

## *In-vitro Pollen Viability and Pollen Germination in Hibiscus Plant*

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### **Abstract**

*Pollen grain is important for help in growers and breeders a new plant. Pollen grains of Hibiscus are used to study the pollen viability and pollen germination. Two pollen viability tests are uses like 2, 3, 5-triphenyl tetrazolium chloride (TTC) and Aniline blue (AB). Pollen grain germinates are studying by using a nutrient medium which containing sucrose (5%, 10%, 15%, 20% and 25%) and to define as the best concentrations of sucrose for the pollen germination. After that, the germinated pollen was counted 24 hours later until there was no more germination. The rate of pollen viability depends upon the time, provided conditions and tests used (TTC and AB).TTC staining test was better result than that of the AB staining test and the rates recorded for 24 hours in all the sucrose concentration (5%, 10%, 15%, 20% and 25%) and we saw that, at the 20% sucrose concentration are gives the more germinated pollen and the pollen germinated after 5 hours and the most germinating period after the 8 hours.*

**Keywords:** *Aniline blue, Pollen grain, Pollen viability and germination, Sucrose, Tetrazolium chloride*

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### **Introduction**

Hibiscus is an ornamental shrub and found in tropical and sub-tropical areas. It has been cultivated since ancient times (1). Hibiscus is one of the flower crop species better adapted to different environmental areas and soils. Hibiscus plantations are widespread over the country, and traditional methods of cultivation are still used (2).

Germination capability of pollen depends on various factors, namely, nutrition conditions of species, varieties used, and environmental factors (3). To investigate pollination potential, estimates should be

made of pollen quantity and viability, as well as of pollen germination capability (4).

The pollen viability and pollen germination check by the using direct methods (5), by the in-vitro experiments (by the using some reagents like TTC and AB and some indirect methods are based on the cytological parameters, such as colour and the inflorescences of flower (6). We discuss the direct method of pollen viability and germinating of pollen grain of Hibiscus. Firstly use the TTC reagent and pollen treated with it and if the pollen shows the some colour like as red that means pollen are viable and germinating and we clearly saw the pollen tube after 5 hours if the pollen not shows any colour that mean

pollen are not viable and don't germinated. A other test for pollen viability like Aniline blue test, pollen treated with it show colour like first test but it show a different colour like Blue if pollen show colour that mean pollen are viable and germinating and if not colour is appear its mean pollen are non viable and do not germinated

In this study, we examined pollen grains for viability, germinability. This information is expected to be useful to plant breeders, or genebank who need pollen viability tests (7).

### ***Materials Required***

Petri-dish, 1% TTC Solution, 1% glycerin, Sucrose Solution, Aniline Blue Solution, Distilled Water, Microscope etc.

### ***Procedure***

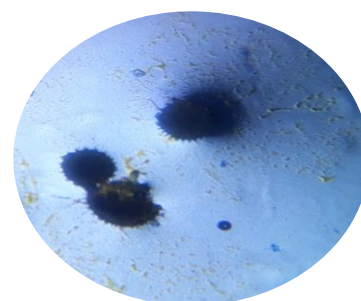
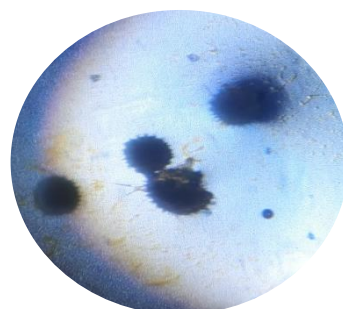
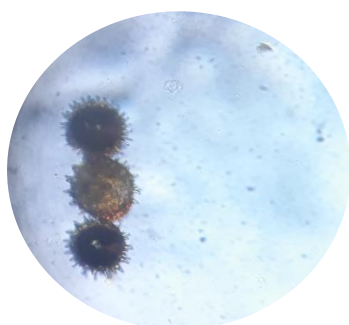
Check the pollen viability and germination by the help of direct and indirect methods. We used the direct method for it by the help of two chemical tests like as TTC and Aniline blue for 24 hour and shows the maximum viability and germination of pollen of Hibiscus.

*First test:-* TTC (2, 3, 5-triphenyl tetrazolium chloride) used for pollen viability and germination

Take a clean and dry Petri-dish and shed some pollen by simple tapping or we can use forceps gently. After collecting pollen add TTC solution prepared early by adding 1g in 10ml distilled water with help of dropper. Wait for four minute till take up stain observe it under microscope and note their viability and germination.

*Second test:-* Aniline blue used for pollen viability and germination. Take a clean and dry glass slide and shade some pollen on glass slide. Add 1-2 drops of Aniline blue solution by the help of dropper. Wait few minutes and take up stain observe it under microscope and note their viability and germination.

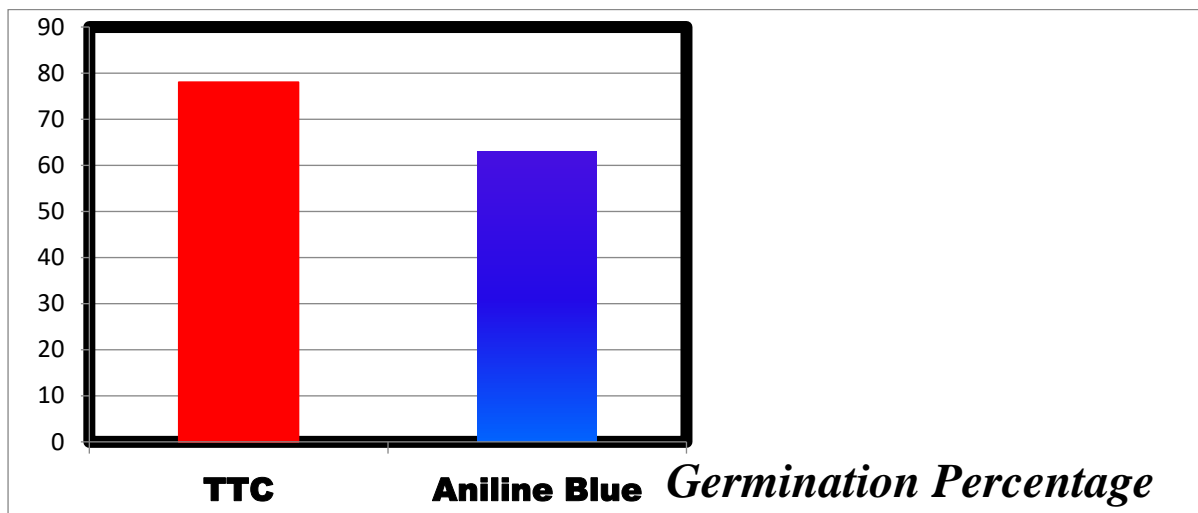
*Germination of Pollen:-* After the pollen viability tests, check the pollen germination by the help of various concentration of sucrose solution (5%, 10%, 15%, 20% and 25%) for 24 hours (7). We saw that, at the 20% sucrose concentration are gives the more germinated pollen and the pollen germinated after 5 hours and the most germinating period after the 8 hours.



***TTC test for Pollen viability    Aniline blue test for Pollen viability    Pollen Germination***

### ***RESULT***

Name of test	Check Viability	Germination Percentage
<i>2, 3, 5-triphenyl tetrazolium chloride</i>	<i>Red</i>	<i>78%</i>
<i>Aniline Blue</i>	<i>Blue</i>	<i>63%</i>



### ***Conclusions***

In analysis the pollen quality of Hibiscus, viability tests to be considered faster and easier methods than the germination tests. The effects of external factors such as temperature, humidity, and germinating media are minimized (9). Either TTC or Aniline Blue can be used in analysis the pollen viability and it may be indicating germination status.

### ***References***

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