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Original Research Article

Avocado (*Persea americana Mill.*) Postharvest Organic Nutritional Conservation Employing Carboxyl Methylcellulose (CMC) Manufactured Out from Moringa Plant

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Abstract

The research concluded that the use of extracts of moringa leaves and moringa seed as an edible coating might help to preserve the shelf life and also the quality of Avocado. The cultivars that were focused on during this research were “Hass” and “Gem”. It may also reduce the risk of infection and inflammation in these cultivars. Methanolic and ethanolic moringa extracts were also examined for their antifungal properties. Shortly, 1% Carboxyl methylcellulose which is shown as CMC and 2% MLE which is Moringa Leaf Extract or MSE which is moringa seed extract, were combined. The fruit was either coated with either (MLE + CMC) or that of (MSE + CMC) and then it was kept at a temperature of 5.5°C and 95% relative humidity (RH) for a period of 21 days. The fruit was kept at ambient temperatures i.e. 21°C and 60% RH, after being refrigerated to approximate retailing conditions. Fruit firmness and postharvest quality indicators including ethylene production and respiration rate were analyzed. In relation to potato dextrose agarose which is shortened as PDA, both coatings were also evaluated for their effectiveness against the fungus which may spoil the fruit quality during postharvest opperations. Compared to uncoated fruit, the coated fruit was noticed to have less mass loss. Respiration rates were also observed to be decreased along with Ethylene production, in a contrast to the uncoated fruit. *A. alternata* and *C. gloeosporioides* were both inhibited by ethanol leaf extract by 43.60% and 42.90%, respectively. All pathogens exposed to coverings had damaged hyphal structures, while uncoated fruit had healthy hyphal structures. Stronger antibacterial activity was observed in ethanolic moringa leaf extracts as compared to the extracts that were derived from methanol. The outcomes of this research showed that carboxyl methylcellulose CMC containing moringa extract reduces illnesses in fruit. Throughout the postharvest supply chain, it also maintains the general quality of avocados. The shelf life of the avocado was remarkably increased. The avocado sector may eventually commercialize the moringa extracts combined with carboxyl methylcellulose, as a new organic edible covering.

Keywords: Organic Moringa extract; Edible coating; Antimicrobial antioxidant; Carboxyl methylcellulose (CMC).

1. INTRODUCTION

The tropical climacteric fruit avocados (*Persea americana*) are in great demand for cultivation across the world. There was a 23% growth from 2017 to 2018 with global production reaching 6.4 million metric tons, or $12.9 billion [1]. It is generally known that ethylene production and respiration during avocado postharvest storage are important. Edible coatings have recently emerged as a cutting-edge, effective, and green method for extending the shelf life of fresh horticultural goods after harvest. With the exception of those advertised as organic, avocados are often waxed in the commercial world to improve appearance, stop moisture loss, and apply fungicides to lengthen shelf life [2] and protecting the surface of the fruit, edible coatings are applied. These coatings are biodegradable, soluble forms that are then

eaten together with the fruit [3]. Avocado postharvest losses may be caused in part by the high frequency of biological activity and fungus infections that occur after harvest. For avocados, the fungal disease anthracnose is caused by *Colletotrichum gloeosporioides*, and end rot of stem is instigated by various species of the *Colletotrichum* and *Botryodiplodia* genus, the *Dothiorella*, *Phomopsis*, and *Lasiodiplodia* family [4]. Sanders and Korsten discovered an infection rate of up to 80%. (2000). A variety of post-harvest fruit treatments have decreased these losses. Copper oxychloride and copper hydroxide, as well as prochloraz, are examples of synthetic fungicides that may be used before and after harvest [4]. After a year, as a result of worries about the health risks posed by the presence of chemical residues on treated fruit and the negative environmental effect they have, chemical treatments have lost favour with consumers [4]. The use of synthetic waxes on avocados destined for the European Union is likewise forbidden. Fruit that has not been waxed is more likely to shrivel, which has a significant impact on its quality. An increase in post-harvest research was required to better inform consumers about edible coatings' health and environmental benefits over fresh fruit's water, gas exchange, and oxidation processes [5]. According to [6-8] edible coatings and essential oils may help prevent post-harvest infections and increase the shelf life of avocado fruit. Due to its high vitamin B and C, amino acid, and crude protein content, as well as its low anti-nutritional and antimicrobial agents with film organic and shelf-life enhancing qualities, M. oleifera is in high demand for its nutritional and therapeutic benefits [9]. Coatings and films, in addition to providing a physical barrier, may also include active components that provide benefits including antioxidant and antibacterial activity. Coatings of chitosan, candelilla wax, pectin, and carboxymethyl cellulose, with and without essential oils and polyphenols, have all been tried on avocados before [10]. Two of the most common edible coatings are chitosan and carboxymethyl cellulose (CMC). Studies have shown that adding chitosan to litchi may slow down the rate at which the fruit loses moisture, hence increasing its storage life [11]. Carboxymethylcellulose (CMC), a food coating that has long dominated the market, may enhance and potentially increase the shelf life of avocados [12]. There is significant potential for CMC films with antioxidant and antibacterial capabilities to lessen food waste and environmental pollution while also enhancing food quality, safety, and security [13]. Multiple fruits, including pomegranates, have had their post-harvest quality improved by applying a coating made of carnauba and carboxymethylcellulose (CMC) [14]. According to the authors, this standard commercial treatment for postharvest fruit diseases is governed by regulatory restrictions associated with various maximum residue levels (MRLs), which must be met if the fruit is to be transported to the international markets for exports [12]. To meet this demand while minimizing post-harvest losses, new post-harvest treatments must be developed. Since these conditions, so much effort has been put into developing more effective and long-lasting systems of control [15]. Moringa (*Moringa oleifera Lam.*) is a common sight in the tropical and subtropical regions of the world. Moringa leaf extracts have been proven to have oxidative and antibacterial properties in a number of investigations [16]. Simestrol, niacin, sitosterol, and quercetin are active phytochemicals found in moringa leaf extract [17]. Everyone that worked on the project as part of their research on avocado 'Fuertas' quality and storage qualities utilized moringa products [18]. According to [19] Academia fruit treated with CMC containing MLE had better quality and longer shelf life. (MLE). No Moringa leaf or seed extracts have ever been tested on the 'Gem' late-season cultivar. According to our knowledge, Moringa's best extraction method has yet to be developed. The use of a CMC coating has been shown to effectively preserve fruit stability and increase the storage life of Avocados [20]. Since different cultivars of avocado may differ in wide ways, i.e. from their physical appearance to their fruit quality, it's crucial to have this information at hand when making recommendations to the industry. So far, moringa extracts haven't been linked to any postharvest diseases in avocados. The quality and longevity of 'Gem' avocados were studied after being treated with (CMC, leaf and seed extracts of moringa. Postharvest infections in avocados were studied to learn more about the efficacy of Moringa extracts as antifungals.

2. MATERIAL AND METHOD

2.1. Fruit cross-selection

Throughout this experiment, Westfalia Fruits TM, a Hans Merensky Holdings (Pvt) LTD company, provided the "Gem" and "Hass" avocado cultivars sourced from Everdon Estate. Scientists are working on an experimental farm in the Howick zone of KwaZulu-Natal Domain, South Africa (latitude 29.451 degrees south, longitude 30.251 degrees east). Open-top display cases were used to collect 360 fruits with an average weight of 211 to 235 grams (180 "Hass," 180 "Gem"). Once the fruit was harvested, it was transported to the university's Postharvest Laboratory in a well-ventilated vehicle.

2.2. Postharvest handlings and storage

MLE and MSE were tested at the Postharvest Laboratory (PHL), as well as control fruit. A total of 20 fruits were used in each of the three treatments. To remove the dipped fruit, all that was required was one minute of drying at room temperature (21°C). It was then set to 5.5°C (95-96%) and 95-96 percent humidity to approximate export conditions for 21 days. To simulate the ripening process, the fruit was held at 21°C and 60 percent relative humidity for seven days [21].

2.3. Abstraction of plant tissues

According to [22], Leaves and Seeds of Moringa were harvested, described as following:

2.3.1. a. Abstraction of methanol

In order to extract 100 grams of moringa plant tissue, one liter of methanol/HCl 1-percent (v/v) was needed. A rotary evaporator was used to collect the supernatant, which was then combined with distilled water and stored in a separate container (20 mL). It was then put through a series of liquid-liquid extractions using hexane and chloroform and eventually Ethanol as the last stage in the process.

2.3.2. b. Ethanol abstraction (method A)

Ethanol 70 percent was used to extract 100 grams of moringa plant tissue for two hours at 4 °C. For this study, we used the Rotary Evaporator (RE) to remove the water from the extracts, which were then diluted with chloroform and then Hexane and finally with Ethyl Acetate.

2.3.3. c. Method B

Ethanol 70 percent was used for two hours to extract 100 grams of moringa plant tissue. The extracts were condensed using a rotary evaporator and 20 mL of distilled water. To complete the procedure, hexane, chloroform, ethyl acetate, and crude extract were employed to extract successive liquid-liquid extracts from the crude extract. Antifungal in-vitro testing was done on the extracts stored in the freezer.

2.4. Quality evaluation of postharvest

Experiments on fruit hardness, ethylene generation, respiration rate, and mass loss at ambient temperatures were conducted after harvesting.

2.4.1. Production of Ethylene and respiration rate in fruit

Ethylene production in fruit was quantified using a 15 mL headspace and a Felix Instruments F-950 portable ethylene analyzer. Following 15 minutes of exposure, kg1 h1 of ethylene was detected in a 1 L jar. On a weekly basis, the amount of carbon dioxide (CO^2) produced by the fruit was measured using a gas meter (EGM-1, PP Systems, Hitchin, UK). The CO^2 concentration in the headspace was determined by using the fruit's mass, the headspace pressure, and the ambient CO^2 level [19].

2.4.2. Firmness and Mass

Each week, portable Hardness testers were used to check the fruit's firmness after a week in cold storage. After two tests, the results ranged from 100 (very hard, unripe) to 60 (safe for human consumption) [23, 24]. A Mettler Toledo digital balance was used to determine the avocado's mass (0.010g).

2.5. Analysis of plant extracts and their antifungal features

2.5.1. Pathogenic organisms' extraction

C. gloeosporides, *A. alternata*, and *L. theobromae* were recovered as of avocados. Tissues were cleaned in 70 percent ethanol aimed at 30 seconds, washed twice in distilled water, and then kept on a PDA plate to stimulate fungal growth. 28°C week-long plates. Potato dextrose and distilled water created PDA medium. The mixture was cooled to 50°C after 15 minutes in an autoclave. In the medium before plating, 100 mg chloramphenicol was dispersed in 20 mL of alcohol. Mycelia were picked and replanted after seven days. Colony arrangement may help detect isolates under the microscope.

2.5.2. Testing the Pathogenicity

A non-ripen Avocado fruit was tested with the pathogenicity effect of the *C. gloeosporiodes*, *L. theobromae*, and *A. alternata*. After harvesting the mycelium from the pure cultures, the sterile knife was used to extract the mycelium from the healthy fruit and transplant it onto the incision. Sterilized cotton wool was applied to the inoculation wounds to keep them clean and dry. For two days, the fruit was allowed to mature in an incubator set to 25°C. It took five days of sitting at room temperature before the tape was peeled off. After seven days of testing, it was shown that *L. theobromae* and *C. gloeosporiodes* were the most harmful isolates for both severe and mild fruit infections. Some *Alternaria* isolates injected into avocado fruits resulted in modest infections, while the others were not observed to be the reason of any noticeable symptoms.

2.5.3. *In vitro* testing

In a petri dish *C. gloeosporiodes*, *L. theodorae*, and *A. alternate* were examined. It worked. Moringa extracts were added to PDA medium. Sterilized Petri plates contained a 20 mL PDA-adjusted solution. 7-day-old mycelium was utilized to make 3-mm mycelium discs on a modified PDA. The control was sterile PDA water. The radial progression of cultures was evaluated after 10 days at 28°C.

2.5.4. Scanning electron microscopy

Tesfay and Magwaza employed scanning electron microscopy to examine the growing medium containing plant extracts vs the PDA alone in their study (2017) for fungal samples that had been exposed to the growth environment, SEM (Zeiss EVO LS15) was used for analysis [25]. The fungal samples had to be fixed and rinsed in cacodylate buffer before they could be seen. The fungus was then dehydrated for an hour in varying concentrations of ethanol. To further dry the same tissue, it was essential to utilize a freeze drier. A sputter coater was used to coat the dry samples before they were examined under an electron microscope.

2.5.5. *In vivo* testing

One in vivo trial assessed the antifungal effects of edible coatings by coating 90 'Gem' fruits with Carboxyl Methyl Cellulose 1 percent + Moringa Leaf Extract, Carboxyl Methyl Cellulose 1 percent + Moringa Seed Extract, and one in which no coatings were used (control). Each fruit was infected with *C. gloeosporiodes* and *A. alternata* by making a 5-mm incision.

2.6. Data analysis

GenStat 17.1 (VSN International, Hemel Hempstead, UK) was used to analyze the data (GenStat 17.1, VSN International, Hemel Hempstead, United Kingdom). At a 5% level of significance, we utilized Fischer's least significant method differences to separate the means.

3. RESULTS AND DISCUSSION

3.1. Edible coating amalgamated influences on fruit qualitative aspects

3.1.1. Rate of respiration and Ethylene formation in produce

There was a significant reduction in ethylene generation in avocados treated with leaf extract and seed extracts of moringa (p 0.05) (Fig. 1A). The lowest quantities of ethylene were found in moringa leaf extract and in CMC. There were an 11.4 kg^{-1} h^{-1} difference between the Gem variety and Hass variety of avocados when it came to their end-of-shelf-life average. Ethylene production in fruit tissues increases as the temperature and metabolic activity increase [22]. They are known as the Magwazas. By lowering the shelf life of ethylene in edible coatings, metabolic activities may be inhibited.

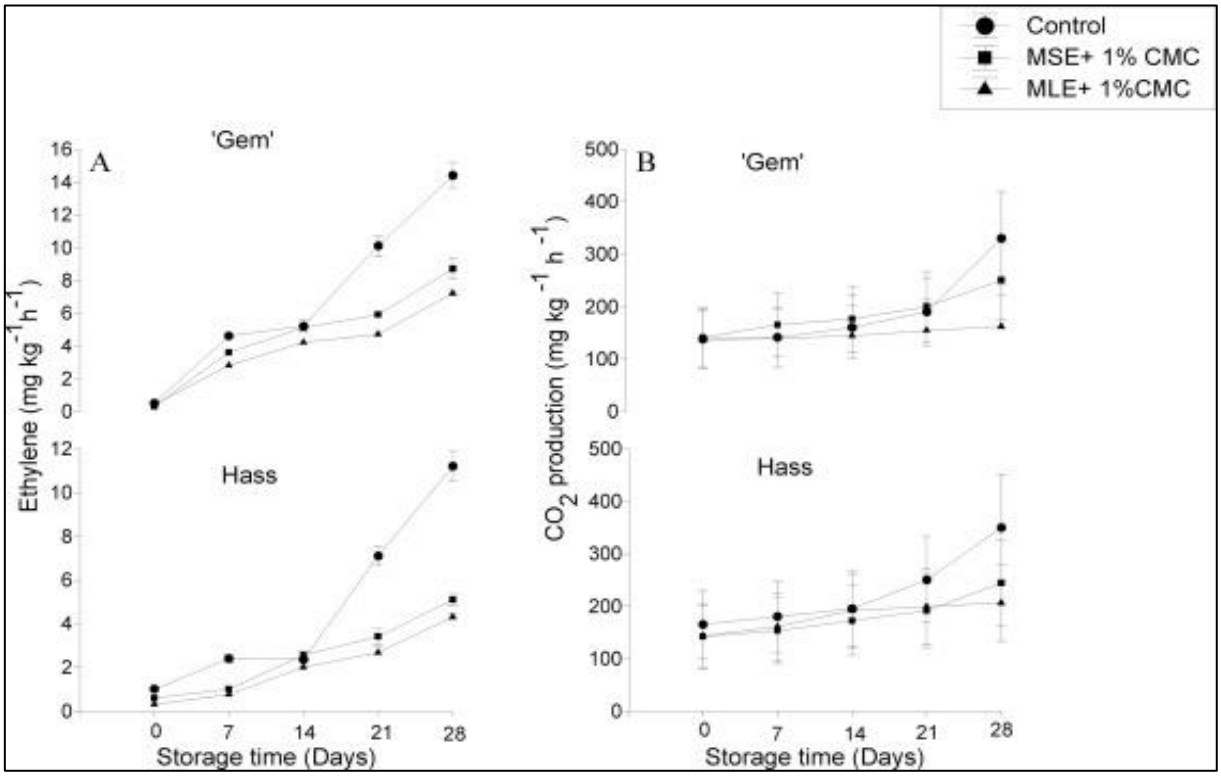


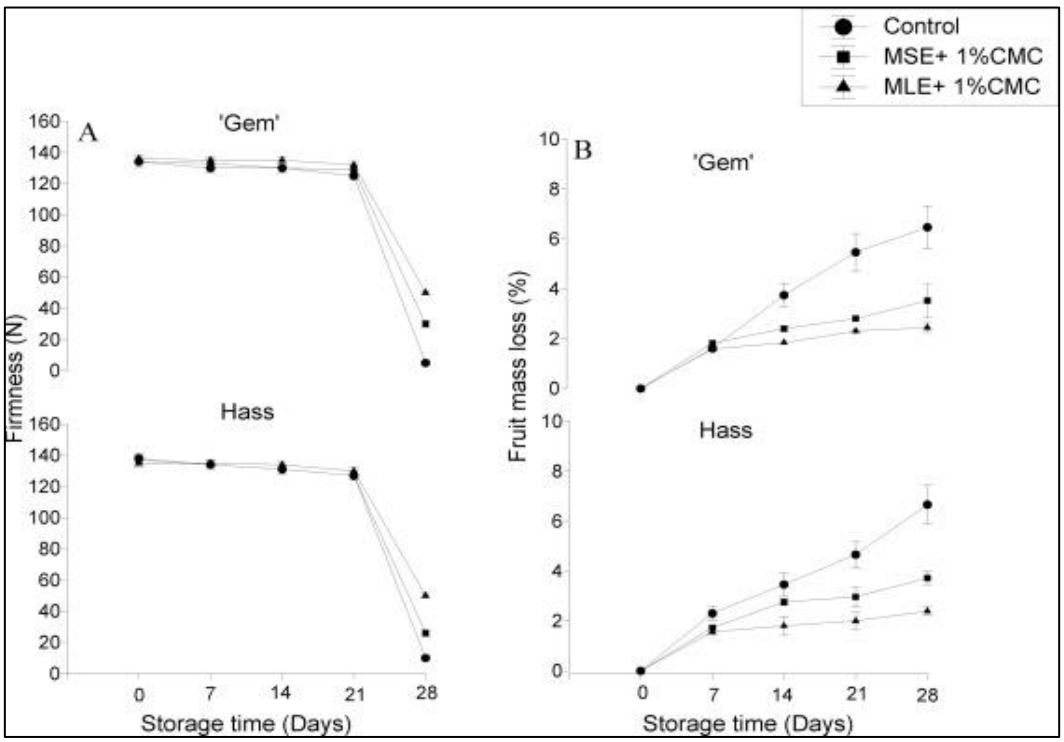
Fig. 1A shows that ethylene production increases with storage time while 1B shows that Co^2 production also increases storage time which shows how edible coatings affect the production of ethylene and rate of respiration of avocados, specifically the "Gem" and "Hass" varieties. (n =+5) The erect bars characterize the standard error of mean. A moringa leaf extract is known as MLE, while a seed extract is known as MSE. CMC stands for Carboxyl Methylcellulose

Ethylene production was reduced in both Hass and Gem variety of avocado fruits (Fig. 1B) when CMC was combined with seed or leaf extract of moringa (Fig. 1B). Comparing respiration rates of MSE + 1 percent CMC treated fruit with gem avocados, which ranged from 126 to 195 mg kg^{-1} h^{-1}, Gem avocados exhibited the lowest respiration rate of 126 mg kg^{-1} h^{-1} (Fig. 1B). Untreated fruit, on the other hand, had a substantially higher respiration rate. At the end of their shelf life, fruit that had not been treated had a respiration rate of up to 300 mg kg^{-1} h^{-1}. Acacia's rate of respiration was shown to be influenced by temperature, according to research. During this time of year, avocados produce more ethylene, which increases respiration and softens the fruit. As storage time and temperature rose after coating treatments, [26] discovered a drop-in respiration rates and an increase in CO^2 production. This study's findings are in line with those of earlier ones. The respiration rate of CMC + MLE coated avocado fruit is lower than that of untreated avocado fruit [19]. They are Tesfay Magwaza and Noel Magwaza. Fruits covered with edible coatings, such as avocados [27], peaches, and kiwis, have lower ethylene production and respiration rates [28, 29].

3.1.2. Firmness and weight loss

Both "Gem" and "Hass" avocados were found to be firmer (P 0.05) when edible covering treatments were applied. Ethylene production and respiration rate

increased as the fruit softened, suggesting that the fruit's shelf life was reaching its end (Figs. 1A and 2A) (Fig. 1B). The avocado Coated with 1% CMC + MLE and 1% CMC + MSE were found to be the most stable (50 N) (30 N). After applying edible coatings, the 'Hass' avocados maintained a large level of firmness. Due to its enhanced barrier to gaseous and moisture diffusion, coated fruit may retain its firmness better than uncoated fruit. The ripening process in fruits and vegetables is governed by volatile ethylene gas [22]. The coating treatments utilized in this investigation may have enhanced the integrity and stiffness retention of the membranes. Fruit that has not been treated becomes mushy as a result of pectin or starch depolymerization in pectin and cellulose fibers [30]. Many enzymes, such as polygalacturonase, pectin methyl esterase, pectatelyase, and b-galactosidase, hydrolyze and depolymerize these components [31]. The less O^2 concentration and greater concentration of CO^2 in coated fruit may have subdued the oxidizing enzymes, enabling the fruit's firmness to be kept throughout storage and shelf life after harvest. 1-MCP or wax-treated avocados lose their firmness more quickly and have a shorter shelf-life, according to the research results. Avocados soften during shipping, which degrades the fruit's quality. These coatings help to maintain the fruit's freshness and flavour by extending its shelf life. Post-harvest treatment with Carboxyl Methyl Cellulose and Moringa leaf or seed extract (Fig. 2B) decreased mass loss (a crucial quality feature in avocados) (Fig. 2A). Moringa leaf extract + 1 percent Carboxyl Methyl Cellulose and Moringa Seed Extract + 1 percent Carboxyl Methyl Cellulose coatings reduced mass by more than 5% compared to the control treatment. Avocado fruit membrane integrity and cell turbidity are negatively impacted by moisture loss. Aside from that, shrivelling damages the fruit's visual appeal. Fruit moisture has a significant impact on shelf life, with greater fruit moisture often leading to longer shelf life than lower fruit moisture. Consequently, Avocado fruit treated with methyl-cellulose lost less moisture than untreated fruit, according to [12]. Because expert brushing and washing remove and reorganizes the fruit's natural wax, untreated control fruit loses more bulk after harvest [32].



As shown in Fig. 2 (A) and (B), edible coatings may impact the fruit's firmness and weight loss (B). As can be seen from the error bars, the mean's standard deviation is rather large (n = 5). They stand straight up. Carboxymethylcellulose is abbreviated as "CMC," "MLE," and "MSE."

3.2. Antifungal attributes in extracts of moringa

3.2.1. In(vitro)screening

Moringa tissue type and extraction procedure affected isolate formation (Table 1, Table 2, Table 3). Both moringa extracts affected isolate growth compared to controls. After seven days, only methanolic extracts of the seed killed the pathogen *L. theobromae* (Table 3). 7 days later, other extracts' antiviral activity was reduced. *C. gloeosporioides* and *A. alternata* grew slower in ethanol leaf and seed extracts. 43.6% and 42.9% of *C. gloesporiodes* were inhibited after 10 days of ethanolic leaf extract (method two). *A. alternata* and *C. gloesporiodes* dropped by 30.1% and 31.6% after 10 days of *A. alternata* seed extract treatment [33].

Table 1: Shows that C. gloeosporioides on PDA plates with moringa extracts developed faster than the control for 10 days. At a 0.05 significance level, Fisher's least significant difference test reveals that the means within columns that begin with the same letter do not vary statistically

Pathogen	Plant tissue	Extraction method	Mycelial growth (cm)		
Empty Cell	Empty Cell	Empty Cell	Day (5)	Day (7)	Day (10)
	Leaf	Methanol	3.16cd	5.26c	6.71c
		Ethanol A	3.22c	5.28c	6.66c
Colletotrichum		Ethanol B	2.66d	4.03d	4.77e
	Seed	Methanol	3.27c	5.80b	7.55b
		Ethanol A	2.67e	4.75d	5.91d
		Ethanol B	3.06d	5.17c	6.87c
	Control	Water	4.75b	7.57a	8.46a
		p-value	<0.001	<0.001	<0.001

Table 2: PDA plates containing moringa extracts were used to change the A. alternata growth rate for 10 days, and the results are shown in Table 2. At a 0.05 significance level, Fisher's least significant difference test reveals that the means within columns that begin with the same letter do not vary statistically

Pathogen	Plant tissue	Extraction method	Mycelial growth (cm)		
Empty Cell	Empty Cell	Empty Cell	Day 5	Day 7	Day 10
	Leaf	Methanol	4.79 c	6.82 b	7.99 a
		Ethanol A	3.56 d	4.74 c	5.81 bc
Alternaria		Ethanol B	3.22 d	4.16 c	4.85 c
	Seed	Methanol	3.27 d	4.81 c	6.64 b
		Ethanol A	3.24 d	4.42 c	5.67 b
		Ethanol B	2.66 e	4.52 c	6.49 b
	Control	Water	5.64 b	8.00 a	8.50 a
		p-value	<0.001	<0.001	<0.001

Table 3: L. theobromae grew faster on PDA plates treated with moringa extracts compared to the control during a 10-day period. At a 0.05 significance level, Fisher's least significant difference test reveals that the means within columns that begin with the same letter do not vary statistically.

Pathogen	Plant tissue	Extraction method	Mycelial growth (cm)		
Empty Cell	Empty Cell	Empty Cell	Day 5	Day 7	Day 10
	Leaf	Methanol	7.06 b	8.51 a	8.51 a
		Ethanol A	8.27 a	8.38 a	8.50 a
Lasiodiplodia		Ethanol B	6.88 c	8.24 ab	8.50 a
	Seed	Methanol	5.22 d	7.62 b	8.04 b
		Ethanol A	6.11 c	8.44 a	8.53 a
		Ethanol B	6.46 c	8.11 ab	8.53 a
	Control	Water	8.50 a	8.50 a	8.51 a
		p-value	<0.001	<0.001	<0.001

L. theobromae was the only methanolic extract that worked against the isolates (Table 3). Moringa leaf extracts were reported to be very efficient in treating *Phytopthora parasitica*, *Fusarium oxysporum*, and *Alternaria solani* infections [34]. Leaf extracts' potent antifungal properties are attributed in part to the tissue's high concentration of phenolic chemicals [34].

3.2.2. Scanning through an electron microscope

C. gloeosporioides,) *A. alternative*, and *L. theobromae* were all shown to be affected by Moringa extracts utilizing scanning electron microscope images. Additionally, the treated samples showed hyphal shrinkage, hyphal breakdown, and even hypoplasia (hyphal constriction). Moringa extracts may have inhibitory characteristics associated with plant biochemical components like phenols. *Fusarium solani* strains' fungus hyphae were killed by moringa extracts, according to research [35]. Moringa extracts include lipophilic chemicals that have the ability to bind with the cytoplasmic membrane and then it may permeabilize the fungal cell membrane, thereby stopping the development of the fungus [35, 36].

3.2.3. *In vivo* testing

When studied in vivo, a significant influence on the occurrence and severity of sickness was observed in coating treatments. MLE or MSE + 1 percent CMC treated avocados exhibited a lower frequency and severity of disease than untreated avocados, proving that (Table 4).

Table 4. Moringa leaf and seed extracts are CMC and MLE

Treatments	***C. gloeosporioides***		***A. alternate***	
Empty Cell	**Incidence (%)**	**Severity (%)**	**Incidence (%)**	**Severity (%)**
Control	66.67 a	39.40 a	61.22 a	32.12 a
CMC 1% + MLE	19.21 b	8.23 b	10.21 b	8.13 b
CMC 1% + MSE	29.55 b	19.41 b	26.12 b	12.42 b

30 'Gem' fruits per treatment had A. alternata and C. gloeosporioides. Duncan's test at a 5% probability level found no statistically significant differences between mean values.

4. CONCLUSIONS

It has been shown that preserving avocados with 1% CMC and extracts from moringa seeds and leaves results in a reduction in ethylene production, respiration rate, and hardness. The study's findings showed that covered fruits also had less illnesses and water losses. Both types of avocados benefited from coating treatments that extended their shelf lives and maintained their quality. Moringa ethanolic extracts were discovered to be superior than moringa methanolic extracts in preventing outbreaks of post-harvest disease. This study showed that an organic post-harvest treatment for avocado fruit may be made using moringa extracts on seeds and leaves to produce a 1-percent CMC edible coating.

Highlight:

1. The study reported the use of extracts of moringa leaves and moringa seed as an edible coating might help to preserve the shelf life and the quality of Avocado.
2. The outcomes of this research showed that carboxyl methylcellulose CMC containing moringa extract reduces illnesses in fruit.

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