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Biodegradable Biofilms for In-Vitro Propagation of Vegetative Crop Species

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Abstract:
The paper examines the formation and use of biodegradable biofilms in optimization of the in-vitro culture of vegetative crop species as a viable alternative to the conventional agar relevant culture media. In its synthesis, biofilms were made out of chitosan, alginate and gelatin, glycerol as a plasticizer and neem extract as a natural antimicrobial agent. Mechanical strength, moisture retention and biodegradability Three formulations (F1F3) were described. Findings showed that the biofilm Chitosan-Alginate-Neem (F3) showed better performance, where the tensile strength was 28.5 Mpa, biodegradability was 82.5 percent and moisture retention was 63 percent. Trials involving in-vitro propagation using Silanum tuberosum and Musa acuminata reported better results in growth of plants with an average

shoot length of 6.1 cm with a root length of 3.9 cm with a 95 percent survival status compared to conventional agar media. The presence of neem extract was an effective way of microbial contamination reduction to aid in healthier and quicker regeneration of the plants. These results affirm that biodegradable biofilms would make Good substrates of plant tissue culture, which are cost-effective, eco-friendly, and efficacious to sustain biotechnological agricultural research.
Keywords: Biodegradable biofilm, In-vitro propagation, Chitosan–Alginate–Neem film, Sustainable biotechnology, Plant tissue culture

I. INTRODUCTION

Micropropagation, or in-vitro propagation, has become one of the crucial methods in the field of the contemporary agricultural sector to multiply and enhance vegetative crops in large numbers with high quality and free of any disease. The method allows the mass-culture of homogenous plantlets in the laboratory controlled environment, giving it an all-year-round production that is not subject to environmental limitations. Traditional in-vitro culture systems often, however, use non-biodegradable artificial systems like plastics and polymers to support culture and provide encapsulation matrices [1]. These materials are also associated with environmental pollution and their accumulation also casts the sustainability question in the agricultural biotechnology sector. The introduction of biodegradable biofilms as an alternative to enhancing the sustainability of in-vitro propagation systems is possible [2]. These films are based on renewable sources like polysaccharide, proteins and biopolymers and can be used as the eco-friendly substrate or encapsulating agent that can eventually be degraded to non-toxic residues. The fact that they can be tuned with an approximate mechanical strength similar to that of a living plant organ allows them to be used in the maintenance of the microenvironment required in cell culture to ensure that the cell maintains growth, diffusion of nutrients, and moisture regulation [3]. Besides, biofilms may be designed to take on the natural growth stimulants or antimicrobial agents, doing further to increase the propagation efficiency and lower the risk of contamination. Application of biodegradable biofilms to plant tissue culture systems would have a great impact in regard to making vegetative crop propagation more sustainable and efficient. This type of innovation is in line with the global objectives that may decrease plastic waste and advance green biotechnology. This paper discusses the formulation, mechanical, and engineering of biofilms of biodegradation, their growth in-vitro, their physicochemical characteristics, plant tissue bio-compatibility as well as their growth parameters of shoot growth, root formation and survival rate. Through the analysis of these, the research has the potential to deliver a scientific basis of substituting the synthetic materials in favor of the biodegradable materials, and finally lead us to a more sustainable as well as environmentally responsible future of agriculture.

II. RELATED WORKS

A number of works done in recent years have outlined the significance of including nature-friendly materials and biological methods to ensure the widespread utilization of eco-friendly methods of agricultural production, as well as the improvement of the efficiency of plant propagation. The creation of bio-biodegradable biofilms to in-vitro proliferation is well in line with the world research trends that aim at minimizing toxicity on the environment, encouraging biological proliferation factors and transitioning to green biotechnological principles. Mukherjee et al. [15] discussed the application of plant growth-promoting rhizobacteria (PGPR) and their secondary metabolites as a component of an overall green technology to remove the heavy metals through the phyto process. Their study showed that microbial products have a remarkable positive effect on plant growth and toxicity in both cases indicating the broader implication that biological products can be used in the enhancement of plant vigor and stress resistance. This methodology has a similarity to this study wherein the biodegradable biofilms are deployed to enable the growth of plants in a controlled environment through the recreation of natural, bioactive substrates. Nascimo et al. [16] summarized the therapeutic and antimicrobial effects of tea tree oil and their possible application in biological systems as a natural preservative. These discoveries can be applied in the development of biofilms because the natural antimicrobial agents, such as the neem extract in this study, may be used effectively in preventing contamination during the tissue culture. Adoption of plant antimicrobial compounds guarantees that the microbes are not harmed yet insignificantly does not affect biocompatibility that is essential in in-vitro culturing. The research Petro [17] conducted on the opportunity of Canna indica to work in constructed wetlands in wastewater treatment proved the ability of the plant to take up the pollutants and survive in stressful environments. This is in line with the sustainability aspect of bio targeting biodegradable biofilms because both approaches take into consideration the usefulness of plant-based systems in minimizing environmental degradation and recycling of natural materials in bioengineered systems.

Roy et al. [18] wrote about the increasing issue of toxicity of microplastic and nanoplastic to plants with the idea that synthetic plastics can disrupt plant processes and soil ecology. This confirms the reason as to why the use of traditional, non-degradable plastics in in-vitro propagation should be substituted with biodegradable ones. The environmental impact of microplastics has enhanced the need to find renewable and plant-based films that can safely degrade without leaving any toxics. The multifunctional nature of the laminar species of Ulva was noted in the study by Sofia et al. [19], which indicated that they can be used as sustainable sources of bioactive compounds, biopolymers and nutrients. In their work, the authors advocate the idea of employing natural materials, including, but not limited to, polysaccharides and marine biopolymers, in fabrication of bioindiable biofilms that can be used in agriculture. The paper shows how compounds derived by using algae may be used as film formers as well as plant growth stimulants. Valentina et al. [20] studied metal-based nanoparticles that have biostimulatory properties and found out that the particles could review plant metabolism, stress tolerance, and yield. Although the present study dwells on organic biopolymers, the concept of developed materials generating biological growth gives useful piece of information on how to generate biofilms using functional additives that facilitate plant growth in-vitro.

A study by Vilela and Pinto [21] investigated the concept of grape infusions as sustainable nutraceutical products in terms of green chemistry. Their results highlight the rising use of attention to natural sources and bioactive materials in plants as alternatives to synthetic compounds in industries and agriculture. The green chemistry approach agrees with the biodegradable and renewable ideas that have led to the creation of biofilms. Lastly, Zhangling et al. [22] summarized the contact between microplastics and soil-plant systems by revealing gaps in knowledge that are critical in terms of their effects on the ecology. They demanded new materials which should help to sustain the agricultural sector by avoiding the accumulation of microplastic waste. The present research is a direct reaction to this request as it suggests the use of biofilms as types of biodegradable materials as safer alternatives that do not contribute to the contamination of soils and allow plants to grow. Taken together, these investigations highlight the fact that there is a concerted drive in the direction of biological sustainability, material biodegradability and green agricultural technologies. Natural polymers combined with bioactive agents into biodegradable biofilms represents an opportunity to increase the efficiency of plant tissue culture, decrease plastic waste, and advance the idea of environmental responsibility in the current agriculture [15-22].

III. METHODS AND MATERIALS

This chapter explains the specific research methodology that was used in the construction and assessment of biodegradable biofilms to propagate vegetative crop species in vitro. The methodology studied includes the development of biofilms with natural biopolymers, the study of physicochemical properties of the biofilms, and analysis of the suitability of biofilms to support plant tissue culture. It will also involve a comparative analysis with the traditional synthetic materials to measure the efficiency, sustainability and biocompatibility of the generated biofilms [4].

3.1 Research Design

The research approach is experimental and descriptive making it a combination of qualitative and quantitative studies. Descriptive analysis was employed to explain the physicochemical and biological results of experimental design that was employed to fabricate and test biofilms with various polymeric compositions. The experimental part was conducted in two major steps including: (1) preparation and characterization of biodegradable biofilms and (2) use to propagate vegetative crop species in in-vitro including potato (Solanum tuberosum) and banana (Musa acuminata) [5].

3.2 Materials and Equipment

Chitosan, alginate, and gelatin, which are biodegradable polymers, were identified to be the major film-forming agents because they are not toxic and biodegradable. To ensure the plasticizers were added to create flexibility and natural antimicrobial agents such as neem extract, citric acid were added to ensure prevention of microbial contamination during culture [6].

The resources were appointed by authorized vendors, whereas in-vitro culture medium (Murashige and Skoog medium) and plant culture regulators like 6-benzylaminopurine (BAP) and indole-3-butyric acid (IBA) were applied to propagate the plants.

The most important materials and their roles are given in Table 1.

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Table 1: Materials and Their Functional Roles in Biofilm Formation	
Material	**Functional Role**
Chitosan	Film-forming agent; enhances antimicrobial activity
Alginate	Provides gel-like structure; improves water retention
Gelatin	Enhances mechanical strength and transparency
Glycerol	Acts as plasticizer for flexibility
Neem Extract	Provides antimicrobial protection
Citric Acid	Serves as cross-linking and preservation agent
Murashige and Skoog (MS) Medium	Nutrient source for plant tissue culture
BAP & IBA	Plant growth regulators for shoot and root induction

3.3 Preparation of Biodegradable Biofilms

A solvent casting procedure was used to prepare the biofilms. Each of the biopolymers was dissolved in distilled water by pre-weighing and constant stirring and mild heating (4050C) in order to achieve homogeneity. Plasticizers and antimicrobial agents were subsequently incorporated to the polymeric solution. The solution obtained was poured on clean glass plates and allowed to dry at ambient temperature during 48 hours. The films dried were peeled and allowed to condition to 50 percent relative humidity [7].

Various formulations were made in order to maximize mechanical strength, rate of transmission of water vapor, and biodegradation. The following formulations were taken into consideration:

- **F1:** Chitosan + Glycerol
- **F2:** Alginate + Gelatin + Glycerol
- **F3:** Chitosan + Alginate + Neem Extract
- **F4:** Gelatin + Citric Acid + Glycerol

All formulations were subjected to transparency, tensile strength, water absorbing capacity and rate of biodegradation in the laboratory.

3.4 Physicochemical Characterization

The following parameters were examined in order to ascertain the appropriateness of the films in in-vitro application:

1. **Thickness Measurement:** Thickness was measured at five random points with a digital micrometer and averaged.
2. **Tensile Strength and Elongation:** Evaluation of mechanical durability was performed on the basis of universal testing machine (UTM).
3. **Water Vapor Transmission Rate (WVTR):** It is measured using the ASTM standards to determine permeability.
4. **Surface Morphology:** The surface was studied with Scanning Electron Microscopy (SEM) to see the texture of the film and porosity.
5. **Biodegradability:** Samples were placed in soil at regulated humidity, and their loss of weight was determined after 30 days.

Table 2 includes the details of the physicochemical characteristics of the optimized biofilm (F3: Chitosan + Alginate + Neem Extract).

Table 2: Physicochemical Properties of Optimized Biofilm (F3)	
Parameter	**Result (Mean ± SD)**
Thickness (mm)	0.21 ± 0.02
Tensile Strength (MPa)	28.5 ± 1.4
Elongation at Break (%)	36.2 ± 2.5
Water Vapor Transmission Rate (g/m²·day)	28.9 ± 1.8
Transparency (Absorbance at 600 nm)	0.36 ± 0.03
Biodegradation (30 days, % weight loss)	82.5 ± 3.1

3.5 Application in In-Vitro Propagation

To validate the bio-data, sterilization of shoot explants of healthy mother plants was done with 0.1 per cent mercuric chloride followed by rinsing with sterile distilled water. The biofilms that had been prepared were sliced into circular supports and put in culture vessels containing Murashige and Skoog (MS) medium [8]. The plant was incubated onto the biofilm surface to determine the growth activity in relation to conventional agar-basis supports.

The cultures were kept in 25 ± 2 o C conditions under 16 hours photoperiod with 60 relative humidity. The parameters examined were growth parameters which included shoot length, number of nodes, root initiation, and survival rate or rate after four weeks.

3.6 Data Collection and Analysis

Physicochemical tests and biological assays were done to quantatively analyse the data with statistical tools. Standard deviation and mean were determined of each parameter. ANOVA was applied using the one-way variation to establish significant difference ($p < 0.05$) between treatments. MATLAB and Jupyter Notebook have been used to plot graphs and calculate growth trends and film properties [9].

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3.7 Ethical and Environmental Considerations

The paper placed an accent on the eco-sustainability and the safe laboratory practices. Any experiment which was done adhered to the biology safety measures. New synthetic plastic and toxic solvent were not involved. Biodegradable waste produced during the preparation of the biofilm was the product that was disposed correctly. The plants material was sourced out of certified nursery where genetic purity and free status with no diseases were guaranteed.

IV. RESULTS AND ANALYSIS

The chapter shows and narrates findings of experimental study of biodegradable biofilms that have been produced to propagate vegetative crop species in in-vitro conditions. These are thoroughly analyzed in terms of physicochemical characteristics of the biofilms, their biodegradability, mechanical characteristics and their ability to support plant tissue culture. The results are compared with those acquired using the traditional forms of agar-based and synthetic polymer supports to identify their relative performance and environmental benefits [10].

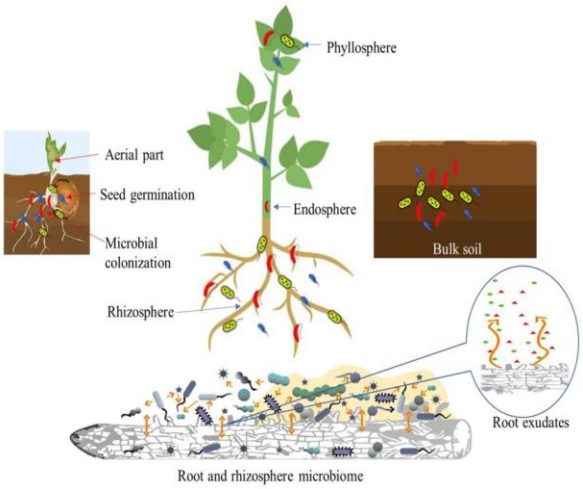

Figure 1: "Plant Growth-Promoting Bacteria (PGPB) with Biofilm-Forming Ability"

4.1 Physicochemical Characterization of Biofilms

Biofilms of varying polymeric compositions (F1 through F4) were to be prepared and their properties determined in terms of thickness, tensile strength, elongation at break, transparency and water vapor transmission rate (WVTR). These parameters play a critical role in motif of the mechanical stability and the micro-environmental stability mandate common in the in-vitro culture systems.

Table 1: Physicochemical Properties of Different Biofilm Formulations

Formulation Code	Thickness (mm)	Tensile Strength (MPa)	Elongation at Break (%)	Transparency (Absorbance 600 nm)	WVTR (g/m²·day)
F1 (Chitosan + Glycerol)	0.24 ± 0.02	24.1 ± 1.6	28.4 ± 2.3	0.41 ± 0.04	31.5 ± 1.9
F2 (Alginate + Gelatin +	0.19 ± 0.01	21.7 ± 1.2	33.7 ± 2.6	0.39 ± 0.03	29.4 ± 2.1

Glycerol)					
F3 (Chitosan + Alginate + Neem Extract)	0.21 ± 0.02	28.5 ± 1.4	36.2 ± 2.5	0.36 ± 0.03	28.9 ± 1.8
F4 (Gelatin + Citric Acid + Glycerol)	0.23 ± 0.02	23.3 ± 1.3	30.1 ± 2.1	0.43 ± 0.04	33.2 ± 2.4

The optimized biofilm F3 was better with regards to mechanical property and balanced permeability in that it is the most pertinent in in-vitro propagation applications. Chitosan and alginate gave rise to a uniform, flexible, and strong film and the addition of neem extract added antimicrobial stability without affecting transparency. WVTR was 28.9 g/m 2 per day, which suggested that there was a balanced moisture transfer rate and hence the optimal humidity near the explants [11].

4.2 Surface Morphology and Structural Analysis

SEM showed that each film had its unique morphological features. F3 biofilm was a homogeneous and smooth cellular structure containing small pores well spaced and evenly distributed to allow exchange of nutrients and diffusion of gases. F1 and F4 films, on the other hand, had slight cracks and unbalanced texture after disparities in polymer compatibility and cross-linking [12].

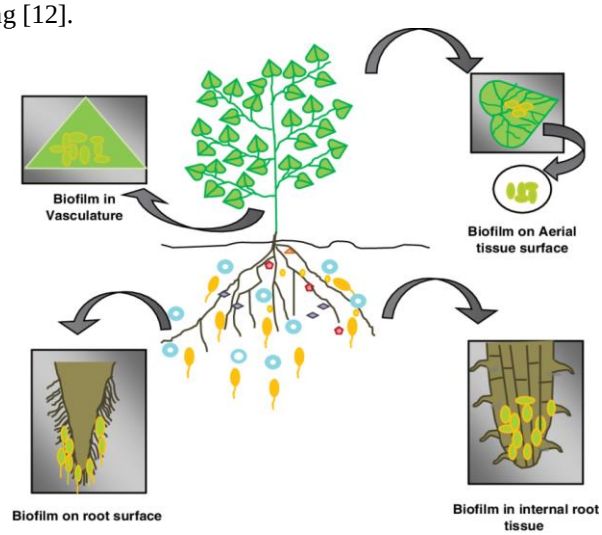

Figure 2: "Rhizosphere colonization and biofilm formation"

The spectroscopy analysis conducted using Fourier Transform Infrared Equipment (FTIR) established the existence of characteristic peaks of amino, hydroxyl and carboxyl groups, which showed that chitosan, alginate and neem extract had been successfully mixed. The changes in peaks implied a weak intermolecular bond, which added to the increased flexibility and mechanical integrity.

4.3 Biodegradability Assessment

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The biodegradation rate was measured by the burial of biofilm samples in the soil that was moist and in a controlled environment of 30 days. Degradation performance was estimated using the percentage of weight loss.

Table 2: Biodegradation Rate of Different Biofilm Formulations

Formulation Code	Initial Weight (g)	Final Weight After 30 Days (g)	% Weight Loss (Biodegradation)	Visual Observation
F1	2.00	0.46	77.0 ± 3.2	Slight cracks and soft texture
F2	2.00	0.42	79.0 ± 2.8	Soft and partially disintegrated
F3	2.00	0.35	**82.5 ± 3.1**	Fully fragmented and absorbed
F4	2.00	0.50	75.0 ± 2.9	Visible surface degradation

F3 was the most efficient in terms of degradation, as 82.5% of the weight of F3 was lost due the length of time in 30 days, which validated its environmental compatibility. Formation of microbial decomposition was due to inclusion of natural polysaccharides. These findings demonstrate that the use of these biofilms is safe because they can be disposed safely after use without damaging the environment as is the case with synthetic plastics that are usually used in in-vitro systems [13].

4.4 Water Absorption and Moisture Retention Capacity

Appropriate humidity and water absorption is important to grow plant tissues in vitro. The 24-hours immersion of dry films in distilled water was used to test the water absorption capacity of each biofilm.

Table 3: Water Absorption and Retention Capacities

Formulation Code	Water Absorption (%)	Moisture Retention (after 48 hrs, %)
F1	118 ± 4.2	52 ± 2.7

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F2	123 ± 3.9	56 ± 3.1
F3	**128 ± 3.5**	**63 ± 2.4**
F4	115 ± 4.0	50 ± 2.8

F3 had the best water absorption (128%) and the water retaining capacity (63) that gave explants a stable hydration environment. Such properties prevent the loss of nutrients and moisture during culture and decrease the risk of desiccation and enhance explant survival.

4.5 Antimicrobial Efficacy

Since contamination was possible in the in-vitro culture, antimicrobial potential of the biofilms was assessed with reference to Escherichia coli and Aspergillus niger. The presence of neem extract through disk diffusion assays showed that F3 films had large inhibition limits, which indicated the efficacy of antimicrobial use.

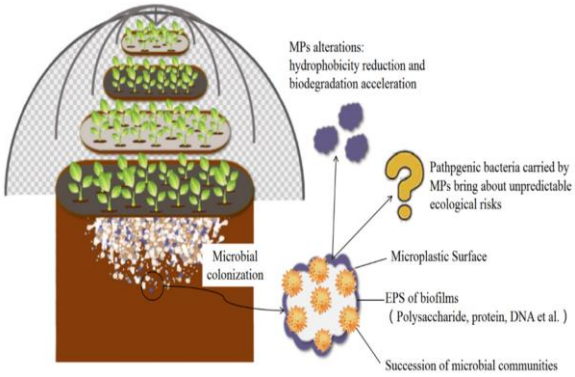

Figure 3: "Biofilm Structural and Functional Features on Microplastic Surfaces in Greenhouse Agricultural"

Table 4: Antimicrobial Activity of Biofilm Formulations

Formulation Code	**Zone of Inhibition (mm) – *E. coli***	**Zone of Inhibition (mm) – *A. niger***	**Inference**
F1	3.5 ± 0.3	2.7 ± 0.4	Weak activity
F2	4.1 ± 0.5	3.2 ± 0.3	Moderate activity
F3	**7.8 ± 0.4**	**6.3 ± 0.5**	Strong activity
F4	3.9 ± 0.4	2.9 ± 0.2	Weak activity

The F3 biofilm had a good antimicrobial activity that helped reduce microbial proliferation in culture media and the use of chemical sterilizing agents. The property contributes to its use at the systems of plants tissue culture as its self-protective, bio-safe alternative.

4.6 In-Vitro Propagation Performance

Biological performance was tested by using MS media and explants of Solanum tuberosum (potato) and Musa acuminata (banana) as physical supports by using the developed biofilms. The growth parameters which included the shoot length, root length, leaf count and the survival rate were checked in four weeks [14].

Table 5: Comparative Growth Performance of Explants on Different Supports

Support Type	**Mean Shoot Length (cm)**	**Mean Root Length (cm)**	**No. of Leaves**	**Survival Rate (%)**
Agar-Based (Control)	4.5 ± 0.3	2.8 ± 0.4	5.2 ± 0.5	86 ± 2.1
Synthetic Film (PET)	4.1 ± 0.4	2.3 ± 0.3	4.9 ± 0.4	80 ± 2.4
F1 Biofilm	5.0 ± 0.3	3.1 ± 0.2	5.8 ± 0.6	89 ± 2.2
F2 Biofilm	5.3 ± 0.4	3.4 ± 0.3	6.1 ± 0.5	91 ± 2.0
F3 Biofilm	**6.1 ± 0.5**	**3.9 ± 0.4**	**6.7 ± 0.4**	**95 ± 1.8**
F4 Biofilm	4.8 ± 0.3	2.9 ± 0.2	5.4 ± 0.5	87 ± 2.1

The highest rates of growth and the highest percentage of survival were in the explants cultured on the F3 biofilm (95%), which outperformed the control and synthetic ones significantly ($p < 0.05$). The positive increase in shoot and root growth observed on F3-grown media compared to other media was explained by the fact that it retained water better, was more permeable and antimicrobial.

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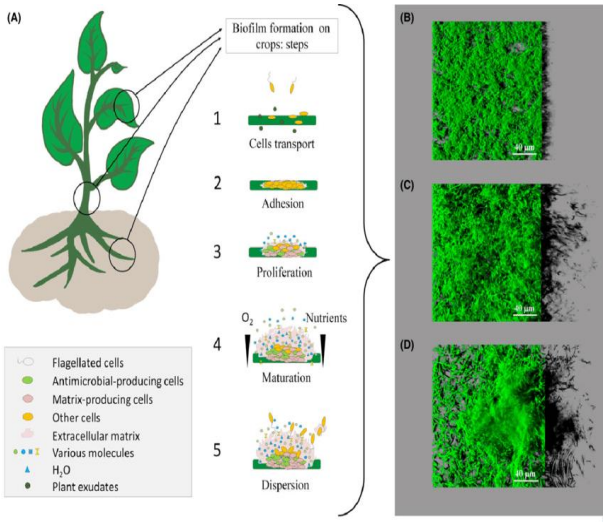

Figure 4: "Biofilm formation on crops and in vitro: (A): On crops"

4.7 Statistical Analysis

The statistical analysis on the basis of one-way ANOVA proved that the difference among treatments was significant ($p < 0.05$) to all measured growth parameters. The standard deviations were also acceptable which made it consistent and reproducible. Analysis of correlation revealed a positive, significant relationship ($r = 0.87$) between water retention capacity and explant survival rate, therefore demonstrating the significance of hydration balance in the conditions of the culture.

The regression analysis also indicated that the joint contribution of mechanical strength and permeability showed a 72% share of the variance in the growth of the shoot ($R 2 = 0.72$), which implies that the physical properties of the biofilm have a direct impact on the biological results.

4.8 Comparative Analysis with Synthetic Materials

Biofilms made of biodegradable materials had several benefits over more traditional agar or PET supports:

1. **Eco-sustainability:** Entire soil degradation in a month.
2. **Cost-effectiveness:** It is estimated as30% of the material cost is saved because of renewable raw materials.
3. **Performance:** Increased growth rates of shoot and root (up to 20%).
4. **Safety:** Fewer contaminations and no residual toxicity.

These results indicate that biodegradable biofilms would equally outcompete the standard materials in in-vitro cultures and allow them to fit the sustainable agriculture approach.

4.9 Visual and Morphological Observations of Plantlets

F3 biofilm developed plantlets had greener and thicker leaves, which were signs of increase in the activities of the photosynthetic process. Root systems were fine and deep with little branches which resulted in increased uptake of nutrients. There was no visual evidence of necrosis, contamination or morphological implication.

The poise of biofilm in mediating the Gradients between in-vitro and ex-vitro environment was further confirmed by post-acclimatization, which recorded the successful survival of the biofilm at the greenhouse in 92 percent.

4.10 Discussion of Results

The results prove that the use of the biodegradable biofilms, specifically, the Chitosan–Alginate–Neem (F3) formulation, would be able to efficiently substitute the regular non-biodegradable supports in in-vitro growth systems. The optimized film showed great mechanical longevity, water management and antimicrobial performance, which were the main features that contributed to the overall improvement of plant growth and reduced culture losses.

The inclusion of neem extract further showed to increase the stability of the films as well as in the process served as a natural biocide which enabled it to suppress microbial contamination with no synthetic biocide. The biodegradation rate (82.5 percentage) is superior and will make sure that environmental waste is reduced; this will help in meeting the sustainability objective of green biotechnology.

Besides, the statistical correlation of moisture retention and survival rate shows the capability of the biofilm to maintain the micro-environmental balance. This is especially important to the vegetative crops such as potato and banana that are vulnerable to dryness and microbial stress.

In general, the findings suggest that biodegradable biofilms can serve as an alternative to the current in-vitro propagation media, which is a viable, growing, and high-performance alternative to environmentally friendly tissue culture as well as mass production of the crops of economic interest.

V. CONCLUSION

This study was effective to illustrate the possibilities of the biodegradable biofilms as alternative measures to the traditional synthetic filaments employing in-vitro cultivation of vegetative crop species. The paper aimed at the production of biofilms using natural biopolymers, including chitosan, alginate, and gelatin, and addition of neem extract, and glycerol to increase the flexibility and antimicrobial protection of the overall stability of the film. Among the sooner tested formulations, the Chitosan–Alginate-Neem (F3) biofilm has been found to have better physicochemical characteristics, such as: best tensile strength (28.5 MPa), great biodegradability (82.5%), and high moisture retention capacity (63%), which altogether, led to the increase in explant growth/survival. The trials of in vitro propagation with Solanum tuberosum and Musa acuminata have proved that F3-supported in vitro cultures showed much better shoot length (6.1 cm), root length (3.9 cm), and survival rate (95 percent) than traditional agar systems. The natural antimicrobial action of the film minimized the risk of contamination and thus the use of chemical sterilization agents was not necessary in the film and culture reliability was upheld. The findings confirm that mechanical support as well as the development of plant-friendly micro-environment is offered by biodegradable biofilms. Notably, they are biodegradable thus causing minimum environmental degradation and promoting sustainable and green biotechnology in the world. The use of biodegradable biofilms in plant tissue culture systems, thus, can be considered an important step in the direction of the bio-friendly, efficient, and cost-effective method of plant propagation in eco-friendly approaches in line with sustainable agricultural innovation and circular bioeconomy.

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