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Microbial degradation of plant packaging biofilms with phytochemical potential

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Abstract

The threat of plastic pollution is a global environmental concern. Bioremediation has become an important challenge in the search for sustainable measures to reduce the harmful effects of this pollution. The aim of this study was to evaluate the level of microbial degradation of plant-based packaging biofilms formulated from agro-resource materials. Three types of plant-based packaging biofilms with a starch matrix supplemented with plant fibers from T. daniellii, M. paradisiaca, and combined fibers were subjected to biodegradation. Methods based on weight loss estimation and cell abundance were used to determine the level of biodegradability of these biofilms with the species P. aeruginosa, S. aureus, and A. niger. A phytochemical screening analysis was used to qualitatively detect the presence of bioactive compounds. Before day 20, biodegradation was faster for packaging biofilms supplemented with T. daniellii + M. paradisiaca, with weight loss percentages ranging from 54.7% to 74.5%. Biodegradability rates ranged from 0.4 to 1 and from 0.5 to 0.9 DO units for P. aeruginosa and A. niger strains, respectively. On day 10, in order of growth importance, this cell abundance was most significant for P. aeruginosa with 60,103 CFU/mL (T. daniellii and M. paradisiaca), 210,105 (T. daniellii), and 680,107 CFU/mL (M. paradisiaca). The biodegradation of M. paradisiaca biofilms indicated higher biomasses ranging from 230,107 CFU/mL (S. aureus) to 680,107 CFU/mL (P. aeruginosa). Phytochemical screening showed the presence of sterols, polyterpenes, polyphenols, flavonoids, alkaloids, catechin tannins, and gallic tannins in packaging biofilms. Qualitative research into phytochemical molecules and toxicity tests may determine the potential of these biofilms in the design of modern, environmentally friendly food packaging.

Keywords: P. aeruginosa, S. aureus; A. niger, packaging biofilms, biodegradatio, T. daniellii, M. paradisiaca

1. Introduction

The degradation time for plastic packaging made from petrochemicals is very long. It takes ten (10) to twenty (20) years for a plastic bag to degrade, and no less than 450 years for a plastic bottle (Macheca *et al.*, 2024; Preda *et al.*, 2024) [13, 19]. Conventional plastics, such as petrochemical polymers, take between 500 and 1,000 years to biodegrade (Macheca *et al.*, 2024) [13]. In addition, the microparticles and nanoparticles resulting from this natural degradation cause invisible pollution and have enormous consequences for animal, human, and environmental health (Preda *et al.*, 2024; Habumugisha *et al.*, 2024) [19, 10].

Indeed, the degradation of plastics generates microparticles and nanoparticles which, when ingested by humans and animals, can cause serious health problems such as oxidative stress, toxin accumulation, organ dysfunction, and inflammation (Preda *et al.*, 2024; Habumugisha *et al.*, 2024) ^[19, 10]. In animals, this can cause intestinal obstruction and death, while in humans, ingestion of microparticles can contribute to cardiovascular disease, neurodegenerative disorders, digestive problems, and potentially cancer (Preda *et al.*, 2024) ^[19]. In terms of the environment, the products resulting from the degradation of these plastic packaging materials affect or contaminate water, soil, and the food chain (Habumugisha *et al.*, 2024, Xu *et al.*, 2023) ^[10, 28].

Given all these consequences of non-biodegradable packaging on animal, human, and environmental health (One Health), the use of bioplastics or biodegradable packaging remains a sustainable alternative. Bioplastics are biodegradable packaging made from biological resources that reduce dependence on synthetic plastics (Preda *et al.*, 2024) [19].

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The appeal of these bioplastics or packaging biofilms made from agricultural resources such as starch and/or plant leaves lies in their ability and flexibility to be used for food packaging while reducing carcinogenic and toxicological effects (Larisa and Alexandre, 2024) [12] proliferation. These bioplastics or packaging biofilms are made of materials that can be degraded by microorganisms such as bacteria, fungi, and algae (Mahesh *et al.*, 2024) [14]. Various methods such as biodegradation and bioremediation potential make these microorganisms suitable for green chemistry to remove harmful plastics from the environment (Anwaruzzaman *et al.*, 2022; Soares *et al.*, 2020) [4, 22].

Among these bacteria, *P. aeruginosa*, *E. coli*, *S aureus*, *A flavus*, and *A. niger* develop different metabolic capabilities in economical, ecological, and renewable bioremediation methods to degrade soil and water contaminants (Mahesh *et al.*, 2024; Anwaruzzaman *et al.*, 2022) [14, 4].

In Africa, specifically in Côte d'Ivoire, to ensure a healthy environment and improve the health of the population, Decree No. 2013-327 of May 22, 2013, was issued to ban the use of non-biodegradable plastic bags in favor of biodegradable bioplastics or biofilms (Benie *et al.*, 2025a). Biodegradable biofilms refer to a type of material designed to decompose naturally in the environment (Zhu and Wang, 2020) [30].

In Côte d'Ivoire, work has been carried out on the recovery and recycling of packaging using local materials (Benie *et al.*, 2024; Onzo *et al.*, 2014) ^[6, 18]. Other work has been carried out on the formulation of packaging biofilms based on starch supplemented with *T. daniellii* and *M. paradisiaca* fibers that could replace non-biodegradable plastics (Benie *et al.*, 2025b) ^[5].

In Côte d'Ivoire, work on the formulation of eco-friendly packaging and its biodegradation remains less well documented. The objective of this study is to evaluate the level of microbial degradation of plant-based packaging biofilms formulated from agro-resource materials.

2. Materials and Methods

Materials: The material consisted of three (3) categories of starch-based packaging biofilms supplemented with plant fibers formulated in our previous study (Benie *et al.*, 2025b) ^[5] (Figure 1). The first category was formulated using only *Thaumatococcus daniellii* leaf fibers; the second using *Musa paradisiaca* leaf fibers; and the last using both leaf fibers simultaneously (Benie *et al.*, 2025b) ^[5] (Figure 1). In addition to the packaging biofilms, the biological material consisted of reference strains used for quality control, including *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 25923, and *A. niger* ATCC 22342 from the Pasteur Institute of Côte d'Ivoire Collection. Other strains of *P. aeruginosa*, *S. aureus*, and *A. niger* isolated from the environment were used to estimate the level of biodegradability of plant-based packaging biofilms.

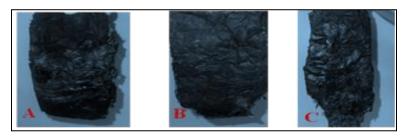


Fig 1: Starch-based plant packaging biofilms

A: Biofilms from M. paradisiaca; B: Biofilms from T. daniellii; C: Biofilms from T. daniellii + M. paradisiaca

2. Methods

2.1 Estimating the Level of Biodegradability of Packaging Biofilms

2.1.1 Criteria for Selecting Microorganisms

The choice of microorganisms was guided by their ability to use numerous carbon substrates as their sole source of carbon and energy in a simple mineral medium. They were also selected for their ubiquity, wide growth temperature range, and potential to produce enzymes that promote biodegradation (Rose *et al.*, 2020) [20].

2.1.2 Biodegradation of Packaging Biofilms

Two main methods were used to assess the biodegradability of plant-based packaging biofilms by fungal and bacterial strains (Min *et al.*, 2020; Montazer *et al.*, 2020) [15, 16]. The first method is the quantitative estimation of the weight loss of polymer or biofilm fragments in the presence of microorganisms. The second is the estimation of cell abundance.

2.1.2.1 Biodegradability by Quantitative Estimation of Weight Loss: The biodegradability monitoring was extended over a period of 30 days. Starting on the day of

seeding (D0), further manipulations were carried out after 10 days, 20 days, and 30 days. Three different masses of 5 grams (initial weight) of each type of packaging biofilm were cut out. These were then rinsed with distilled water, dried, and exposed to bacterial or fungal culture after being immersed in ethanol (70%) for five minutes (Min *et al.*, 2020) [15].

Thus, a volume of 100 mL of nutrient broth was introduced into three different conical flasks and seeded with 1000 µL (mL) of bacterial inoculum of *P. aeruginosa*, *S. aureus*, or fungal strain of *A. niger*. The flasks or vials were incubated at 37°C for thirty days and kept on a rotary shaker at 120 rpm. Every ten days, a mass of packaging biofilms from each flask was removed from the solution using tweezers. This mass was then rinsed thoroughly with distilled water to remove any excess microbial cells still attached to the polymer biofilms, then dried in an oven at 37°C for 6 hours and its final weight was determined using a balance. The weight loss of the polymer biofilms was calculated using the following formula (Montazer *et al.*, 2020) [16]:

$$Percentage of weight loss = \frac{initial \ weight - Final \ weight}{initial \ weight} *100$$

With: Initial weight, the mass of the sample before testing, and Final weight, its mass after testing.

2.1.2.2 Biodegradability by Estimating Cell Abundance

The abundance of bacterial and fungal cells in 1 mL of each biodegradation stock solution was assessed every 10 days. Under aseptic conditions, serial dilutions ranging from 10^{-1} to 10^{-5} were made from the biodegradability stock solution. Different volumes of 100 μ L of the last two (2) respective dilutions (10^{-4} and 10^{-5}) were taken and seeded using the surface spread method on PCA agar and incubated for 24-48 hours at 37° C. The results obtained were expressed in CFU/mL of the biodegradation stock solution (Rose *et al.*, 2020). To obtain the number of CFU in 1 mL of the seeded dilution, the correspondence $100~\mu$ L = $0.1~\mu$ L was applied (Min *et al.*, 2020) [15]. The number of CFU counted on the Petri dishes was multiplied by 10 to obtain the number of CFU/mL of the seeded dilution. The following formula was then applied:

 $y=10^a \times b$

y is the CFU/mL number of the biodegradation solution, **a** is the dilution factor considered or evaluated, and **b** is the CFU/mL number counted in the dilution considered.

2.2 Phytochemical Characterization of Packaging Biofilms: The main chemical groups contained in plant-based packaging biofilms were subject to qualitative detection.

2.2.1 Determination of Flavonoids

Flavonoids were analyzed using the method described by Koudoro *et al.* (2014) ^[11] in packaging biofilm samples for their neutralizing action against free radicals. Thus, a volume of 5 ml of plant packaging biofilm in a 5% solution was introduced into a test tube. Next, a few drops of HCl and three (3) magnesium chips were added. The appearance of a pink or red color indicates the presence of flavonoids.

2.2.2 Determination of Sterols and Polyterpenes

In a test tube, a mass of 2 mg of plant packaging biofilms was dissolved in 2 mL of acetic anhydride with 1 mL of sulfuric acid. The appearance of a purple ring at the interface, turning blue and then green, indicates the presence of sterols and polyterpenes (Yandjou *et al.*, 2023; Agbangnan *et al.*, 2012)^[29, 2].

2.2.3 Determination of Polyphenols

A drop of an alcoholic ferric chloride solution (2% FeCl3) was added to 2 mL of the 5% plant packaging biofilm solution. In the presence of polyphenolic derivatives, ferric chloride generates a blue-black or green color of varying darkness, indicating the presence of these polyphenolic derivatives. A control test is performed using an alcoholbased gallic acid solution (Yandjou *et al.*, 2023; Agbangnan *et al.*, 2012) [29, 2].

2.2.4 Testing for Tannins

Tannins exist in two forms. The first is composed of catechin tannins, which are non-hydrolyzable and formed from condensed catechol polymers. The second consists of gallic tannins, derived from gallic acid and combined in the form of hydrolyzable glycosides (Yandjou *et al.*, 2023;

Agbangnan *et al.*, 2012) ^[29, 2]. Thus, they were detected by two different reactions.

2.2.4.1 Determination of Catechin Tannins

To the remaining 5 mL of evaporated plant packaging biofilm solution, 15 mL of Stiasny's reagent (30% formalin, concentrated HCl) was added. The mixture was kept in a water bath at 80°C for 30 minutes, then cooled (Yandjou *et al.*, 2023; Agbangnan *et al.*, 2012) [29, 2]. The observation of a large-flocculated precipitate indicates the presence of catechin tannins. Catechin served as a control.

2.2.4.2 Determination of Gallic Tannins

An additional 5 mL of the previous solution of plant packaging biofilms is filtered and saturated with sodium acetate. The addition of three drops of ferric chloride (2% FeCl3) causes a bright blue-black color to appear, indicating the presence of gallic tannins in the medium (Yandjou *et al.*, 2023; Agbangnan *et al.*, 2012) [29, 2]. An experimental test is carried out using gallic acid.

2.2.5 Determination of Quinone Compounds

Free or combined quinone compounds are detected using the Borntraeger reaction. A 2 mL volume of the plant packaging biofilm solution is evaporated to dryness in a capsule and the residue is taken up with 5 mL of 1/5 diluted hydrochloric acid. The resulting solution is poured into a test tube and kept for 30 minutes in a boiling water bath. After complete cooling, 20 mL of chloroform is added. The chloroform phase is then recovered and 0.5 mL of half-diluted ammonia (Borntraeger reagent) is added. The appearance of a red to purple color indicates the presence of quinone compounds (Yandjou *et al.*, 2023; Agbangnan *et al.*, 2012) [29, 2]. A control test is performed with vitamin E.

2.2.6 Determination of Alkaloids

The characterization of alkaloids begins with the dry evaporation of 6 mL of the plant packaging biofilm solution in a capsule. The residue is recovered with 6 mL of 60° alcohol. The resulting alcoholic solution is distributed into two test tubes. Two drops of Dragendorff's reagent are added to the first tube. The presence of a brown-red precipitate indicates a positive reaction. In the second tube, two drops of Bouchardât's reagent are added. The presence of a brown-red precipitate indicates a positive reaction (Yandjou *et al.*, 2023; Agbangnan *et al.*, 2012) [29, 2]. In both experiments, a control test is performed using quinine.

2.2.7 Determination of saponosides

Saponosides are detected by placing 15 mL of the aqueous solution of plant packaging biofilms in a test tube with a diameter of 16 mm and a height of 16 cm. The tube is sealed before being shaken vigorously in a vertical position for about ten seconds, then left for 10 minutes. If the foam exceeds 1 cm, this indicates the presence of saponosides (Yandjou *et al.*, 2023; Agbangnan *et al.*, 2012) [29, 2].

2.2.8 Statistical analyses

The information obtained from the surveys was processed manually and electronically. SPSS 20.0 software was used to analyze the questionnaire data. Excel was used to plot the graphs. SPSS 20.0 software was used for statistical analysis.

3. Results

3.1 Level of biodegradability of packaging biofilms by bacterial and fungal strains

3.1.1 Biodegradability of packaging biofilms by weight loss: Analysis of the biodegradability results for packaging biofilms supplemented with plant fibers shows that for all

microorganisms (*P. aeruginosa*, *S. aureus*, and *A. niger*), biodegradation is complete (100%) from day 20 to day 30 (Figure 2). Before the 20th day, biodegradation is faster for packaging biofilms supplemented with fibers from both plant species (*T. daniellii* + *M. paradisiaca*), with weight loss percentages ranging from 54.7% to 74.5% (Figure 2).

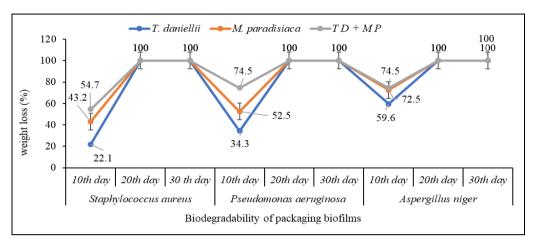


Fig 2: Biodegradability of packaging biofilms by weight loss

3.1.2 Biodegradability Rate of Packaging Biofilms

The results for *S. aureus* show biodegradability rates below 0.5 OD units (optical density) for all packaging biofilms supplemented with plant fibers (Figure 3). These

biodegradability rates range from 0.4 to 1 and from 0.5 to 0.9 OD units for *P. aeruginosa* and *A. niger* strains, respectively (Figure 3).

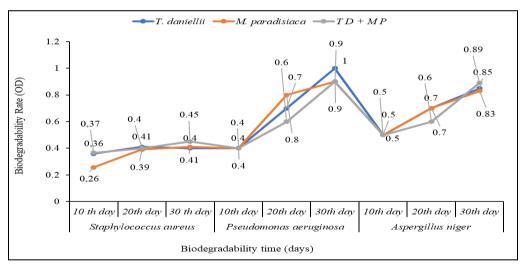


Fig 3: Changes in Biodegradability

3.1.3 Biodegradability of Packaging Biofilms by Estimating Cell Abundance Cell Abundance

Cell abundance is reflected in a variation in microbial biomass ranging from 20,10³ to 680,10⁷ in biodegradability solutions. On day 10, in order of growth importance, this cell abundance was most significant for *P. aeruginosa* with 60.10³ CFU/mL (*T. daniellii* and *M. paradisiaca*), 210.10⁵ (*T. daniellii*) and 680.10⁷ CFU/mL (*M. paradisiaca*) (Table

1). The different biological degradations of packaging biofilms supplemented with *M. paradisiaca* fibers indicate higher biomasses ranging from 230,10⁷ CFU/mL (*S. aureus*) to 680,10⁷ CFU/mL (*P. aeruginosa*) (Table 1). This biomass, ranging from 20.10³ CFU/mL (*A. niger*) to 60.10³ CFU/mL (*P. aeruginosa*), is lower in biodegradability solutions supplemented with *T. daniellii* and *M. paradisiaca* (Table 1).

Table 1: Biodegradability by cell abundance

Types of germs	Biodegradability of biofilms in CFU/mL on day 10				
	T. daniellii	M. paradisiaca	T. daniellii + M. paradisiaca		
P. aeruginosa	210.10 ⁵	680.10 ⁷	60.10^3		
A. niger	50.10 ⁵	470.10^7	20.10^3		
S. aureus	70.10^5	230.10 ⁷	30.10^3		

3.2 Phytochemical characteristics of plant packaging biofilms

Phytochemical screening analysis showed the presence of sterols and polyterpenes, polyphenols, flavonoids, and alkaloids in packaging biofilms supplemented with *M. paradisiaca*, *T. daniellii*, and *M. paradisiaca* + *T. daniellii* fibers (Table 2). Catechin and gallic tannins were only present in the biofilms of

packaging supplemented with *M. paradisiaca* fibers and those supplemented with both plant fibers (Table 2). Saponins were present in biofilms supplemented with *M. paradisiaca* fibers and *T. daniellii* fibers only, while quinone compounds were present in biofilms supplemented with a mixture of plant fibers (Table 2).

Table 2: Phytochemical	compounds	present in f	formulated	nackaging	biofilms
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Phytochemical compounds		Fiber-supplemented packaging biofilms				
		M. paradisiaca T. daniellii		M. paradisiaca + T. daniellii		
Sterols and polyterpenes		+	+	+		
Polyphenols		+ +		+		
Flavonoids		+	+	+		
Saponins		+	+	-		
Quinone compounds		•	-	+		
Alkaloids		+	+	+		
Tannins	Catechins	+	-	+		
	Gallic acids	+	-	+		

^{+:} Presence of phytochemical compound; -: Absence of phytochemical compound

4. Discussion

Reducing plastic pollution and its harmful effects by seeking sustainable measures is a global environmental concern (Macheca et al., 2024; Preda et al., 2024) [13, 19]. Among renewable ecological measures, bioremediation is a method that uses microorganisms to degrade contaminants in soil and water (Mahesh et al., 2024) [14]. This study focuses on the microbial degradation of plant-based packaging biofilms using fungal and bacterial strains. On the other hand, it highlights the potential of these packaging biofilms to be used in the design of modern, ecofriendly packaging that respects the environment.

Three different types of plant-based packaging biofilms with a starch matrix supplemented with plant fibers from *T. daniellii* and *M. paradisiaca*, as well as pre-formulated combined fibers, were subjected to biodegradation (Benie *et al.*, 2025b) ^[5].

This work indicated that the biodegradability of these packaging biofilms supplemented with plant fibers is complete with the species *P. aeruginosa*, *S. aureus*, and *A. niger* from the 20th to the 30th day. This result shows that these species of bacteria and fungi could develop metabolic and enzymatic capabilities enabling them to degrade these packaging biofilms (Mahesh *et al.*, 2024; Habumugisha *et al.*, 2024) [14, 10].

This microbial degradation involves the use of extracellular and intracellular depolymerases by organisms to break down the organic substances contained in these packaging biofilms (Tamnou, 2022) [23]. Several studies have demonstrated the potential of microorganisms to degrade biofilms, particularly synthetic plastics (Mahesh *et al.*, 2024) [14]. Exoenzymes produced by these microorganisms could cause the polymer chain to break down, generating oligomers and monomers that can be absorbed and used for microbial metabolism (Dela Torre *et al.*, 2018) [8].

The cellular abundance observed in this study also indicated an exponential rate of biodegradability of packaging biofilms with *P. aeruginosa* and *A. niger* strains (Tamnou, 2022; Dela Torre *et al.*, 2018) [23, 8]. This biodegradability rate ranged from 0.4 to 1 and 0.5 to 0.9 DO units for *P. aeruginosa* and *A. niger* strains, respectively. This result shows that the *P. aeruginosa* and *A. niger* strains studied are capable of using the starch or plant fibers in packaging biofilms as their sole source of carbon at varying temperatures and pH levels (Tamnou, 2023; Tamnou, 2022) [24].

Indeed, the species *P. aeruginosa*, *A. niger*, and *S. aureus* are known to be present in a wide range of habitats due to

their ubiquity, high versatility, and extreme metabolic diversity (Tamnou, 2022) $^{[23]}$.

These same findings were reported by Thomas *et al.* (2015) [25], who reported the potential of *P. aeruginosa*, *S. aureus*, and *A. niger* in the bioremediation of sites contaminated with plastic pollutants. These species have the ability to adapt to many physicochemical conditions, allowing them to survive or adapt to these different environments (Tamnou, 2023; Dela Torre *et al.*, 2018) [24, 8].

In addition, the various biological degradations of packaging biofilms supplemented with *M. paradisiaca* fibers indicated higher biomasses with *P. aeruginosa* (680,107 CFU/mL). The synthesis of large quantities of extracellular esterases and lipases by *P. aeruginosa* could explain this high biomass and the speed of biodegradation of packaging biofilms supplemented with *M. paradisiaca* fibers (Novotny *et al.*, 2015) [17].

In addition, the biosynthesis of biosurfactants could also be an important inducer of bioremediation potential in P. aeruginosa (Saruni et al., 2019) [21]. Indeed, this biosynthesis of biosurfactants by P. aeruginosa could play an important role in the biodegradation of packaging biofilms (Montazer *et al.*, 2020) [16]. These biosurfactants are microbial secondary metabolites that stimulate the activity of the surface or pollutant to be degraded or cleaned. In their study, Wilkes and Aristilde (2017) [27] indicated that P. aeruginosa is one of the best and most sought-after bioremediation and bioremediation agents due to its extensive metabolism in plastic polymers. The potential of these microbial strains to degrade these formulated biofilms indicates that T. daniellii and M. paradisiaca leaf fibers could be ideal materials with phytochemical properties for the design of modern and environmentally friendly packaging.

In this study, phytochemical screening also revealed the presence of sterols and polyterpenes, polyphenols, flavonoids, and alkaloids in the biofilms of packaging supplemented with plant fibers. The presence of these phytochemical compounds, such as flavonoids and alkaloids, could enhance the antimicrobial action of these packaging biofilms. This could extend the shelf life of packaged foodstuffs (Dela Torre *et al.*, 2018; Tamnou, 2023) ^[8, 24]. These phytochemicals could also confer antioxidant properties to packaging biofilms during substance transfer from packaging biofilms to hot foods

(Agarwal et al., 2022) [1].

Therefore, the presence of these compounds could protect the cells of packaged food consumers from damage caused by free radicals (Tsvetozara and Tsvetelina, 2025) [26]. In addition, these phytochemicals may positively influence cardiovascular health, strengthen the immune system, and have potential effects on the prevention of certain cancers in consumers of packaged foods (Tsvetozara and Tsvetelina, 2025) [26].

The presence of catechin and gallic tannins could also give packaging biofilms antioxidant and antimicrobial properties, which could help prevent foodborne illness (Duda-Chodak *et al.*, 2023; Ahari *et al.*, 2022) ^[9, 3]. In addition to the pharmacological potential of the various chemical groups found in these packaging biofilms, their use as biodegradable packaging materials could also have therapeutic benefits.

This study has therefore demonstrated the potential of these biofilms, formulated from agri-resource materials, to be used in the design of biodegradable and environmentally friendly food packaging.

5. Conclusion

Cette étude a montré la potentialité de biodégradabilité des biofilms d'emballages supplémentés de fibres Thaumatococcus daniellii, de Musa paradisiaca et de fibres combinées de ces deux espèces végétales. L'estimation par perte de poids et par l'abondance cellulaire ont montré une vitesse exponentielle de biodégradabilité avec les espèces de P. aeruginosa, S. aureus et A. niger. La biodégradabilité des biofilms d'emballage supplémentés de fibres de M. paradisiaca a indiqué les plus grandes biomasses avec P. aeruginosa. La détection de composés phytochimiques a indiqué la présence de stérols et de polyterpènes, de polyphénols, de flavonoïdes et d'alcaloïdes et de tanins catechiques et de tanins galliques dans les biofilms d'emballages supplémentés de fibres végétales. Cette étude a indiqué que ces biofilms supplémentés de fibres peuvent être utilisés pour la conception d'emballages alimentaires modernes biodégradables et respectueux des conditions écologiques.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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