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Use of medicinal plant extracts and chitosan as an alternative to chemicals to control mango postharvest anthracnose

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Abstract

Anthracnose caused by *Colletotrichum gloeosporioides* is one of the most important post-harvest diseases affecting mangoes' post-harvest management, resulting in huge post-harvest losses. However, the present research aims to study the antifungal activity of chitosan and aqueous extracts of *Tectona grandis* L. and *Artemisia annua* L. leaves on the development of *C. gloeosporioides*. In the "*in vitro*" evaluation, chitosan at the concentration of 2.5% (v/v) and the two extracts evaluated at 10% (w/v) showed a fungicidal effect on *C. gloeosporioides*. While for the evaluation of mango fruit ("*in situ*"), the anthracnose disease was controlled with 2.5% (v/v) chitosan and 10% (w/v) aqueous extracts applied after inoculation of *C. gloeosporioides*. The chitosan and aqueous extract treatments did not affect the acidity and pH of the mango. However, all products used on mango fruit reduced mass loss and delayed the decline in firmness, skin greenness and ascorbic acid content after ripening. They also showed a beneficial effect on total soluble solids content. A concentration of 2.5% chitosan and 10% aqueous extracts effectively inhibited anthracnose disease and improved some ripening parameters of mangoes. Therefore, Chitosan and aqueous extracts of *A. annua* and Teak (*Tectona grandis* L.) leaves can be used as an alternative to synthetic chemicals to substantially reduce postharvest mango anthracnose.

Keywords: Antifungal activity; Aqueous extract; *Artemisia annua* L; Mango anthracnose; *Tectona grandis* L

1. Introduction

Mango (*Mangifera indica* L.) is one of the most popular fruits grown and consumed in tropical and subtropical regions worldwide [1]. Mango is highly valued for its delicious taste, unique aroma, and nutritional value as Ascorbic acid, Vitamin A, fibres, etc. [2], [3]. However, mangoes face huge storage problems due to their high perishability and various diseases caused by flies (*Diptera Tephritidae*), bacteria (*Xanthomonas citri pv. Mangiferaeindicae*) and especially fungi (*C. gloeosporioides*, *L. theobromae).* These diseases affect the production and storage of mangoes leading to significant losses in the pre-harvest and post-harvest periods estimated at around 40-60% in Côte d'Ivoire [4]. Among them, there is anthracnose caused by *Colletotrichum gloeosporioides* as a most important field and post-harvest disease of mango. This fungal pathogen can affect leaves, fruit (immature and ripe) and flowers [5]. Controlling pre- and post-harvest diseases is essentially based on chemical fungicides. Still, the use of these synthetic fungicides has become a relevant

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problem due to regulatory and consumer requirements. These products are also limited by their impacts on the environment (ecosystems' pollution), and animal and human health [6]. The increasing demand for mangoes worldwide has increased the need to develop new, effective, eco-friend and low-cost technologies to extend shelf life and reduce losses. Thus, an alternative to manage post-harvest mango diseases could be the use of natural materials such as chitosan, cellulose, starch, microorganisms, etc. Chitosan is a biopolymer (polysaccharide) generated by the deacetylation of chitin [7]. It is biodegradable, non-toxic and has multiple biological and chemical abilities such as antimicrobial properties [2], [8]. Chitosan is used in many fields, especially in food packaging as an edible coating or biofilm.

In addition, it has been widely reported that postharvest diseases can be controlled with medicinal plants such as *Moringa oleifera*, *Ocimum gratissimum*, *Lippia multiflora*, *Eucalyptus camaldulensis*, *Azadirachta indica.* However, some of these plants are almost exclusively used in folk medicine. This is the case for *Tectona grandis* and *Artemisia annua* L. which are only used in folk medicine [9], [10]. *A. annua* L. is an aromatic annual herb widespread in many parts of the world. The antimicrobial potency of extracts of this plant and its essential oil against human pathogens has been studied in different parts of the world [11], [12]. *T. grandis* (commonly known as teak) is a forest species with a wide range of pharmacological properties such as wound healing, antimicrobial, antioxidant, antiplasmodial and cytotoxic properties, etc. [13]–[15].

However, despite their strong properties, these species are exclusively used in medicine and pharmacopoeia. Yet, their properties can be exploited in agriculture to control microbial diseases as an alternative to synthetic chemicals. The present study aims to investigate the antifungal activity of a solution of chitosan and aqueous extracts of *A. annua* and *T. grandis* leaves on phytopathogen: *C. gloeosporioides*, the causal agent of mango anthracnose.

2. Material and methods

2.1. Plant material

Leaves of *Artemisia annua* and *Tectona grandis* were collected locally from the botanical garden of the University Peleforo Gon Coulibaly, Korhogo, Côte d'Ivoire. The Chitosan, commercially named "Kitae", was provided by Green Impulse (Angers, France). It is constituted of 11.5% (w/w) Chitosan hydrochloride (with 10% chitosan) and is water soluble with a pH of 2,1 – 2,4. The fungal strain was isolated from infected mangoes var. *'Kent'*. For the "*in vivo"* experiment, mangoes var. '*Kent'* was used in this study and purchased in May 2020 at the market of Korhogo, Côte d'Ivoire.

2.2. Preparation of the aqueous extract

The leaves (*A. annua* and *T. grandis),* harvested early in the morning before sunrise, were carefully washed with tap water to remove sand and other solids. Then they were dried under shade at 27±2 °C for seven days. The dried leaves were ground with a grinder and stored in polyethene jars at room temperature until use. For each plant species, the aqueous extract was obtained by adding 10 g of leaf powder to 100 mL of boiling water (100 °C). The mixture was macerated for 24 hours and aseptically filtered. The filtrate obtained (10%, w/v) was used for the analyses.

2.3. Isolation and identification of Colletotrichum sp.

The method described by [16] was used with slight modification to isolate and identify the *Colletotrichum gloeosporioides* strain from infected mangoes. Three ripe mangoes with anthracnose symptoms were first washed with soapy water containing sodium hypochlorite (1%). They were then rinsed twice with sterile distilled water and air-dried. Finally, the mangoes were wiped with paper towels soaked in 70% ethanol and dried with sterile blotting paper. Small portions of the epicarp of the infected fruit were taken from the edges of the disease necroses and placed separately in Petri dishes containing Sabouraud dextrose agar amended with chloramphenicol. Three Petri dishes were made up, with one dish per mango. The plates were then incubated at 27 °C. The cultures obtained were purified by successive subculturing in fresh Petri dishes until pure cultures were obtained. Conidia were visually inspected using a light microscope and identification based on conidial and colony morphology was performed according to the methods described by Pandey et al [17] and Rivera-Vargas et al. [18].

2.4. *"In vitro"* evaluation of the antifungal activity of plant extracts and chitosan

The effect of chitosan and the two plant extracts on the mycelial growth of the *Colletotrichum gloeosporioides* strain was studied using the poisoned food technique described by Bussaman et al. [19] with slight modifications. It consisted of direct diffusion of the treatment solution into the Sabouraud Dextrose Agar (SDA) medium. Each crude aqueous plant

extract was diluted to have concentrations of 2.5; 5 and 10% (v/v). The chitosan solution (10%) was also diluted to obtain different concentrations (0.5; 1; 2.5 and 5%). Then, for each concentration, 1mL of each extract was used for mixing with 15 mL of hot SDA and poured into a sterile 9 cm Petri dish. After solidification, 10 µL of *Colletotrichum gloeosporioides* strain suspension (10^5 spores / mL) prepared from a seven-day-old culture was aseptically deposited in the centre of each Petri dish with treatments in a sterilised area using a Bunsen burner. The control Petri dishes were prepared exclusively with sterile distilled water. For each concentration, the experiment was performed in five replicates (plates). The inoculated Petri dishes were sealed with adhesive parafilm after diffusion of the suspension and incubated at 30 °C for 10 days. Colony diameter was measured at 4 and 10 days after incubation along two perpendicular axes drawn on the bottom of each Petri dish. Fungal susceptibility was calculated as the percentage of radial mycelial growth inhibition compared to the control according to the following formula [20]:

$$PIG(\%) = 1 - \frac{t}{c}\ x\ 100$$

Where PGI = Percentage of growth inhibition (%); c = average surface area of the pathogen growth in the control plate and t = average surface area of pathogen growth in the treated plaque

2.5. *"In vivo"* evaluation of the antifungal activity of plant extracts and chitosan

The effect of the treatment on fungal development was carried out according to the Cissé et al. [2] method. Fifty healthy mature mangoes were selected and disinfected with chlorinated water (1%). They were then rinsed with distilled water and air-dried at room temperature. The mangoes were divided into ten groups of five fruit, one group per treatment and concentration. On each mango, eight lesions (diameter 1 mm and depth 2 mm) were made with a sterile needle, with four per cheek. The lesions on the same mango cheek are approximately 6 cm apart. Each mango is considered a replicate. The fruits were individually inoculated by dipping for 2 min in 500 mL of fungal solution (10^5 spore/mL). The inoculated mangoes were treated by dipping in the different solutions at different concentrations for 2 min and then stored at 28 ± 2 °C. After 4 days and 10 days, the diameter of the necrosis was measured and the percentage of fungal inhibition by the different treatments was assessed against the control according to the formula of Attrassi et al. [21]

$$IP(\%) = 1 - \frac{d_t}{d_c}\ x\ 100$$

Where d_t is the diameter of the lesion area in the treated mango and d_c is the diameter of the lesion area in the control.

2.6. Assessment of fruit quality attributes

The influence of the different treatments on the quality attributes of the mango was assessed 10 days after treatment. For the plant extracts, the concentration of 10% (v/v) was used whereas the concentration of 2.5% (v/v) for chitosan. Fruit mass loss was measured on 25 mangoes following the Cissé et al. (2015) method using an electronic scale (KCC300sx, 600 x 800 mm). Mass loss was expressed as a percentage of the initial mass. Firmness was measured using a digital penetrometer (FC GAUGE PCE - FM 200, Milan, Italy) according to the following Silué et al. [22] method. The colour of the fruit skin was measured using a Minolta colourimeter (CR-10 Plus, Japan). The colour was measured in the Lab system and was represented by a* (greenness) and b* (yellowness). For soluble solids content, titratable acidity and pH, the pulp juice of ten mangoes was extracted individually using a juice extractor (Kuvings D9900, South Korea). Soluble solids content and titratable acidity were measured with a Brix-acid dual-scale digital refractometer (PAL-BX-ACID91, Atago, Japan) at 25 °C following the manufacturer's instructions. The pH was measured with a hand-held digital pH meter (Testo 206-pH1) calibrated with pH 4 and 7 buffer solutions. The ascorbic acid content was determined using a 2,6-dichlorophenolindophenol titration according to the following procedure of Pongracz [23].

2.7. Statistical analysis

Statistical analysis was performed using RStudio software version 4.1.1 (2021-08-10) for Windows. All results represent the mean and the standard error (mean ± se) which indicates the estimate of variability. Experimental data were subjected to analysis of variance (ANOVA) to identify differences between treatments. The means of the treatments were compared using the Least Significant Difference (LSD) test at the 5% significance level.

3. Results

3.1. *"In vitro"* antifungal activity of plant extracts and chitosan

Figure 1 shows the results of the different treatments and concentrations on mycelial growth after 10 days of incubation. The results showed that chitosan, *Tectona grandis* L and *Artemisia annua* L had antifungal properties against the pathogen *Colletotrichum gloeosporioides* at all concentrations tested. Moreover, this inhibitory activity increased with the concentration of extract or chitosan. Thus, the inhibition rate of chitosan varies from 27.22 ± 0.79% (0.5% v/v) to be total and constant from the 2.5% concentration. While *T. grandis* L and *A. annua* L. achieved total inhibition of the strain at the concentration of 10% (v/v).

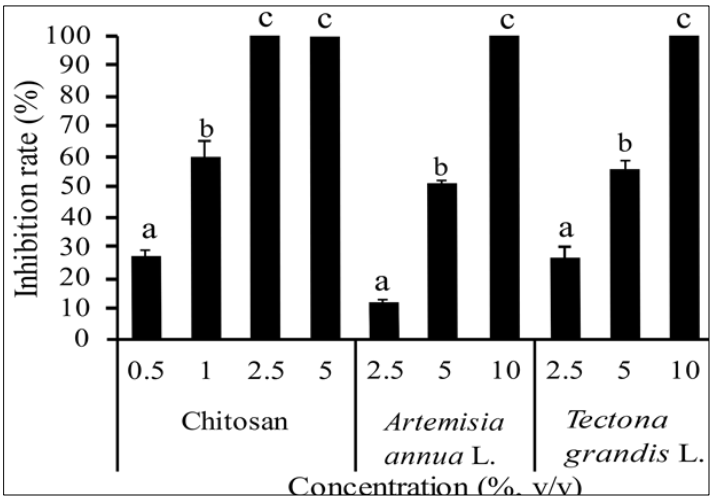

Figure 1 "*In vitro"* antifungal activity of chitosan solution, *Artemisia annua* and *Tectona grandis* extracts against *Colletotrichum gloeosporioides*. Different letters indicate a significant difference (P <0.05)

3.2. *"In situ"* effect of chitosan and extracts of *T. grandis* L. and *A. annua* L. on anthracnose disease

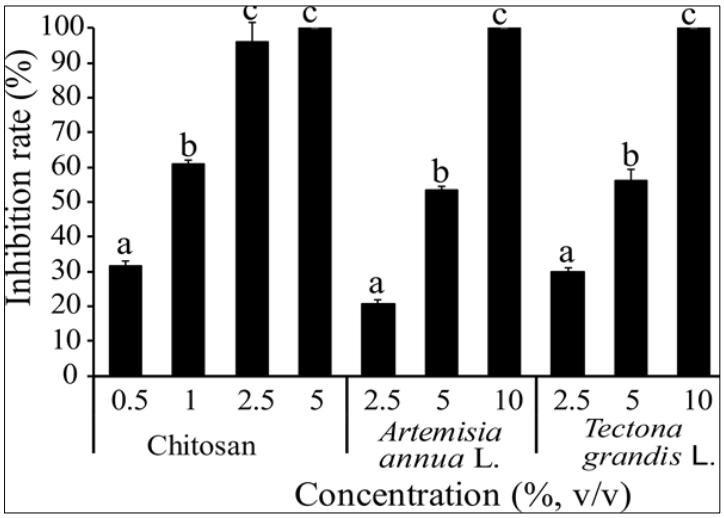

Figure 2 Antifungal activity of the chitosan solution, *Artemisia annua* and *Tectona grandis* extracts against the anthracnose disease on the mango cv '*Kent*'. Different letters indicate a significant difference (P <0.05)

Figure 2 shows the sensitivity results of the pathogen to the different treatments applied to mangoes after 10 days of storage. The presence of chitosan or aqueous extract affected the development of the fungus on the fruit. As "*in vitro"*, the treatment was significantly effective against the isolate at higher concentrations (10% for aqueous extracts and 2.5% for chitosan) with complete inhibition of the strain and consequently of the disease. Chitosan recorded 96.15 ±

5.44% and 100% at 2.5% and 5% concentrations, respectively. However, no statistical difference was observed between these concentrations.

3.3. Changes in quality attributes after treatment

3.3.1. Loss of mass

Figure 3 shows the weight loss of treated mangoes compared to untreated fruit after 10 days of storage. The mass loss of the control fruit was significantly greater (19.48 ± 0.61%) than that of the treated fruit. The lowest water loss was recorded with the aqueous extract of *Tectona grandis* L (4.6 ± 0.02%).

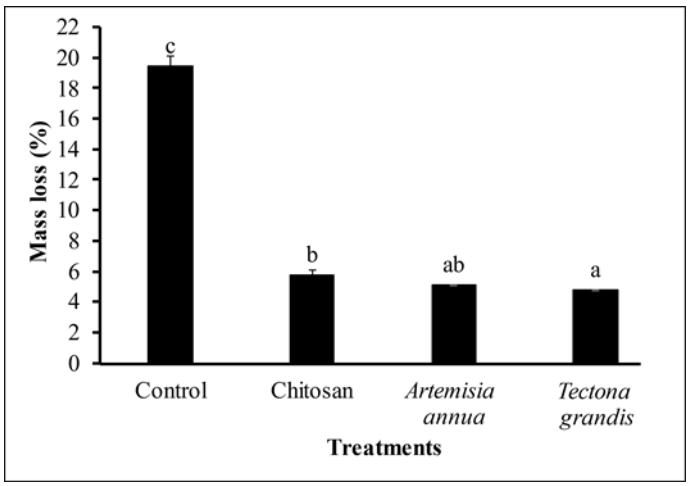

Figure 3 Effect of chitosan solution, *Artemisia annua* and *Tectona grandis* extracts on the mass loss of '*Kent*' mango at 10d of storage. Different letters indicate a significant difference (P<0.05)

3.3.2. Firmness

Figure 4 shows the variation in mango firmness after storage. The results revealed that irrespective of the treatment, the firmness of the fruit decreased with ripening. Regarding epicarp firmness, a significant difference was observed between all treated samples and untreated fruit (control). However, no significant difference was observed in the firmness of the pulp between the different samples.

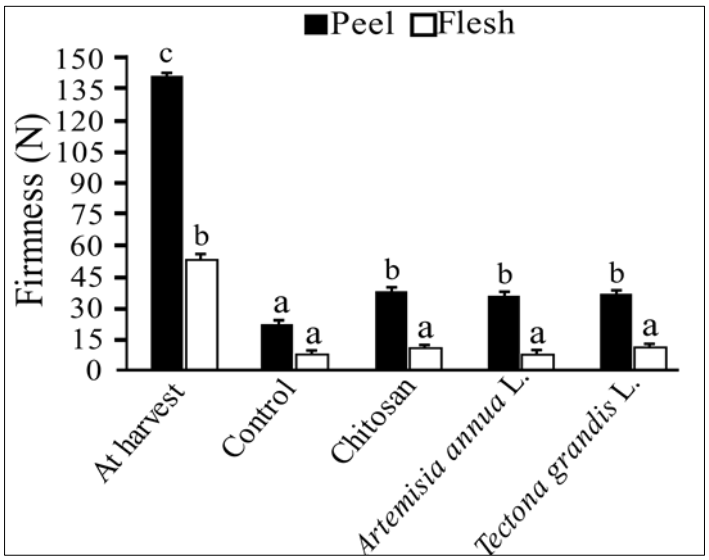

Figure 4 Effect of the chitosan, *Artemisia annua* and *Tectona grandis* on the firmness of '*Kent*' mango at 10d of storage. Different letters indicate a significant difference (P <0.05)

3.3.3. Colour

Figure 5 presents the variation of colour coordinates after the ripening of the fruit. The results show that the a*-coordinate indicating greenness increased significantly while the b*-coordinate decreased with ripening. For these two coordinates, no significant difference was observed between treatments. But the difference is statically significant between treated and control samples except for *T. grandis* for the b* coordinate. The loss of greenness of the control fruit is greater (high a*) than that of the treated fruit after storage.

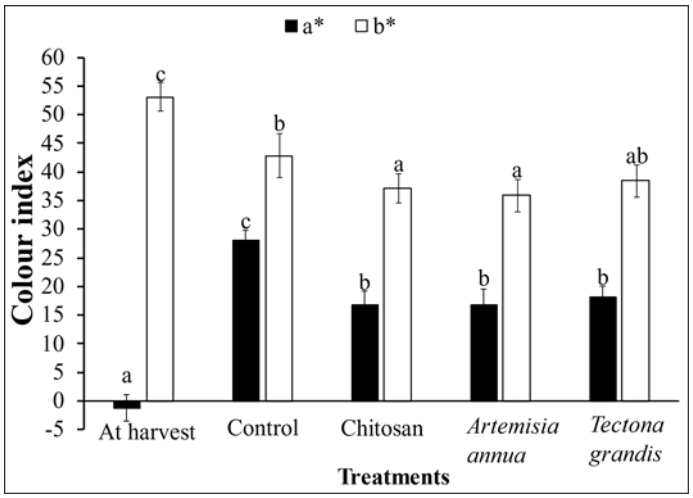

Figure 5 Effect of the chitosan solution, *Artemisia annua* and *Tectona grandis* extracts on the colour of '*Kent*' mango at 10d of storage. Different letters indicate a significant difference (P< 0.05)

3.3.4. Variations in biochemical parameters

Table 1 exhibits the biochemical changes in mango after treatment and storage. The results show that soluble solids content and pH increase while the acidity and ascorbic acid content decrease during storage. The control sample showed higher pH and soluble solids content than the other samples after storage. In addition, statistical analysis showed a significant difference in the soluble solids content between the control and treated samples. On the other hand, the ascorbic acid content of the control mangoes (118.00 ± 7.15 mg/kg FP) was lower than that of the treated mangoes. A significant difference in ascorbic acid content was observed between all mango samples whereas no significant difference in acidity was recorded between them.

Table 1 Variations of '*Kent*' mango's biochemical characteristics at ten days after application of chitosan solution, *Artemisia annua* L. and *Tectona grandis* L

Treatments	Soluble solids content (%)	Acidity (%)	pH	Ascorbic acid (mg/kg pulp)
Day of treatment	6.32 ± 0.48^{a}	0.57 ± 0.17^{b}	3.68 ± 0.08^{a}	548.90 ± 3.12^{e}
Control	18.94 ± 0.58^{c}	0.37 ± 0.06^{a}	4.86 ± 0.06^{c}	118.00 ± 7.15^{a}
Chitosan	17.16 ± 0.95^{b}	0.34 ± 0.04^{a}	4.79 ± 0.18^{c}	384.67 ± 9.39^{d}
Artemisia annua L.	16.54 ± 0.48^{b}	0.30 ± 0.05^{a}	4.67 ± 0.08^{bc}	229.20 ± 8.04^{b}
Tectona grandis L.	17.04 ± 1.42^{b}	0.27 ± 0.09^{a}	4.71 ± 0.17^{b}	321.33 ± 8.25^{c}

Values (mean ± se) within a column with the same letter are not significantly different (P> 0.05).

4. Discussion

The use of natural products is widespread and recognised in the treatment of various human diseases through folk medicine and pharmacopoeia. However, due to their high antimicrobial potential, they can be used as an alternative approach to synthetic chemicals used in agriculture. Our results showed that chitosan solution and leaves of teak (*Tectona grandis* L.) and *Artemisia annua* L. have significant antifungal properties. In the "*in vitro*" experiment, chitosan and plant extracts had significant activity against the pathogen *Colletotrichum gloeosporioides* at different

concentrations. Also, the inhibition of anthracnose disease in mangoes ("*in situ*") strengthens the idea that the extracts used have strong antifungal activity. Furthermore, it was observed that the sensitivity of *C gloeosporioides* to these extracts increases with concentration. However, no difference was observed amongst concentrations of 2.5% and 5% against the pathogen. Therefore, a concentration of 2.5% may be economically more efficient. This variation in the susceptibility of the pathogen indicates that their antifungal activities are dose-dependent. The mechanism of action of the extracts was not investigated in this study. But some authors, like Li et al. [24], reported that the antifungal effect of chitosan could be attributed to the interaction of its positive charges and the negative charges of the cell membrane thus altering the permeability of the cell. Furthermore, the control of anthracnose by chitosan could be associated with the production of certain metabolites with antifungal properties that strengthen the natural defence system of the fruit.

The antifungal activity of aqueous plant extracts is thought to be due to their various phytochemical constituents, including tannins, flavonoids, terpenoids, and steroids. According to He e al. [25], the most important chemical component of the aqueous extract of *Artemisia annua* L. is artemisinin. This compound is widely known for its antimicrobial activity against various pathogens [26], [27]. The mode of action of artemisinin on malaria agents is reported in the literature. However, its mechanism of action on fungi remains unknown. Concerning *Tectona grandis* L., the major compounds would be deoxylapachol and tectoquinone which can induce fungi cell wall stress [28]. Neamatallah et al. (2005) have identified the 5-hydroxy-1,4-naphthalenedione from teak extract (*T. grandis*) as the molecule which is responsible for the inhibition of *Listeria monocytogenes*.

Based on the results of the antifungal activity of these natural products, it can be confirmed that chitosan and the aqueous extracts of *T. grandis* and *A. annua* are an interesting alternative to synthetic fungicides in the post-harvest treatment of mangoes to control fungal diseases.

In addition to being a better disease control agent, the aqueous plant extracts and the chitosan solution also affected some metabolic pathways of mango degradation. Thus, their actions allow for extending the shelf life of the mango without altering the quality of the fruit. Indeed, our results showed that the mass loss of the control fruit was significantly higher than that of all treated fruit. This indicates that the chitosan and the aqueous plant extracts have favourited the maintaining of freshness during mango storage.

Moreover, mango skin firmness and colour, two important selection criteria for the consumer, were significantly impacted by the treatments. The results showed a significant difference in mango skin firmness and colour between treated and untreated samples. The loss of firmness and green colour during fruit storage was greater in control mangoes than in treated mangoes. This is an indication that the action of the applied treatments would be concentrated on the skin (potentially on the cell wall or membrane). This hypothesis on the action of the treatments was consolidated by the non-significance of the difference in firmness and colour of the pulp between control and treated mangoes. Some authors such as Bautista-Baños et al.[30]; Cissé et al.[2]; Shah & Hashmi [31] have reported similar results on the effects of chitosan and plant extracts on mango.

There is a difference in variation of soluble solids content, pH and titratable acidity between control and treated fruit. This confirms that the treatments would interact with certain metabolic pathways such as polysaccharide degradation into sugars (glucose, fructose, etc.) or organic acids (citric acid) conversion throughout the photosynthesis process. Sumthong et al.[32] have reported that teak extract inhibits cellulase enzymes. Finally, the study showed that the ascorbic acid content decreased significantly after storage no matter which treatment. The ascorbic acid decrease could be related to its involvement in multiple pathways as the biosynthesis of plant hormones (ethylene, gibberellin) and other compounds [33]. However, the rate of decrease in vitamin C was much greater in the untreated fruit (control) than in the treated. The lowest ascorbic acid content in untreated fruit could be attributed to a partial disintegration of the cell wall. This observation implies a positive effect of the treatments on both the anthracnose disease and the quality of the mango.

5. Conclusion

This study proved that the use of chitosan (from a concentration of 2.5%) and aqueous extracts of teak (*Tectona grandis* L.) and *Artemisia annua* L. (at a concentration of 10%) in the post-harvest treatment of anthracnose in mango is a credible and promising option. Moreover, these products have the potential to interact with ripening without affecting the final quality of the mango. However, further studies are needed to verify their antifungal potential for other mango pathogens.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors have declared that no competing interests exist.

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