

# The Effectiveness of Different Sterilisation Techniques Used For Micropropagation.

Mathilda Hudson

## Introduction

The variety of disinfectant used vary between methodologies and that can be seen within the methodology used at University Centre Askham Bryan (Binda *et al.*, 2020; Estrella-Maldonado *et al.*, 2022). The research conducted aims to refine the methodology to select a disinfectant that can be used by the students when undertaking the process of micropropagation within the science laboratories. The areas of specimens used for the explant and the medium used to grow the substrate vary (Gao *et al.*, 2021; Safari *et al.*, 2023). Explants can include the root, shoot tip or axillary bud (Lemma *et al.*, 2020).

## Aims and Objectives

- Aim - To explore the effectiveness of five different disinfectant methods used on plant specimens to help refine the process of micropropagation undertaken by lecturers and students of University Centre Askham Bryan.
- Objectives – To test different sterilisation techniques, including UV, sterile water, Fairy®, Bleach, and Milton®. To investigate how effective these disinfectant techniques are when used for the micropropagation of the Tansy Plant and Lavender. To find the most appropriate sterilisation technique to refine the process for University Centre Askham Bryan.

## Hypothesis

- H<sub>0</sub> – There will be no statistically significant difference for Tansy Plant between the disinfectants used and pathogenic growth.
- H<sub>1</sub> – There will be a statistically significant difference for Tansy Plant between the disinfectants used and pathogenic growth.
- H<sub>0</sub> – There will be no statistically significant difference for Lavender between the disinfectants used and pathogenic growth.
- H<sub>1</sub> – There will be a statistically significant difference for Lavender between the disinfectants used and pathogenic growth.

## Literature Review

Science allows for the exploration and research into the unknown and provides results that can require further investigation into the research (Boguslav *et al.*, 2023). Science analyses data that is previously collected, or the collection of new data, adding to scientific research and knowledge. Refining micropropagation methodology can allow for a higher quality product in commercial sectors and a reliable and accurate result in the educational and research sector (Patil *et al.*, 2021; Cheung and Tai, 2023). Micropropagation must be conducted under sterile conditions, both the environment and the explant itself (Ma *et al.*, 2021). Disinfectants used in micropropagation allow for the surface sterilisation of the plant and is needed in both small and large scale micropropagation (Babu *et al.*, 2022).

## Results

### Statistical Analysis for Disinfectants Used On Lavender and Tansy Plant

The five disinfectants used for surface disinfection on Lavender and Tansy Plant were Bleach, Milton®, Fairy®, Sterile Water and UV. Four had a highly significant statistical difference between the disinfectants used (Kruskal-Wallis, H<sub>2</sub> = 26.43, p < 0.001, post hoc Mann-Whitney U Bleach and Fairy®, W = 1041.00, N = 36,36, p = 0.001; Bleach and Sterile Water, W = 1007.00, N = 36,36, p = < 0.001; Bleach and UV, W = 965.50, N = 36,36, p = < 0.001; Milton® and UV, W = 1038.50, N = 36,36, p = 0.001.

### Statistical Analysis for Disinfectants Used On Lavender

The five disinfectants used for surface disinfection on Lavender and Tansy Plant were Bleach, Milton®, Fairy®, Sterile Water and UV. Six had a highly significant statistical difference between the disinfectants used (Kruskal-Wallis, H<sub>2</sub> = 49.98, p < 0.001, post hoc Mann-Whitney U Bleach and Fairy®, W = 187.50, N = 18,18, p = < 0.001; Bleach and Sterile Water, W = 189.00, N = 18,18, p = < 0.001; Bleach and UV, W = 187.50, N = 18,18, p = < 0.001; Milton® and Fairy®, W = 207.00, N = 18,18, p = < 0.001; Milton® and Sterile Water, W = 217.50, N = 18,18, p = < 0.001; Milton® and UV, W = 201.00, N = 18,18, p = < 0.001).

### Statistical Analysis for Disinfectants Used On Tansy Plant

The Tansy Plant showed no statistical difference (P>0.05) in pathogenic growth when specimens were surface disinfected with any of the disinfectant techniques. Bleach, Milton®, Sterile Water and UV all had highest pathogenic presence (median = 3), Fairy® had the lowest signs of pathogenic presence (median = 2).

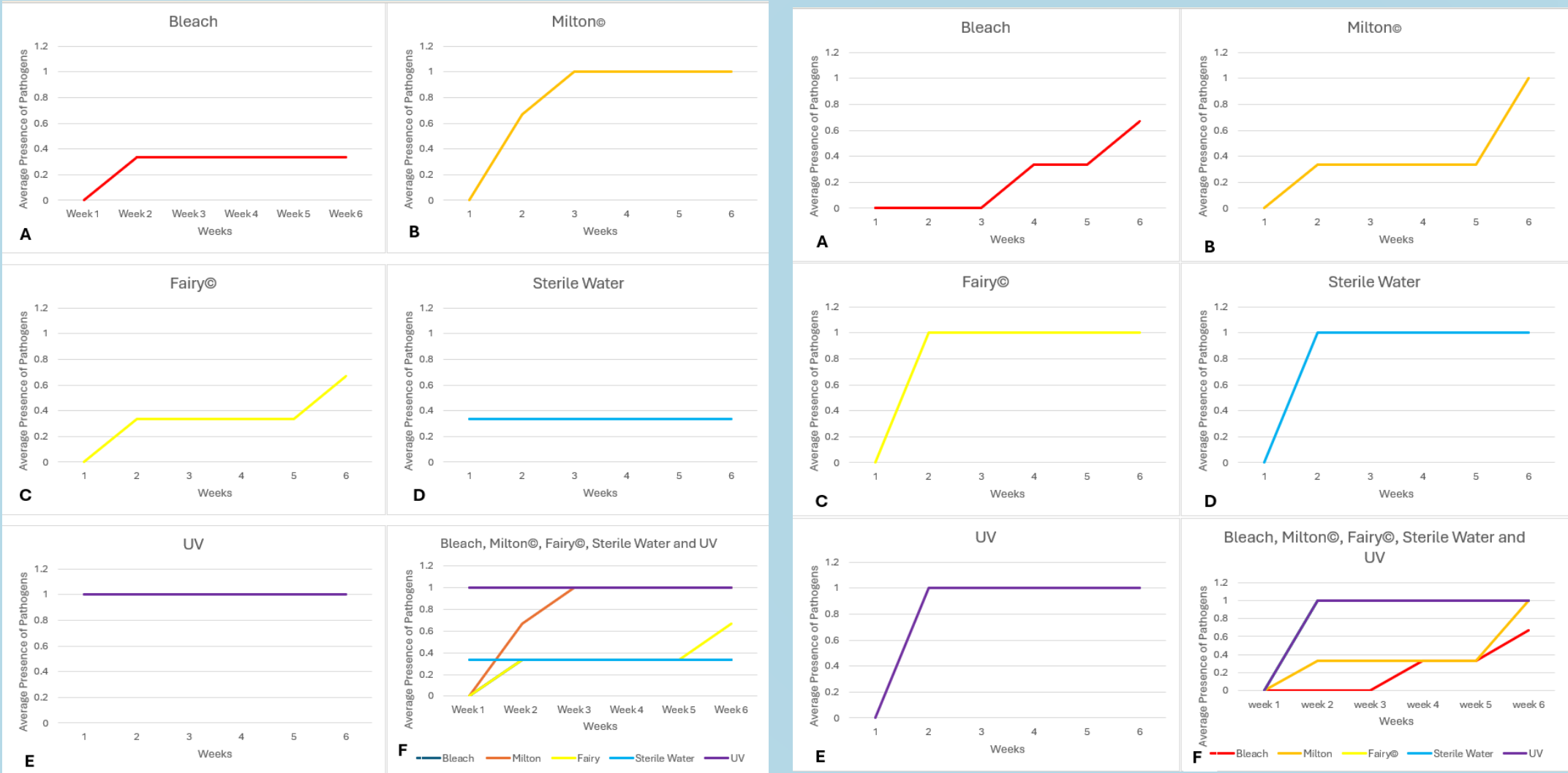


Figure 1: The Average presence of pathogens within the Tansy Plant Specimens.

A – Average number of explants with the presence of pathogens with Bleach as the disinfectant agent on the Tansy Plant specimens.  
B – Average number of explants with the presence of pathogens with Milton® as the disinfectant agent on the Tansy Plant specimens.  
C – Average number of explants with the presence of pathogens with Fairy® as the disinfectant agent on the Tansy Plant specimens.  
D – Average number of explants with the presence of pathogens with Sterile Water as the disinfectant agent on the Tansy Plant specimens.  
E – Average number of explants with the presence of pathogens with UV as the disinfectant agent on the Tansy Plant specimens.  
F – Average number of explants with pathogens for all disinfectant agents used on the Tansy Plant specimens.

Figure 2: The Average presence of pathogens within the Lavender Specimens.

A – Average number of explants with the presence of pathogens with bleach as the disinfectant agent on the Lavender specimens.  
B – Average number of explants with the presence of pathogens presence with Milton® as the disinfectant agent on the Lavender specimens.  
C – Average number of explants with the presence of pathogens presence with Fairy® as the disinfectant agent on the Lavender specimens.  
D – Average number of explants with the presence of pathogens presence with Sterile Water as the disinfectant agent on the Lavender specimens.  
E – Average number of explants with the presence of pathogens presence with UV as the disinfectant agent on the Lavender specimens.  
F – Average number of explants with pathogenic presence for all disinfectant agents used on the Lavender specimens.

Full results available via QR code.



## Methodology

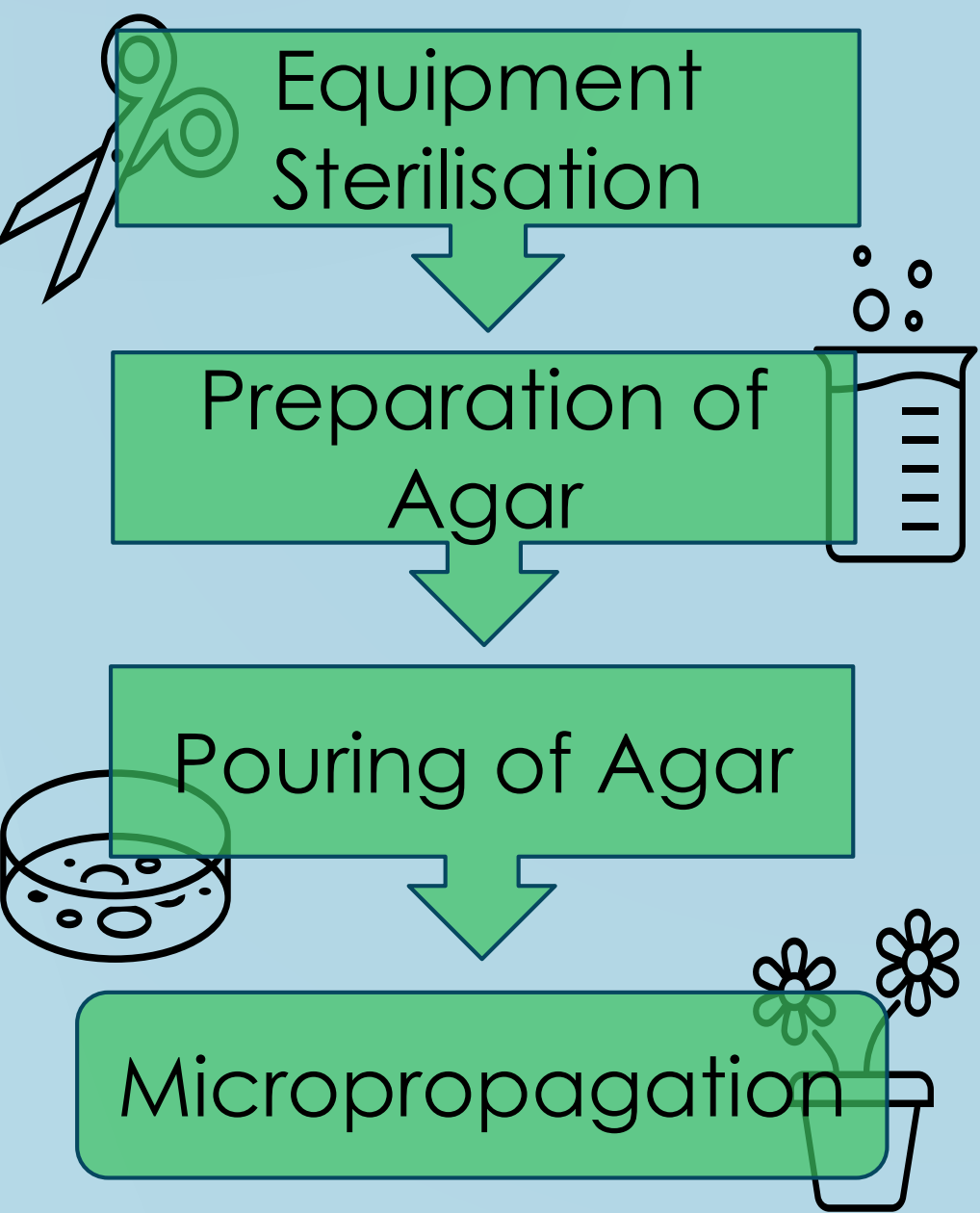


Table 1: Ranking Chart for the specimens, with aids of how to rank specimens.

| Ranking Value | Visual Indication | Descriptive Indication                                                                                                                                        |
|---------------|-------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1-3           |                   | Clear and pale colour of the Agar. Little to no pathogenic presence visible within the agar or on the specimen.                                               |
| 4-6           |                   | Colour of the Agar has darkened, there are signs of pathogenic presence within the specimen tube or on the specimen.                                          |
| 7-10          |                   | Agar has darkened completely, may struggle to see the specimen within the agar, pathogenic presence can be seen over the top of the agar and on the specimen. |

## Discussion

The research conducted found no statistical difference within the Tansy Plant data, so the null hypothesis was accepted. The results for the Lavender Plant found that there was a statistical difference between the data sets, so the null hypothesis was rejected.

In the research conducted a range of explants were used including shoot tips and axillary buds. The plants used should be considered within the experiment, as Tansy Plant is a woody species, which affects the ability to detect if the plant is dying/wilting. The use of a laminar flow cabinet is debated, due to the sterility when removing the plastic shield and the disruption of air flow within. The sterility can be compromised when using a Bunsen burner, the effectiveness and size of the sterile field it creates and the risk of contamination when changing the gas canister.

## Conclusion

The research conducted found a 1% solution of Bleach and Milton® were the most effective disinfectants used in the research as the surface disinfectant of the explants for micropropagation of the Lavender Plant. However, with Tansy Plant there was no statistically significant difference in the disinfectants used in micropropagation. Further research should be conducted.

## References

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