk. and minimum (33%) in *Tridax procumbens* L.

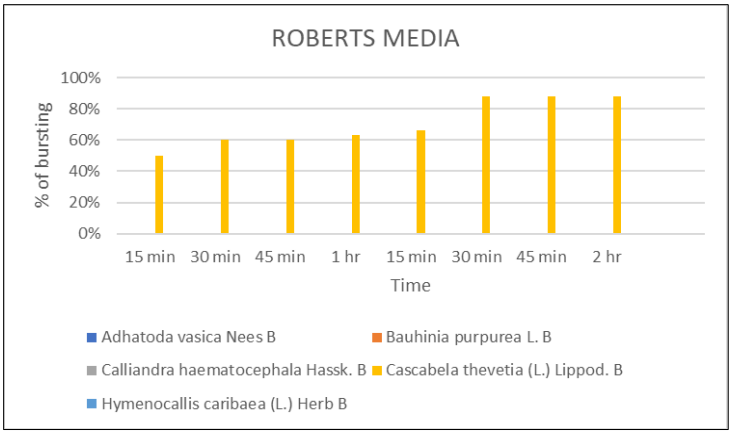

Figure 23 % of bursting in Robert's media

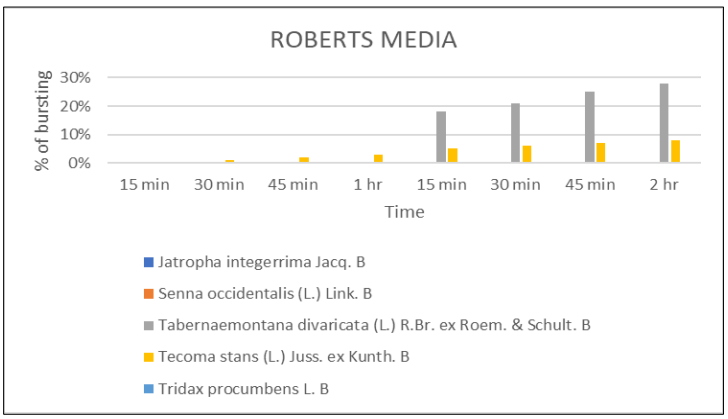

Figure 24 % of bursting in Robert's media

Above graph reveals the maximum bursting shown in *Cascabela thevetia* (L.) Lippod. (88%).

3.7. Pollen Germination and Bursting of selected plants in Periwinkle nectar

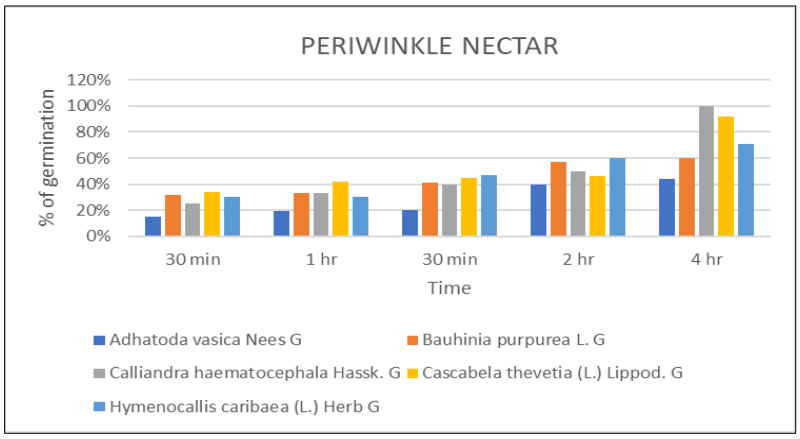

Figure 25 % of germination in Periwinkle nectar

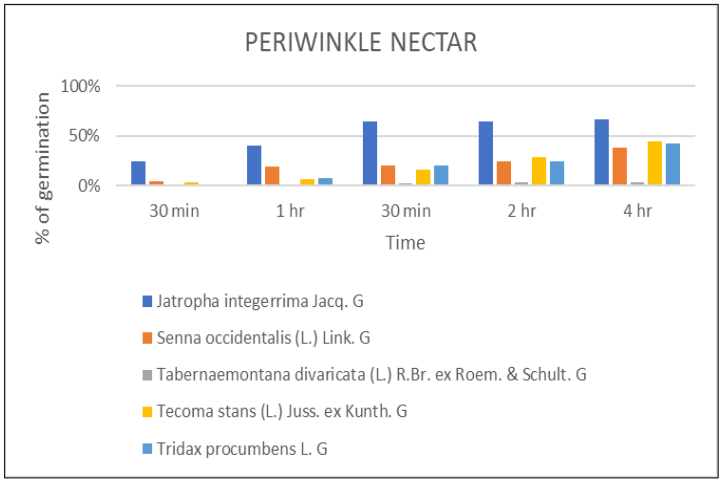

Figure 26 % of germination in Periwinkle nectar

Above graph reveals that the maximum germination (100%) in *Calliandra haematocephala* Hassk. and minimum (4%) in *Tabernaemontana divaricata* (L.) R.Br. ex Roem. & Schult.

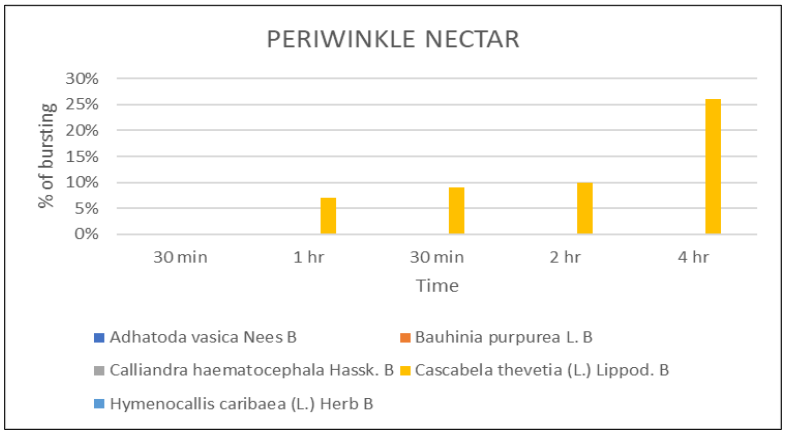

Figure 27 % of bursting in Periwinkle nectar

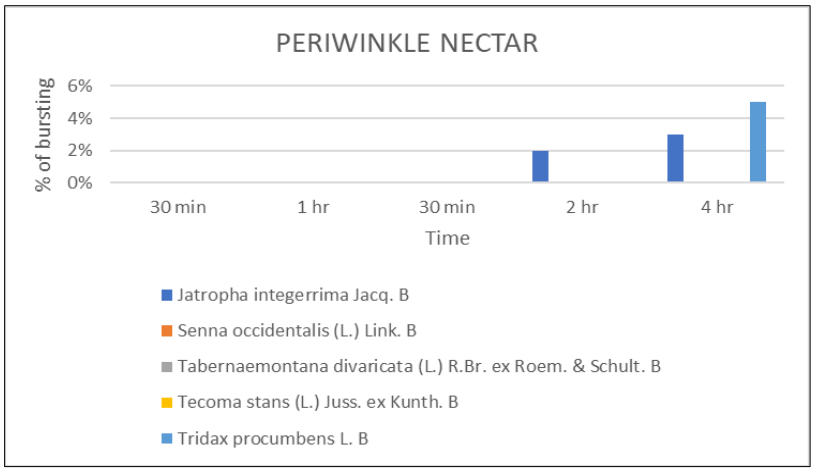

Figure 28 % of bursting in Periwinkle nectar

Above graph reveals the maximum bursting shown in *Cascabela thevetia* (L.) Lippod. (26%).

3.8. Pollen Germination and Bursting of selected plants in Ixora nectar

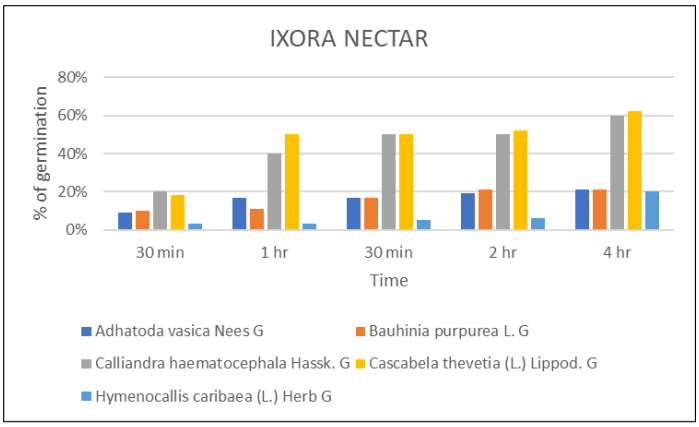

Figure 29 % of germination in Ixora nectar

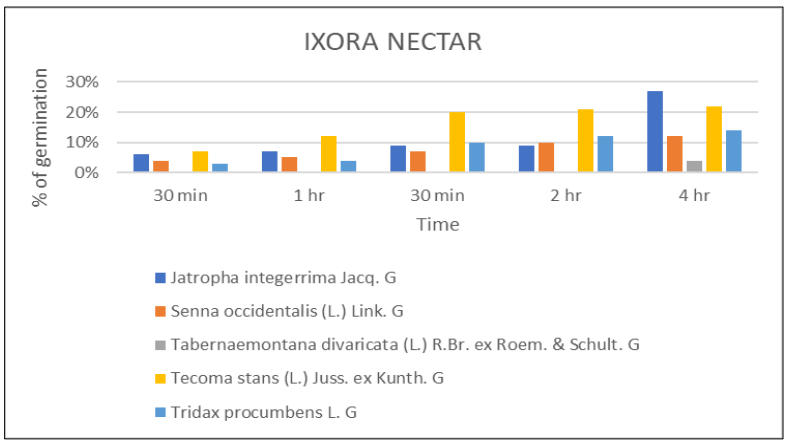

Figure 30 % of germination in Ixora nectar

Above graph reveals that the maximum germination (100%) in *Cascabela thevetia* (L.) Lippod. and minimum (4%) in *Tabernaemontana divaricata* (L.) R.Br. ex Roem. & Schult.

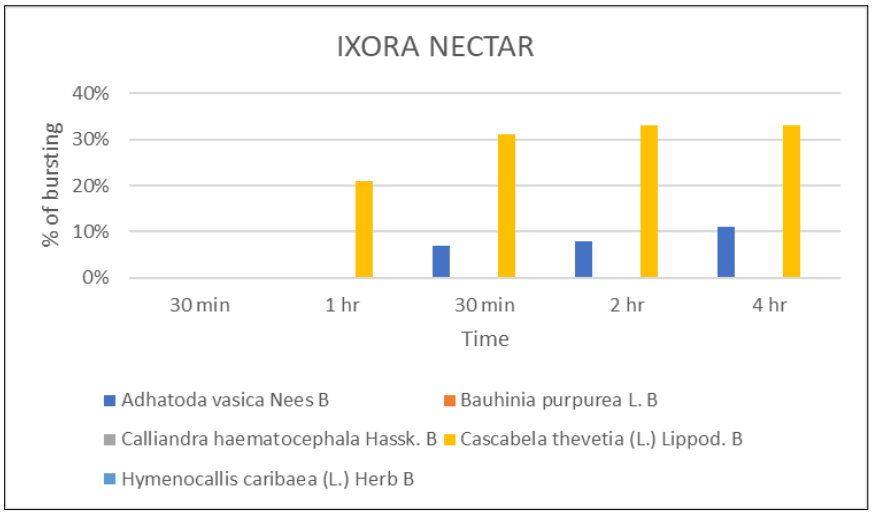

Figure 31 % of bursting in Ixora nectar

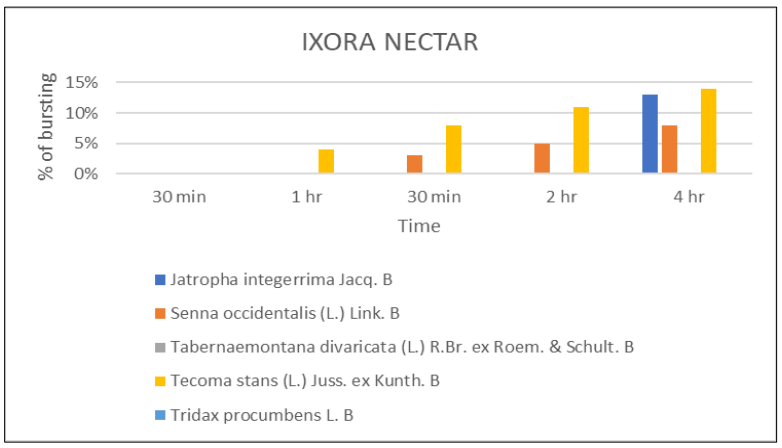

Figure 32 % of bursting in Ixora nectar

Above graph reveals the maximum bursting shown in *Cascabela thevetia* (L.) Lippod. (33%).

4. Conclusion

The study emphasizes the effects of various medium and sucrose concentrations on pollen viability and tube growth, based on the examination of pollen germination in certain plant species. The findings show that different species and media types have differential ideal pollen germination rates. Significant germination rates were obtained from Brewbaker media, Robert's media and natural nectar solutions among the investigated media; under particular circumstances, species such as *Cascabela thevetia* (L.) Lippod. and *Tecoma stans* (L.) Juss. ex Kunth. showed the highest germination rates. Furthermore, *Cascabela thevetia* (L.) Lippod. Had the most noticeable pollen tube bursting, indicating sensitivity to media components. These results advance our knowledge of the variables affecting pollen germination, which is crucial for breeding and plant reproduction initiatives.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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