

Contents lists available at ScienceDirect

I.WT

journal homepage: www.elsevier.com/locate/lwt



Antifungal *in vitro* potential of *Aloe vera* gel as postharvest treatment to maintain blueberry quality during storage

Francisca Sempere-Ferre ^{a,*}, Silvia Giménez-Santamarina ^b, Josefa Roselló ^b, M. Pilar Santamarina ^b

ARTICLE INFO

Keywords: Antifungal activity Aloe vera Blueberries Biofilms

ABSTRACT

The postharvest life of most fruit and vegetables is limited by fungus proliferation. The *Aloe vera* gel was added at different concentrations to test its antifungal potential against five fungi (*Rhizoctonia solani, Curvularia hawaiiensis, Botryotinia fuckeliana, Penicillium italicum, Verticillium dahliae*), which cause significant losses in basic agricultural products included in the world's diet: tuber potato, cereals, fruit and vegetables. The best results were for the fungus *Verticillium dahlie*, with a mycelial growth inhibition of 100% and 70% at 200 and 100 mL/L, respectively. Satisfying results were for fungi *R. solani* and *B. fuckeliana*, where the mycelial growth inhibition exceeded 50% in them all at 200 mL/L. Antifungal activity was maintained in *B. fuckeliana* by lowering the dose to 100 mL/L. The *Aloe vera* extract at 300 mL/L was applied as a coating in the postharvest treatment to blueberry fruit at 21 °C and 85% humidity. When the experiment ended, the percentage of rotted berries was significantly lower in the treated than in the control. The *Aloe vera* gel could be considered a promising postharvest treatment to maintain blueberry quality and turgor during storage.

1. Introduction

In recent years some plant extracts have been successfully tested against different pre- and post-harvest microorganisms that produce significant economic losses worldwide and can cause diseases in humans. The study of different coatings based on these extracts has acquired much importance in the food industry in recent years as their application to foods of plant origin prolongs their useful life, helps to maintain their quality and acts an alternative to other conservation methods because they respect the environment and are also non-toxic at the applied doses (Hasan et al., 2021).

Aloe vera (L.) Burm.f., 1768 is a perennial succulent herbaceous plant of the Asphodelaceae family that grows in different climates around the world and its cultivation is used for different purposes in pharmaceutical, food and cosmetic industries. Aloe vera leaves are arranged in a rosette pattern at the stem and are triangular-shaped with soft spikes. Three parts can be distinguished: cuticle; outer leaf pulp that mainly contains anthraquinones and latex; inner layer that stores aloe gel

(Rahman et al., 2017).

Its leaves are made up of mainly water (95%), and present more than 200 bioactive compounds, such as carbohydrates, proteins, lipids, enzymes, minerals, vitamins and amino acids. Various currents of medicine have used *Aloe vera* for its therapeutic effects, such as antioxidant, antidiabetic, anti-inflammatory, immunomodulatory, antimicrobial, aphrodisiac, etc. (Banjare et al., 2014; Benzidia et al., 2019; Sonawane et al., 2021).

Aloe vera gel has excellent medicinal, functional and nutritional properties, and food values can rise if it is incorporated (Jayabalan & Karthikeyan, 2013). Its antioxidant and antifungal capacity against phytopathogenic and post-harvest fungi makes it optimal for developing coatings and biofilms used to conserve foods of plant and animal origins (Benítez et al., 2015; Benzidia et al., 2019; Ortega-Toro et al., 2017; Shahrezaee et al., 2018; Valverde et al., 2005).

Plant pathogenic fungi cause some of the most devastating crop diseases. In addition, postharvest diseases of fruit and vegetables lead to 30–50% reduced production, and greatest losses occur in developing

E-mail addresses: frasemfe@upvnet.upv.es (F. Sempere-Ferre), silviasantam@upvnet.upv.es (S. Giménez-Santamarina), jrosello@upvnet.upv.es (J. Roselló), mpsantam@eaf.upv.es (M.P. Santamarina).

a Departamento de Estadística e Investigación Operativa Aplicadas y Calidad, Universitat Politècnica de València, Camino de Vera s/n, 46022, Valencia, Spain

^b Departamento de Ecosistemas Agroforestales, Escuela Técnica Superior de Ingeniería Agronómica y del Medio natural, Universitat Politècnica de València, Camino de Vera s/n, 46022, Valencia, Spain

^{*} Corresponding author.

Table 1Growth mean and Mycelial Growth Inhibition (MGI) for each species in *Aloe vera* medium (AVM) at the doses of 200 and 100 mL/L at 7 days of growth.

	Control	MGI					
Species	$\pm SD$	200 mL/L	200 mL/L		100 mL/L		
		AVM 200 ± SD	% Inhibition	AVM 100 ± SD	% Inhibition		
Botriotyna fuckeliana	88.50 ± 1.075	43.70 ± 1.841	50.62	43.80 ± 1.889	50.51		
Curvularia hawaiiensis	47.30 ± 0.823	$\begin{array}{c} 33.70 \pm \\ 1.160 \end{array}$	28.75	-	-		
Rhizoctonia solani	$137.20 \pm \\ 1.476$	$68.40 \pm \\1.350$	50.15	91.40 ± 0.516	33.38		
Penicillium italicum	31.90 ± 0.738	22.80 ± 2.348	28.53	-	-		
Verticillium dahliae	26.30 ± 0.823	0.00 ± 0.00	100	$\begin{array}{c} \textbf{7.20} \pm \\ \textbf{1.1352} \end{array}$	70.62		

SD: standard deviation.

countries (Sanzani et al., 2016; Villa et al., 2017).

The herein studied fungi were Botryotinia fuckeliana, Curvularia hawaiiensis, Penicillium italicum, Rhizoctonia solani and Verticillium dahliae. They have been considered to lie at the top of phytopathogenic and postharvest fungi for their scientific and economic importance. The fungus Rhizoctonia solani is a pathogen of many plants and severely damages crops worldwide. In potato plants, it is able to develop at very different temperatures, and causes considerable damage to emerging tuber shoots when grown in moist, acidic and cold soils (Tsror, 2010). Curvularia is a very destructive pathogen in cereals as it affects mainly the Poaceae family, wheat, rice, corn, sorghum and barley, and results in large agricultural production and grain storage losses. Furthermore, some species are associated with both humans and plants (Manamgoda et al., 2015; Pitt & Hocking, 2009). Botryotinia fuckeliana is the fungus that most frequently causes diseases in fruit and plants in general. Its diseases are probably the most frequent and the most widely distributed in vegetables, ornamental plants, fruit, and even in some field crops, worldwide. They are also the commonest diseases of greenhouse crops (Agrios, 2005; Fillinger & Elad, 2016) Penicillium italicum is a blue mould pathogen that causes vast economic losses in Citrus (Papoutsis et al., 2019). *Verticillium dahliae* is one of the most important pathogenic fungi, is capable of infecting more than 100 dicotyledonous hosts annual and perennial, herbaceous and woody plants, and causes significant losses in olive trees, vegetables and red fruits (Agrios, 2005; Tian et al., 2017).

Blueberry (*Vaccinium* spp.) is a plant of the ericaceous family that produces small edible blue (*Vaccinium corymbosum*) or red (*Vaccinium oxycoccus*) berries (Bell et al., 2021). After being harvested, their shelf life usually lasts 7–40 days, depending on different factors like genotype, harvest method, storage conditions and microbial inocula, which are serious problems for producing countries. The postharvest diseases in these fruits caused primarily by fungi *Colletotrichum* spp. (ripe rot), *Alternaria* spp. (Alternaria fruit rot), and *Botrytis cinerea* (grey mould) have been reported (Nambeesan et al., 2018). However, other microbial inocula in *Vaccinum* spp., such as *Verticillium dahliae*, may proliferate during storage and can affect fruit quality.

The aims of this study were to: I) determine the antifungal capacity under "in vitro" conditions of the Aloe vera gel at different concentrations against Rhizoctonia solani, Curvularia hawaiiensis, Botryotinia fuckeliana Penicillium italicum and Verticillium dahliae; 2) study its activity in blueberry fruits against Verticillium dahliae to develop a natural environmentally friendly film.

2. Materials and methods

2.1. Aloe vera gel

The gel of the *Aloe vera* leaves from organic farming was supplied by the TEIDEALOE (www.pencazabila.net) commercial company (batch 20200401).

To obtain *Aloe vera* gel, the following procedure was followed: after receiving leaves, they were left to rest in pools of cold water for 24 h to remove the aloin and impurities associated with leaves. After cutting lateral peaks and bases, leaves were subjected to basal and lateral peeling, which was when rind was removed and pulp was obtained. Pulp was placed inside a cold tunnel hopper at 5 $^{\circ}$ C without air to avoid oxidation and impurities, where it was crushed during several sequences and emulsified. Finally, the resulting gel was filtered twice and packaged.

99.5% of the gel was composed of water. The dry extract was dominated by carbohydrates (mannose, glucose, fructose, cellulose, glucomannan, acemannan, etc.), while phenols (aloin and aloemodin), enzymes such as amylase and catalase, vitamins A, C and E, the B complex, minerals like calcium, iron and zinc, amino acids such as lysine, cysteine and glycine, fatty acids, among others, were also isolated. In its composition, the concentration of acemannans stood out which, due to the climate conditions in the area, was obtained at a concentration above 2000 ppm when the usual concentration is around 1250 ppm.

The final product consisted of 99.7% gel and the rest was comprised of xanthan gum to thicken the solution. A small amount of additives is approved for organic farming to avoid product deterioration.

2.2. Phytopathogenic and postharvest fungi

Four phytopathogenic fungi, namely *Botryotinia fuckeliana* (BF) CECT 2100, *Penicillium italicum* (PI) CECT 2294 and *Rhizoctonia solani* (RS) CECT 2819 and *Verticillium dahliae* risk group 1 (VD) CECT 2694, were supplied by the Spanish Type Culture Collection (CECT).

Curvularia hawaiiensis (CH) CECT 20934 was isolated in the Botanical Laboratory of the Departamento de Ecosistemas Agroforestales, Universitat Politècnica de València, Spain. This fungal species was morphologically and molecularly identified before being deposited in the culture collection.

2.3. Culture media

Two *Aloe vera* culture media (AVM) at different concentrations (100 mL/L and 200 mL/L) were tested following the methodology of Castillo et al. (2010). For its preparation, Potato Dextrose Agar (PDA), Tween 20 and water were incorporated into flasks and sterilised for 15 min at $\frac{118\,^{\circ}C}{100}$

Subsequently, the *Aloe vera* gel was added under aseptic conditions and homogenised in an orbital shaker at 170 rpm for 10 min. The medium was distributed in 90 mm- and 150 mm-diameter Petri dishes.

2.4. Mycelial growth inhibition

Fungi were inoculated in the centre of AVM plates and control plates in the form of disc-shaped explants (8 mm diameter), which were extracted from the periphery of 7-day-old fungal colonies. Petri dishes were incubated at 25 $^{\circ}\text{C}$ for 7 days. Ten repetitions were performed per experiment. The medium used in the control plates was PDA/Tween 20 (0.1%). Mycelial growth was evaluated by measuring perpendicular colony diameters after 7 days.

Mycelial growth inhibition (MGI) was determined by the f formula (Albuquerque et al., 2006):

$$MGI = [(CD - OD)/CD] \times 100$$

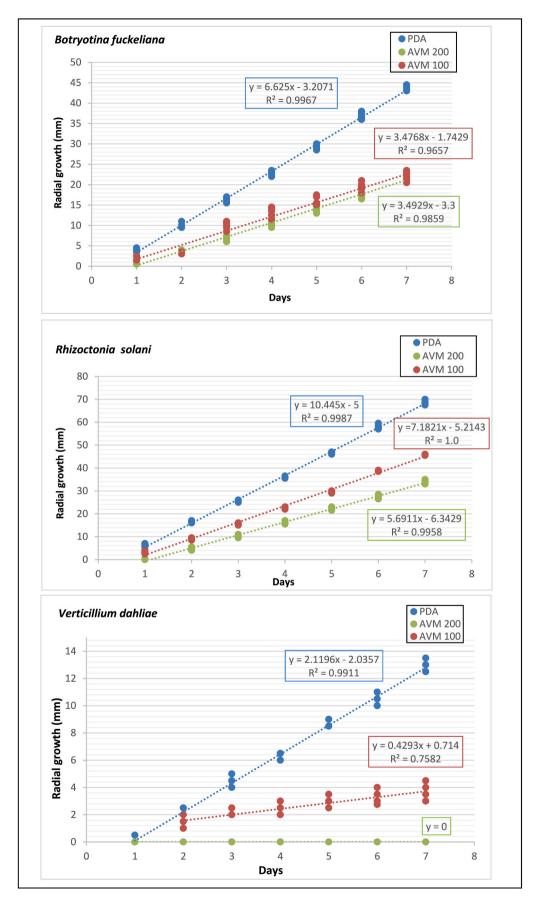


Fig. 1. Growth Rate (mm/day) of Botryotinia fuckeliana, Rhizoctonia solani and Verticillium dahliae at the different concentrations of assayed Aloe vera extract and their comparison to the control. PDA: Potato Dextrose Agar (control). AVM 200: Aloe vera medium 200 mL/L. AVM 100: Aloe vera medium 100 mL/L.

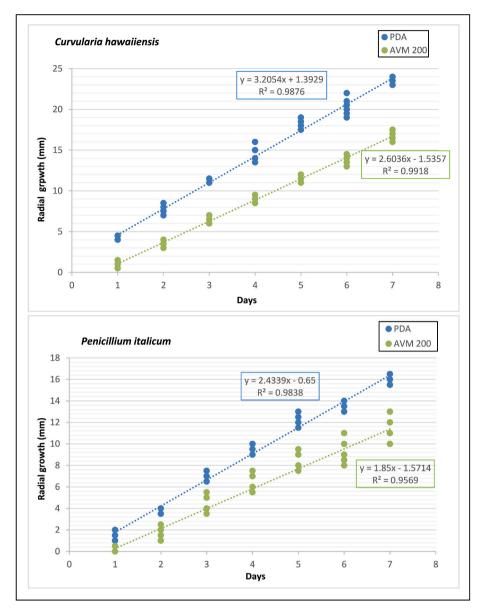


Fig. 2. Growth Rate (mm/day) of Curvularia hawaiiensis and Penicillium italicum at the 200 mL/L concentration of the Aloe vera extract and its comparison to the control. PDA: Potato Dextrose Agar (control). AVM 200: Aloe vera medium 200 mL/L.

where CD is the average of the colonies in the control dishes, DO is the average of the colonies in AVM.

2.5. In vivo studies

2.5.1. Preparing Aloe vera film solution for fruit coating (AVF)

The *Aloe vera* extract solution for coating fruit was prepared at the 300 mL/L concentration. The *Aloe vera* gel was homogenised by orbital shaking at 170 rpm for 10 min in flasks containing a sterile solution of water/Tween 20 (0.1%)/0.25% agar. Agar was used to improve film adherence to fruit, while Tween was employed to favour fungal inoculum dispersion.

A higher gel concentration was used because, unlike *in vitro* experimentation, conditions are less controlled. In addition, previous studies have shown that a higher concentration is needed to obtain a film that is effective under these conditions (Ortega-Toro et al., 2017).

2.5.2. Preparing fungal inoculum film (FIF)

To recovery the blueberry fruit with the fungus, a solution containing

Verticillium dahliae propagules was prepared. To do so, 10 mL of a suspension of 1×10^8 ufc/mL of the fungus were added to 90 mL sterile solution of water/Tween 20 (0.1%)/0.25% agar. The mixture was homogenised by orbital shaking at 170 rpm for 10 min to obtain a homogeneous suspension.

2.5.3. Blueberries coated with the Aloe vera solution and the fungal inoculum

Blueberry fruits (*Vaccinum corymbosum* L. variety legacy) (origin Huelva, Andalusia province, Spain) were sterilised superficially with 1% sodium hypochlorite solution for 2 min and then washed twice with sterile distilled water for 4 min. Fruits were distributed into three batches of 50 units each (2 controls and the *A. vera* film treatment). They were all subjected to a small wound (1 mm depth) on the surface made with a sterile needle (punch).

In the *Aloe vera* film treatment, 50 damaged fruit were immersed in the solution containing the *Aloe vera* extract (AVF) for 4 min before being placed on racks and dried for 24 h at room temperature. They were then bathed in the fungal inoculum (FIF) for 2 min. The fruit covered with the V. *dahliae* suspension were placed on racks.

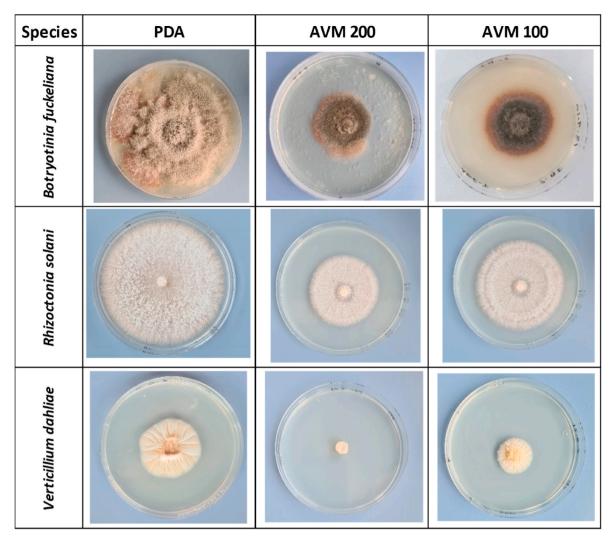


Fig. 3. Mycelial growth of 7th day of Botryotinia fuckeliana, Rhizoctonia solani and Verticillium dahliae on PDA (Control) and different concentrations of Aloe vera medium (AVM) at 25 °C. AVM 200: Aloe vera medium 200 mL/L. AVM 100: Aloe vera medium 100 mL/L.

Table 2 Effects of *Aloe vera* film (100 and 200 mL/L) on radial growth and growth rates of *Botriotyna fuckeliana, Rhizoctonia solani*, and *Verticillium dahliae*. Confidence intervals with a probability of 0.95.

Treatment	Species	Mean	Lower limit	Upper limit	GR	
Control	B. fuckeliana	$\begin{array}{c} 23.29 \pm \\ 1.15 \end{array}$	21.68	24.90	6.63 (0.997)	
	R. solani	36.78 ± 1.93	34.08	39.48	10.45 (0.999)	
	V. dahliae	$\begin{array}{l} \textbf{6.44} \pm \\ \textbf{0.31} \end{array}$	6.01	6.87	2.12 (0.991)	
200 mL/L	B. fuckeliana	$\begin{array}{c} 10.67 \pm \\ 1.15 \end{array}$	9.06	12.28	3.49 (0.986)	
	R. solani	$16.42 \pm \\1.93$	13.73	19.12	5.69 (0.996)	
	V. dahliae	$\begin{array}{c} 0.00\ \pm \\ 0.00 \end{array}$	-0.43	0.43	0.00	
100 mL/L	B. fuckeliana	12.16 ± 1.15	10.56	13.77	3.48 (0.966)	
	R. solani	23.51 ± 1.93	20.82	26.21	7.18 (0.999)	
	V. dahliae	$\begin{array}{c} \textbf{2.27} \pm \\ \textbf{0.31} \end{array}$	1.84	2.70	0.43 (0.758)	

Mean: mean radius (mm) \pm standard deviation, GR: growth rate (R²).

In the assay 'control 1' (50 fruit), damaged fruit were only bathed with the fungal inoculum (FIF). In the assay 'control 2' (50 fruit), damaged fruit were first immersed for 4 min in the coating solution containing only agar and Tween, without the Aloe vera extract, to then be dried for 24 h and later bathed with the fungal inoculum (FIF).

In the blueberry tests, the three lots (control 1, control 2 and the *A. vera-*film treatment) were placed in the chamber at the same time (85% humidity at 21 $^{\circ}$ C). Blackberry evolution was controlled for 7 and 14 days.

2.6. Statistical analysis

The fungal growth results were submitted to an analysis of variance (ANOVA). The HSD Tukey intervals were represented to compare species and treatment, with significant values at p < 0.05. The data analysis was performed by Statgraphics Centurion XVIII.

3. Results

3.1. Calculation of mycelial growth inhibition (MGI) and growth rates in solid media

The *Aloe vera* gel gave excellent results (Table 1) as the 200 mL/L dose in fungus *Verticillium dahliae* totally inhibited its growth with values of MGI 100% and 70% MGI at the 100 mL/L dose. The MGI at

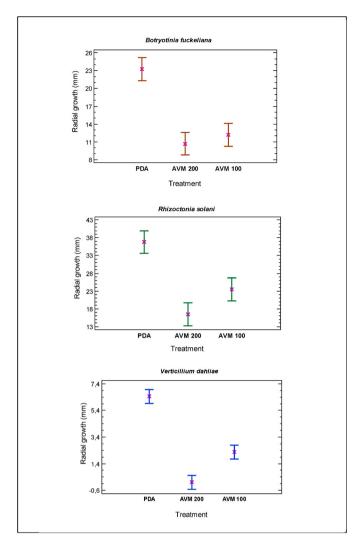


Fig. 4. Means and 95% Tukey's HSD, n=70 of *Botryotinia fuckeliana*, *Rhizoctonia solani* and *Verticillium dahliae* at the different concentrations of the assayed *Aloe vera* extract and their comparison to the control. PDA: Potato Dextrose Agar (control). AVM 200: *Aloe vera* medium 200 mL/L. AVM 100: *Aloe vera* medium 100 mL/L.

200 mL/L was 50% in *Botryotinia fuckeliana* and *Rhizoctonia solani*, while the MGI at this same dose was less than 50% in *Curvularia hawaiiensis* and *Penicillium italicum*. Hence the lowest dose was not tested on these fungi.

When the growth rate was calculated, species were inoculated for 7 days on control PDA medium, *Aloe vera* medium 200 mL/L (AVM 200) and *Aloe vera* medium 100 mL/L (AVM 100), except for *Curvularia hawaiiensis* and *Penicillium italicum*. *Rhizoctonia solani* was the fungus with the highest growth rates, followed by *Botryotinia fuckeliana*,

Curvularia hawaiiensis, Penicillium italicum and Verticillium dahliae (Figs. 1–3). The addition of the Aloe vera gel at the different concentrations brought about a reduction in the growth rates of the tested species in a dose-dependent manner.

Verticillium dahliae was the species in which Aloe vera gel produced the greatest inhibition. The growth rate was 10.45 mm/day on PDA versus 0.43 mm/day at 100 mL/L (80% reduction). No development was recorded at the highest dose (Figs. 1 and 3, and Table 2). When species B. fuckeliana was inoculated in Aloe vera medium at both concentrations, similar results were obtained, with MGI values of 50.51% and 50.62% (Figs. 1 and 3, Tables 1 and 2).

The growth rates of all the treatments were significantly different (P < 0.05). In the representation of Tukey's HSD graphs (Fig. 4), significant differences appeared between the different treatments in the assayed fungal species. In species $\it Rhizoctonia~solani~$ and $\it Verticillium~dahliae,$ statistically significant differences were recorded between the growth means of all the treatments. For $\it Botryotinia~fuckeliana,$ there were no significant differences between the two tested $\it Aloe~vera~$ gel concentrations.

3.2. In vivo test of the antifungal activity of Aloe vera gel against Verticillium dahliae on blueberry fruit

During the trial, three measurements were taken after 7, 15 and 30 days at 21 °C and 85% humidity (Table 3, Fig. 5). After 30 days, the fruit not bathed in *Aloe vera* film, Control 1 (fruit bathed only in the *Verticillium dahliae* fungal solution), the percentage of rotten, stained and healthy fruit was 53%, 0% and 47%, respectively, which was more than half the rotted fruit. In Control 2 (fruit dipped in coating solution, but with no *Aloe*, and then bathed in fungal solution) the percentage of rotten, stained and healthy fruit was 33%, 7% and 60%, respectively, and the film with no *Aloe* had a certain protective effect. In the experiment in which the fruit with the coating containing film and *Aloe* were bathed in fungal inoculum, the fungus poorly developed, only 13% of the fruit rotted, 13% were stained, while 74% remained healthy. This finding demonstrates the protective effect of *Aloe vera* gel.

4. Discussion

This manuscript investigated the effect of *Aloe vera* gel against five pathogenic species under *in vitro* conditions and the effect of a natural biodegradable film against *Verticillium dahliae* in blueberries.

The results obtained in this research agree with those reported by other authors in which the inhibition of different fungal species' development was greater when the gel concentration in the medium increased (Sitara et al., 2011; Zapata et al., 2013). According to Sitara et al. (2011), when Aloe vera gel was tested against fungi Aspergillus niger, Aspergillus flavus, Alternaria alternata, Drechslera hawaiensis and Penicillium digitatum at the concentrations of 150, 250 and 350 mL/L by the agar diffusion plate method in PDA, it inhibited the development of all species, but only had a fungicidal effect against D. hawaiensis and A. alternata at 350 mL/L. In another study in which Fusarium sp., Lasiodiplodia theobromae, A. niger and Colletotrichum gloeosporioides were

Table 3

Efficacy of treatment with *Aloe vera* film (AVF) at 300 mL/L against the fungal development of *Verticillium dahliae* on blueberry fruit at 85% HR and 21 °C.

Test	7 days			15 days			30 days		
	rotten (%)	stained (%)	healthy (%)	rotten (%)	stained (%)	healthy (%)	rotten (%)	stained (%)	healthy (%)
Control 1	13	14	73 b	47	0	53 с	53	0	47 c
Control 2	0	7	93 a	27	7	66 b	33	7	60 b
Aloe film	0	7	93 a	7	13	80 a	13	13	74 a

Control 1. Fruit without film and without Aloe.

Control 2. Fruit with film and without Aloe.

AVF. Fruit with film and Aloe.

Different letters in the same column indicate a significant difference at 95% level probability by Tukey's HSD.

Day	Control 1	Control 2	Aloe film
7			
15			

Fig. 5. Assay of the biofilm of *Aloe vera* at 300 mL/L in blueberries fruits against *Verticillium dahliae* at 7 and 15 days. Control 1, Fruits without film and without *Aloe*; Control 2, fruits with film and without *Aloe*; film, blueberry with film and *Aloe*; r, rotten; s, stained; h, healthy.

also inoculated on PDA, filtered fresh *Aloe vera* gel was able to inhibit fungal growth better than an unfiltered gel (Mendy et al., 2019). It was not very effective against *Aspergillus carbonarius* in reducing its growth in Czapek Yeast Agar medium, with 11.59% at the 50% concentration, but showed marked anti-ochratoxigenic activity (Dammak et al., 2018).

The differences obtained by different researchers in *Aloe vera* gel effectiveness could be due to the fact that the concentration of different potential molecules may vary depending on a plant's harvest time and other factors related to its cultivation: geographical location, soil, etc. Moreover, the processing of gel and its heat treatment can also affect its composition.

Different authors have suggested that the aloin or barbaloin (10-glucopyranosyl-1.8-dihydroxy-3-(hydroxymethyl)-9(10H)-anthracenone) concentration can be involved in its fungicidal activity, while the exerted mechanism of action could be because it affects the phospholipid membrane of the fungal wall and cause its disruption (Zapata et al., 2013). However, other gel compounds like acemannan have also been described as fungicides (Heś et al., 2019). The gel used in this research was characterised by a high acemannan concentration, which is possibly the main compound involved in fungicidal effectiveness against the studied species, although the presence of aloin is also described in its composition.

Aloe vera extracts obtained from different leaf parts have also been tested against several fungal species using various methodologies with contrasting results. The phenolic extracts of the species at different

concentrations brought about a considerable decrease in the development of Fusarium oxysporum f. sp. radicis-lycopersici, Phaeomoniella chlamydospora, Phaeoacremonium aleophilum and Sporisorium scitamineum (Pintos et al., 2019). Jasso de Rodríguez et al. (2005) studied the antifungal activity of *Aloe vera* pulp and liquid fraction on the mycelial growth and development of Rhizoctonia solani, Fusarium oxysporum and Colletotrichum coccodes isolated from a potato crop. Those authors reported that the liquid fraction inhibited the mycelial growth of the three pathogens, whereas pulp inhibited only F. oxysporum. Aqueous extractions of water soluble ingredients were effective in the control of three Alternaria species (Alternaria alternata, A. citri and A. tenuissima) (Bajwa et al., 2007). The minimum fungicidal concentration varied between 80 mL/L and 100 mL/L when hydro alcoholic extracts against the mycelial growth of Botrytis gladiolorum, Fusarium oxysporum sp., gladioli, Heterosporium pruneti and Penicillium gladioli on Czapek-agar medium were tested (Rosca-Casian et al., 2007). Saniasiaya et al. (2017) reported that the antifungal effect of alcohol extracts of Malaysian Aloe vera leaf was better than the aqueous extract for A. niger. Similar results have been obtained when testing different solutions against A. flavus (Babaei et al., 2013).

The natural biofilm obtained from *Aloe vera* gel at a concentration of 300 mL/L was effective in inhibiting V. *dahliae* development when applied to blueberries. Moreover, a higher percentage of healthy fruit was obtained than for other treatments, as well as statistically significant differences. This fungus is an important soilborne fungus that can

remain viable in soil for years which affects fruit and other plant parts (Bell et al., 2021; Serdani et al., 2018).

Under different conditions, gel and other extracts have been successfully tested *in vivo* to fight against different postharvest fungi. Valverde et al. (2005) developed an edible coating from plants as a conservation means to maintain table grape quality. With this coating, they were able to extend the useful life of grapes up to 35 days at 1 $^{\circ}$ C, while the useful life at the same temperature was 7 days in the untreated grapes. In addition, the presence of yeasts and moulds reduced in the treated grapes, whereas they significantly increased during storage in those without coatings. This efficacy has also been shown against different fungi responsible for postharvest fruit disease in papaya.

Aloe vera gel can be an alternative to synthetic preservatives and is being used as an edible coating alone or combined with other substances for whole or fresh-cut fruit and vegetables by acting as a protective layer against chemical, physical or microbiological changes can enhance product shelf life and safety. Prevention of weight loss, firmness and colour changes, their effect on respiration, ripening and enzymatic activity, and the preservation of bioactive compounds and antioxidant activity, have all been reported in different foods like oranges, strawberries, grapes, litchi fruit, mangoes, tomatoes, etc. Studies like this aim to know and improve the effectiveness of the biofilms that derive from this plant species.

5. Conclusions

The natural and biodegradable film created from *Aloe vera* at a concentration of 300 mL/L, applied in the postharvest treatment, was able to extend shelf life and maintain blueberry fruit quality for 30 days at 21 $^{\circ}$ C and 85% humidity. This natural film could be developed as a natural environmentally friendly product that respects consumer health.

Author credit statement

The work herein presented was carried out with the collaboration of all the authors. Conceptualization, M.P.S. and J.R.; methodology, M.P. S., J.R and F.S-F; investigation, J.R., S.G-S and F.S-F.; original draft preparation, F.S-F. and M.P.S.; writing, F.S-F and M.P.S; review and editing, M.P.S., F.S-F.; supervision, M.P.S. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare that they have no competing interests.

References

- Agrios, G. N. (2005). Plant pathology (5^{th} ed.). Elsevier Academic Press.
- Albuquerque, C. C., Camara, T. R., Willadino, R. D. R., & Ulises, C. (2006). Antimicrobial action of essential oil of *Lippia gracilis* Schauer. *Braz Arch Biol Technol*, 49, 527–535.
- Babaei, A., Manafi, M., & Tavafi, H. (2013). Study on effect of Aloe vera leaf extracts on growth of Aspergillus flavus. Annual Research & Review in Biology, 1091–1097.
- Bajwa, R., Shafique, S., & Shafique, S. (2007). Appraisal of antifungal activity of Aloe vera. Mycophat, 5(1), 5–9.
- Banjare, K., Kumar, M., Nanda, H. C., Karthikeyan, S., & Lakpale, R. (2014). Advances in herbal prospects processing and food products researches on aloe vera: A review. *International Journal of Food and Nutritional Sciences*, 3(3), 28.
- Bell, S. R., Hernández Montiel, L. G., González Estrada, R. R., & Gutiérrez Martínez, P. (2021). Main diseases in postharvest blueberries, conventional and eco-friendly control methods: A review. LWT, 149, 112046. https://doi.org/10.1016/j. lwt.2021.112046
- Benítez, S., Achaerandio, I., Pujolà, M., & Sepulcre, F. (2015). Aloe vera as an alternative to traditional edible coatings used in fresh-cut fruits: A case of study with kiwifruit slices. Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 61(1), 184–193. https://doi.org/10.1016/j.lwt.2014.11.036
- Benzidia, B., Barbouchi, M., Hammouch, H., Belahbib, N., Zouarhi, M., Erramli, H., Ait Daoud, N., Badrane, N., & Hajjaji, N. (2019). Chemical composition and antioxidant activity of tannins extract from green rind of Aloe vera (L.) Burm. F. Journal of King Saud University Science, 31(4), 1175–1181. https://doi.org/10.1016/j. iksus.2018.05.022
- Castillo, S., Navarro, D., Zapata, P. J., Guillén, F., Valero, D., Serrano, M., & Martínez-Romero, D. (2010). Antifungal efficacy of *Aloe vera* in vitro and its use as a

- preharvest treatment to maintain postharvest table grape quality. *Postharvest Biology and Technology*, *57*(3), 183–188. https://doi.org/10.1016/j. postharvbio.2010.04.006
- Dammak, I., Lasram, S., Hamdi, Z., Ben Moussa, O., Mkadmini Hammi, K., Trigui, I., Houissa, H., Mliki, A., & Hassouna, M. (2018). In vitro antifungal and antiochratoxigenic activities of Aloe vera gel against Aspergillus carbonarius isolated from grapes. Industrial Crops and Products, 123, 416–423. https://doi.org/10.1016/j. indcrop.2018.07.023
- Fillinger, S., & Elad, Y. (Eds.). (2016). Botrytis The fungus, the pathogen and its management in agricultural systems. Springer International Publishing. https://doi.org/10.1007/978-3-319-23371-0
- Hasan, M. U., Riaz, R., Malik, A. U., Khan, A. S., Anwar, R., Rehman, R. N. U., & Ali, S. (2021). Potential of *Aloe vera* gel coating for storage life extension and quality conservation of fruits and vegetables: An overview. *Journal of Food Biochemistry*, 45 (4), Article e13640. https://doi.org/10.1111/jfbc.13640
- Heé, M., Dziedzic, K., Górecka, D., Jędrusek-Golińska, A., & Gujska, E. (2019). Aloe vera (L.) Webb.: Natural sources of antioxidants – A review. Plant Foods for Human Nutrition, 74(3), 255–265. https://doi.org/10.1007/s11130-019-00747-5
- Jasso de Rodríguez, D., Hernández-Castillo, D., Rodríguez-García, R., & Angulo-Sánchez, J. L. (2005). Antifungal activity in vitro of Aloe vera pulp and liquid fraction against plant pathogenic fungi. Industrial Crops and Products, 21(1), 81–87. https://doi.org/10.1016/j.indcrop.2004.01.002
- Jayabalan, C. K., & Karthikeyan, C. (2013). Optimizations of ingredients for sensory evaluation of Aloe vera jam using preparation response surface methodology. International Journal of Engineering Research and Applications, 3(1), 1224–1234.
- Manamgoda, D. S., Rossman, A. Y., Castlebury, L. A., Chukeatirote, E., & Hyde, K. (2015). A taxonomic and phylogenetic re-appraisal of the genus *Curvularia* (Pleosporaceae): Human and plant pathogens. *Phytotaxa*, 212(3), 175. https://doi.org/10.11646/ phytotaxa.212.3.1
- Mendy, T. K., Misran, A., Mahmud, T. M. M., & Ismail, S. I. (2019). Antifungal properties of *Aloe vera* through in vitro and in vivo screening against postharvest pathogens of papaya fruit. *Scientia Horticulturae*, 257, 108767. https://doi.org/10.1016/j. scienta.2019.108767
- Nambeesan, S., Doyle, J., Capps, H., Starns, C., & Scherm, H. (2018). Effect of electronic cold-pasteurization[™] (ECP[™]) on fruit quality and postharvest diseases during blueberry storage. *Horticulturae*, 4(3), 25. https://doi.org/10.3390/horticulturae4030025
- Ortega-Toro, R., Collazo-Bigliardi, S., Roselló, J., Santamarina, P., & Chiralt, A. (2017). Antifungal starch-based edible films containing *Aloe vera. Food Hydrocolloids*, 72, 1–10. https://doi.org/10.1016/j.foodhyd.2017.05.023
- Papoutsis, K., Mathioudakis, M. M., Hasperué, J. H., & Ziogas, V. (2019). Non-chemical treatments for preventing the postharvest fungal rotting of citrus caused by Penicillium digitatum (green mold) and Penicillium italicum (blue mold). Trends in Food Science & Technology, 86, 479–491. https://doi.org/10.1016/j.tifs.2019.02.053
- Pintos, B., Martín-Calvarro, L., Piñón, D., Esteban, A., Tello, M. L., Pérez-Urria, E., & Gómez-Garay, A. (2019). Antifungal effects of phenolic extract from industrial residues of Aloe vera. Spanish Journal of Agricultural Research, 16(4), 1010. https://doi.org/10.5424/sjar/2018164-12480
- Pitt, J. I., & Hocking, A. D. (2009). Fungi and food spoilage (3rd ed.). Springer US. https://doi.org/10.1007/978-0-387-92207-2
- Rahman, S., Carter, P., & Bhattarai, N. (2017). Aloe vera for tissue engineering applications. Journal of Functional Biomaterials, 8(1), E6. https://doi.org/10.3390/ jfb8010006
- Rosca-Casian, O., Parvu, M., Vlase, L., & Tamas, M. (2007). Antifungal activity of Aloe vera leaves. Fitoterapia, 78(3), 219–222. https://doi.org/10.1016/j. fitote 2006 11 008
- Saniasiaya, J., Salim, R., Mohamad, I., & Harun, A. (2017). Antifungal effect of Malaysian Aloe vera leaf extract on selected fungal species of pathogenic otomycosis species in in vitro culture medium. Oman Medical Journal, 32(1), 41. https://doi.org/10.5001/ omi_2017_08
- Sanzani, S. M., Reverberi, M., & Geisen, R. (2016). Mycotoxins in harvested fruits and vegetables: Insights in producing fungi, biological role, conducive conditions, and tools to manage postharvest contamination. *Postharvest Biology and Technology*, 122, 95–105. https://doi.org/10.1016/j.postharvbio.2016.07.003
- Serdani, M., Wiseman, M. S., Inderbitzin, P., & Putnam, M. L. (2018). First report of Verticillium dahliae causing dieback of highbush blueberry (Vaccinium corymbosum) in Oregon and Washington. Plant Disease, 102(2). https://doi.org/10.1094/PDIS-02-17-0229-PDN, 439-439.
- Shahrezaee, M., Soleimanian-Zad, S., Soltanizadeh, N., & Akbari-Alavijeh, S. (2018). Use of *Aloe vera* gel powder to enhance the shelf life of chicken nugget during refrigeration storage. *LWT*, 95, 380–386. https://doi.org/10.1016/j. lwt.2018.04.066
- Sitara, U., Hassan, N., & Naseem, J. (2011). Antifungal activity of Aloe vera gel against plant pathogenic fungi. Postharvest Biology and Technology, 6(1–2), 159–161. https:// doi.org/10.1016/0925-5214(94)00051-S
- Sonawane, S. K., Gokhale, J. S., Mulla, M. Z., Kandu, V. R., & Patil, S. (2021). A comprehensive overview of functional and rheological properties of *Aloe vera* and its application in foods. *Journal of Food Science & Technology*, 58(4), 1217–1226. https://doi.org/10.1007/s13197-020-04661-6
- Tian, L., Yu, J., Wang, Y., & Tian, C. (2017). The C2H2 transcription factor VdMsn2 controls hyphal growth, microsclerotia formation, and virulence of Verticillium dahliae. Fungal Biology, 121(12), 1001–1010. https://doi.org/10.1016/j.funbio.2017.08.005
- Tsror, L. (2010). Biology, epidemiology and management of *Rhizoctonia solani* on potato. *Journal of Phytopathology, 158*(10), 649–658. https://doi.org/10.1111/j.1439-0434.2010.01671.x

- Valverde, J. M., Valero, D., Martínez-Romero, D., Guillén, F., Castillo, S., & Serrano, M. (2005). Novel edible coating based on *Aloe vera* gel to maintain table grape quality and safety. *Journal of Agricultural and Food Chemistry*, 53(20), 7807–7813. https://doi.org/10.1021/jf050962v
- Villa, F., Cappitelli, F., Cortesi, P., & Kunova, A. (2017). Fungal biofilms: Targets for the development of novel strategies in plant disease management. Frontiers in Microbiology, 8, 654. https://doi.org/10.3389/fmicb.2017.00654
- Zapata, P., Navarro, D., Guillem, F., Castillo, S., Martínez-Romero, D., Valero, D., & Serrano, M. (2013). Characterisation of gels from different *Aloe* spp. as antifungal treatment: Potential crops for industrial application. *Industrial Crops and Products*, 42, 223–230. https://doi.org/10.1016/j.indcrop.2012.06.002