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Antifungal Activity and Synthesis of Nano-Chitosan and Combined with Thyme Essential Oil to Management Anthracnose of Mango Fruits During Storage

Nehal Mohamed Saied

Department of Plant Pathology, Agricultural & Biological Research Institute, National Research Centre (NRC), 33 El-Buhouth St., 12622 Dokki, Egypt.

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ABSTRACT

In order to prevent anthracnose disease in mango fruits during stored, a combination of nano-chitosan and thyme essential oils was evaluated. Thyme or nano-chitosan were tested in vitro for their ability to inhibit *Colletotrichum gloeosporioides*' mycelial growth. Complete suppression of linear growth was achieved with 1.0 g/L of nano-chitosan and 1.0% of thyme. All tested concentrations of thyme EO greatly decreased the anthracnose disease of mango fruits in vivo trials. Thyme, at 1.0%, is the most effective treatment, it lowered the incidence and severity of the disease by 70.0 and 72.0%, respectively. Moreover, the anthracnose disease of mango fruits was considerably decreased by all tested concentrations of nano-chitosan. The best treatments are nano-chitosan at 0.75 and 1.0 g/L, which decreased the disease incidence by 70.0 &72.0 % and disease severity by 70.0 and 74.0 % respectively. To investigate their impact on the occurrence of anthracnose rot in mango fruits during a 30-day storage period, single or combined treatments of thyme EO at 1.0 % and nano-chitosan at 0.75 and 1.0 g/L were used. The anthracnose disease of mango fruits was considerably decreased by all tested treatments, according to the results. The combination of thyme and nano-chitosan treatments proved to be the most successful, as they decreased both the incidence by 86.0 & 88.0 % and disease severity by 84.0 and 86.0 % respectively. The proportion of weight loss was decreased by 55.6 and 50.0%, respectively, by the prior treatments. In terms of TSS, the greatest rise was seen when thyme (at1.0%) and nano-chitosan (at 0.75 or 1.0 g/L) were combined. These treatments raised TSS by 8.6 and11.4%, respectively. Sigle treatments had a moderate impact.

Keywords: Mango, anthracnose, thyme, nano-chitosan, control

1. Introduction

Mangos (*Mangifera indicca* L.), among other fruits, are widely farmed in the tropics and warmer subtropics. It contains essential minerals, vitamins, and fiber for human health (Sethi *et al.,* 2011). After harvesting, fruit ripening and senescence lead to degradation, and shriveling that occurs during storage, transit, and shelf life results in fruit losing market value (Rodov *et al.,* 1997). The most important disease affecting mango-growing countries globally is anthracnose, caused by *Colletotrichum gloeosporioides* and presents a major challenge to the export sector. It resulted in black spots on foliage, blight on blossoms, and black spots and fruit drop on fruits. The tree consequently loses all ability to produce fruit (Arauz, 2000). The main cause of anthracnose disease is *Colletotrichum gloeosporioides*, severely reduces yield and quality of fruits. Postharvest fruit anthracnose was the most damaging type of fruit anthracnose, causing 17–36% of economic losses by decreasing the fruit's marketability and rendering it unusable (Haggag, 2010). The use of coatings to increase the commercial shelf life of different kinds of food has recently attracted a lot of study attention. Fresh fruit preservation is one area in which they excel.

Due to its qualities, chitosan has emerged as one of the most adaptable substitutes for controlling fungal diseases. Applying this substance on its own to prevent fruit diseases has had incredibly positive

Corresponding Author: Nehal Mohamed Saied, Department of Plant Pathology, Agricultural & Biological Research Institute, National Research Centre (NRC), 33 El-Buhouth St., 12622 Dokki, Egypt.

outcomes (Gutiérrez-Martínez *et al.* 2018; Ramos-Guerrero *et al.* 2018; Braga *et al.* 2019; Peralta-Ruiz *et al.* 2020). But more recently, chitosan has been applied on a nanometric scale, which has increased the surface area of interaction and proven to be much more effective (Duan *et al.* 2019). Chitosan has been dubbed the most significant polymer in agricultural nanotechnology because to its qualities, which include its antibacterial qualities, nontoxicity, economic accessibility, and biodegradability (Saharan *et al.* 2015). Because of its larger dispersion ability, chitosan is said to be far more effective when present in nanostructures as opposed to macro-size (Sampathkumar *et al.* 2020). In an effort to replace dangerous synthetic fungicides, chitosan has been utilized for its unique properties in the manufacture of nanomaterials, which are defined as materials with a nanometric size (Sampathkumar *et al.* 2020). In order to control *Colletotrichum gloeosporioides* from attacking avocados, Correa-Pacheco *et al.* (2017) created chitosan nanoparticles and chitosan nanoparticles thyme at 1-3%. When these treatments were tested in vitro, the phytopathogen showed no sporulation or germination at the highest dose and was completely inhibited of linear growth. Another study that examined the effects of chitosan nanoparticles in conjunction with pepper tree essential oil used *C.gloeosporioides* as a case study (Chavez-Magdaleno *et al.* 2018). They showed that the growth of this phytopathogen could be inhibited by 65.6 and 68.7%, respectively, at 0.08 and 0.16 mg/mL when this nano-emulsion was applied in vitro in comparison to the control and the components assessed individually.

Correa-Pacheco *et al.* (2018) reported very promising results when they preparation of chitosan nano-combined with canola, lemon, and thyme against pathogenic fungi *Rhizopus stolonifer*, *C. fragariae*, *A. alternata*, and *Colletotrichum gloeosporioides* that were isolated from tropical fruits.

Arabpoor *et al.* (2021) used the ion gelation process to create a coating made of *Eryngium campestre* L. essential oil capsulated in nano-chitosan (ECO-NPC). The chitosan nanoparticle had the best effects; after 21 days of storage. The effectiveness of a clay-chitosan nanocomposite to control *Penicillium digitatum* was reported by Youssef and Hashim (2020). The clay-chitosan nanocomposite's direct and indirect antifungal efficacy against oranges was assessed by the authors. The results demonstrated that the nanocomposite's direct action totally prevented green mold, but the application of a clay-chitosan (1:2) nanocomposite reduced indirect activity (the induction of resistance).

Essential oils (EOs) are mixtures of hundreds of naturally occurring volatile plant-based compounds, mainly oxygenated derivatives of monoterpenes and sesquiterpenes (Tisserand and Young, 2013; Perumal *et al.,* 2016).

Numerous EOs have demonstrated strong anti-fungal efficacy (Rabari, *et al.,* 2018). Many EOs are commonly regarded as safe (GRAS) and biodegradable, as well as being biodegradable. Several essential oils (EOs) have been shown to effectively prevent anthracnose on mango fruits of various cultivars (Sefu *et al.,* 2015 and Danh *et al.,* 2021). Several essential oils has been shown to be effective on several plant diseases (de Oliveira *et al.,* 2017; Abd-alla and Haggag; Duamkhnmanee 2008; Karunanayake *et al.,* 2020; 2013and Perumal *et al.,* 2017).

We hope that this work will be useful for the application and design of nano-chitosan combined with thyme essential oils to boost the shelf life of mango as well as protection against anthracnose diseases.

2. Materials and Methods

2.1. Pathogenic fungus

Colletotrichum gloeosporioides, the causal organism of fruit anthracnose disease of mango fruits were isolated and identified in a previous study.

2.2. Mango fruits

We acquired the mango fruits (cv. Naoomy) from the National Research Center Station. They were selected based on the absence of fungal diseases and their homogeneity in size, color, and shape**.**

2.3. In vitro, trails

2.3.1. Testing the impact of thyme essential oils on the fungus *Colletotrichum gloeosporioides* linear growth

On PDA medium at 25 ± 2°C, the effects of thyme at 0.0,0.25,0.50,0.75, and 1% against the mycelial growth of *Colletotrichum gloeosporioides* were evaluated. To sterilize the PDA medium, it was diluted into 100 ml portions and placed in 250 ml Erlenmyer flasks. The flasks were autoclaved at

121°C for 15 minutes. Final concentrations were prepared separately, added to PDA medium right before it hardened, and then gradually mixed in 0.1% Tween 80 (Sigma) to increase solubility in order to get the final concentrations. The media in each flask was first broken up in a sterile, 9-cm-diameter Petri plate before it solidified. Ten-day-old *Colletotrichum gloeosporioides* cultures were utilized to inoculate individual plates. A temperature of 25 ±C was maintained for the cultures. The mycelial growth of the fungus was recorded after 10 days. For every treatment, five replicate plates were used.

2.4. Testing the nano-chitosan on the linear growth of *Colletotrichum gloeosporioides*

2.4.1. Chitosan nanoparticle preparation

We modified the prior work (Rodrigues, *et al.,* 2012) and used ionic gelation to create chitosan nanoparticles chemically. Chitosan (0.2% w/v) was dissolved in 0.5% v/v acetic acid and mixed thoroughly using a magnetic stirrer. We added 0.1% v/v of Tween 80.

The solution of tripolyphosphate (TPP) obtained by dissolving sodium tripolyphosphate (NaTTP) in deionized water (1 mg/ml). Using chitosan (0.2% w/v) in 0.5% v/v acetic acid (pH 5.6) as a control, we created the nanoparticle solutions by swirling chitosan solutions containing Tween surfactant and TPP droplets at a volume ratio of 5:1 in a glass beaker for 30 to 60 minutes at room temperature. Nanoparticles will naturally form when TPP is added to chitosan solutions, so the mixture was homogenised in a high-speed homogeniser to reduce the particle size.

2.4.2. Effect on nano-chitosan on linear growth of pathogenic fungus

The effects of nano-chitosan at 0.0,0.25,0.50,0.75 and 1.0 g / L against *Colletotrichum gloeosporioides's* mycelial growth were assessed on PDA medium. The PDA medium was divided into 100 ml sections and put into 250 ml Erlenmyer flasks for sterilization. After that, the flasks were autoclaved for 15 minutes at 121°C.

The final concentrations were produced separately, added to the PDA medium just before it hardened, and then progressively mixed in 0.1% Tween 80 (Sigma) to improve solubility. Before the medium in each flask solidified, it was first broken up in a sterile, 9-cm-diameter Petri plate. To inoculate individual plates, cultures of Colletotrichum gloeosporioides that were ten days old were used. For the cultures, a temperature of 25 ±C was maintained. Once the control plates had reached their full size and the average growth diameter had been determined, the fungus's linear mycelial development was assessed. Five dummy plates were utilized for each treatment.

2.5. In vivo trails

2.5.1. Effect of thyme essential oils on anthracnose of mango fruits during storage for 30 days

2.5.2. Inoculum preparation

For this test, *Colletotrichum gloeosporioides* isolate was cultivated on PDA plates for 10 days at 20±2°C in order to prepare the standard inoculum. Spore suspension was made by brushing the culture's surface with 10 mL of distilled water that had been sterilized for each plate. The spore suspensions were then filtered through muslin. Using a hemocytometer slide, the spore suspension concentration was brought down to roughly 10^6 spore/mL.

Mango fruits cv. Naoomy were subjected to an in vivo assessment of the effects of thyme 0.0,0.25,0.50, 0.75 and 1.0 % . After applying 70% ethanol to the fruit's surface for two minutes at room temperature, it was repeatedly cleaned in sterile water. On the fruits, fictitious wounds were made with a sterile scalpel. The fruits were individually dipped in the previous concentrations and allowed to air dry. The process of inoculating treated fruits involved spraying them with a suspension of 10^6 spores/ml of *Colletotrichum gloeosporioides* spores and allowing them to air dry. A set of fruits that had only received a *Colletotrichum gloeosporioides* inoculation were utilized as a control. Each of the four carton boxes used for each treatment, containing five fruits each, were filled with either treated or untreated (control) fruits in order for them to be evaluated. After that, it was stored for 15 days at 20–2°C and 90–95% relative humidity. Fruits were checked for anthracnose infections on a regular basis. The previously indicated measurements of disease severity and incidence (%) were made.

2.5.3. Effect of nano-chitosan on anthracnose of mango fruits during storage for 30 days

Mango fruits cv. Naoomy were subjected to an in vivo assessment of the effects of nano-chitosan at 0.0, 0.25,0.50, 0.75 and 1.0 g /L. After applying 70% ethanol to the fruit's surface for two minutes at room temperature, it was repeatedly cleaned in sterile water. On the fruits, fictitious wounds were made with a sterile scalpel. The fruits were individually dipped in the previous concentrations and allowed to air dry. The process of inoculating treated fruits involved spraying them with a suspension of 106 spores/ml of *Colletotrichum gloeosporioides* spores and allowing them to air dry. A set of fruits that had only received a *Colletotrichum gloeosporioides* inoculation were utilized as a control. Each of the four carton boxes used for each treatment, containing five fruits each, were filled with either treated or untreated (control) fruits in order for them to be evaluated. After that, it was stored for 15 days at 20–2°C and 90–95% relative humidity. Fruits were checked for anthracnose infections on a regular basis. The previously indicated measurements of disease severity and incidence (%) were made.

2.6. Disease assessment

The percentage of infected fruits to total fruits was used to calculate the disease incidence. On mango fruits, the disease severity (0–5) is used to determine the percentage of fruit infected with anthracnose disease (Rose, 1974).0= zero infection, 1= 1-10, 2= 11-25, 3=26-50, 4= 51-75 and 5 more than 75 percent of fruit area infected.

The percent disease index was recorded according Rose (1974)

$$\text{Percent Disease Index (PDI)} = \frac{\text{Sum of all individual rating}}{\text{Total number of fruits grate x Maximum disease grate}} \times 100$$

2.6.1. Testing of thyme and nano-chitosan alone or in combination on anthracnose of mango fruits during storage for 30 days

Thyme EO at 1.0 % and nano-chitosan at 0.75 and 1.0 g/L were applied as single or combined treatments to study their effect on anthracnose rot incidence of mango fruits during storage for 30 days. Utilizing a sterile scalpel, navel orange fruits were wounded. Fruits that were injured were given the following flowing treatments: Navel orange fruits were surface-sterilized and wounded using sterilized scalpel. Wounded fruits were subjected to the flowing treatments: (1) dipping in Thyme at1.0 % for 1.0 minute, (2) Dipping nano-chitosan 0.75 g/L for 1.0 minute (3) dipping nano-chitosan at 1.0 g/Lfor 1.0 minutes (4) control (untreated fruits). Meanwhile combined treatment was done by dipping fruits in thyme at 1.0 % for 1.0 min. followed by dipping fruits in nano-chitosan ant 0.75 or 1.0 g/L for 1.0 min. then are dried at room temperature. Fruits were inoculated by spraying them with a suspension of *Colletotrichum gloeosporioides* spores (10^6 spores/ml) and letting them air dry. There were three replicates of the control and each treatment was used. To be evaluated, all treated or untreated (control) fruits were packed into carton boxes at a rate of 10 fruits per box and kept in storage for 15 days at a temperature of 20–2°C and a relative humidity of 90–95%. Fruits were periodically inspected for mold infection.

2.6.2. Effect of thyme and nano-chitosan alone or in combination on mango fruits quality during storage without artificial infection

Thyme EO at 1.0 % and nano-chitosan at 0.75 and 1.0 g/L were applied as single or combined treatments to study their effect on mango fruits quality during storage for 30 days. The treatments were assessed to find out how they affected the quality of the mango fruit, namely the fruit weight loss, total soluble solids (TSS), and tratable acidity (TA%). Each treatment consisted of three carton boxes (60 x 40 x 15 cm) with one layer of roughly 10 kg weight. For 21 days, the experimental boxes were kept at 20±2˚C and 90% relative humidity without any artificial infections.

The following findings were used to assess the impact of the tested treatments on mango fruits: -

2.7. Fruit weight loss percentage

Mango fruit weights were initially recorded for each treatment. Fruit weight loss percentage was then calculated by weighing the same fruits at the end of the cold storage duration using the following formula:

$$\text{Weight loss \%} = \frac{\text{Initial weight} - \text{Weight at end experiment}}{\text{Initial weight}} \times 100$$

2.8. Total soluble solids percentage (TSS)

Chen and Mellenthin (1981) state that a hand refractometer was used to determine the TSS% in orange fruit juice.

2.9. Titratable Acidity (TA %)

Fruit juice was titrated against 0.1 N NaOH to pH 8.1 in order to estimate total titratable acidity (TA), which was then calculated as a percentage of citric (AOAC 1990).

2.10. Statistical analysis

Neler *et al.* (1984) used the Tukey test for multiple comparison among means.

3. Results

3.1. Impact of thyme essential oils on the fungus *Colletotrichum gloeosporioides* linear growth

Thyme at concentrations of 0.0,0.25,0.50,0.75, and 1% was tested for its ability to inhibit *Colletotrichum gloeosporioides' mycelial* growth. The results shown in Fig (1) indicate that a considerable reduction in the linear growth of *Colletotrichum gloeosporioides* at all tested doses of thyme EO. When thyme was used at 1.0%, linear growth was completely inhibited. Thyme at 0.75% produced the largest reduction, which reducing the linear growth by 82.0%. A moderate effect was shown by other concentrations.

3.2. Effect the nano-chitosan on the linear growth of *Colletotrichum gloeosporioides*

The study assessed the impact of nano-chitosan at concentrations of 0.0,0.25,0.50,0.75, and 1.0 g/L on the mycelial growth of *Colletotrichum gloeosporioides*. The results presented in Fig (2) demonstrate a considerable reduction in *Colletotrichum gloeosporioides'* linear growth at all tested concentrations. Using nano-chitosan at 1.0 g/L, linear growth was completely inhibited mycelial growth. While, at 0.75g/L, nano-chitosan produced the largest reduction a 91.0% decrease in linear growth. A moderate effect was shown by other concentrations.

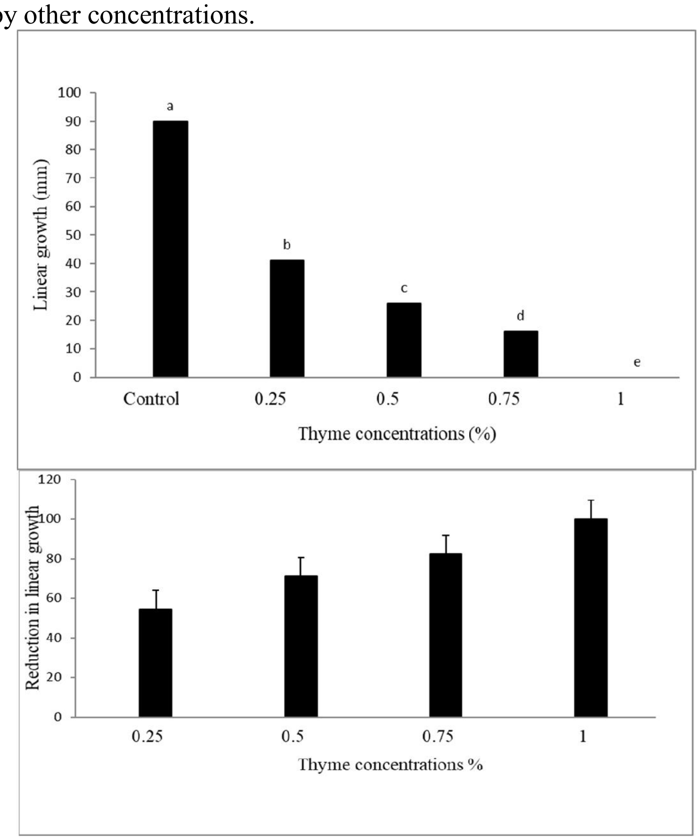

Fig. 1: Impact of various concentrations of thyme essential oils on linear growth and reduction of *Colletotrichum gloeosporioides*

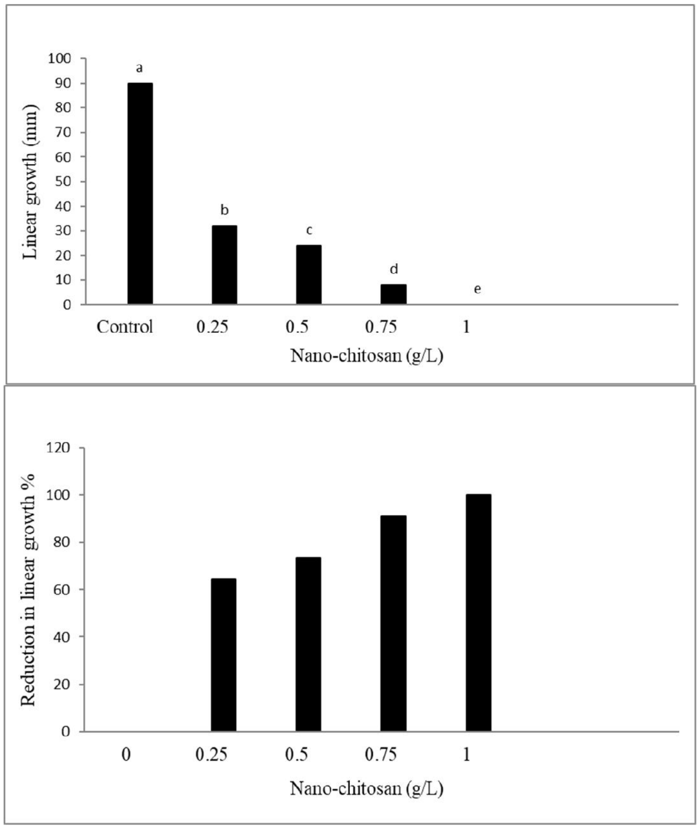

Fig. 2: Impact of various concentrations of nano-chitosan on linear growth and reduction of *Colletotrichum gloeosporioides*

3.3. In vivo trails

3.3.1. Effect of thyme essential oils on anthracnose of mango fruits during storage for 30 days

Impact of thyme EOs at 0.0,0.25,0.50,0.75, and 1% against mango fruit anthracnose disease was tested. The results shown in Table (3) demonstrate a significant reduction in mango fruit anthracnose disease at all tested concentrations of thyme EO. Thyme, at 1.0%, is the most effective treatment; it lowered the incidence and severity of the disease by 70.0 and 72.0%, respectively. Thyme, at 0.75%, had the biggest reduction, lowering the disease's incidence and severity by 60.0 and 62.0 %, respectively. A moderate effect was shown by other concentrations.

Table 3: Impact of various concentrations of thyme(EO) on anthracnose rot incidence of mango fruits during 30 days of storage

Thyme essential oils (%)	Mango anthracnose disease			
	Disease incidence	Reduction %	Disease severity	Reduction %
0.25	54.0 b	46.0	50.0 b	50.0
0.50	48.0 c	56.0	46.0 b	54.0
0.75	40.0 d	60.0	38.0 c	62.0
1.0	30.0 d	70.0	28.0 d	72.0
Control (un treated)	100.0 a	100.0	100.0 a	0.0

Figures with the same letter are not significantly different (P=0.5)

3.3.2. Effect of nano-chitosan on anthracnose of mango fruits during storage for 30 days

In order to investigate the effects of nano-chitosan at 0.0,0.25,0.50, 0.75, and 1.0 g /L on anthracnose disease of mango fruits cv. Naoomy were tested in vivo assessment. The results shown in Table (4) demonstrate a significant reduction in the anthracnose disease of mango fruits at all tested dosages of nano-chitosan. The most effective treatment was nano-chitosan at 0.75 and 1.0 g/L, which decreased the disease severity by 74.0% and the disease incidence by 72.0%, respectively. The greatest reduction was achieved at 0.50% with nano-chitosan, which decreased the disease's incidence and severity by 60.0 and 64.0%, respectively. Moderate effects were seen at other concentrations.

Table 4: Effect of different concentrations of nano-chitosan on anthracnose rot incidence of mango fruits during 30 days of storage

Nano -chitosan (g/L)	Mango anthracnose disease			
	Disease incidence	Reduction %	Disease severity	Reduction %
0.25	46.0 b	54.0	45.0 b	55.0
0.50	40.0 c	60.0	36.0 c	64.0
0.75	30.0 d	70.0	30.0 d	70.0
1.0	28.0 d	72.0	26.0 d	74.0
Control (un treated)	100.0 a	0.0	100.0 a	0.0

3.3.3. Testing of thyme and nano-chitosan alone or in combination on anthracnose of mango fruits during storage for 30 days

Mango fruit anthracnose rot incidence was studied during a 30-day storage period using thyme EO at 1.0 % and nano-chitosan at 0.75 and 1.0 g/L, applied either alone or in combination. All of the tested therapies effectively decreased the anthracnose illness of mango fruits, according to Table (5) results. The most successful therapies are combined treatments between thyme and nano-chitosan which reduced the disease incidence by 86.0 & 88.0 % and disease severity by 84.0 and 86.0 % respectively. Sigle treatments showed moderate effect.

3.3.4. Effect of thyme and nano-chitosan alone or in combination on mango fruits quality during storage without artificial infection

Mango fruits were stored for 30 days without artificial infection. The effects of thyme EO at 1% and nano-chitosan at 0.75 and 1.0 g/L were studied individually or in combination. Table (5) results show that every therapy that was tried considerably decreased the anthracnose illness that affects mango fruits. The best therapies include combinations of 1.0% thyme and 0.75–1.0 g/L nano-chitosan, which decreased the percentage of weight loss by 55.6 and 50.0%, respectively. In terms of TSS, the greatest rise was seen when thyme (at1.0%) and nano-chitosan (at 0.75 or 1.0 g/L) were combined. These treatments raised TSS by 8.6 and11.4%, respectively. Treatments in isolation had a moderate impact.

Table 5: Effect of combined treatments between thyme essential oil and nano-chitosan on anthracnose of mango fruits during 30 days of storage

Applications	Mango anthracnose disease			
	Disease incidence	Reduction %	Disease severity	Reduction %
	Single treatments			
Thyme 10.0ml/L	36.0 b	64.0	38.0 b	62.0
Nano- chitosan 0.75g/L	34.0 bc	66.0	32.0 c	68.0
Nano- chitosan 1.0g/L	30.0 c	70.0	28.0 c	72.0
	Combined treatments			
Thyme 10.0+nano-chitosan 0.75	14.0 d	86.0	16.0 d	84.0
Thyme 10.0 +nano-chitosan 1.0	12.0 d	88.0	14.0 d	86.0
Control (un treated)	100.0 a	0.0	100.0 a	0.0

Table 6: Effect of combined treatments between thyme essential oil and nano-chitosan on fruit quality of mango fruits after 40 days of storage without artificial infection

Treatments	Fruit quality		
	Weight loss %	Total soluble solids (TSS)	Titratable Acidity (TA%)
		Single treatments	
Thyme 10.0 ml/L	14.0b	18.0c	0.42a
Nano- chitosan 0.75 g/L	12.0b	18.5b	0.43a
Nano- chitosan 1.0 g/L	12.0b	18.7b	0.42a
		Combined treatments	
Thyme 10.0+nano-chitosan 0.75	9.0c	19.0a	0.43a
Thyme 10.0 +nano-chitosan 1.0	8.0c	19.5a	0.44a
Control (un-treated)	18.0 a	17.5	0.41a

Figures with the same letter are not significantly different (P=0.5)

Plate 1: Effect of combined treatments between thyme essential oil and nano-chitosan on anthracnose of mango fruits during 30 days of storage(a- Thyme 10.0ml/L +nano-chitosan 0.75g/L, b- Thyme 10.0ml/L +nano-chitosan 1.0 g/l , c-control fruits).

4. Discussion

The most important fugus affecting mango-growing countries globally called C*olletotrichum gloeosporioides*, which causes anthracnose disease and is a major hindrance to the export sector (Arauz, 2000). According to Tisserand and Young (2013) and Perumal *et al.* (2016), hundreds of naturally occurring volatile plant-based chemicals, mostly oxygenated derivatives of monoterpenes and sesquiterpenes, combine to form essential oils (EOs). Results in the present study showed that complete suppression of mycelial growth was achieved with thyme at 1.0%. While, in vivo trails, all concentrations of thyme EO decreased the anthracnose disease of mango fruits. Thyme, at 1.0%, is the most effective treatment, reducing the incidence and severity by 70.0% and 72.0%, respectively. The most effective treatments are combined treatments between thyme and nano-chitosan which reduced the disease incidence by 86.0 & 88.0 % and disease severity by 84.0 and 86.0 % respectively. The previous treatments reduced the percentage of wight loss and increased the TSS. In this regard, a number of successful studies have shown that the use of biological agents, such as essential oils, can be used to manage postharvest diseases of several fruits (Amiri *et al.,* 2008; Liu, *et al.,* 2009). According to Bakkali *et al.* (2008), essential oils (EOs) have been demonstrated to be a useful control measure for reducing the environmental impact of fruit production; however, most of these studies were carried out in greenhouse environments (Lopez-Reyes *et al.,* 2010 and 2013). The molecular structure of essential oils (EOs) such as aldehydes, phenols, and ketones influences how they interact with pathogens. These compounds effectively stop infections from growing. According to studies conducted by Daferera *et al.* (2000) and Barrera-Nacha *et al.* (2008), environmental factors that are abundant in fungicidal compounds, including thymol, carvacrol, and b-anisaldehyde, exhibit the best inhibitory effect against *Penicillium digitatum (*Daferera *et al.,* 2000) and *Colletotrichum gloeosporioides* (Barrera-Nacha *et al.,* 2008).A number of researchers have reported that EO exhibits antifungal activity in vitro against a range of postharvest fungi, such as Aspergillus spp. (Tang *et al.,* 2018), Penicillium spp. (Xing *et al.,* 2016), Alternaria spp. (Chen *et al.,* 2016 a, b), Colletotrichum spp. (Bill *et al.,* 2016), and Botrytis cinerea (Banani, *et al.,* 2018).Typically, dipping, spraying, or direct contact with the fruit are the techniques employed to confirm the efficacy of EOs (Elshafie *et al.,* 2016). Furthermore, it has been noted that chitosan, the most malleable biopolymer, has antibacterial action against a variety of foodborne pathogens, raising interest in it as a possible preservative (Ganan *et al.,* 2009).

According to Abd-El-Kareem *et al.* (2022), attempts were made to test the effects of thyme, nerol and chitosan against *Penicillium digitatum* mycelial growth and spore germination in vitro. The results demonstrated that using thyme and nerol at 1.5% as well as 8.0 g/L of chitosan completely suppressed the mycelial growth and spore germination of all fungi that were investigated. They added that in order to investigate their potential protection against green and blue molds as well as sour rot diseases of Washington navel orange fruits, thyme or nerol at a concentration of 1.5% and chitosan at 8.0 g/L were administered separately or in combination. The greatest inhibition, according to the results, was achieved when thyme or nerol at 1.5% was mixed with 8.0g/L of chitosan. This combination significantly decrease of incidence and severity of green, blue mold, and sour rot diseases. Fruit quality was unaffected negatively by any of the studied treatments. Due to its unique qualities, chitosan has emerged as one of the most effective substitutes for controlling plant diseases (Gutiérrez-Martínez *et al.* 2018; Ramos-Guerrero *et al.* 2018; Braga *et al.* 2019; Peralta-Ruiz *et al.* 2020).

Nevertheless, more recently, chitosan has been applied on a nanometric scale, which has shown to be even more successful because it increases the surface area of contact (Duan *et al.* 2019). The current study's results showed that *Colletotrichum gloeosporioides'* linear growth was considerably inhibited at all nano-chitosan dosages examined. Using nano-chitosan at 1.0 g/L, linear growth was completely inhibited. Furthermore, the anthracnose disease of mango fruits was considerably decreased by all tested concentrations of nano-chitosan. The best therapies include nano-chitosan at 0.75 and 1.0 g/L, which decreased the disease severity by 74.0% and the disease incidence by 72.0%, respectively. Additionally, the disease severity decreased by 84.0 and 86.0 % and the disease incidence by 86.0 and 88.0 %, respectively, when thyme at 1.0% and nano-chitosan at 0.75 or 1.0 g/L were combined as therapies. The proportion of weight loss was decreased by 55.6 and 50.0%, respectively, by the prior therapies. also caused an 8.6% and 11.4% increase in TSS, respectively. In order to control *Colletotrichum gloeosporioides* from attacking avocados, Correa-Pacheco *et al.* (2017) created chitosan nanoparticles and chitosan essential oil nanoparticles (thyme) at concentrations of 1-3%. When these treatments were tested in vitro, the phytopathogen showed no sporulation or germination at the highest dose and was completely inhibited from growing mycelially. Chavez-Magdaleno *et al.* (2018) showed that the application of nano-emulsion in vitro could inhibit the growth of Colletotrichum gloeosporioides.

To inhibit the growth of the *Colletotrichum gloeosporioides*, avocado fruit was coated with chitosan nanoparticles and chitosan-thyme essential oil nanoparticles (Correa-Pacheco *et al.* 2017). The bacteria populations on pomegranate arils were decreased by applying savory essential oil-loaded chitosan nanoparticles and chitosan nanoparticles without any flavor. After 18 storage days, both treatments postpone the appearance of mold and yeast in their entirety (Amiri *et al.* 2020). The effects of chitosan nanoparticles as environmentally friendly edible coatings on table grapes were assessed by Castelo Branco Melo *et al.* (2018). When chitosan nanoparticles were applied to grapes, the best outcomes were seen when the grapes were kept at 12 °C and had minimal inhibitory concentration values between 2 and 3 g L^{-1}. For grapes kept at 12 and 25 °C, the percentage of deterioration was 0% and 12.5%, respectively. Generally speaking, the coats shielded the grapes from harmful foodborne pathogens. The impact of coatings with chitosan, titanium, and silicon dioxide nanoparticles on blueberries was examined by Li *et al.* (2021) in order to assess the postharvest quality that extends shelf life during storage. films made of chitosan, nanosilicon dioxide, and nanotitanium dioxide. For nano-TiO2 and nano-SiO2, the coating treatments regulated the decay rates at 5.99% and 9.04%, respectively.

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