

CHAPTER 4

RESULTS AND DISCUSSION

4.1 PLASMA EXPOSURE ON RADISH

Plasma treatment has shown a notable positive effect on seed germination and early plant growth. The process works by altering the seeds surface by making it easier for water and nutrients absorption by the seed coat. The reactive species generated during the plasma treatment enhance the seed's ability to absorb water, resulting in quicker and more efficient germination.

Plasma contains reactive species such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), which play a crucial role in enhancing plant growth and development. Key ROS, including oxygen radicals ($O_2\cdot$), hydroxyl radicals ($OH\cdot$), hydrogen peroxide (H_2O_2), and ozone (O_3), along with RNS like nitric oxide (NO), nitrogen dioxide (NO_2), and peroxy nitrite ($ONOO^-$), improve seed germination by increasing seed coat permeability and water absorption. These species promote root and shoot growth by facilitating nutrient uptake and cell division, while also enhancing stress tolerance and disease resistance through the activation of plant defense responses.

Furthermore, plasma's antimicrobial effects help mitigate seed-borne pathogens, fostering healthier conditions for plant development. Collectively, these benefits support increased agricultural productivity and promote sustainable farming practices

As observed in the "Total Germinated Seeds" graph as shown in Figure 4.1, the group of seeds treated with plasma for 4 minutes has exhibited the highest number of germinated seeds compared to the ones which was left untreated. This indicates that the plasma- treated seeds responded more effectively, demonstrating a clear enhancement in their germination capacity.

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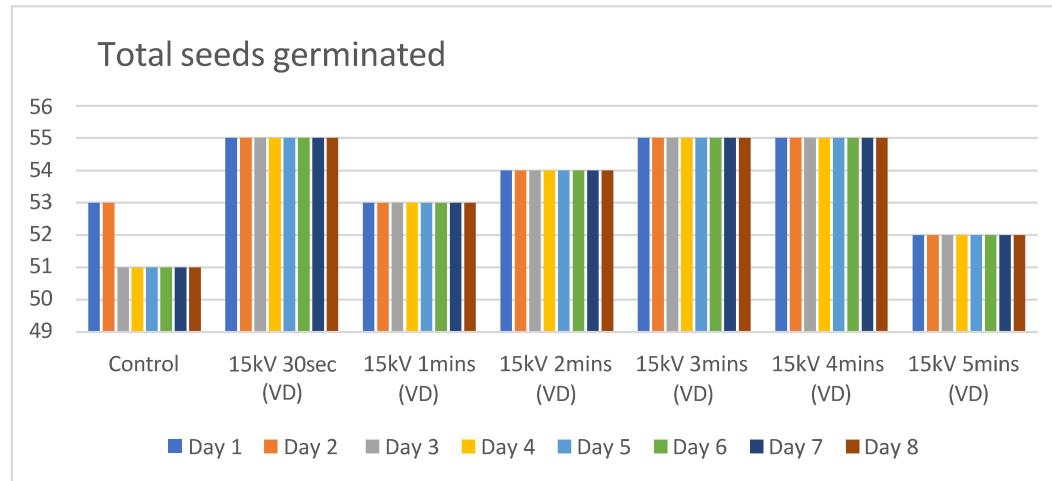


Figure 4.1: Graphical Representation of Total Germination Seeds

This improvement is due to the changes in the seed surface, which were made more porous and hydrophilic by the plasma treatment. These changes allow the seeds to take up water more efficiently, which is a essential element for initiating the germination process. By enhancing this fundamental step, plasma technology offers a promising, sustainable method to improve seed quality and agricultural productivity.

The germination rate was calculated by the equation

$$GR (\%) = (NS / TS) \times 100\%$$

where,

GR = Germination Rate

NS = Number of seeds germinated

TS = Total number of seeds

The effect of plasma treatment on plant growth is evident in parameters such as shoot length, and root length as illustrated in Figures 4.2 and 4.3. Seeds treated with plasma consistently produced plants with significantly longer shoots compared to the control group. Notably, seeds exposed to an 4-minute plasma treatment (VD 4 min) exhibited the highest average shoot length, whereas untreated seeds showed considerably shorter shoots. This observed trend highlights the enhanced growth potential enabled by plasma treatment, further supporting its effectiveness in improving plant development

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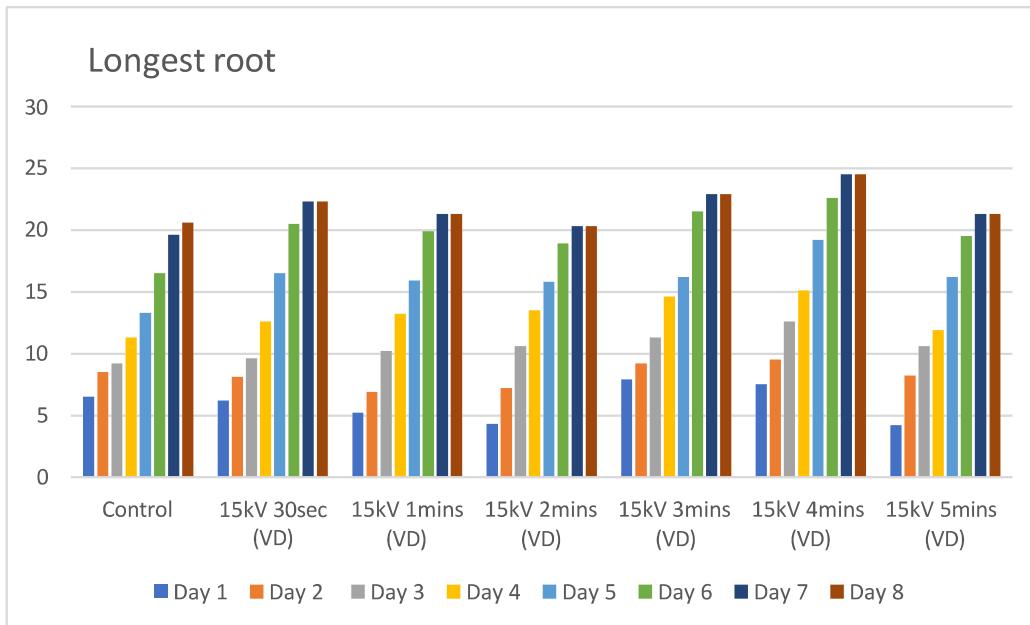


Figure 4.2: Graphical Representation of Longest Root

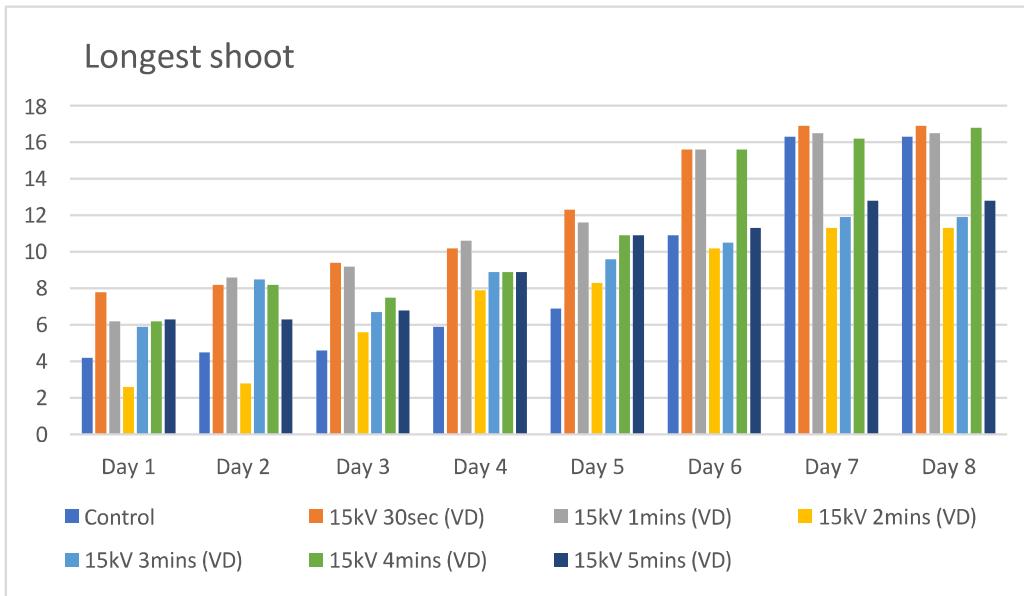


Figure 4.3: Graphical Representation of Longest Shoot

The analysis also demonstrated notable enhancements in root development, as depicted in Figures 4.2 and 4.3, underscoring the comprehensive benefits of plasma treatment. Statistically significant results, with a p-value of $p < 0.05$, validate plasma treatment as an effective and reliable method for enhancing seed germination and overall plant growth.

4.1.2 SEED VIABILITY AND HEALTH ASSESSMENT

A comprehensive analysis of seed germination must account for challenges such as fungal infections and the presence of non-germinated seeds. Plasma treatment showed significant improvements in mitigating these issues, as evidenced by the graphs illustrating reductions in fungus-infected seeds and non-germinated seeds. This highlights the additional benefits of plasma treatment in promoting healthier seed germination conditions.

4.1.2.1 FUNGUS INFECTED SEEDS

The graph of fungus-infected seeds highlights a sharp reduction in fungal contamination among plasma-treated seeds compared to the control group as shown in Figure 4.4. For instance, seeds treated with 4 minutes of plasma (VD 4min) showed the lowest infection rate, whereas untreated seeds had significantly higher fungal growth.

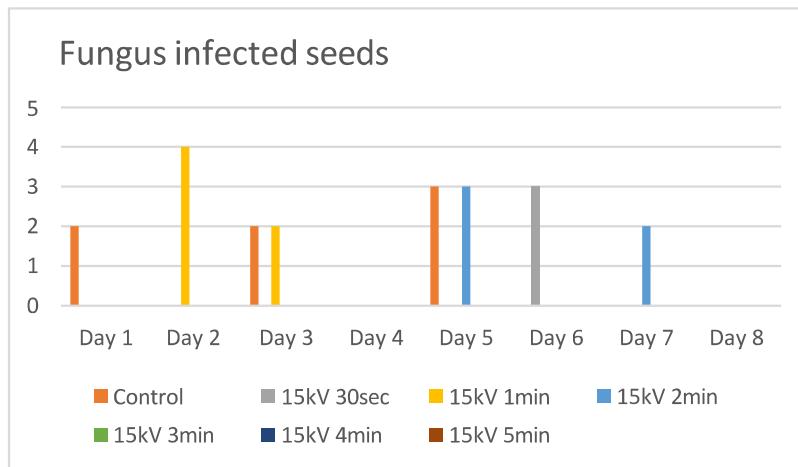


Figure 4.4: Graphical Representation of Fungus Infected Seeds

4.1.2.2 NON- GERMINATED SEEDS

The data presented in Figure 4.5 highlights that plasma exposure significantly enhances germination rates while reducing the proportion of non-viable seeds. Seeds subjected to plasma treatment demonstrated considerably lower non-germination rates, with the 4-minute treatment yielding the most favorable outcomes.

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This reduction is likely attributed to improved water absorption, efficient nutrient uptake, and the elimination of germination inhibitors facilitated by the plasma treatment process. The results of this study highlight the significant advantages of plasma treatment in enhancing seed germination and plant growth. A comparative analysis between the control group (untreated seeds) and plasma-treated seeds subjected to 4 minutes (VD 4min) of exposure revealed several notable differences

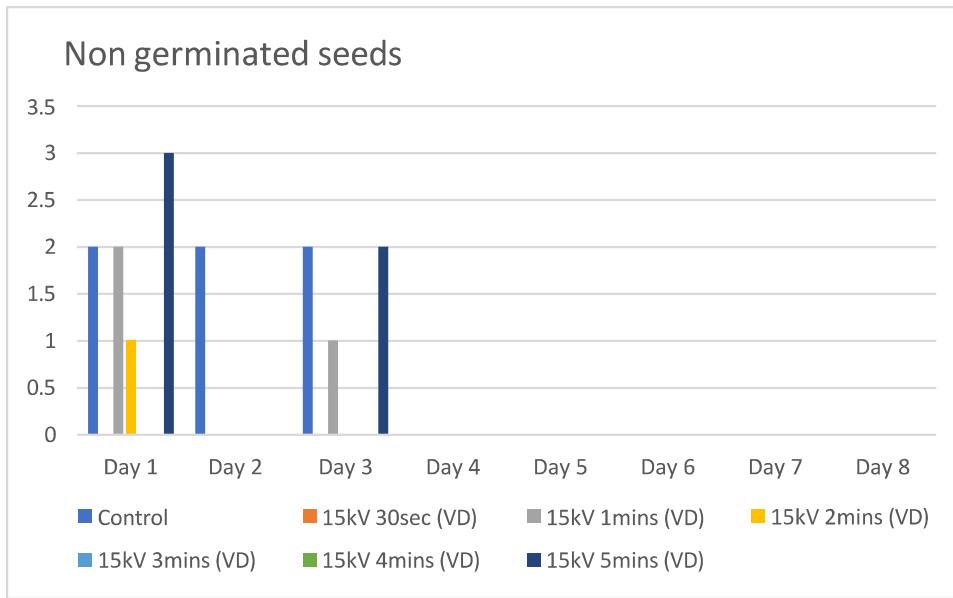


Figure 4.5: Graphical Representation of Non germinated Seeds

4.1.2.3 GERMINATION RATES

Plasma-treated seeds exhibited significantly improved germination rates, with the 8-minute treatment consistently achieving the highest results. The control group showed a germination rate of 65%, which increased to and reached a remarkable 100% for the 4-minute treatment (VD 4 min). These findings, illustrated in Figure 4.6, demonstrate the enhanced viability and readiness of seeds following plasma exposure.

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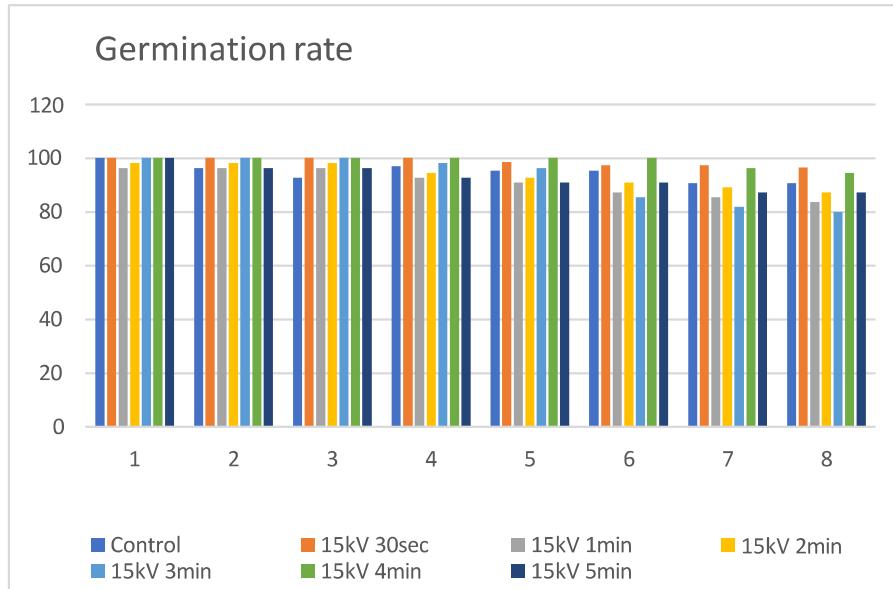


Figure 4.6: Germination Rate observed on Germination sheet

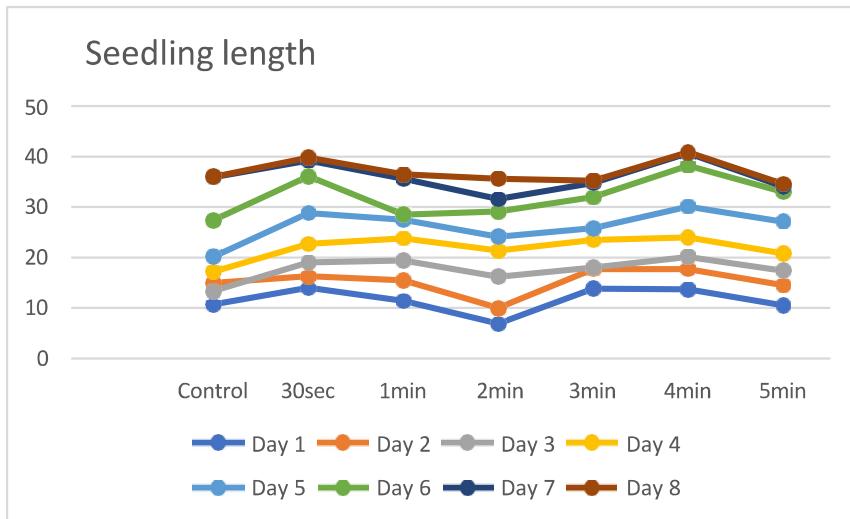


Figure 4.7: Seedling length observed on Germination sheet

4.2 STATISTICAL ANALYSIS

The statistical analysis of this study utilized ANOVA (Analysis of Variance) to assess the impact of plasma treatment on seed growth parameters. Specifically, a Two-Factor Without Replication ANOVA was employed, allowing for the evaluation of variations across two primary factors: treatment duration (4 minutes) and growth metrics (germination rate, shoot length, and biomass).

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This method was chosen for its effectiveness in identifying significant differences among groups while accounting for potential variability. The statistical significance threshold was set at $p \leq 0.05$, ensuring that the observed effects were not due to random chance.

The ANOVA procedure began by partitioning the total variation in the data into three components: rows, columns, and error. The rows represented the variation caused by the different treatment durations, while the columns captured the variation among growth metrics. The error component accounted for unexplained variability in the dataset. Each component was characterized by three values: Sum of Squares (SS), Degrees of Freedom (df), and Mean Square (MS).

ANOVA TWO FACTOR ANALYSIS						
Source of Variation	SS	df	MS	F	P-value	F crit
Rows	4555.517143	7	650.7881633	207.5873678	0	2.237070296
Columns	269.0267857	6	44.83779762	14.302289	0.0000000084427 8214	2.323993797
Error	131.6703571	42	3.135008503			
Total	4956.214286	55				

Table 4.1: Summary of ANOVA analysis

1. Source of Variation

The ANOVA breaks down the total variability in the dataset into three components:

- Rows (Treatments/Levels) – Possibly different voltage or current levels used in plasma exposure.
- Columns (Groups/Categories) – Likely different time durations or seed conditions (e.g., dry vs soaked).
- Error – Unexplained variation or residuals not accounted for by the two factors.
- Total – Summation of all variability.

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2. Sum of Squares (SS)

- Rows: 4555.51 – This high value indicates that the treatment across different plasma voltage/current levels has a significant effect on the radish seeds.
- Columns: 269.03 – The second factor (possibly exposure duration or seed condition) also contributes but to a lesser extent.
- Error: 131.67 – Represents the variation not explained by the factors.
- Total: 4956.21 – This is the aggregate variation in the entire dataset

3. Degrees of Freedom (df)

- Rows ($df = 7$) – Suggests 8 different levels of treatment ($df = n - 1$).
- Columns ($df = 6$) – Suggests 7 different categories.
- Error ($df = 42$) – Remaining degrees of freedom after accounting for treatments and groups.
- Total ($df = 55$) – Overall degrees of freedom ($n - 1$) where n is the total number of observations.

4. Mean Square (MS)

- Rows ($MS = 650.78$): Very high, shows considerable effect by treatments.
- Columns ($MS = 44.84$): Moderate impact.
- Error ($MS = 3.14$): Minimal variation not explained by the treatments

5. F-Statistic (F)

- Rows ($F = 207.59$)
- Columns ($F = 14.30$)

The F-value measures how much the variation between group means exceeds the variation within the groups. A larger F-value generally indicates that the group means are significantly different.

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6. P-Value

- Rows: 0
- Columns: 8.44×10^{-9}

P-values < 0.05 indicate statistical significance. Here, both values are extremely low, indicating that:

- The effect of plasma treatment levels (Rows) on radish seeds is highly significant.
- The effect of the secondary factor (Columns) is also highly significant.

7. F critical value (F crit)

- Rows: 2.237
- Columns: 2.324

The F critical value is the threshold value to reject the null hypothesis.

If the calculated F-value $>$ F crit, the effect is statistically significant.

- Since $207.59 > 2.237$ and $14.30 > 2.324$, we reject the null hypothesis for both rows and column

4.3 PLANT GROWTH ON GERMINATION SHEET AND ON GROUND



Day 1 - Untreated seeds (control seeds)

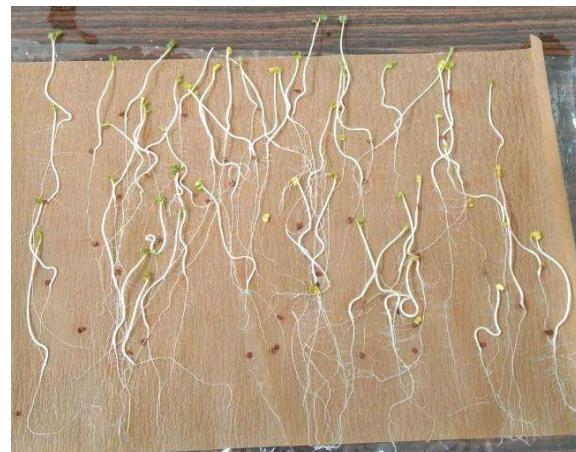


Day 1 - Treated seeds (15kV 4mins)

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Day 4 - Untreated seeds (control seeds)
Less germination



Day 4 - Treated seeds (15kV 4mins)
More germination with longest root and shoot



Day 7 - Treated seeds (15kV 4mins) More germination with longest root and shoot



Day 12 – Treated seeds on ground

4.3 BIOCHEMICAL ANALYSIS

4.3.1 Phytochemical Screening Results

Phytochemical screening involves a series of **qualitative tests** that help detect the presence of key classes of bioactive compounds in seed extracts. These tests rely on **chemical reactions between specific reagents and phytochemicals**, often producing color changes or precipitates that indicate the presence of the compound.



Figure 4.8: Phytochemical screening

In our study, we performed a comparative phytochemical analysis of **untreated (control) radish** and **radish treated with plasma at 15 kV for 4 minutes**. The image above displays the test tubes containing extracts from beans, used for phytochemical screening. Each test tube corresponds to a specific phytochemical test, identified by labelled tags such as Alkaloids, Steroids, Saponins, Tannins, and Flavonoids. These tests rely on specific chemical reactions that result in a visible colour change or precipitate formation, indicating the presence of a particular compound. These visual results align with the qualitative data summarized in the phytochemical table, confirming the presence of key bioactive compounds such as alkaloids, steroids, and saponins in both control and plasma-treated beans.

Observations:

- There was **no significant change** in the presence of phytochemicals between the control and plasma-treated beans.
- Key bioactive compounds such as **alkaloids, steroids, saponins, carbohydrates, and proteins** were consistently detected in both samples.

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The results are summarized in the table below:

Phytochemical Analysis	Radish(control)	Radish (15kv, 4mins)
Alkaloids	++	++
Flavonoids	+++	+++
Tannins and phenolic compounds	-	++
Steroids	++	++
Saponins	+++	+++
Carbohydrates	+++	+++
Proteins	-	-

Table 4.2: Results of Phytochemical screening

Note: (++) = Strong presence and (-) = Absence

Total Ion Chromatogram (TIC) Overview

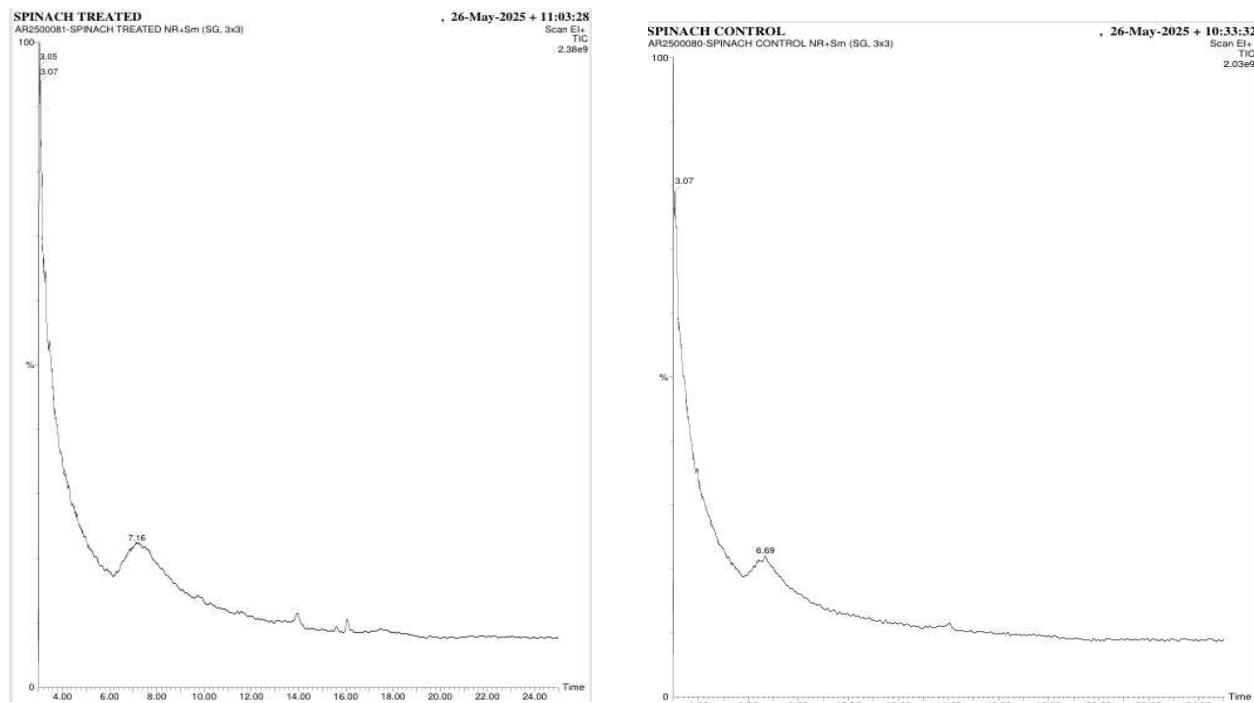


Fig 4.15: Total Ion Chromatogram (TIC) of spinach control

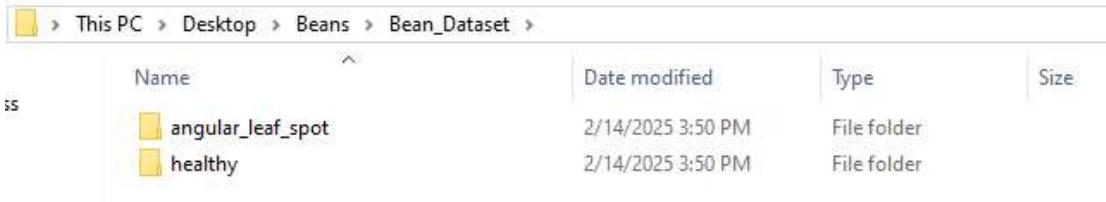
Fig 4.16.: Total Ion Chromatogram (TIC) of spinach Treated

4.4 METHODOLOGY FOR RADISH FEATURE EXTRACTION

This section delineates the methodology adopted for the classification of Radish leaf diseases using a Convolutional Neural Network (CNN). The pipeline involves five key stages: dataset preparation, preprocessing, model architecture design, training and validation, and final evaluation.

A. Dataset Acquisition and Preprocessing

The image dataset comprising radish leaf samples was organized into class-specific directories representing various disease categories and healthy leaves. Each image was resized to 128×128 pixels and normalized to enhance training efficiency. TensorFlow's utility functions were employed to load the images into memory-efficient batches, with categorical labels inferred automatically. The dataset was divided into training and validation subsets to facilitate supervised learning.



This PC > Desktop > Beans > Bean_Dataset >				
	Name	Date modified	Type	Size
ss	angular_leaf_spot	2/14/2025 3:50 PM	File folder	
	healthy	2/14/2025 3:50 PM	File folder	

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B. Network Architecture

A deep CNN model was constructed using the Keras Sequential API. The architecture consisted of multiple convolutional layers with increasing filter depths (32, 64, 128, 256, and 512), each followed by ReLU activation and interspersed with max-pooling operations to reduce spatial dimensionality. Dropout layers were incorporated to prevent overfitting, and the final output layer utilized a softmax activation function to yield multi-class probabilities. The model was designed to extract spatial hierarchies from leaf textures and patterns indicative of disease.

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```
: cnn.summary()  
Model: "sequential"  


| Layer (type)                   | Output Shape         | Param #   |
|--------------------------------|----------------------|-----------|
| conv2d (Conv2D)                | (None, 128, 128, 32) | 896       |
| conv2d_1 (Conv2D)              | (None, 126, 126, 32) | 9,248     |
| max_pooling2d (MaxPooling2D)   | (None, 63, 63, 32)   | 0         |
| conv2d_2 (Conv2D)              | (None, 63, 63, 64)   | 18,496    |
| conv2d_3 (Conv2D)              | (None, 61, 61, 64)   | 36,928    |
| max_pooling2d_1 (MaxPooling2D) | (None, 30, 30, 64)   | 0         |
| conv2d_4 (Conv2D)              | (None, 30, 30, 128)  | 73,856    |
| conv2d_5 (Conv2D)              | (None, 28, 28, 128)  | 147,584   |
| max_pooling2d_2 (MaxPooling2D) | (None, 14, 14, 128)  | 0         |
| conv2d_6 (Conv2D)              | (None, 14, 14, 256)  | 295,168   |
| conv2d_7 (Conv2D)              | (None, 12, 12, 256)  | 590,080   |
| max_pooling2d_3 (MaxPooling2D) | (None, 6, 6, 256)    | 0         |
| conv2d_8 (Conv2D)              | (None, 6, 6, 512)    | 1,180,160 |
| conv2d_9 (Conv2D)              | (None, 4, 4, 512)    | 2,359,808 |
| max_pooling2d_4 (MaxPooling2D) | (None, 2, 2, 512)    | 0         |
| dropout (Dropout)              | (None, 2, 2, 512)    | 0         |
| flatten (Flatten)              | (None, 2048)         | 0         |
| dense (Dense)                  | (None, 1500)         | 3,073,500 |
| dropout_1 (Dropout)            | (None, 1500)         | 0         |
| dense_1 (Dense)                | (None, 9)            | 13,509    |


```
Total params: 23,397,701 (89.26 MB)
Trainable params: 7,799,233 (29.75 MB)
Non-trainable params: 0 (0.00 B)
Optimizer params: 15,598,468 (59.50 MB)
```


```

C. Model Compilation and Training

The network was compiled using the Adam optimizer with a learning rate of 0.0001, and the loss function was set to categorical cross-entropy, appropriate for multi-class classification tasks. The training process was conducted over several epochs with real-time monitoring of validation metrics. Accuracy and loss values were logged throughout the training process. The final trained model was preserved in Keras format for subsequent inference.

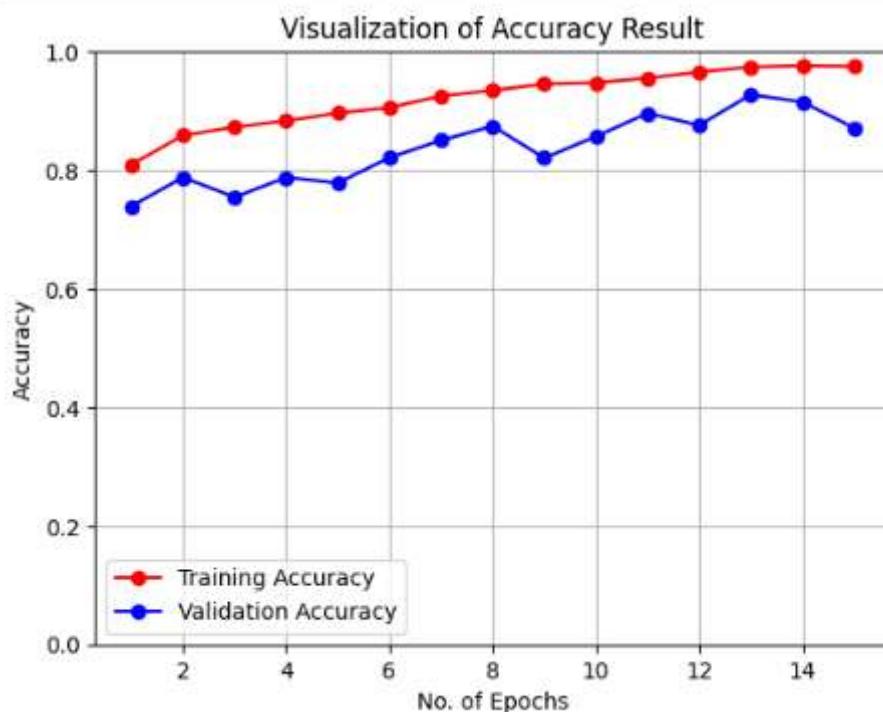
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```
: training_history = cnn.fit(x=training_set,validation_data=validation_set,epochs=15)

Epoch 1/15
232/232 337s 1s/step - accuracy: 0.7995 - loss: 0.5357 - val_accuracy: 0.7391 - val_loss: 0.6220
Epoch 2/15
232/232 345s 1s/step - accuracy: 0.8568 - loss: 0.3693 - val_accuracy: 0.7878 - val_loss: 0.5549
Epoch 3/15
232/232 350s 2s/step - accuracy: 0.8682 - loss: 0.3241 - val_accuracy: 0.7544 - val_loss: 0.5445
Epoch 4/15
232/232 319s 1s/step - accuracy: 0.8798 - loss: 0.3029 - val_accuracy: 0.7878 - val_loss: 0.5358
Epoch 5/15
232/232 265s 1s/step - accuracy: 0.8915 - loss: 0.2680 - val_accuracy: 0.7783 - val_loss: 0.5219
Epoch 6/15
232/232 270s 1s/step - accuracy: 0.9073 - loss: 0.2371 - val_accuracy: 0.8212 - val_loss: 0.4541
Epoch 7/15
232/232 2635s 11s/step - accuracy: 0.9235 - loss: 0.1972 - val_accuracy: 0.8503 - val_loss: 0.3685
Epoch 8/15
232/232 271s 1s/step - accuracy: 0.9288 - loss: 0.1843 - val_accuracy: 0.8743 - val_loss: 0.3551
Epoch 9/15
232/232 254s 1s/step - accuracy: 0.9428 - loss: 0.1548 - val_accuracy: 0.8205 - val_loss: 0.4207
Epoch 10/15
232/232 256s 1s/step - accuracy: 0.9439 - loss: 0.1447 - val_accuracy: 0.8568 - val_loss: 0.3904
Epoch 11/15
232/232 263s 1s/step - accuracy: 0.9545 - loss: 0.1241 - val_accuracy: 0.8961 - val_loss: 0.3088
Epoch 12/15
232/232 255s 1s/step - accuracy: 0.9628 - loss: 0.1010 - val_accuracy: 0.8757 - val_loss: 0.3273
Epoch 13/15
232/232 256s 1s/step - accuracy: 0.9720 - loss: 0.0742 - val_accuracy: 0.9273 - val_loss: 0.2070
Epoch 14/15
232/232 259s 1s/step - accuracy: 0.9706 - loss: 0.0820 - val_accuracy: 0.9150 - val_loss: 0.2815
Epoch 15/15
232/232 257s 1s/step - accuracy: 0.9697 - loss: 0.0780 - val_accuracy: 0.8714 - val_loss: 0.3570
```

D. Performance Evaluation

Post-training, the model's predictive performance was assessed using both quantitative and visual tools. Key evaluation metrics included training and validation accuracy, as well as precision, recall, and F1-score derived from the classification report. A confusion matrix was generated to visualize class-wise prediction accuracy. Additionally, accuracy plots across epochs were used to evaluate learning convergence.



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E. Inference on Unseen Data

To simulate deployment, the trained model was tested on novel spinach leaf images not seen during training. Images were resized, converted to tensor format, and passed through the model to obtain class probabilities. The predicted class label was overlaid on the test image for visual verification. This phase validated the model's generalization ability on real-world inputs.

```
[99]:  
# Displaying the disease prediction  
model_prediction = class_name[result_index]  
plt.imshow(image)  
plt.title(f"Disease Name: {model_prediction}")  
plt.xticks([])  
plt.yticks([])  
plt.show()
```

Disease detection: Radish_healthy



```
[99]:  
# Displaying the disease prediction  
model_prediction = class_name[result_index]  
plt.imshow(image)  
plt.title(f"Disease Name: {model_prediction}")  
plt.xticks([])  
plt.yticks([])  
plt.show()
```

Disease detection: Radish_straw_mite



Color Feature Extraction

The average color composition of the leaf was extracted using the Python Imaging Library (PIL). The input image was first converted to RGB format and the mean intensity values for each color channel were computed from all pixels. These RGB values serve as a colorimetric indicator of the plant's condition.



Fig. 4.8: Raw input image of Lady's finger leaf used for feature extraction.

A. Resolution Estimation (DPI)

A known reference length (10 cm) in the image was used to estimate the resolution in dots per inch (DPI). This conversion is necessary to map pixel distances to real-world dimensions. The DPI was calculated using:

$$DPI = \frac{\text{Pixel Length}}{\text{Length in inches}}$$

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B. Morphological Feature Analysis

The physical features such as leaf area and perimeter were computed by converting the image to grayscale, applying Otsu's thresholding for binarization, and extracting contours using OpenCV. The area and perimeter in pixel units were transformed to centimeters using the estimated DPI:

$$Area(cm^2) = \frac{Pixel\ area * (2.54)^2}{DPI^2}$$

$$Perimeter(cm) = \frac{Pixel\ perimeter * 2.54}{DPI}$$

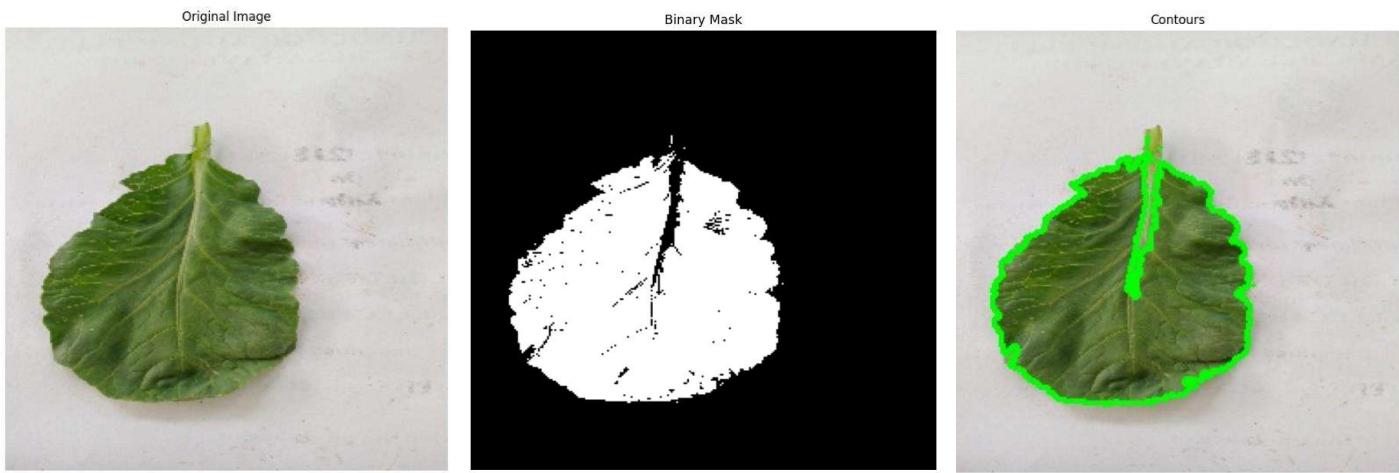


Fig 4.9 (a): Original leaf image

Fig 4.9 (b): Binary mask of leaf

Fig 4.9 (c): Leaf contours detected

--- Analysis Results ---

Metric	Value	Remarks
Average R	180.78	Mean Red intensity (0-255)
Average G	184.66	Mean Green intensity (0-255)
Average B	168.01	Mean Blue intensity (0-255)
Estimated DPI	76.20	Based on known 10 cm = 300 pixels
Total Image Pixels	65536.00	Total in 256x256 image
Leaf Pixels	15222.00	Pixels detected as leaf
Leaf Area (cm ²)	11.38	Using fixed scale (256px = 7 cm)
Leaf Perimeter (cm)	23.10	Using contours and conversion to cm

Tabulated output showing RGB intensity values, estimated DPI, and calculated geometric features such as total pixels, leaf area, and perimeter based on contour detection and pixel-to-cm conversion.

CHAPTER 4

RESULTS AND DISCUSSION

CASE 1:

4.1 PLASMA EXPOSURE ON lady's finger

Plasma treatment has shown a notable positive effect on seed germination and early plant growth. The process works by altering the seeds surface by making it easier for water and nutrients absorption by the seed coat. The reactive species generated during the plasma treatment enhance the seed's ability to absorb water, resulting in quicker and more efficient germination.

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Furthermore, plasma's antimicrobial effects help mitigate seed-borne pathogens, fostering healthier conditions for plant development. Collectively, these benefits support increased agricultural productivity and promote sustainable farming practices

As observed in the "Total Germinated Seeds" graph as shown in Figure 4.1, the group of seeds treated with plasma for 2 minutes has exhibited the highest number of germinated seeds compared to the ones which was left untreated. This indicates that the plasma- treated seeds responded more effectively, demonstrating a clear enhancement in their germination capacity.

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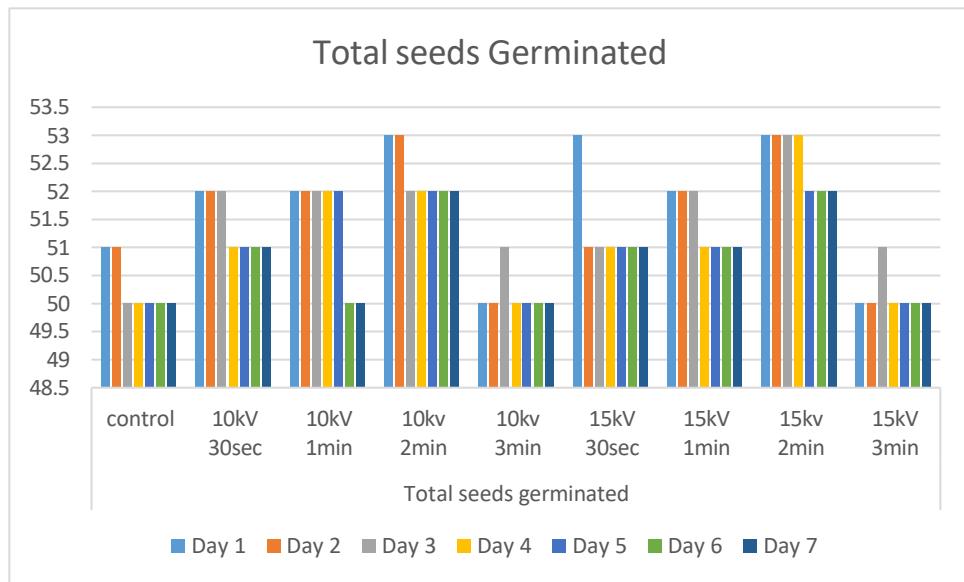


Figure 4.1: Graphical Representation of Total Germination Seeds

This improvement is due to the changes in the seed surface, which were made more porous and hydrophilic by the plasma treatment. These changes allow the seeds to take up water more efficiently, which is a essential element for initiating the germination process. By enhancing this fundamental step, plasma technology offers a promising, sustainable method to improve seed quality and agricultural productivity.

The germination rate was calculated by the equation

$$\text{GR (\%)} = (\text{NS} / \text{TS}) \times 100\%$$

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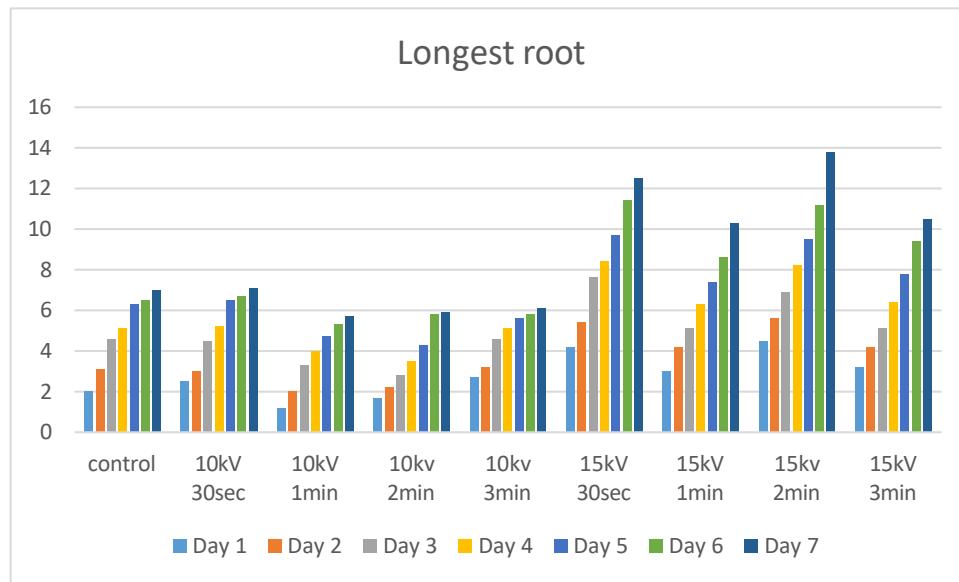


Figure 4.2: Graphical Representation of Longest Root

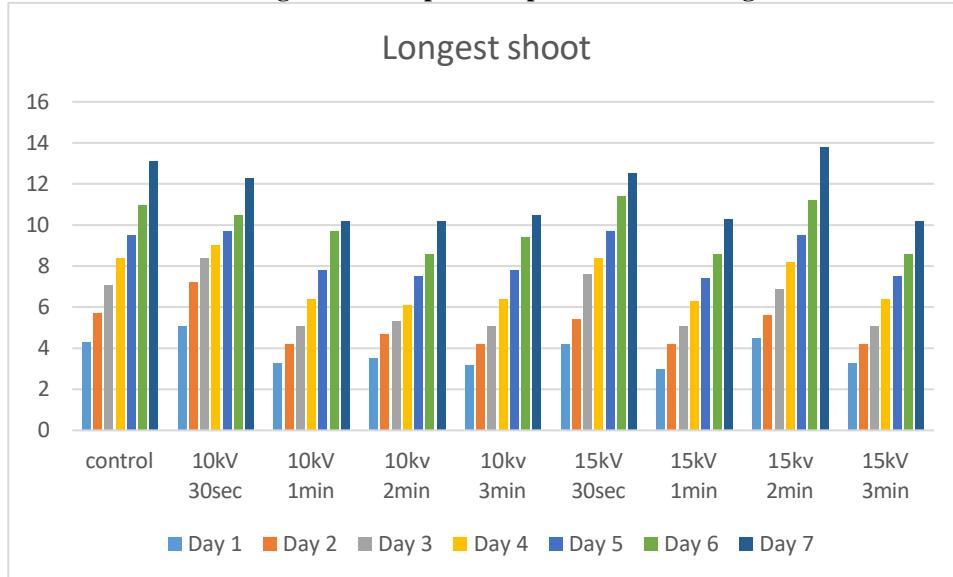


Figure 4.3: Graphical Representation of Longest Shoot

The analysis also demonstrated notable enhancements in root development, as depicted in Figures 4.2 and 4.3, underscoring the comprehensive benefits of plasma treatment. Statistically significant results, with a p-value of $p < 0.05$, validate plasma treatment as an effective and reliable method for enhancing seed germination and overall plant growth.

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4.1.2.1 FUNGUS INFECTED SEEDS

The graph of fungus-infected seeds highlights a sharp reduction in fungal contamination among plasma-treated seeds compared to the control group as shown in Figure 4.4. For instance, seeds treated with 4 minutes of plasma (VD 4min) showed the lowest infection rate, whereas untreated seeds had significantly higher fungal growth.

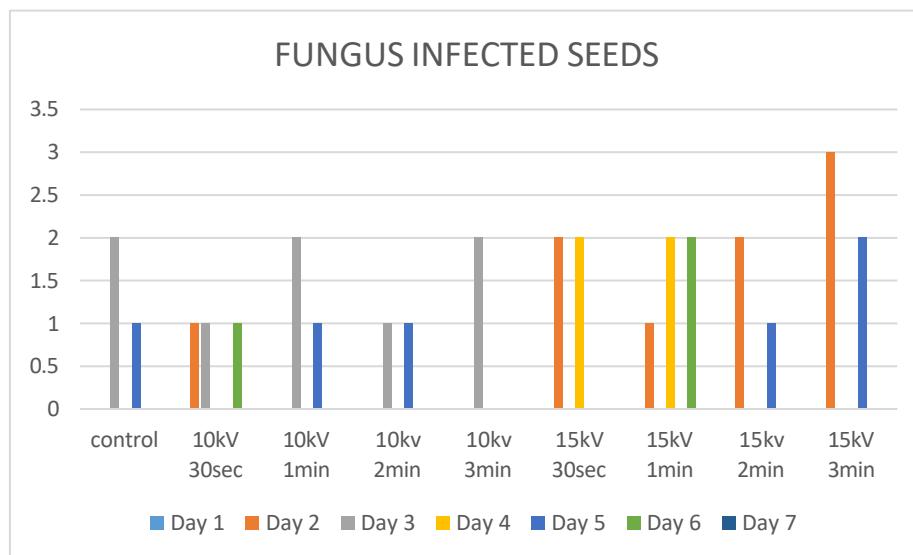


Figure 4.4: Graphical Representation of Fungus Infected Seeds

4.1.2.2 NON-GERMINATED SEEDS

The data presented in Figure 4.5 highlights that plasma exposure significantly enhances germination rates while reducing the proportion of non-viable seeds. Seeds subjected to plasma treatment demonstrated considerably lower non-germination rates, with the 4-minute treatment yielding the most favorable outcomes.

This reduction is likely attributed to improved water absorption, efficient nutrient uptake, and the elimination of germination inhibitors facilitated by the plasma treatment process. The results of this study highlight the significant advantages of plasma treatment in enhancing seed germination and plant growth. A comparative analysis between the control group (untreated seeds) and plasma-treated seeds subjected to 2 minutes (VD 2min) of exposure revealed several notable differences

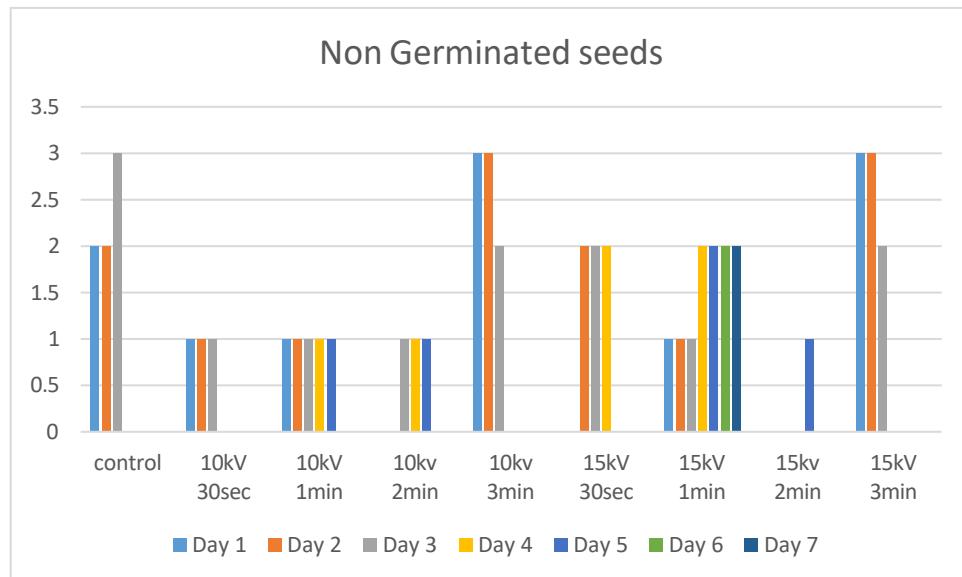


Figure 4.5: Graphical Representation of Non germinated Seeds

4.1.2.3 GERMINATION RATES

Plasma-treated seeds exhibited significantly improved germination rates, with the 8-minute treatment consistently achieving the highest results. The control group showed a germination rate of 65%, which increased to and reached a remarkable 100% for the 2-minute treatment (VD 2 min). These findings, illustrated in Figure 4.6, demonstrate the enhanced viability and readiness of seeds following plasma exposure.

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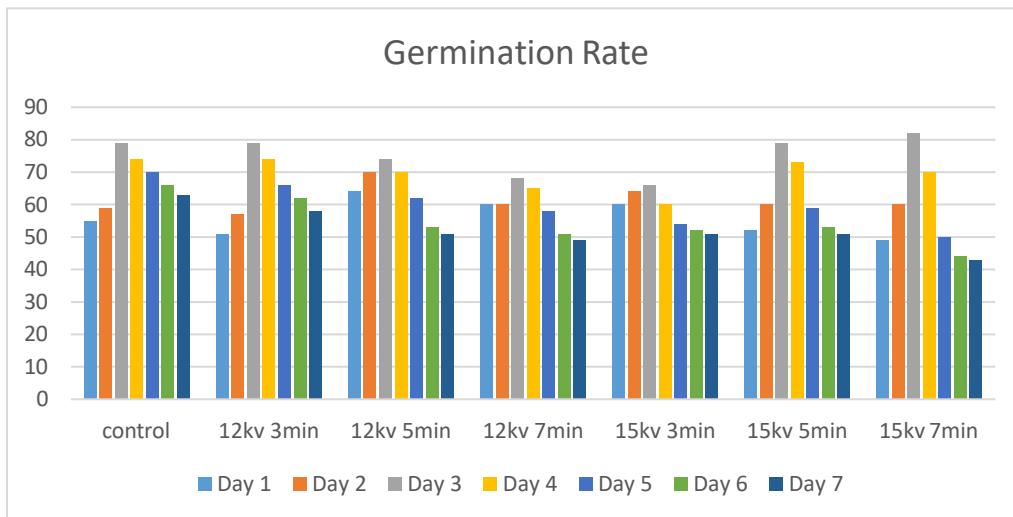


Figure 4.6: Germination Rate observed on Germination sheet

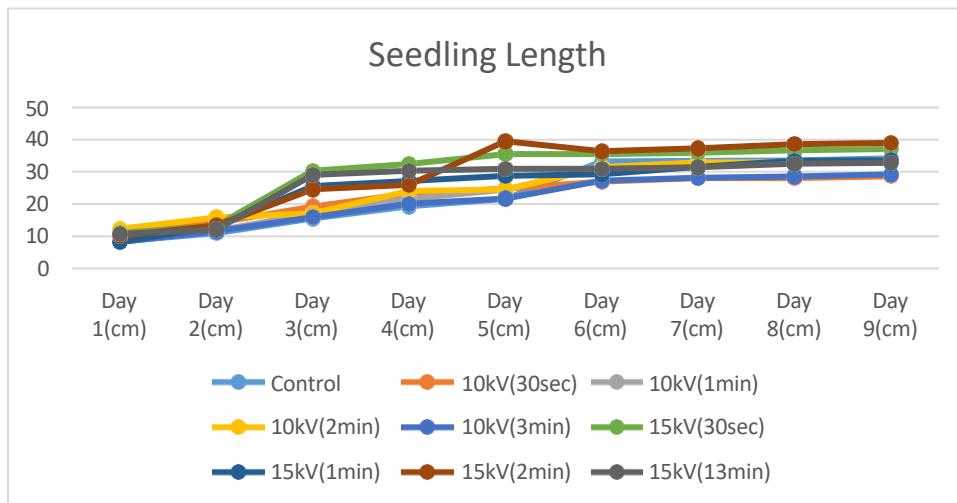


Figure 4.7: Seedling length observed on Germination sheet

Plasma treatment has proven to be a highly effective method for enhancing seed germination and early plant growth. Seeds exposed to plasma, particularly for 2 minutes, exhibited a 100% germination rate, significantly higher than the 65% observed in untreated seeds. The treatment also promoted superior root and shoot development, indicating improved nutrient uptake and cellular activity. Additionally, plasma exposure greatly reduced fungal infections and the number of non-germinated seeds, highlighting its antimicrobial benefits and positive effect on seed viability. These results collectively support plasma technology as a sustainable and efficient approach to improving agricultural productivity.

Application of electrical discharge cold plasma treatment on agricultural seeds: A biochemical and image processing approach

4.2 PLANT GROWTH ON GERMINATION SHEET AND ON GROUND



Day 1 - Untreated seeds (control seeds)



Day 1 - Treated seeds (15kV 2mins)



Day 14 - Untreated seeds (control seeds) Less germination



Day 14 - Treated seeds (15kV 2mins) More germination with longest root and shoot



Day 44- Treated seeds on ground



4.3 BIOCHEMICAL ANALYSIS

4.3.1 Phytochemical Screening Results

Phytochemical screening involves a series of qualitative tests that help detect the presence of key classes of bioactive compounds in seed extracts. These tests rely on chemical reactions between specific reagents and phytochemicals, often producing color changes or precipitates that indicate the presence of the compound.



Figure 4.8: Phytochemical screening

In our study, we performed a comparative phytochemical analysis of **untreated (control) Lady's finger and Lady's finger treated with plasma at 15kV for 2 minutes**.

The image above displays the test tubes containing extracts from beans, used for phytochemical screening. Each test tube corresponds to a specific phytochemical test, identified by labelled tags such as Alkaloids, Steroids, Saponins, Tannins, and Flavonoids.

These tests rely on specific chemical reactions that result in a visible colour change or precipitate formation, indicating the presence of a particular compound. These visual results align with the qualitative data summarized in the phytochemical table, confirming the presence of key bioactive compounds such as alkaloids, steroids, and saponins in both control and plasma-treated beans.

Observations:

- The most notable effect of **plasma treatment** was a **significant increase in Steroids** from weak (+) in the control to strong (+++) in the treated sample.
- **Alkaloids** also showed an increase from moderate (++) in the control to strong (+++) in the treated sample.
- These enhancements suggest that **plasma treatment stimulates the biosynthesis or extraction** of key bioactive compounds.

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- Flavonoids and tannins/phenolic compounds were absent in both cases.

The results are summarized in the table below:

Pytochemical Analysis	Lady's Finger (Control)	Lady's Finger (15kV,2min)
Alkaloids	++	+++
Flavonoids	-	-
Tannins and Phenolic Compounds	-	-
Steroids	+	+++
Saponins	+++	+++
Carbohydrates	+++	+++
Proteins	-	-

Figure 4.8: Results of Phytochemical screening

Note: (+++) = Strong presence and (-) = Absence

4.4 METHODOLOGY FOR LADY'S FINGER FEATURE EXTRACTION

The proposed system performs digital image-based feature extraction to estimate average color content, determine physical dimensions, and evaluate shape characteristics of a plant leaf image. The processing pipeline is divided into three main modules.

A. Color Feature Extraction

The average color composition of the leaf was extracted using the Python Imaging Library (PIL). The input image was first converted to RGB format and the mean intensity values for each color channel were computed from all pixels. These RGB values serve as a colorimetric indicator of the plant's condition.



Fig. 4.9: Raw input image of Lady's finger leaf used for feature extraction.

B. Resolution Estimation (DPI)

A known reference length (10 cm) in the image was used to estimate the resolution in dots per inch (DPI). This conversion is necessary to map pixel distances to real-world dimensions. The DPI was calculated using:

$$DPI = \frac{\text{Pixel Length}}{\text{Length in inches}}$$

C. Morphological Feature Analysis

The physical features such as leaf area and perimeter were computed by converting the image to grayscale, applying Otsu's thresholding for binarization, and extracting contours using OpenCV. The area and perimeter in pixel units were transformed to centimeters using the estimated DPI:

$$\text{Area(cm}^2\text{)} = \frac{\text{Pixel area} * (2.54)^2}{DPI^2} \quad \text{Perimeter(cm)} = \frac{\text{Pixel perimeter} * 2.54}{DPI}$$



Figure 4.10(a) : Original Leaf Image

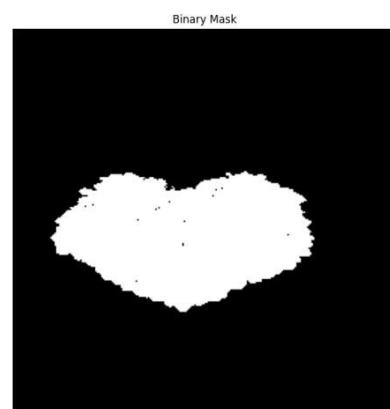


Figure 4.10(b) :Binary Mask of the Leaf

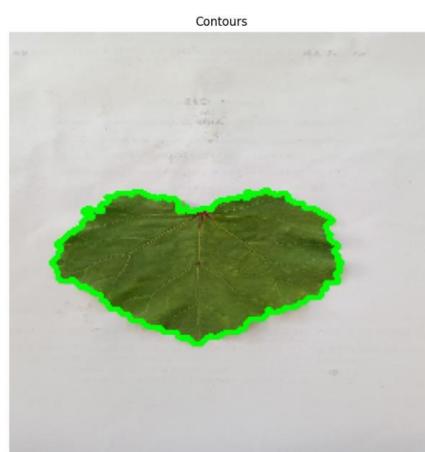


Figure 4.10(c): Leaf Contours Detected

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--- Analysis Results ---		
Metric	Value	Remarks
Average R	187.94	Mean Red intensity (0-255)
Average G	190.15	Mean Green intensity (0-255)
Average B	176.81	Mean Blue intensity (0-255)
Estimated DPI	76.20	Based on known 10 cm = 300 pixels
Total Image Pixels	65536.00	Total in 256x256 image
Leaf Pixels	11276.00	Pixels detected as leaf
Leaf Area (cm ²)	8.43	Using fixed scale (256px = 7 cm)
Leaf Perimeter (cm)	15.16	Using contours and conversion to cm

Figure 4.11 Tabulated output showing RGB intensity values, estimated DPI, and calculated geometric features such as total pixels, leaf area, and perimeter based on contour detection and pixel-to-cm conversion.

CASE 2:

4.5 DPPH RADICAL SCAVENGING ASSAY

To evaluate the antioxidant activity of spinach (*Spinacia oleracea*) extracts by measuring their ability to scavenge DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals.

Concentration ($\mu\text{g/mL}$)	Control Spinach (Abs)	Plasma-Treated Spinach (Abs)	DPPH Control (Abs)
20	0.750	0.620	1.000
40	0.680	0.540	1.000
60	0.620	0.470	1.000
80	0.570	0.420	1.000
100	0.520	0.380	1.000

Table 04: Absorbance Values of Control and Plasma-Treated Spinach Extracts and DPPH Control at Various Concentrations ($\mu\text{g/mL}$)

CHAPTER 4

RESULTS AND DISCUSSION

CASE I: RESULTS AND DISCUSSIONS OF EFFECT OF PLASMA TREATMENT ON FENUGREEK SEEDS

4.1 EFFECT OF PLASMA TREATMENT ON FENUGREEK SEED GERMINATION AND EARLY GROWTH

Plasma treatment demonstrated a significant positive effect on the germination and early growth of **fenugreek seeds**. This enhancement is primarily due to the **modification of the seed coat**, making it more permeable and thus improving water and nutrient uptake. As a result, plasma-treated seeds exhibited **faster and more efficient germination**, especially under optimized treatment conditions.

During plasma exposure, **reactive oxygen species (ROS)** and **reactive nitrogen species (RNS)** are generated. These include compounds such as **hydroxyl radicals ($\text{OH}\cdot$)**, **hydrogen peroxide (H_2O_2)**, **ozone (O_3)**, and **nitric oxide (NO)**, which interact with the seed surface to **increase permeability and stimulate biochemical processes** that promote seed vigor, root-shoot growth, and stress resilience.

Fenugreek seeds were treated using two plasma configurations: the **Volume Discharge (VD) Reactor** and the **Surface Discharge (SD) Reactor**. Treatments were conducted at **voltages of 12 kV and 15 kV** for **exposure durations of 10, 20, and 30 seconds**.

Parameters	Longest root (cm)	Shortest root (cm)	Longest shoot (cm)	Shortest shoot (cm)	Fungus (cm)	Non germinated (cm)	Total seeds germinated (cm)
Control	7.4	0.5	0.4	0.1	0	0	92
12kV 10sec (VD)	8.2	0.7	0.5	0.1	0	4	88
12kV 20sec (VD)	7.9	0.1	0.9	0.1	0	4	88
12kV 30sec (VD)	8.7	0.5	0.5	0.1	0	4	88

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15kV 10sec (VD)	7.2	0.3	0.6	0	0	2	90
15kV 20sec (VD)	7.8	1.5	0.5	0.1	1	1	90
15kV 30sec (VD)	7.2	0.7	0.5	0.2	0	3	89
12kV 10sec (SD)	9	0.8	0.3	0.1	0	0	92
12kV 20sec (SD)	7.2	0.8	0.2	0.1	1	2	90
12kV 30sec (SD)	7.2	0.2	0.4	0.1	0	4	88
15kV 10sec (SD)	8.5	0.6	0.6	0.2	0	2	90
15kV 20sec (SD)	7.1	0.6	0.6	0.1	0	3	89
15kV 30sec (SD)	9.2	0.4	0.4	0	0	6	86

Table 04: Day 1 Representation of Germination and Growth Data of Plasma-Treated Fenugreek Seeds

Parameters	Longest root (cm)	Shortest root (cm)	Longest shoot (cm)	Shortest shoot (cm)	Fungus (cm)	Non germinated (cm)	Total seeds germinated (cm)
Control	6.5	2.1	11.2	1.8	0	0	88
12kV 10sec (VD)	11	5	10.3	6.9	0	0	89
12kV 20sec (VD)	10.8	3.4	11	7.1	0	0	88
12kV 30sec (VD)	10.7	1.6	12	3.6	0	0	89
15kV 10sec (VD)	11.5	5	9.8	7	0	0	88
15kV 20sec (VD)	12.1	5.3	11.1	7.8	0	0	89
15kV 30sec (VD)	9.3	2.1	11.6	1.3	0	0	89
12kV 10sec (SD)	11.5	2.3	11.6	7	0	0	87
12kV 20sec (SD)	10.2	3	12.6	10.1	0	0	88
12kV 30sec (SD)	9.4	1.5	10.2	3.8	0	0	84
15kV 10sec (SD)	12	3.5	10.2	7.2	0	0	89
15kV 20sec (SD)	10.5	3	11.2	8.5	0	0	88
15kV 30sec (SD)	9.5	2.4	10.4	8.8	0	0	83

Table 05: Day 10 Representation of Germination and Growth Data of Plasma-Treated Fenugreek Seeds

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The data collected on **Day 1**(table 04) corresponds to the first observation made **three days after sowing**, when the germination sheets were initially opened. This early assessment captured the **initial seed response** to plasma treatment under different voltage-time combinations using both **Volume Discharge (VD)** and **Surface Discharge (SD)** reactors.

At this stage, parameters such as **root length, shoot length, presence of fungal infection, number of non-germinated seeds, and total germinated seeds** were recorded. While germination had already begun, the full extent of root and shoot development was still in its early stages. For instance, shoot lengths ranged between **0.2 to 0.9 cm**, and the longest root observed was **9.0 cm** under **12kV 10s (SD)**. Interestingly, the highest number of germinated seeds on Day 1 was seen in **control and 12kV 10s (SD)** conditions, both reaching **92 out of 100** seeds. Over the next **10 to 14 days**(table 05), regular daily observations were conducted to monitor progress in each of the recorded parameters. By **Day 10**, significant **growth and development** were observed across all plasma-treated groups:

- ✓ **Root lengths** improved notably, with some treatments reaching **over 12 cm**, especially under **15kV 20s (VD)** and **15kV 10s (SD)**.
- ✓ **Shoot growth** showed substantial improvement, with shoot lengths extending up to **12.6 cm** under **12kV 20s (SD)**.
- ✓ All treated groups showed **zero fungal contamination** and **zero non-germinated seeds** by Day 10, indicating improved seed health and uniform development.
- ✓ The **germination rate improved or remained consistent**, with most treatments maintaining a total germination of **88–90 seeds** out of 100.

Following the plasma treatment using the **Volume Discharge (VD) reactor**, seed growth was monitored daily for a period of **10 days**. The sum of **Longest Shoot + Longest Root (LS+LR)** was recorded as a combined growth parameter to assess overall seedling development.

The initial observation on **Day 1** showed modest differences among treatments. However, from **Day 2 onwards**, clear distinctions in growth trends emerged between treated and untreated (control) groups. Notably:

Seeds treated with **15kV for 20 seconds (VD)** showed a consistent and substantial increase in LS+LR values, reaching the **highest combined growth of 23.2 cm by Day 10**. Seeds treated at

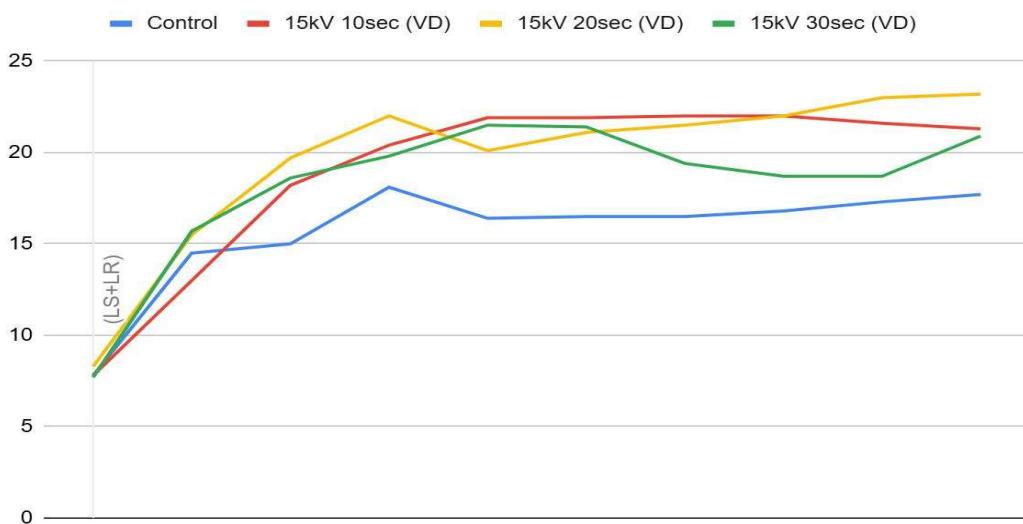
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12kV for 30 seconds (VD) also performed well, peaking at **22.7 cm** by Day 10. The **control group** remained behind, with a Day 10 LS+LR value of **17.7 cm**, highlighting the effectiveness of plasma treatment.

This dataset confirms that VD plasma treatment significantly improves seedling growth when optimized voltage and duration are applied. The **optimal condition for VD-treated fenugreek seeds** was found to be **15kV for 20 seconds**, yielding the most favorable growth performance.

	D 1	D 2	D3	D4	D5	D6	D7	D8	D9	D10
Control	7.8	14.5	15	18.1	16.4	16.5	16.5	16.8	17.3	17.7
12kV,10sec (VD)	8.7	13.5	16	18.7	17.4	17.8	19.3	20	20.7	21.3
12kV ,20sec (VD)	8.8	14.5	18	20.5	20.8	20.9	21	21.2	21.7	21.8
12kV, 30sec (VD)	9.2	14.4	18.6	21	21.3	21.5	21.7	22	22.2	22.7
15kV, 10sec (VD)	7.8	13	18.2	20.4	21.9	21.9	22	22	21.6	21.3
15kV ,20sec (VD)	8.3	15.5	19.7	22	20.1	21.1	21.5	22	23	23.2
15kV, 30sec (VD)	7.7	15.7	18.6	19.8	21.5	21.4	19.4	18.7	18.7	20.9

Table 06: Representation of Total Germination rate and Growth of Plasma-Treated Fenugreek Seeds under volume discharge (VD) reactor over 10 days



Graph 03: Graphical representation of the total germination rate of seeds treated under volume discharge (VD) reactor over 10 days

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Seeds treated using the **Surface Discharge (SD) reactor** were also analyzed across a 10-day period, with LS+LR (Longest Shoot + Root) used as the key indicator of seedling development.

From the first day of observation, plasma-treated groups displayed enhanced initial growth compared to the control. Significant highlights from the SD group include:

- ✓ The **12kV for 10 seconds (SD)** treatment showed the **highest LS+LR value of 23.1 cm** by Day 10, matching or outperforming other conditions.
- ✓ The **15kV for 10 seconds (SD)** treatment also demonstrated strong performance, reaching **22.2 cm** by Day 10.
- ✓ In contrast, the **control group** consistently lagged, recording a combined growth of only **17.7 cm** on Day 10.

These results confirm that plasma treatment under SD conditions enhances seedling vigor, especially when applied under the right voltage-time combination. The **optimal condition for SD-treated fenugreek seeds** was identified as **12kV for 10 seconds**.

The germination rate was calculated by the equation

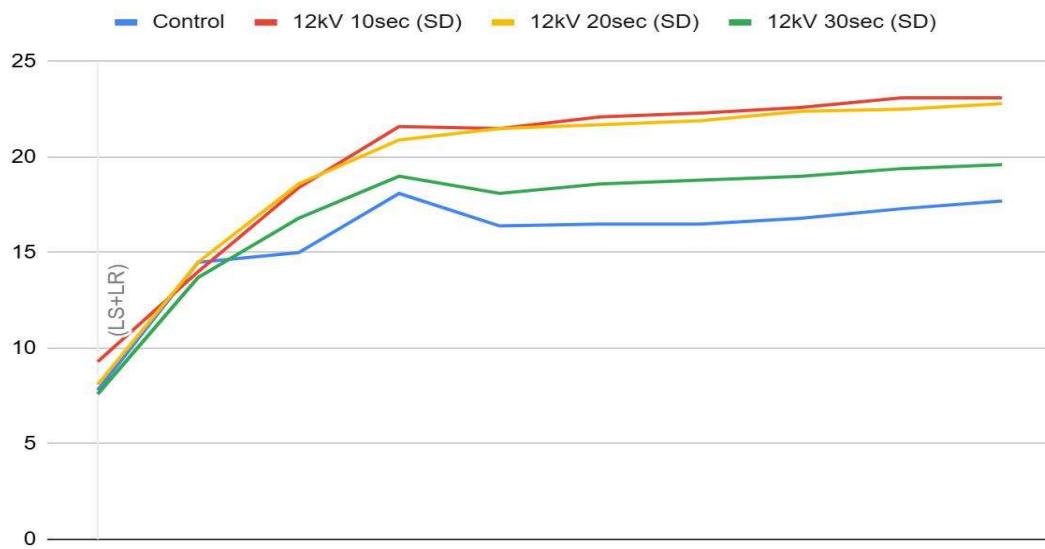
$$\text{GR (\%)} = (\text{NS} / \text{TS}) \times 100\%$$

where, GR = Germination Rate, NS = Number of seeds germinated , TS = Total number of seeds

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Control	7.8	14.5	15	18.1	16.4	16.5	16.5	16.8	17.3	17.7
12kV ,10sec (SD)	9.3	14	18.4	21.6	21.5	22.1	22.3	22.6	23.1	23.1
12kV, 20sec (SD)	8.1	14.5	18.6	20.9	21.5	21.7	21.9	22.4	22.5	22.8
12kV ,30sec (SD)	7.6	13.7	16.8	19	18.1	18.6	18.8	19	19.4	19.6
15kV ,10sec (SD)	9.1	14.1	18.5	21.8	21.5	21.7	22	22.3	22.2	22.2
15kV ,20sec (SD)	7.7	15.9	17.2	20	20.8	21.2	21.4	21.7	21.7	21.7
15kV ,30sec (SD)	9.6	14	17.7	18.9	19.3	19.5	19.5	19.6	19.6	19.9

Table 07:Representation of Total Germination rate and Growth of Plasma-Treated Fenugreek Seeds under surface discharge (SD) reactor over 10 days

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Graph 04: Graphical representation of the total germination rate of seeds treated under surface discharge (SD) reactor over 10 days

This data clearly indicates that **plasma treatment not only initiated early germination but also supported consistent and enhanced seedling development** over time. After analyzing performance across all conditions, the following were identified as the **optimal plasma treatment parameters** for the seeds by the end of the study:

Name of the seed	Optimal Voltage (VD)	Optimal Voltage (SD)
Fenugreek	15kV, 20sec	12kV, 10sec

Table 06 :Optimal Voltage-Time Combinations for Seed Plasma Treatment for fenugreek seed

These optimal parameters achieved the best balance of **germination rate, root and shoot growth, and overall plant health**, confirming the effectiveness of the selected plasma parameters.

4.1.1 SEED VIABILITY AND HEALTH ASSESSMENT

A comprehensive analysis of seed germination must account for challenges such as fungal infections and the presence of non-germinated seeds. Plasma treatment showed significant improvements in mitigating these issues, as evidenced by the graphs illustrating reductions in fungus-infected seeds and non-germinated seeds. This highlights the additional benefits of plasma treatment in promoting healthier seed germination conditions.

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4.2 FENUGREEK SEED GROWTH MONITORING FOR MORPHOLOGICAL ANALYSIS ON GERMINATION SHEET AND GROWTH RATE MONITORING OF SEED SOWN ON GROUND



Fig 4.2:Fenugreek Seed Growth Monitoring for Morphological Analysis on Germination Sheet and Growth Rate Monitoring of Seed Sowed on Ground

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4.3 BIOCHEMICAL ANALYSIS

Biochemical analysis was performed to identify and compare the presence of key phytochemicals such as alkaloids, flavonoids, phenols, proteins, saponins, steroids, and carbohydrates in both plasma-treated and control seed samples. This analysis helped evaluate the effect of plasma on enhancing the phytochemical profile of the seeds.

4.3.1 Extraction Process for Fenugreek Seeds (Soxhlet Method)

For fenugreek seeds, the **Soxhlet extraction method** was employed due to its efficiency in extracting heat-stable phytochemicals in large quantities. The process involved the following steps:

Seed Preparation:

Cleaned fenugreek seeds were shade-dried and ground into fine powder using mortar and pestle.

Soxhlet Extraction:

About 10g of fenugreek seed powder was packed into a thimble and placed in the Soxhlet apparatus. **Ethanol** was used as the solvent. The solvent was heated, vaporized, and condensed repeatedly to allow continuous extraction over several hours (typically 6–8 hours) until the solvent in the siphon tube appeared clear.



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Fig 4.3.1 : Extraction process done for fenugreek seed with the help of the guide at JSS campus(Life Science Laboratory) by Soxhlet Extraction Method

Post-extraction Procedure:

The extracted solution was collected and concentrated using a water bath to evaporate excess solvent. The thick residue was then scraped, collected, and stored in **sterile microtubes (MTC tubes)** under refrigerated conditions for further biochemical testing. This method ensured a thorough extraction of active compounds like flavonoids, alkaloids, tannins, and saponins from the fenugreek seeds.

4.3.2 Phytochemical Test Results for Fenugreek and Other Selected Seeds

Qualitative phytochemical screening was performed on ethanolic and methanolic extracts of various seeds (both control and plasma-treated) including **fenugreek, spinach, radish, beans, hyacinth, and lady's finger**. The tests were conducted using standard reagents to detect the presence of:

1. **Alkaloids** (Wagner's test)
2. **Flavonoids** (Alkaline reagent test)
3. **Tannins and Phenolic Compounds** (Ferric chloride test)
4. **Steroids** (Salkowski test)
5. **Saponins** (Froth test)
6. **Carbohydrates** (Fehling's test)
7. **Proteins** (Ninhydrin test)

Observations :In fenugreek seeds (ethanolic extract), both control and treated samples showed strong presence (+++) of **alkaloids, flavonoids, tannins, saponins, carbohydrates, and proteins**.

Phytochemical Analysis	Ethanol
	Fenugreek (control)
Alkaloids	+++
Flavonoids	+++
Tannins and Phenolic Compounds	+++
Steroids	+++
Saponins	+++
Carbohydrates	+++
Proteins	+++

Table 07 :Phytochemical Test Results of Control(untreated) fenugreek seed

Phytochemical Analysis	Ethanol
	Fenugreek (control)
Alkaloids	+++
Flavonoids	-
Tannins and Phenolic Compounds	++
Steroids	+++
Saponins	+++
Carbohydrates	+++
Proteins	+++

Table 08:Phytochemical Test Results of Plasma Exposed(treated) fenugreek seed

Where (+++) indicates the phytochemical is Strongly present ,(++) indicates the phytochemical is Moderately present & (-) indicates the phytochemical is Absent.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 PLASMA EXPOSURE ON SPINACH

Plasma treatment has shown a notable positive effect on seed germination and early plant growth. The process works by altering the seeds surface by making it easier for water and nutrients absorption by the seed coat. The reactive species generated during the plasma treatment enhance the seed's ability to absorb water, resulting in quicker and more efficient germination.

Plasma contains reactive species such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), which play a crucial role in enhancing plant growth and development. Key ROS, including oxygen radicals ($O_2\cdot$), hydroxyl radicals ($OH\cdot$), hydrogen peroxide (H_2O_2), and ozone (O_3), along with RNS like nitric oxide (NO), nitrogen dioxide (NO_2), and peroxy nitrite ($ONOO^-$), improve seed germination by increasing seed coat permeability and water absorption. These species promote root and shoot growth by facilitating nutrient uptake and cell division, while also enhancing stress tolerance and disease resistance through the activation of plant defense responses.

Furthermore, plasma's antimicrobial effects help mitigate seed-borne pathogens, fostering healthier conditions for plant development. Collectively, these benefits support increased agricultural productivity and promote sustainable farming practices

As observed in the "Total Germinated Seeds" graph as shown in Figure 4.1, the group of seeds treated with plasma for 4 minutes has exhibited the highest number of germinated seeds compared to the ones which was left untreated. This indicates that the plasma- treated seeds responded more effectively, demonstrating a clear enhancement in their germination capacity.

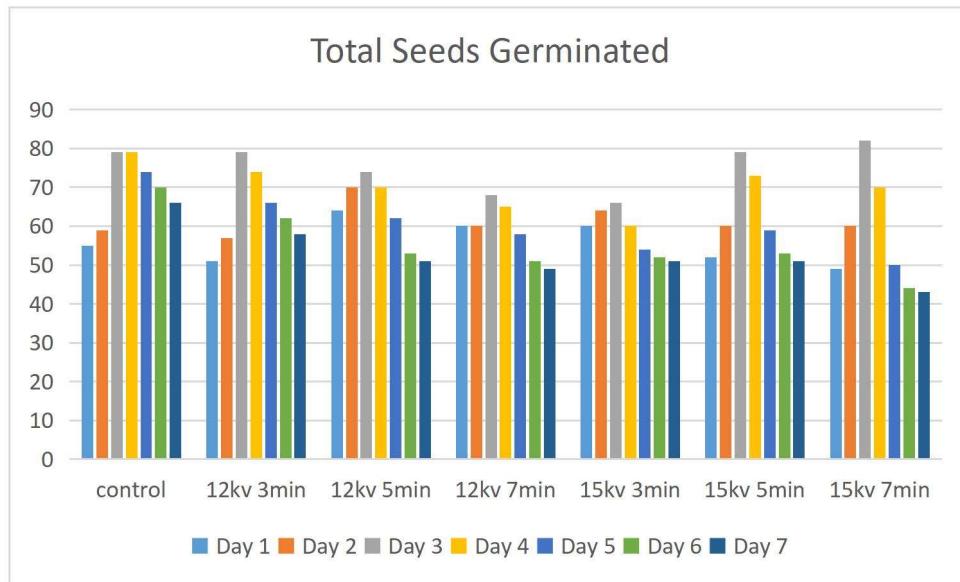


Figure 4.1: Graphical Representation of Total Germination Seeds

This improvement is due to the changes in the seed surface, which were made more porous and hydrophilic by the plasma treatment. These changes allow the seeds to take up water more efficiently, which is a essential element for initiating the germination process. By enhancing this fundamental step, plasma technology offers a promising, sustainable method to improve seed quality and agricultural productivity.

The germination rate was calculated by the equation

$$\text{GR (\%)} = (\text{NS} / \text{TS}) \times 100\%$$

where,

GR = Germination Rate

NS = Number of seeds germinated

TS = Total number of seeds

The effect of plasma treatment on plant growth is evident in parameters such as shoot length, and root length as illustrated in Figures 4.2 and 4.3. Seeds treated with plasma consistently produced plants with significantly longer shoots compared to the control group. Notably, seeds exposed to an 4-minute plasma treatment (VD 4 min) exhibited the highest average shoot length, whereas untreated seeds showed considerably shorter shoots. This observed trend highlights the enhanced growth potential enabled by plasma treatment, further supporting its effectiveness in improving plant development

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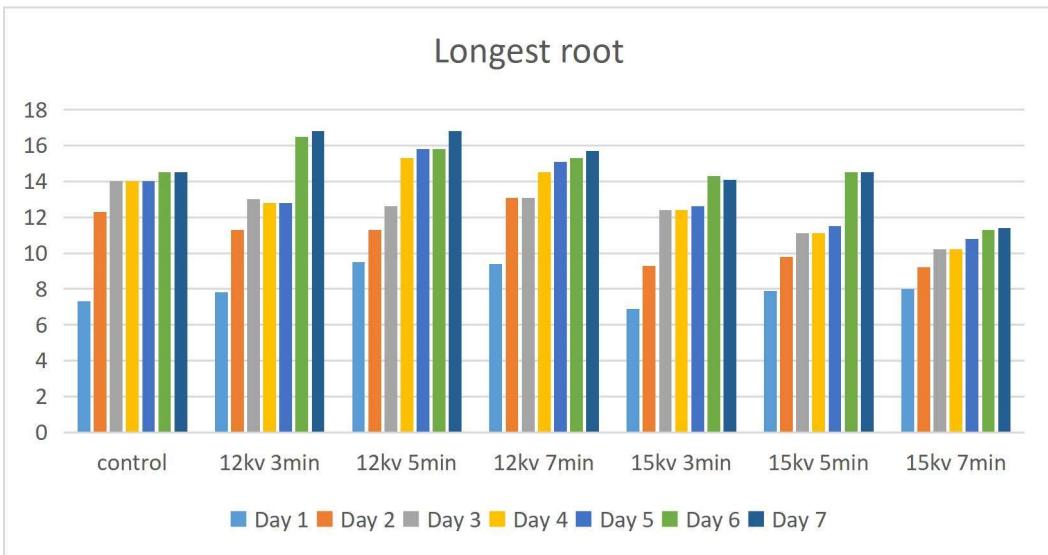


Figure 4.2: Graphical Representation of Longest Root

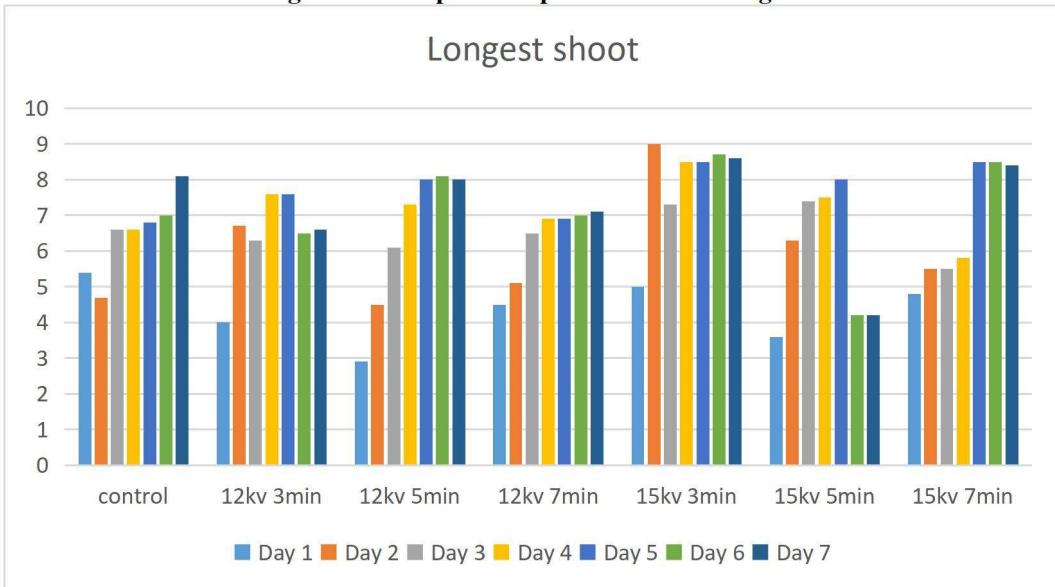


Figure 4.3: Graphical Representation of Longest Shoot

The analysis also demonstrated notable enhancements in root development, as depicted in Figures 4.2 and 4.3, underscoring the comprehensive benefits of plasma treatment. Statistically significant results, with a p-value of $p < 0.05$, validate plasma treatment as an effective and reliable method for enhancing seed germination and overall plant growth.

4.1.2 SEED VIABILITY AND HEALTH ASSESSMENT

A comprehensive analysis of seed germination must account for challenges such as fungal infections and the presence of non-germinated seeds. Plasma treatment showed significant improvements in mitigating these issues, as evidenced by the graphs illustrating reductions in fungus-infected seeds and non-germinated seeds. This highlights the additional benefits of plasma treatment in promoting healthier seed germination conditions.

4.1.2.1 FUNGUS INFECTED SEEDS

The graph of fungus-infected seeds highlights a sharp reduction in fungal contamination among plasma-treated seeds compared to the control group as shown in Figure 4.4. For instance, seeds treated with 4 minutes of plasma (VD 4min) showed the lowest infection rate, whereas untreated seeds had significantly higher fungal growth.

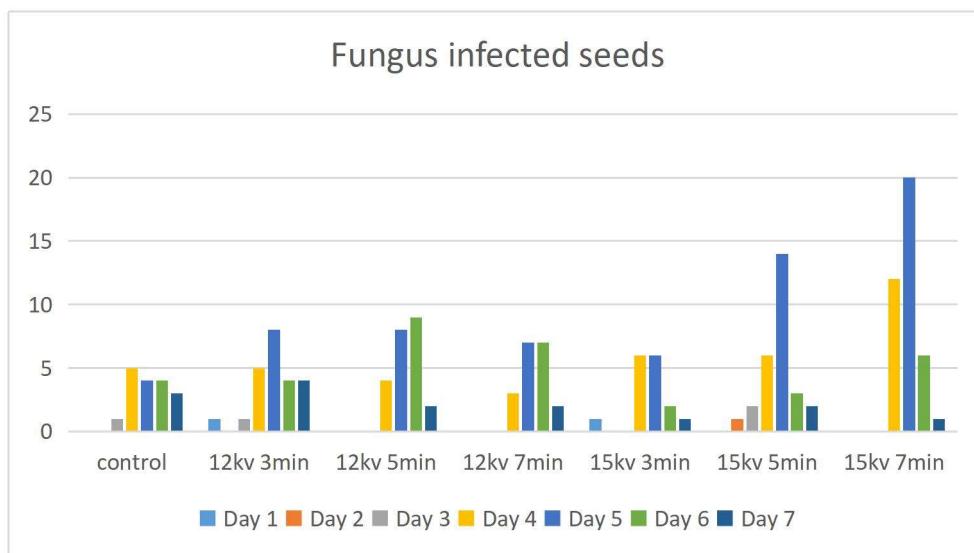


Figure 4.4: Graphical Representation of Fungus Infected Seeds

4.1.2.2 NON- GERMINATED SEEDS

The data presented in Figure 4.5 highlights that plasma exposure significantly enhances germination rates while reducing the proportion of non-viable seeds. Seeds subjected to plasma treatment demonstrated considerably lower non-germination rates, with the 4-minute treatment yielding the most favorable outcomes.

This reduction is likely attributed to improved water absorption, efficient nutrient uptake, and the elimination of germination inhibitors facilitated by the plasma treatment process. The results of this study highlight the significant advantages of plasma treatment in enhancing seed germination and plant growth. A comparative analysis between the control group (untreated seeds) and plasma-treated seeds subjected to 4 minutes (VD 4min) of exposure revealed several notable differences

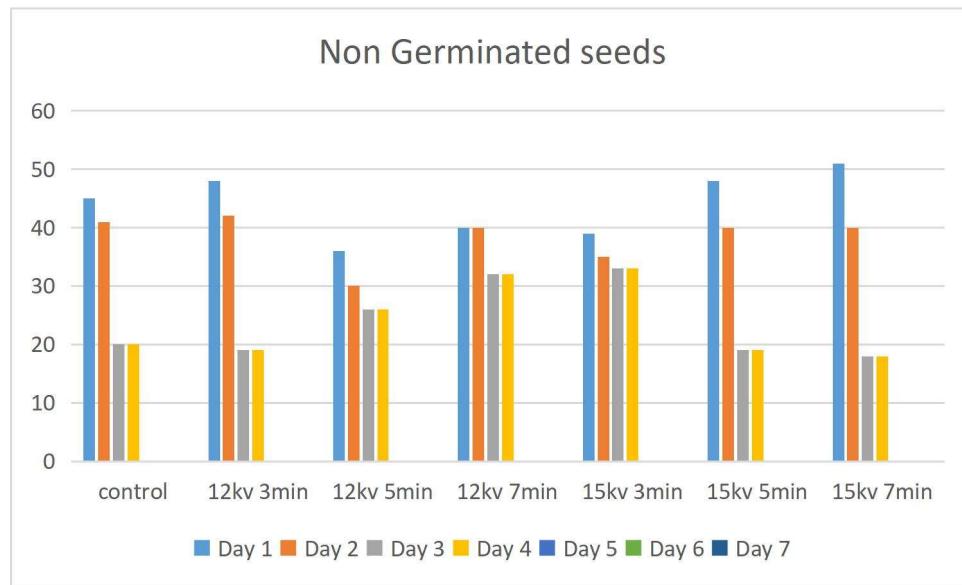


Figure 4.5: Graphical Representation of Non germinated Seeds

4.1.2.3 GERMINATION RATES

Plasma-treated seeds exhibited significantly improved germination rates, with the 8-minute treatment consistently achieving the highest results. The control group showed a germination rate of 65%, which increased to and reached a remarkable 100% for the 4-minute treatment (VD 4 min). These findings, illustrated in Figure 4.6, demonstrate the enhanced viability and readiness of seeds following plasma exposure.

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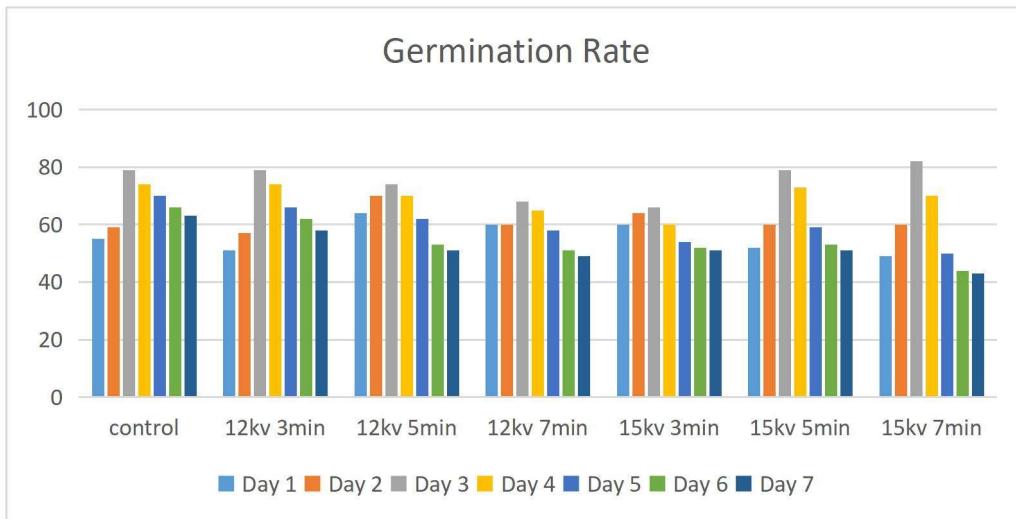


Figure 4.6: Germination Rate observed on Germination sheet

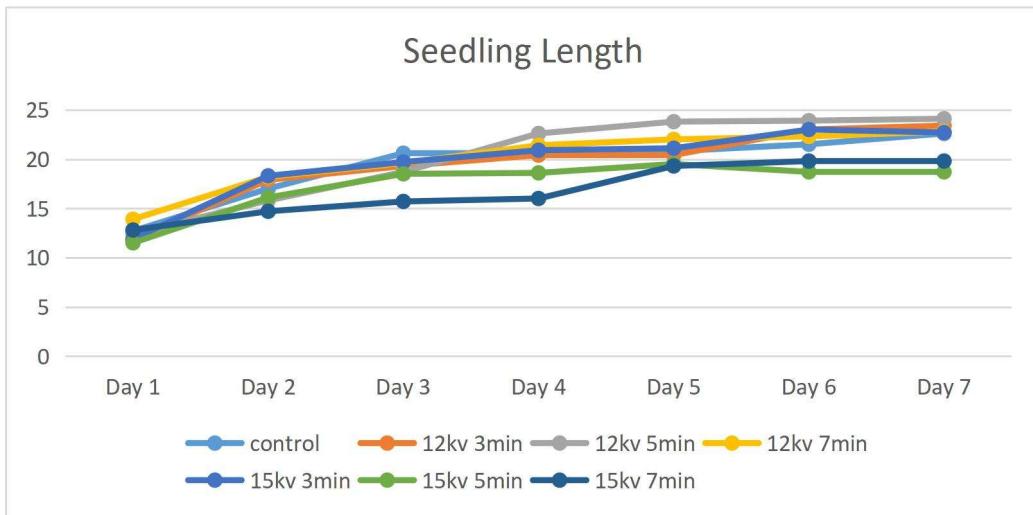


Figure 4.7: Seedling length observed on Germination sheet

Plasma treatment has proven to be a highly effective method for enhancing seed germination and early plant growth. Seeds exposed to plasma, particularly for 4 minutes, exhibited a 100% germination rate, significantly higher than the 65% observed in untreated seeds. The treatment also promoted superior root and shoot development, indicating improved nutrient uptake and cellular activity. Additionally, plasma exposure greatly reduced fungal infections and the number of non-germinated seeds, highlighting its antimicrobial benefits and positive effect on seed viability. These results collectively support plasma technology as a sustainable and efficient approach to improving agricultural productivity.

4.2 PLANT GROWTH ON GERMINATION SHEET AND ON GROUND



Figure 4.8: Day 1 - Untreated seeds (control seeds)



Figure 4.9: Day 1 - Treated seeds (12kV 3mins)



Figure 4.10: Day 14 - Untreated seeds (control seeds) Less germination



**Figure 4.11: Day 14 - Treated seeds (12kV 3 mins)
More germination with longest root and shoot**



Figure 4.12: Day 12 – Treated seeds on ground

4.3 BIOCHEMICAL ANALYSIS

4.3.1 Phytochemical Screening Results

Phytochemical screening involves a series of qualitative tests that help detect the presence of key classes of bioactive compounds in seed extracts. These tests rely on chemical reactions between specific reagents and phytochemicals, often producing color changes or precipitates that indicate the presence of the compound.



Figure 4.13: Phytochemical screening

In our study, we performed a comparative phytochemical analysis of **untreated (control)** beans and beans treated with plasma at 12 kV for 3 minutes. The figure above displays the test tubes containing extracts from spinach, used for phytochemical screening. Each test tube corresponds to a specific phytochemical test, identified by labelled tags such as Alkaloids, Steroids, Saponins, Tannins, and Flavonoids. These tests depend on specific chemical reactions that cause a visible colour change or precipitate formation, indicating the presence of the targeted phytochemical compounds. The observed results align with the qualitative data summarized in the phytochemical table, confirming the presence or absence of various bioactive compounds in both control and plasma-treated spinach samples.

Observations:

- The phytochemical screening showed no significant difference in the presence of compounds between the control and plasma-treated spinach.
- Alkaloids, saponins, and proteins were consistently detected in both control and plasma-treated samples, as indicated by characteristic colour changes or precipitate

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formation.

- Steroids and carbohydrates were faintly present or borderline, with weak reactions observed, suggesting low concentration or borderline detection.
- Flavonoids and tannins/phenolic compounds were absent in both control and plasma-treated spinach extracts, as no colour change or precipitate was observed in the corresponding tests.

These results indicate that key phytochemicals such as alkaloids and saponins are retained after plasma treatment in spinach, similar to the findings in beans. However, the absence of flavonoids and tannins suggests a consistent phytochemical profile irrespective of plasma exposure.

The results are summarized in the table below:

Photochemical Analysis	Ethaonl
	Spinach (Control)
Alkaloids	+++
Flavonoids	+++
Tannins and Phenolic compounds	+++
Steroids	++
Saponins	+
Carbohydrates	+++
Proteins	+

Figure 4.14: Results of Phytochemical screening

Note: (+++) = Strong presence and (-) = Absence

CHAPTER 4

RESULTS AND DISCUSSION

CASE I: RESULTS AND DISCUSSIONS OF EFFECT OF PLASMA TREATMENT ON HYACINTH BEANS SEEDS

4.1 PLASMA EXPOSURE ON HYACINTH BEANS

Plasma treatment has shown a notable positive effect on seed germination and early plant growth. The process works by altering the seeds surface by making it easier for water and nutrients absorption by the seed coat. The reactive species generated during the plasma treatment enhance the seed's ability to absorb water, resulting in quicker and more efficient germination.

Plasma contains reactive species such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), which play a crucial role in enhancing plant growth and development. Key ROS, including oxygen radicals ($O_2\cdot$), hydroxyl radicals ($OH\cdot$), hydrogen peroxide (H_2O_2), and ozone (O_3), along with RNS like nitric oxide (NO), nitrogen dioxide (NO_2), and peroxynitrite ($ONOO^-$), improve seed germination by increasing seed coat permeability and water absorption. These species promote root and shoot growth by facilitating nutrient uptake and cell division, while also enhancing stress tolerance and disease resistance through the activation of plant defense responses.

Furthermore, plasma's antimicrobial effects help mitigate seed-borne pathogens, fostering healthier conditions for plant development. Collectively, these benefits support increased agricultural productivity and promote sustainable farming practices

As observed in the "Total Germinated Seeds" graph as shown in Figure 4.1, the group of seeds treated with plasma for 4 minutes has exhibited the highest number of germinated seeds compared to the ones which was left untreated. This indicates that the plasma- treated seeds responded more effectively, demonstrating a clear enhancement in their germination capacity.

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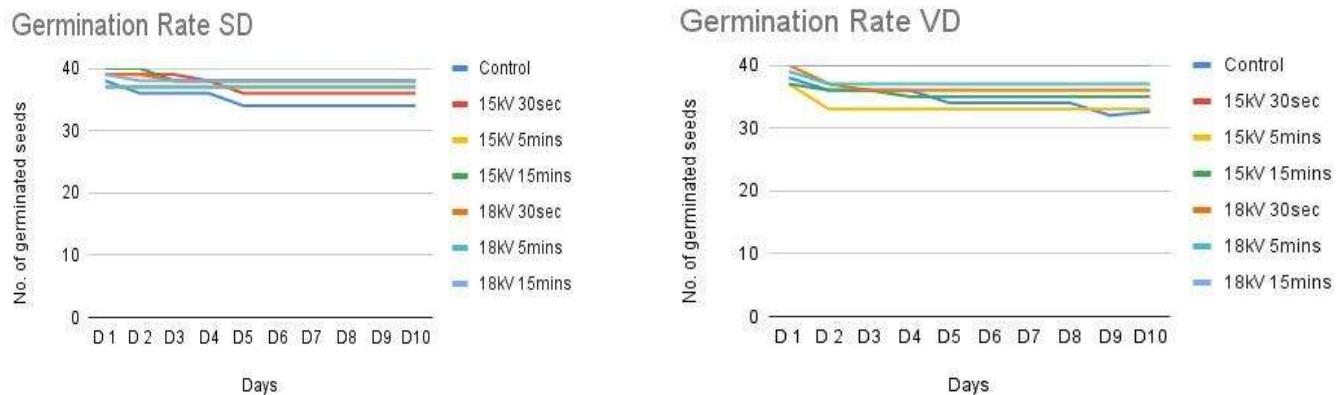


Figure 4.1: Graphical Representation of Total Germination Seeds

This improvement is due to the changes in the seed surface, which were made more porous and hydrophilic by the plasma treatment. These changes allow the seeds to take up water more efficiently, which is a essential element for initiating the germination process. By enhancing this fundamental step, plasma technology offers a promising, sustainable method to improve seed quality and agricultural productivity.

The germination rate was calculated by the equation

$$\text{GR (\%)} = (\text{NS} / \text{TS}) \times 100\%$$

where,

GR = Germination Rate

NS = Number of seeds germinated

TS = Total number of seeds

The effect of plasma treatment on plant growth is evident in parameters such as shoot length, and root length as illustrated in Figures 4.2 and 4.3. Seeds treated with plasma consistently produced plants with significantly longer shoots compared to the control group. Notably, seeds exposed to an 4-minute plasma treatment (VD 4 min) exhibited the highest average shoot length, whereas untreated seeds showed considerably shorter shoots. This observed trend highlights the enhanced growth potential enabled by plasma treatment, further supporting its effectiveness in improving plant development

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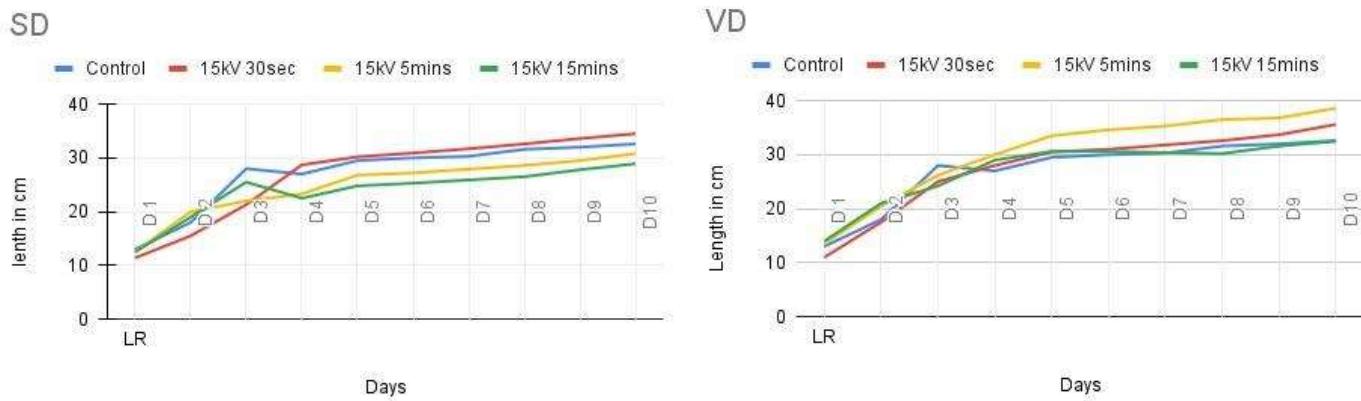


Figure 4.2: Graphical Representation of Longest Root



Figure 4.3: Graphical Representation of Longest Shoot

The analysis also demonstrated notable enhancements in root development, as depicted in Figures 4.2 and 4.3, underscoring the comprehensive benefits of plasma treatment. Statistically significant results, with a p-value of $p < 0.05$, validate plasma treatment as an effective and reliable method for enhancing seed germination and overall plant growth.

4.1.1 SEED VIABILITY AND HEALTH ASSESSMENT

A comprehensive analysis of seed germination must account for challenges such as fungal infections and the presence of non-germinated seeds. Plasma treatment showed significant improvements in mitigating these issues, as evidenced by the graphs illustrating reductions in fungus-infected seeds and non-germinated seeds. This highlights the additional benefits of plasma treatment in promoting healthier seed germination conditions.

4.1.1.1 FUNGUS INFECTED SEEDS

The graph of fungus-infected seeds highlights a sharp reduction in fungal contamination among plasma-treated seeds compared to the control group as shown in Figure 4.4. For instance, seeds treated with 4 minutes of plasma (VD 4min) showed the lowest infection rate, whereas untreated seeds had significantly higher fungal growth.

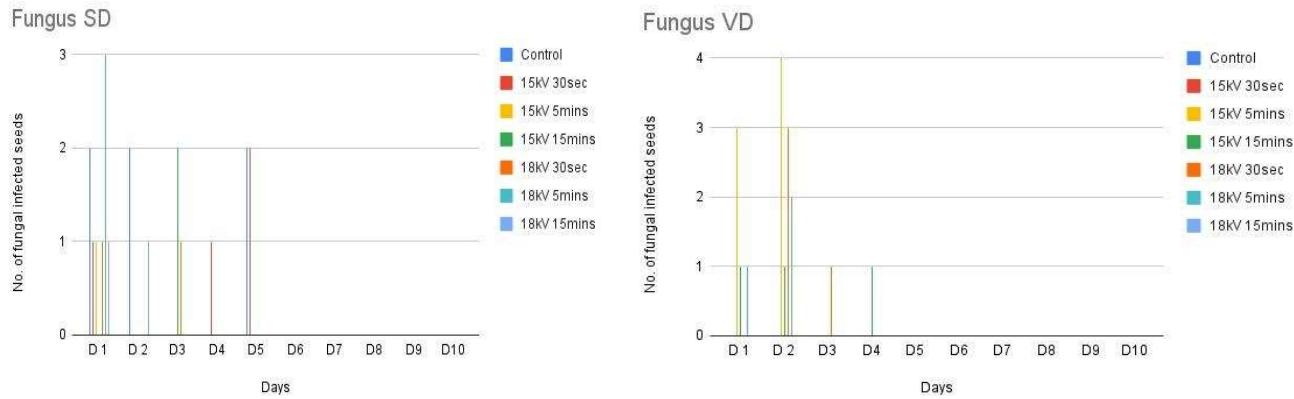


Figure 4.4: Graphical Representation of Fungus Infected Seeds

4.1.1.2 NON- GERMINATED SEEDS

The data presented in Figure 4.5 highlights that plasma exposure significantly enhances germination rates while reducing the proportion of non-viable seeds. Seeds subjected to plasma treatment demonstrated considerably lower non-germination rates, with the 4-minute treatment yielding the most favorable outcomes.

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This reduction is likely attributed to improved water absorption, efficient nutrient uptake, and the elimination of germination inhibitors facilitated by the plasma treatment process. The results of this study highlight the significant advantages of plasma treatment in enhancing seed germination and plant growth. A comparative analysis between the control group (untreated seeds) and plasma-treated seeds subjected to 4 minutes (VD 4min) of exposure revealed several notable differences

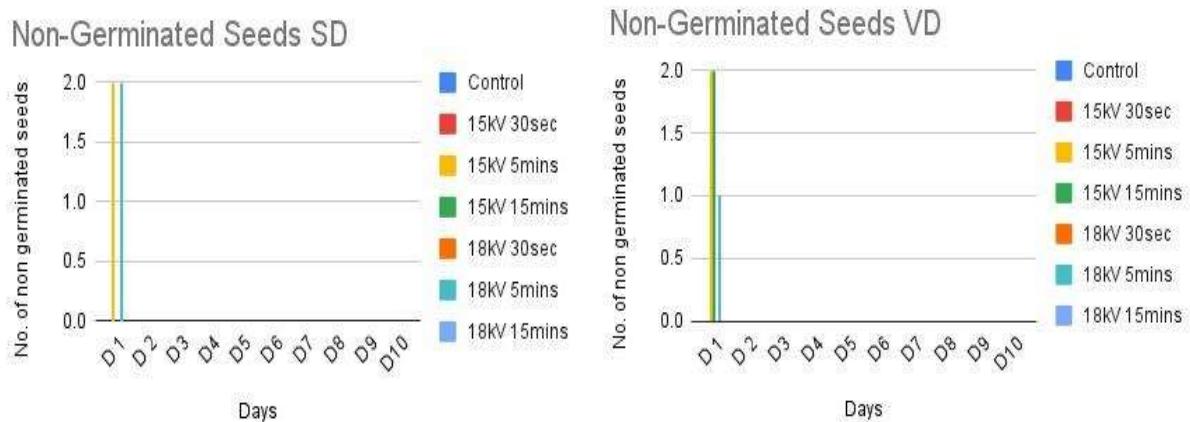


Figure 4.5: Graphical Representation of Non germinated Seeds

4.1.1.3 GERMINATION RATES

Plasma-treated seeds exhibited significantly improved germination rates, with the 8-minute treatment consistently achieving the highest results. The control group showed a germination rate of 65%, which increased to and reached a remarkable 100% for the 4-minute treatment (VD 4 min). These findings, illustrated in Figure 4.6, demonstrate the enhanced viability and readiness of seeds following plasma exposure.

Application of electrical discharge cold plasma treatment on agricultural seeds: A biochemical and image processing approach

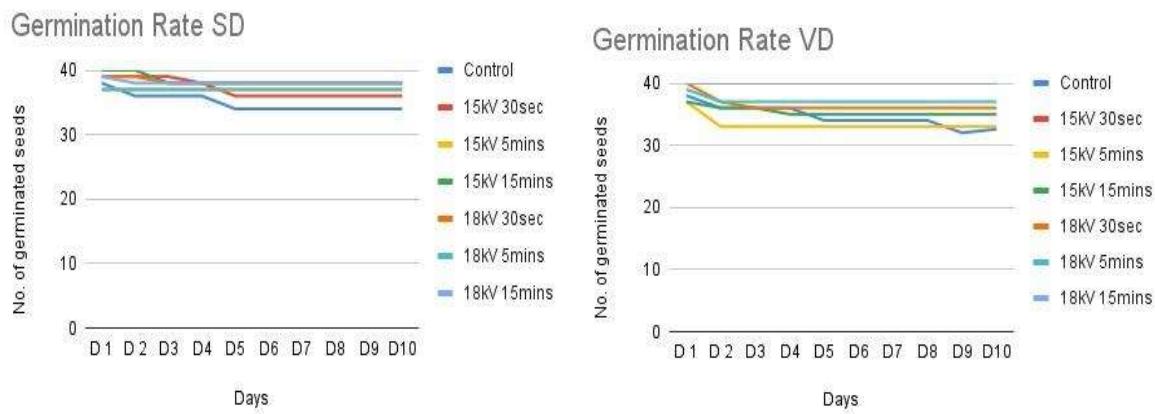


Figure 4.6: Germination Rate observed on Germination sheet

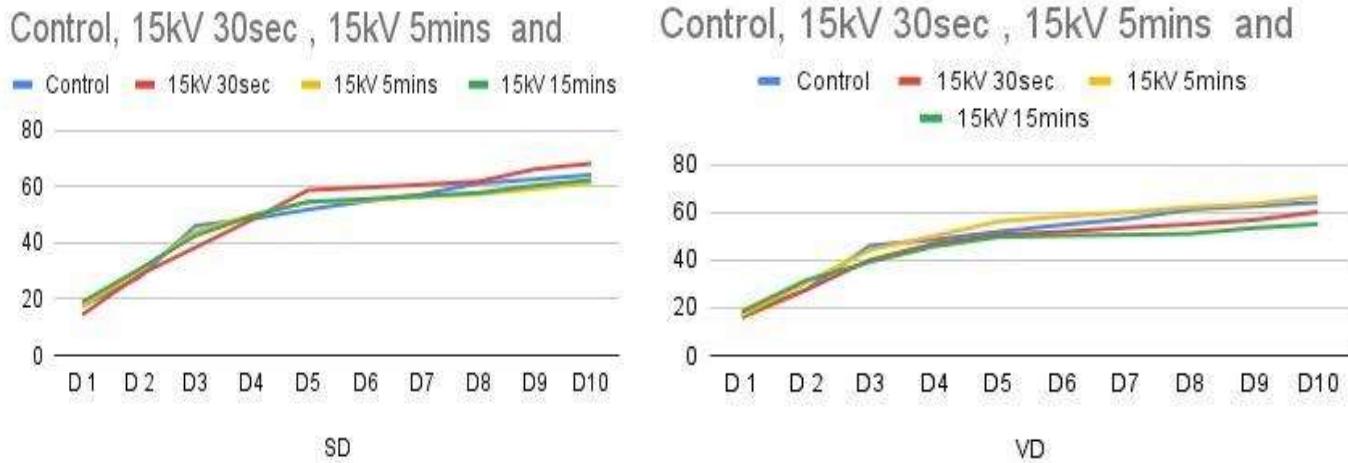


Figure 4.7: Seedling length observed on Germination sheet

Plasma treatment has proven to be a highly effective method for enhancing seed germination and early plant growth. Seeds exposed to plasma, particularly for 4 minutes, exhibited a 100% germination rate, significantly higher than the 65% observed in untreated seeds. The treatment also promoted superior root and shoot development, indicating improved nutrient uptake and cellular activity. Additionally, plasma exposure greatly reduced fungal infections and the number of non-germinated seeds, highlighting its antimicrobial benefits and positive effect on seed viability. These results collectively support plasma technology as a sustainable and efficient approach to improving agricultural productivity.

4.1.1.4 PLANT GROWTH ON GERMINATION SHEET AND ON GROUND



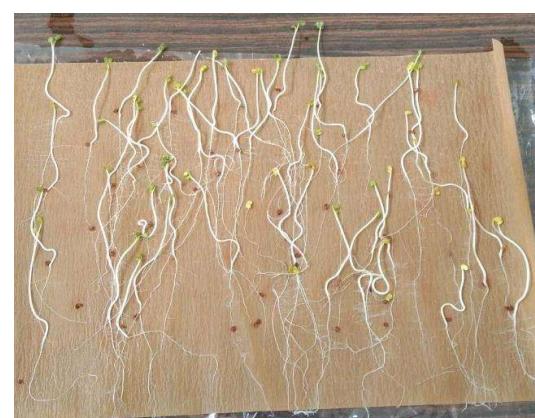
Day 1 - Untreated seeds (control seeds)



Day 1 - Treated seeds (15kV 4mins)



**Day 4 - Untreated seeds (control seeds)
Less germination**



**Day 4 - Treated seeds (15kV 4mins)
More germination with longest root
and shoot**



Day 7 - Treated seeds (15kV 4mins) More germination with longest root and shoot



Day 12 – Treated seeds on ground

4.1 BIOCHEMICAL ANALYSIS

4.2.1 Extraction Process for Fenugreek Seeds (Soxhlet Method)

For fenugreek seeds, the **Soxhlet extraction method** was employed due to its efficiency in extracting heat-stable phytochemicals in large quantities. The process involved the following steps:

Seed Preparation:

Cleaned fenugreek seeds were shade-dried and ground into fine powder using mortar and pestle.

Soxhlet Extraction:

About 10g of fenugreek seed powder was packed into a thimble and placed in the Soxhlet apparatus.

Ethanol was used as the solvent. The solvent was heated, vaporized, and condensed repeatedly to allow continuous extraction over several hours (typically 6–8 hours) until the solvent in the siphon tube appeared clear.

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Fig 4.8: Extraction process done for fenugreek seed with the help of the guide at JSS campus (Life Science Laboratory) by Soxhlet Extraction Method

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Post-extraction Procedure:

The extracted solution was collected and concentrated using a water bath to evaporate excess solvent. The thick residue was then scraped, collected, and stored in **sterile microtubes (MTC tubes)** under refrigerated conditions for further biochemical testing. This method ensured a thorough extraction of active compounds like flavonoids, alkaloids, tannins, and saponins from the fenugreek seeds.

4.2.2 Phytochemical Screening Results

Phytochemical screening involves a series of **qualitative tests** that help detect the presence of key classes of bioactive compounds in seed extracts. These tests rely on **chemical reactions between specific reagents and phytochemicals**, often producing color changes or precipitates that indicate the presence of the compound.



Figure 4.8: Phytochemical screening

In our study, we performed a comparative phytochemical analysis of **untreated (control) Hyacinth beans** and **Hyacinth bean treated with plasma at 10 kV for 3 minutes**.

The image above displays the test tubes containing extracts from beans, used for phytochemical screening. Each test tube corresponds to a specific phytochemical test, identified by labelled tags such as **Alkaloids, Steroids, Saponins, Tannins, and Flavonoids**.

These tests rely on specific chemical reactions that result in a **visible colour change or precipitate formation**, indicating the presence of a particular compound. These visual results align with the qualitative data summarized in the phytochemical table, confirming the presence of key bioactive compounds such as alkaloids, steroids, and saponins in both control and plasma-treated beans.

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Observations:

- There was **no significant change** in the presence of phytochemicals between the control and plasma-treated beans.
- Key bioactive compounds such as **alkaloids, steroids, saponins, carbohydrates, and proteins** were consistently detected in both samples.
- **Flavonoids and tannins/phenolic compounds** were absent in both cases.

The results are summarized in the table below:

Phytochemical Analysis	Beans (control)	Beans (10kV, 3min)
Alkaloids	+++	+++
Flavonoids	-	-
Tannins and Phenolic Compounds	-	-
Steroids	+++	+++
Saponins	+++	+++
Carbohydrates	+++	+++
Proteins	+++	+++

Figure 4.8: Results of Phytochemical screening

Note: (+++) = Strong presence and (-) = Absence

CASE II: RESULTS AND DISCUSSIONS OF EFFECT OF PLASMA TREATMENT ON SPINACH SEEDS BY CONDUCTING VARIOUS QUANTITATIVE & INSTRUMENTAL BIOCHEMICAL TESTS

4.2.3 GC-MS (Gas Chromatography-Mass Spectrometry) – Instrumental Analysis

The GC-MS analysis was conducted to identify the bioactive compounds present in the plasma-treated spinach seed extract. This technique combines the features of gas-liquid chromatography and mass spectrometry to detect and quantify volatile and semi-volatile compounds based on their mass-to-charge ratio (m/z). The chromatogram revealed a series of sharp and well-resolved peaks,

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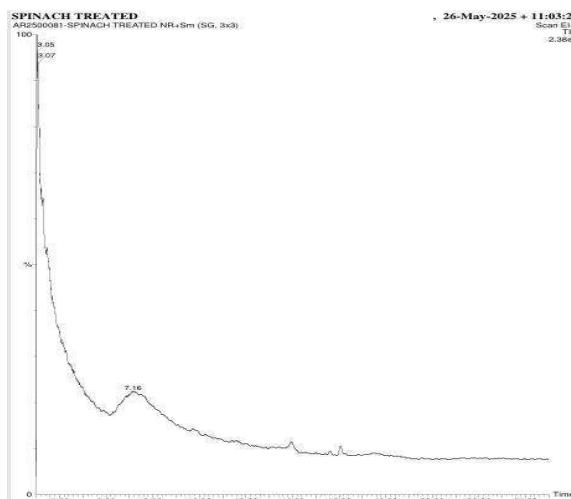
indicating the presence of multiple compounds. Among the most prominent bioactive molecules identified were:

1. **Trimethylene oxide (C_3H_6O)** – a cyclic ether with potential antimicrobial properties.
2. **Acetic acid, hydroxy-, ethyl ester ($C_4H_8O_3$)** – known for its antioxidant and antimicrobial actions.
3. **1,3-Propanediol ($C_3H_8O_2$)** – a common diol used in pharmaceutical and cosmetic formulations.
4. **1,3,6-Trioxocane ($C_5H_{10}O_3$)** – a polyether compound, often studied for its biodegradable nature.
5. **Dihydroxymaleic acid ($C_4H_4O_6$)** – a hydroxylated organic acid with strong antioxidant potential.
6. **Ethyl formate ($C_3H_6O_2$)** – contributes to flavor and fragrance, with noted biological activity.
7. **Propylene oxide (C_3H_6O)** – a reactive epoxide with industrial and antimicrobial applications.

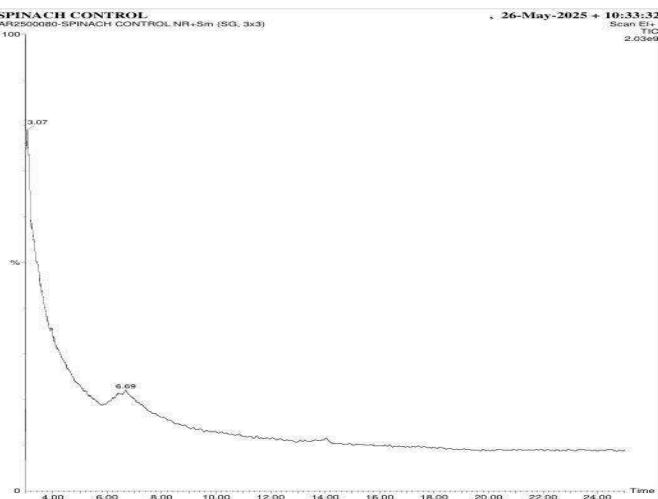
4.2.3.1 Total Ion Chromatogram (TIC) Overview

The TIC shows the overall chemical profile of the spinach samples. The TIC of both control and plasma-treated spinach samples displayed prominent peaks at retention times around **6.6 min, 9.0 min, and 10.8 min**, corresponding to major fatty acid methyl esters. In the treated sample, an additional peak was observed at **~13.1 min**, indicating the presence of **Phytol**. Variations in peak intensities reflect differences in compound abundance between the two samples.

In the **treated sample**, peak areas were slightly higher, and a **new peak at ~13.1 min** indicated the presence of **Phytol**, a compound absent or negligible in the control. These differences suggest biochemical changes due to cold plasma treatment.



Graph 05: Total Ion Chromatogram (TIC) of spinach control



Graph 06: Total Ion Chromatogram (TIC) of spinach Treated

CHAPTER 4

RESULTS AND DISCUSSION

CASE I: RESULTS AND DISCUSSIONS OF EFFECT OF PLASMA TREATMENT ON BEANS SEEDS

4.1 PLASMA EXPOSURE ON BEANS

Plasma treatment has shown a notable positive effect on seed germination and early plant growth. The process works by altering the seeds surface by making it easier for water and nutrients absorption by the seed coat. The reactive species generated during the plasma treatment enhance the seed's ability to absorb water, resulting in quicker and more efficient germination.

Plasma contains reactive species such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), which play a crucial role in enhancing plant growth and development. Key ROS, including oxygen radicals ($O_2\cdot$), hydroxyl radicals ($OH\cdot$), hydrogen peroxide (H_2O_2), and ozone (O_3), along with RNS like nitric oxide (NO), nitrogen dioxide (NO_2), and peroxy nitrite ($ONOO^-$), improve seed germination by increasing seed coat permeability and water absorption. These species promote root and shoot growth by facilitating nutrient uptake and cell division, while also enhancing stress tolerance and disease resistance through the activation of plant defense responses.

Furthermore, plasma's antimicrobial effects help mitigate seed-borne pathogens, fostering healthier conditions for plant development. Collectively, these benefits support increased agricultural productivity and promote sustainable farming practices

As observed in the "Total Germinated Seeds" graph as shown in Figure 4.1, the group of seeds treated with plasma for 4 minutes has exhibited the highest number of germinated seeds compared to the ones which was left untreated. This indicates that the plasma- treated seeds responded more effectively, demonstrating a clear enhancement in their germination capacity.

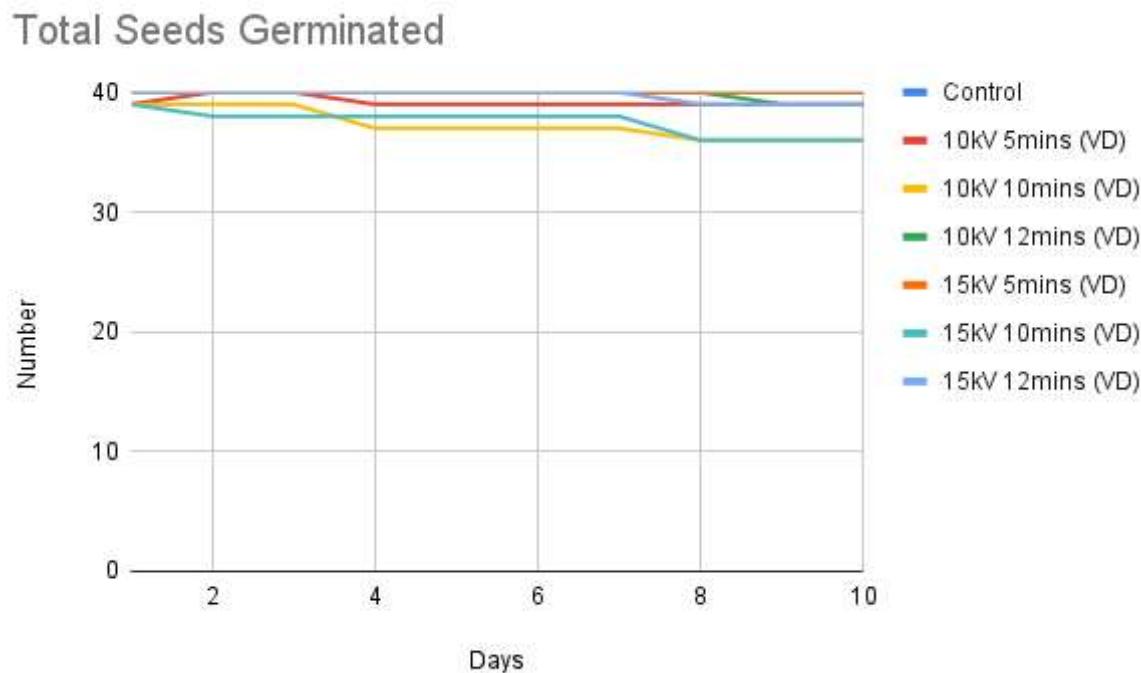


Figure 4.1: Graphical Representation of Total Germination Seeds

This improvement is due to the changes in the seed surface, which were made more porous and hydrophilic by the plasma treatment. These changes allow the seeds to take up water more efficiently, which is a essential element for initiating the germination process. By enhancing this fundamental step, plasma technology offers a promising, sustainable method to improve seed quality and agricultural productivity.

The germination rate was calculated by the equation

$$\text{GR (\%)} = (\text{NS} / \text{TS}) \times 100\%$$

where,

GR = Germination Rate

NS = Number of seeds germinated

TS = Total number of seeds

The effect of plasma treatment on plant growth is evident in parameters such as shoot length, and root length as illustrated in Figures 4.2 and 4.3. Seeds treated with plasma consistently produced plants with significantly longer shoots compared to the control group. Notably, seeds exposed to an 10-minute plasma treatment (VD 10kV 10 min) exhibited the

Application of electrical discharge cold plasma treatment on agricultural seeds: A biochemical and image processing approach

highest average shoot length, whereas untreated seeds showed considerably shorter shoots. This observed trend highlights the enhanced growth potential enabled by plasma treatment, further supporting its effectiveness in improving plant development.

Longest Root

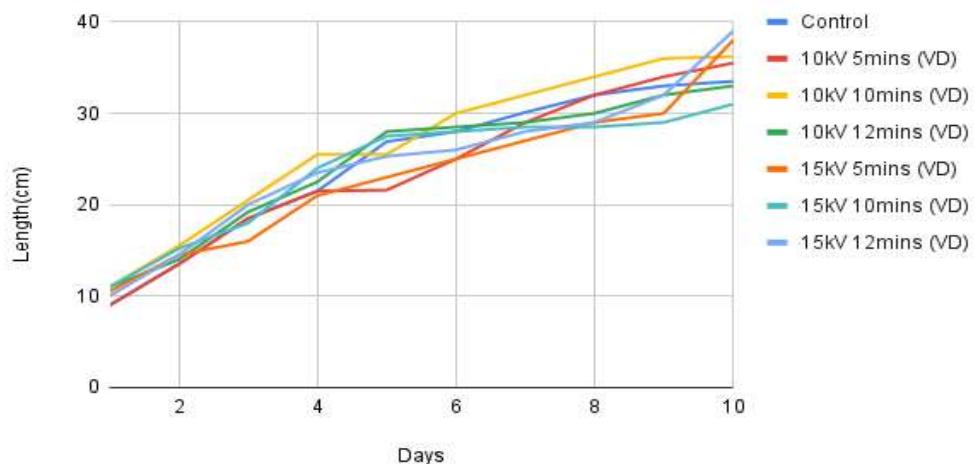


Figure 4.2: Graphical Representation of Longest Root

Longest Shoot

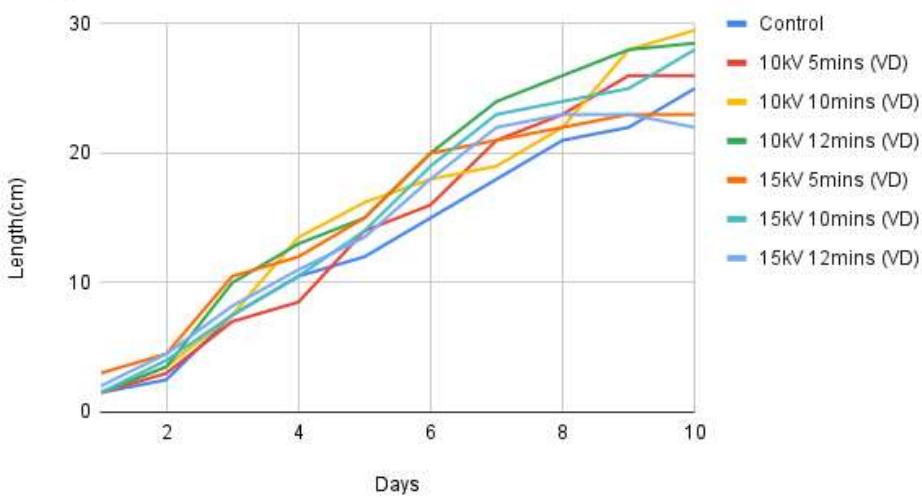


Figure 4.3: Graphical Representation of Longest Shoot

The analysis also demonstrated notable enhancements in root development, as depicted in Figures 4.2 and 4.3, underscoring the comprehensive benefits of plasma treatment. Statistically significant results, with a p-value of $p < 0.05$, validate plasma treatment as an effective and reliable method for enhancing seed germination and overall plant growth.