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SAFE INTEGRATED CONTROL OF POSTHARVEST ROT DISEASES ON BANANA FRUIT

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Abstract

The integrative management of banana postharvest diseases *i.e.,* crown, neck, finger and flower end rots caused by *Colletotrichum musae* (Berk & M.A.Curtis) Arx and *Fusarium moniliforme* J. Sheld was carried out by dipping banana fruits in each suspensions for 10 minutes of clove oil (2.0%), agar suspension (0.2%) and their combinations compared with hot water and untreated fruits. Results declared that all dipping treatments significantly reduced the incidence and severity of all postharvest rot diseases in banana fruits compared with untreated fruits. Interestingly, the single application of clove oil (2.0%) or agar suspension (0.2%) were the most effective and significants treatments reduced the incidence and severity of postharvest rot diseases. However, no significant effects on fruit firmness loss (%) and soluble solid concentration (SSC) in treated fruits and untreated. Meanwhile, individual application of each clove oil (2.0%) or agar suspension (0.2%) greatly decreased fruit weight loss percentage in stored fruits, and thus, had effective role in maintaining fruit quality.

Key words*:* banana, fruit rot, fungi, disease, agar-agar, clove oil.

Introduction

Banana (*Musa* species), belongs to family Musaceae, is an economic and important fruit crop and a popular worldwide staple food for more than 400 million people (Zhang *et al.*, 2005). Due to fungal infection, ripening fruits are characterized by relatively short postharvest shelf-life (Hossain and Iqbal, 2016). Postharvest diseases destroy 10 to 30% of the total yield of crops during handling, transportation, storage and marketing (Agrios, 2005). *Colletotrichum musae* and *Fusarium* species including *F. solani, F. semitectum, F. moniliforme* and *F. musae* are the major fungi causing postharvest complex diseases of banana fruits (Krauss and Johanson, 1998; Abd-Alla *et al.*, 2014; Diedhiou *et al.*, 2014; Abdullah *et al.*, 2016 and Kamel *et al.*, 2016). *Colletotrichum musae* and *F. moniliforme* are the major fungi causing postharvest diseases of banana fruits *i.e.,* crown rot, finger rot, neck rot and flower end rot (Zoeir *et al*., 2017 a). Application of synthetic fungicides for controlling plant diseases brings several hazard effects such as residues in edible plant parts, resistant strains and environmental pollution. In addition, consumers prefer natural products than synthetic ones. Therefore, there are increasing interests for using safe eco-friendly antifungal agents such as plant-based essential oils (Tabassum and Vidyasagar, 2013 and Moghaddam and Mehdizadeh, 2016). Essential oil of clove (*Syzygium aromaticum* L.) Merrill & Perry (Family Myrtaceae) has strong antifungal activity against postharvest pathogenic fungi in peanuts (Kishore *et al.,* 2007), grapefruits (Sukatta *et al.* 2008), citrus (Anjum and Akhtar, 2012), and bananas (Ranasinghe *et al.* 2002; Zoeir *et al*., 2017 b). The most common edible coating agents including waxes, chitosan, gelatin and gums have beneficial effects to suppress decay during postharvest storage and improve fruit quality (Ali *et al.*, 2010; Cháfer *et al*., 2012 and Dhall, 2013). Agar-agar has been popular across Asia as an ingredient used in desserts, cooking and used as a laxative, a vegetarian gelatin substitute, to thicken soups, sauces, and preserves (Armisen and Galatas, 2000). Agar, a nontoxic and non-degradable gelling agent, is recently used for the first time as coating agent to protect banana fruits against fungal postharvest diseases (Ziedan *et al.*, 2018). This study aimed to integrative management of banana fruits against postharvest fungal rots with maintenance fruit quality

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during storage by using cost-effective safe eco-friendly agents including clove oil, agar suspension and their combination.

Materials and methods

Banana fruit samples

Banana fruit samples of Balady cultivar were purchased from private orchard in El-Gharbiya Governorate Egypt, at maturity stage, and directly transported to the laboratory of the Agricultural Botany Department, Faculty of Agriculture, Tanta University, Egypt. The selected healthy ripe banana fruits were washed thoroughly several times by tap water, left to air dry at room temperature (27± 2^{0}C), then surface sterilized by dipping in 2% of 70% ethyl alcohol solution for one minute immediately before inoculation according (Zoeir *et al.*, 2017 b).

Source and preparation of agar-agar and clove oil

Agar-agar is a polymer made up of subunit of galactose sugar extracted from various seawood, red-purple marine algae mainly from *Gelidium amansii*. Agar-agar and clove oil were provided from Chemical Industrial Development Company (CID), Egypt. Clove oil at 2% (v/v) was prepared by dissolving 20 ml of concentrated clove oil in 1 L of 0.1 % tween 80 (Zoeir *et al.*, 2017 b). while, the concentration of agar at 2 g/L (w/v) was prepared by dissolving 2 g agar in boiling distilled water (1 L) for 1-2 min, and then cold at 50°C (Ziedan *et al.,* 2018).

Pathogenic fungal and inocula preparation

Highly aggressive isolates of *Colletotrichum musae* and *Fusarium moniliforme*, causing major postharvest diseases of banana fruit (Zoeir *et al*., 2017 a), were provided by Agricultural Botany Department, Faculty of Agriculture, Tanta University, Egypt. Inoculum of each fungal isolate was prepared from 10 days old cultures growing on potato dextrose agar (PDA) medium. Conidia were dislodged from the surface of the medium by flooding plates with sterile distilled water and gentle rubbing with sterile glass rod. Suspensions of each fungal isolate were filtered through sterilized cotton wool to remove mycelial fragments and adjusted to 10^6 conidia ml^{-1} by using Hemocytometer slide according to (Zoeir *et al.*, 2017 a)

Effect of agar and clove essential oil suspensions and their combination on postharvest rot diseases of banana fruit

Fruit samples were disinfected by double immersion in 70% ethanol for 5 min and allowed to dry at room temperature under sterile conditions, and then separated in polyethylene bags that previously disinfected and exposed to UV light for 20 min. The effect of the promising protective agents (clove oil at 2.0% and agar at 2 g L^{-1}) were tested, singly or in combination, on the incidence and severity of postharvest rot diseases of banana fruit (Cv. Balady) was dipping for 10 min. Banana fruit samples were infested by mixture (1:1) of 10^6 ml^{-1} spore suspensions of either *C. musae* and *F. moniliforme*. Ten fruits were used as replicates for each treatment, *i.e.* hot water, agar suspension (2 g L^{-1}), clove oil (2.0%), and agar + clove oil, and ten fruits free treatment were served as a control. Fruit samples were incubated at 23±2ºC for 10 and 15 days.

Determination of postharvest diseases incidence and disease severity

The incidence (%) of postharvest diseases (crown rot, neck rot, finger rot, and flower end rot) on banana fruit was calculated as follows:

Disease incidence (DI %) = No. of infected fruits/total number of fruits × 100.

The disease severity was ranked by observing percentage of rotten symptoms based on linear scale (0-4) (Zoeir *et al*., 2017 a) as follows:

0 = healthy fruit free rotten and discoloration.

1= 1-25% rotten and discoloration area.

2 = 26-50% rotten and discoloration area.

3= 51-75% rotten and discoloration area.

4 = 76-100% rotten and discoloration area.

The disease severity (%) was calculated as follows:

Disease severity (DS %) = $\Sigma(n\times r) \times 100/N$

Where n= number of fruits in each numerical disease grade; r = number of the disease grade and N= total number of infected fruits multiplied by the maximum numerical disease grade.

Determination of some physical and chemical criteria of banana fruit

Fruit weight loss

For the weight loss determination, five fruit samples in each replicate for each treatment were marked before storage and weighed using a digital balance. The same fruit samples were weighed at the beginning of the experiment and after 5, 10 and 15 days storage period. The fruit weight loss percentage was calculated using the following formula (El-Sharony and Amin, 2015):

Weight loss (%) = $(W_1 - W_2)/W_1 \times 100$.

Where W_1 and W_2 are initial weight and weight at specific interval (5, 10 and 15 days), respectively.

Fruit firmness

Peel tissues from one side of banana fingers were removed and pulp firmness measurements taken at three different points using a fruit firmness tester (Fruit Pressure Tester). Firmness values were the force required (kg) for complete penetration of 1 cm (Ranasinghe *et al*., 2005). Firmness loss (%) was calculated as follows:

Firmness loss (%) = $(F_1 - F_2)/F_1 \times 100$.

Where F_1 and F_2 are initial firmness and firmness at specific interval (5, 10 and 15 days), respectively.

Soluble solid concentration

Soluble solid concentration (SSC) content of banana fruit pulp was estimated using Abbe's refractometer. According to Akter *et al.* (2013), a drop of banana juice squeezed from the fruit pulp on the prism of the refractometer and the percent of soluble solid concentration were recorded as % Brix from direct reading of the instrument. Temperature corrections were made using the temperature correction chart that accompanied the instrument.

Statistical analysis

Data set was statistically analysed by analysis of variance (ANOVA) technique using computer software SAS program (SAS Institute, Cary, NC, USA). The values presented are the means of all measurements, and comparisons of means were determined by Duncan's multiple range tests, at $P = 0.05$ (Snedecor and Cocharn, 1980).

Results

Effect of clove oil, agar suspensions and their combination on postharvest rot diseases on banana fruit

Significant reduction in the incidence and severity of postharvest rot diseases in banana fruit was obtained by all dipping treatments (clove oil at 2.0% and agar at 2 g L^{-1}, clove oil + agar, and hot water) compared to control after two periods of storage (10 and 15 days) at 23-25ºC (table 1 and 2). Moreover, the single application of each clove oil (2.0%) and agar suspension (0.2%) was more effective than their combination in the reduction of the incidence and severity of all postharvest rot diseases in banana fruit. Where, in these individual treatments banana fruit did not show any symptoms of postharvest diseases after 10 days of storage period. After storage period (15 days), the symptoms of all rot diseases (crown, neck, finger and flower end rots) appeared in all treated fruit. However, the reduction in diseases incidence and severity can be arranged descendingly as follows: clove oil (2.0%) > agar (0.2%) > clove oil + agar > hot water > control. This means that the single application of clove oil followed by the single application of agar suspension, as dipping treatment for 10 min, were the best agents for reducing the incidence and severity of banana postharvest rot diseases.

Effect of clove oil, agar suspension and their combination on some physical and chemical properties of banana fruit at different storage periods

Fruit weight loss percentage

Data presented in Table 3 illustrated that, fruit weight loss percentage was increased with advancing the storage

Table 1: Effect of clove oil agar suspension and their combination on postharvest rot diseases of banana fruit after storage 10 days at 23 -25 ºC.

Treatment	Postharvest rot diseases incidence (%) and severity (DS)							
	crown rot		neck rot		finger rot		flower end rot	
	%	DS	%	DS	%	DS	%	DS
Control	100 a	4.0 a	70.0 a	3.0 a	70.0 a	3.0 a	100 a	4.0 a
Hot water	30.0 c	2.0 b	20.0 c	2.0 b	20.0 c	1.0 c	20.0 c	1.0 c
Clove (2.0 %)	0.0 d	0.0 c	0.0 d	0.0 c	0.0 d	0.0 d	0.0 d	0.0 d
Agar (0.2 %)	0.0 d	0.0 c	0.0 d	0.0 c	0.0 d	0.0 d	0.0 d	0.0 d
Clove +Agar	40.0 b	2.0 b	40.0 b	2.0 b	30.0 b	2.0 b	30.0 b	2.0 b

Data are mean values of n = 10. Columns with different letters are considered significantly different at P≤0.05 according to Duncan's multiple rang test

Table 2: Effect of clove oil and agar suspension and their combination on postharvest rot diseases of banana fruit after storage 15 days at 23 -25 ºC.

Treatment	Postharvest rot diseases incidence (%) and severity (DS)							
	crown rot		neck rot		finger rot		flower end rot	
	%	DS	%	DS	%	DS	%	DS
Control	100 a	4.0 a	70.0 a	4.0 a	100 a	4.0 a	100 a	4.0 a
Hot water	70.0 b	3.0 b	60.0 b	3.0 b	60.0 b	3.0 b	50.0 b	2.0 b
Clove (2.0 %)	30.0 e	2.0 c	20.0 d	1.0 d	20.0 d	1.0 d	25.0 d	1.0 c
Agar (0.2 %)	50.0 d	2.0 c	40.0 c	2.0 c	20.0 d	2.0 c	30.0 c	2.0 b
Clove +Agar	60.0 c	3.0 b	60.0 b	3.0 b	40.0 c	2.0 c	50.0 b	2.0 b

Data are mean values of n = 10. Columns with different letters are considered significantly different at P≤0.05 according to Duncan's multiple rang test

Table 3: Effect of clove oil, agar suspension and their combination on weight loss (%) of banana fruit during different storage periods at 23-25ºC.

Treatment	Storage period (days)			Mean
	5	10	15	
Control	24.65 cde	33.33 c	55.03 a	37.67 A
Hot water	24.73 cde	30.05 cd	48.94 ab	34.57 A
Clove (2.0%)	10.64 g	14.76 fg	29.98 cd	18.46 C
Agar (0.2%)	18.81 efg	24.55 cde	42.31 b	28.56 B
Clove + Agar	22.86 def	28.59 cd	48.24 ab	33.23 A
Mean	**20.34 C**	**26.26 B**	**44.90 A**	

Data are mean values of n = 3. The different capital letters indicated the significant difference at $P \leq 0.05$ according to Duncan's multiple rang test among mean values of treatments (right column) or storage periods (lower row). The different small letters indicated the significant difference at $P \leq 0.05$ according to Duncan's multiple rang test among the interaction between treatments and storage periods.

Table 4: Effect of clove oil, agar suspension and their combination on the firmness loss (%) of banana fruit during different storage periods at 23-25ºC.

Treatment	Storage period (days)			Mean
	5	10	15	
Control	19.04 f	43.38 bcd	57.16 a	39.86 A
Hot water	18.82 f	39.59 cd	53.44 ab	37.28 A
Clove (2.0%)	25.80 ef	37.30 de	49.14 a-d	37.41 A
Agar (0.2%)	25.84 ef	39.85 cd	51.47 abc	39.05 A
Clove + Agar	21.75 f	36.55 de	51.00 abc	36.43 A
Mean	**22.25 C**	**39.33 B**	**52.44 A**	

Data are mean values of n = 3. The different capital letters indicated the significant difference at $P \leq 0.05$ according to Duncan's multiple rang test among mean values of treatments (right column) or storage periods (lower row). The different small letters indicated the significant difference at $P \leq 0.05$ according to Duncan's multiple rang test among the interaction between treatments and storage periods.

Table 5: Effect of integrated treatments of clove and agar suspension on SSC of banana fruit during different storage periods at 23-25ºC.

Treatment	Storage periods (days)				Mean
	0	5	10	15	
Control	5.67 c-f	7.67 a-d	9.67 ab	10.00 a	8.25 A
Hot water	2.00 f	4.33 def	7.00 a-d	7.00 a-d	5.08 B
Clove (2.0%)	5.00 c-f	7.00 a-d	8.33 abc	8.33 abc	7.17 A
Agar (0.2%)	4.33 def	6.33 a-e	7.67 a-d	7.67 a-d	6.50 AB
Clove + Agar	2.67 ef	4.67 c-f	6.00 b-f	7.33 a-d	5.17 B
Mean	3.93 C	6.00 B	7.73 A	8.07 A	

Data are mean values of n = 3. The different capital letters indicated the significant difference at $P \leq 0.05$ according to Duncan's multiple rang test among mean values of treatments (right column) or storage periods (lower row). The different small letters indicated the significant difference at $P \leq 0.05$ according to Duncan's multiple rang test among the interaction between treatments and storage periods.

period. Control treatment scored significantly the highest fruit weight loss (%) after different storage periods (5, 10 and 15 days). After 5 days of storage, all tested treatments, except hot water, significantly reduced weight loss (%) of banana fruit samples compared to control. After 10 and 15 days of fruit storage, the effective treatments for reducing weight loss (%) were the single application of clove oil (2.0%) and agar suspension (0.2%). Meanwhile, weight loss (%) was not significantly affected by hot water and the combination of clove oil and agar suspension.

Fruit firmness loss (%)

The firmness loss (%) of banana fruit pulps was significantly decreased with increasing storage periods. Meanwhile, all treatments did not significantly affect fruit firmness loss (%) compared with control (table 4).

Soluble solids content (SSC)

Results in (table 5) demonstrated that, SSC (ºBrix) of banana fruit samples was affected by storage periods, where it was significantly increased with increasing storage period till reach the maximum value after 15 days from storage particularly in control treatment. Both clove oil (2.0%) and agar suspension (0.2%), as single application, had no effect on SSC compared to control. While, hot water and clove oil + agar suspension significantly decreased SSC in banana fruit.

Discussion

Due to the adverse effects of fungicides on human health and environment, safe eco-friendly and cost-effective alternatives that protect and maintain quality of fruits are being investigated (Maqbool *et al*., 2010). The present study revealed that, dipping banana fruit samples in hot water (50°C for 10 min) exhibited higher reduction in disease incidence and severity than untreated fruits. Several studies proved that, hot water (45-50°C) is an effective non-chemical method for controlling postharvest diseases (Reyes *et al.*, 1998; Alvindia, 2012 and Mirshekari *et al.*, 2013) if suitable combinations of temperatures and exposure times are selected to prevent quality loss (Lurie 1998). Application of essential oil is a very attractive method for controlling postharvest diseases (El-Sharony and Amin 2015). The present study demonstrated, clove essential oil (2%) was effective in reducing the incidence and severity of banana postharvest rot diseases (crown, neck, finger and flower end rots) compared to control. In agreement with this result, Ranasinghe *et al.* (2002) declared that, clove essential oil could be used as antifungal agent to control postharvest fungal diseases of banana fruit caused by *Colletotrichum*

musae and *Fusarium proliferatum*. Additionally, clove oil at 1% completely reduced the linear growth of *C. musae* and *F. moniliforme* associated with postharvest rots in banana fruit (Zoeir *et al.*, 2017 b). The antifungal activity of clove oil could be attributed to its components particularly a phenolic compound eugenol (about 83%) that has a strong antimicrobial activity (Tabassum and Vidyasagar, 2013; Xing *et al.*, 2012 and Moghaddam and Mehdizadeh, 2016). The antimicrobial activity of eugenol can be ascribed to the presence of an aromatic nucleus and a phenolic OH group that are known to be reactive and can form hydrogen bonds with –SH groups in the active sites of target enzymes, resulting in the deactivation of enzymes in fungi (Sukatta *et al.*, 2008). However, it is difficult to attribute the activity of natural essential oils which are complex mixtures to a particular constituent (Tabassum and Vidyasagar, 2013). Over the last two decades, the development and use of edible coatings to prolong the shelf-life and to improve fruit quality has been receiving increased attention (Dhall, 2013). Agar-agar, a hydrophilic colloid extracted from marine red algae (Davidson, 2006), has multiple applications particularly in the human food industry. It is also used in the preparation of microbiological and plant tissue cultures media since it is non-degradable by microorganisms (Armisen and Galatas, 2000). The present study indicated the efficacy of agar suspension at 2.0 g L^{-1} in controlling the postharvest rot deceases in banana fruit. Scanning electron microscopy observation of coated banana fruit samples with agar suspension had very fewer cracks and smooth surface as well on the limitation of mycelia of pathogenic fungi compared with the untreated fruit samples (Ziedan *et al*., 2018). Agar as a polysaccharide coating agent resembles chitosan and gum Arabic for controlling postharvest diseases litchi (Zhang and Quantick, 1997), sweet cherries (Romanazzi *et al*., 2012) and banana fruits (Hossain and Iqbal, 2016). Interestingly, the combination between clove oil (2%) and agar suspension (0.2%) was less effective in reducing the incidence and severity of banana postharvest rot diseases compared with their single application. The antagonistic effect of clove oil and agar suspension is may be due to mainly phenolic compounds (eugenol) that inhibit agar gelation. This result was agreement with (Armisen and Galatas, 2009) reported that, the presence of tannic acid (phenolic compound) may inhibit agar gelation. The present study revealed that single application of clove oil or agar suspension significantly reduced the loss in fruit weight percentage compared to other treatments. However, the weight loss (%) in fruits is mainly due to water loss as a result of evaporation and transpiration, while the amount of dry matter was lost by respiration (Abdel-Gayed *et al.*, 2017). It was also observed that, water loss increased with increasing disease severity, where the pathogenic fungi raised fruit transpiration and respiration. Thus, the decrease in disease severity due to clove oil (2%) and agar suspension (0.2%) treatments deceased fruit weight loss. The weight loss reduction of agar-coated banana fruit compared to control fruit was probably due to the effect of agar as a barrier against gas exchange (O_2 and CO_2) and water vapor (Baldwin *et al*., 1999 and Park, 1999). Accordingly, the mechanism of the extended storage life of banana fruit coated with agar suspension could be due to reduction in transpiration and respiration as well as the modification of the internal atmosphere and water loss reduction (Ali *et al*., 2010). SEM observation on banana fruit coated with agar suspension indicted that, agar-treated fruit had fewer cracks and smooth surface, and had limited mycelial growth. Therefore, agar reduced fruit transpiration and decreased fruit infections by pathogenic fungi, and in turn, significantly deceased fruit weight loss percentage (Ziedan *et al.*, 2018). The present study demonstrated that, fruit firmness loss (softening) significantly increased with fruit ripening progresses during storage. This can be attributed to cell wall hydrolases such as pectinesterase and polygalacturonase (Yaman and Bayoindirli, 2002). Limited respiration in coated fruit decreases the activity of cell wall hydrolases, thereby, delays fruit ripening and reduces fruit firmness loss (Tanada-Palmu and Grosso, 2005). However, all treatment have not significant effects on fruit firmness loss (%). Additionally, soluble solid concentration (SSC) was increased with increasing storage periods due to ripening progress. However, SSC was lower in treated fruits with single application of clove oil or coated fruit with agar suspension than other treatments particularly at the end of storage period. As mentioned earlier, coated fruit limits respiration rate compared to control fruit. In addition, the decrease in respiration rate causes a slowdown of ethylene production resulting in lower SSC (Yaman and Bayoindirli, 2002). So, present study concluded that clove essential oil or agar suspensions as coating agent could be applied as a promising, safe and cheap approach to extend shelf-life of banana fruit, enhancing fruit physicochemical properties and reducing incidence and severity of postharvest diseases of banana fruit during storage.

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