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STUDY ON AQUEOUS KAFFIR LIME EXTRACT AS TREATMENT AGENT ON FRESH TOMATO FRUITS

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Abstract: Tomatoes have a short shelf life thus they pose a big challenge for growers to maintain the quality of tomatoes to increase customer acceptance. In this study, fungi associated with tomato disease symptoms were isolated and the potential of kaffir lime aqueous extract was evaluated in maintaining post-harvest quality of tomatoes. For this purpose, healthy tomatoes were dipped in 10% aqueous kaffir lime extract before evaluating the post-harvest parameters namely weight loss and firmness. A fungus namely *Rhizophus stolonifer* was isolated from the symptomatic tomatoes. Subsequently, it was confirmed to be pathogenic on healthy tomato fruits with 100% disease severity. Application of aqueous kaffir lime extract showed that tomato fruits dipped in 10% aqueous kaffir lime extract recorded higher weight loss and higher firmness as compared to untreated tomato fruits. The results showed that treatment with this concentration of plant extract did not help to reduce the weight loss, but it retained the firmness of the tomato fruits stored at room temperature at 27 ± 2^{o}C. Higher transpiration process would lead to shrinkage, weight loss, changes in texture and appearance of the fruits. Therefore, this study suggested an increased concentration of aqueous kaffir lime extract as a treatment agent in order to have a better effect in maintaining the quality of tomato fruits.

Keywords: Kaffir lime extract, post-harvest quality, tomato

Introduction

The tomato fruit (*Solanum lycopersicum* L.) belongs to the Solanaceae family. It is widely consumed as raw fruits globally (Agrios, 2005). Tomatoes are rich in calcium, phosphorus, magnesium, copper, niacin, iron, folate, vitamin A, B6, vitamin E, vitamin B2, vitamin C, iron and carbohydrates (Wamache, 2005 not in the reference). Due to the high nutritional value, tomatoes are commonly consumed as salads, cooked in soups or processed into juice, ketchup and paste (Adedeji *et al*., 2006). The fruits are highly perishable due to the short storage life. Therefore, post-harvest handling, transportation and marketing along the supply chain should be conducted properly because it gives a huge impact on the quality and shelf life of tomatoes. Rough handling during harvesting of tomatoes can cause mechanical injuries like bruising (Milczarek *et al*., 2009). To date, post-harvest decay due to fungi and bacteria has become a major challenge for the storage of tomatoes. Fungal deterioration will eventually change the fruits' appearance, which later reduces the customer's acceptance, their marketing value and most importantly reduce the shelf life of the fruit (Prusky & Keen, 1993). Synthetic fungicides are commonly used as a fruit treatment. However, it has become a crucial issue among the producers since it has harmful effects on the human health (Norman, 1998). Various alternatives have been studied which focused on natural plant products that are considered safe, low cost

and eco-friendly. To achieve this objective, the current study was conducted to evaluate the potential of aqueous kaffir lime extract as treatment agent on fresh tomatoes. The data of this study provide information on the fungal pathogen causing post-harvest disease and the potential of aqueous kaffir lime extract in maintaining the postharvest quality of tomatoes.

Materials and Methods

Isolation of pathogenic fungal isolate

Plant tissues of infected tomatoes were cut into 5mm x 5mm x 5 mm at the margin, and surface sterilized using 3% sodium hypochlorite (NaOCI), rinsed with sterile distilled water and air-dried prior to incubation on potato dextrose agar (PDA), Merck, Germany. All PDA plates were incubated at room temperature (27°C± 2°C) until the presence of mycelium. The mycelium ~~was~~ underwent single spore process to obtain a pure culture isolate. Then, the isolate was observed under microscope to identify its morphological characteristics according to key identification suggested by Watanabe (2010).

Pathogenicity test

For pathogenicity test, healthy tomatoes were washed, surface sterilized using 3% NaOCI and air-dried. Then, the fruits were wounded at 1mm depth and 5mm length using a sterile cutter before inoculated with 7-day PDA plug containing fungal isolate. Control fruits were inoculated with PDA agar without any mycelium. Development of lesion on the inoculated areas on the tomato was recorded. Growing of fungal mycelium on the inoculated area was re-isolated to confirm the Koch's postulate.

Aqueous kaffir lime extract

Kaffir lime fruits were thoroughly washed under running tap water. Then the peel of the fruits were cut into small pieces, blotted dried and macerated in 500 ml sterile distilled water in a blender, Waring USA for 15 minutes. After that, the solution was filtered through double-layered muslin cloth and kept overnight at room temperature and filtered again using a filter paper (Whatman filter paper No. 1) and adjusted to 10% concentration prior to use.

In vitro antifungal assay

For *in vitro* antifungal assay, poison food technique was applied by incorporating the PDA with kaffir lime extract at 10% concentration at temperatures ranging from 45°C to 50°C, following Mishra and Tiwari (1992). Then, the pathogenic fungal disc was placed at the center of the plate and incubated for 7 days at room temperature 27 ± 2°C. PDA plates without incorporating with the plant extract served as the negative control. Percentage of fungal inhibition was calculated based on the following formula:

Percentage of inhibition (%) = (C - T) / C x 100

Where;

C= Average increase in mycelial growth in control plate

T= Average increase in mycelial growth in treatment plate

In vivo antifungal assay

Before applying the plant extract on the tomatoes, the fruits were washed thoroughly and air-dried. Then, the tomato fruits were immersed for 2 minutes in ~~to~~ aqueous kaffir lime extract mixed with 1% Tween 80, Sigma-Aldrich until a thin film layer was formed. All the fruits were air-dried for 5 minutes before being arranged in a container. Then, all the fruits were incubated at room temperature, 27 ± 2°C for 8 days and the data on weight loss and fruit firmness were evaluated at a 2 day-interval.

Determination of weight loss

~~Each~~ Treated and untreated fruits were weighed individually at a two-day interval. Percentage of weight loss was calculated using the following formula:

$$\% (WL) = \frac{IW - FW}{IW} \times 100$$

Where;

WL= The percentage weight loss of the sample

IW= Initial weight of the sample

FW= Final weight of the sample

Determination of firmness

The firmness of tomato fruits was determined by using Texture Analyzer (TA-TX) Plus Texture Analyzer (Texture Technologies Corp, USA). The texture analyzer was equipped with P/2N needle probe that interfaced with a computer with speed 1.0 mm/s and 5 kg load cell. The data obtained were expressed in force, Newton (N).

Statistical analysis

For *in vitro* study, three replicates were used for each treatment with three plates in each replicate. For *in vivo* study, each treatment had three replicates with three fruits in each replicate. The treatments were conducted in a completely randomized design (CRD) throughout the experiment. The data were analysed using t-test using Statistical Package for the Social Sciences (SPSS) software version 23.

Results and Discussion

Major tomato cultivation areas in Malaysia are mainly found the in highland areas such as Cameron Highlands in Pahang and Kundasang in Sabah, due to the suitable temperature. The plant is ready to be harvested after 60 to 80 days from transplanting depending on the variety, surrounding factors and management. After harvesting, tomato fruits are ready to be sold either at the fresh market or being treated prior to long-distance transportation. The tomato fruits have ~~a~~ soft flesh thus they easily encounter mechanical injuries which can cause fungal infection later. Fungal infection can fasten the deterioration process of tomato fruits and lead to marketable losses. According to Droby (2005), about 20-25% of the harvested fruits could not be sold due to fungal infection. Changes in the appearance of the fruit surface area will affect the customer's acceptance, reduce its marketing value, and most importantly reduce the shelf life of the fruit (Prusky & Keen, 1993). In this study, a fungus namely *Rhizophus stolonifer* has been isolated from the infected tomatoes with Rhizophus rot symptoms. Based on pathogenicity test, the fungus has been confirmed to be pathogenic on healthy tomato fruits with disease severity, 100% (Figure 1). On the inoculated area, the infected tissue appeared water-soaked lesion and covered with thin, cotton-like fungal structure.

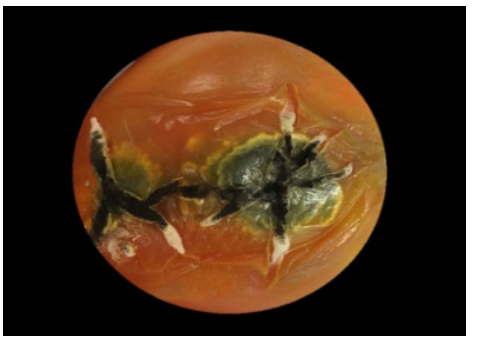

Figure 1: Disease symptoms on a tomato fruit caused by *Rhizophus stolonife.*

According to Bautista-Baños et al. (2008), *R. stolonifer* is a major factor limiting the postharvest life of this commodity. Once infection takes place, the symptoms are easily developed and spread to the adjacent parts of the tomato fruits which cause severe infection and economic loss. Other

common fungi that have been reported to be pathogenic on tomato fruits are *Botrytis cinerea* (Pers.: Fr) and *Alternaria alternata* (Fr.: Fr.) Keissl. According to Sommer (1982 not in the reference list), rotten due to *R. stolonifer* could be increased at 27 ºC with high relative humidity. However, no spore germination occurs at 4 ºC. Infected tomatoes with severe infection can be observed particularly during the rainy season. Tomatoes infected by *R. stolonifer* normally colonized by coarse, grey, hairy mycelia that form a mass of black sporangia at their tips.

In order to maintain the fruit quality and reduce fungal infection on tomato fruits, this study investigated the potential of aqueous kaffir lime extract as a treatment agent for fresh tomatoes. In terms of weight loss, the result shows that tomato fruits dipped in aqueous kaffir lime extract has significantly higher weight loss as compared to untreated tomato fruits (control) with p-value, 0.19 (Figure 2).

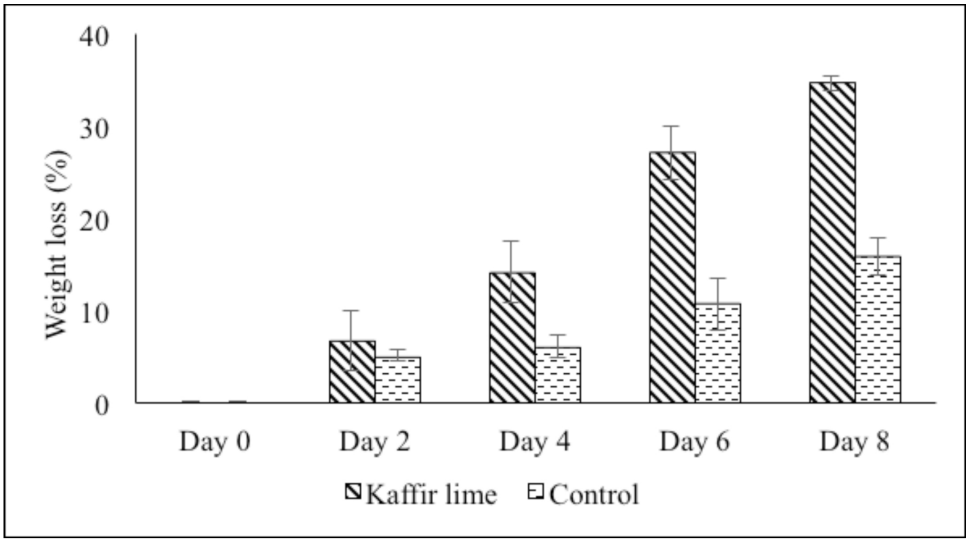

Figure 2: Weight loss of treated and untreated (control) tomato fruits.

For firmness, tomato fruits treated with 10% aqueous kaffir lime extract demonstrated significantly higher firmness as compared to the untreated fruits with *p*-value, 0.00 (Figure 3).

Typically, the percentages of weight loss in tomato fruits gradually increased as days of incubation time increased. This indicated that the fruits had undergone senescence or desiccation processes which would affect shelf life. According to Ali *et al.* (2010), the weight loss of tomatoes will occur at senescence stage due to metabolic degradation of the cell wall and low water retention capacity. From the results of the current study, treatment with 10% kaffir lime aqueous extract did not help ~~to~~ prevent the weight loss. It ~~is~~ indicated that 10% aqueous kaffir lime extract did not enhance the protective barrier within pectin to minimize the moisture loss of the tomato fruits. Higher percentage of weight loss in treated fruits was also attributed to higher transpiration process during incubation time. Transpiration occurs when the fruits lose their water from the tissues after harvesting and would not be replaced afterwards. Bhowmilk and Pan (1992) reported that transpiration and loss

of carbon atom from the tomato fruits in each cycle of respiration contributes to the weight loss. Generally, fresh produce will start to wilt when they lose 5-10% of the fresh weight (Burden, 1989). This condition will result in shrivelling, shrinkage, weight loss and changes in texture of the fruits due to water loss (Burden, 1989).

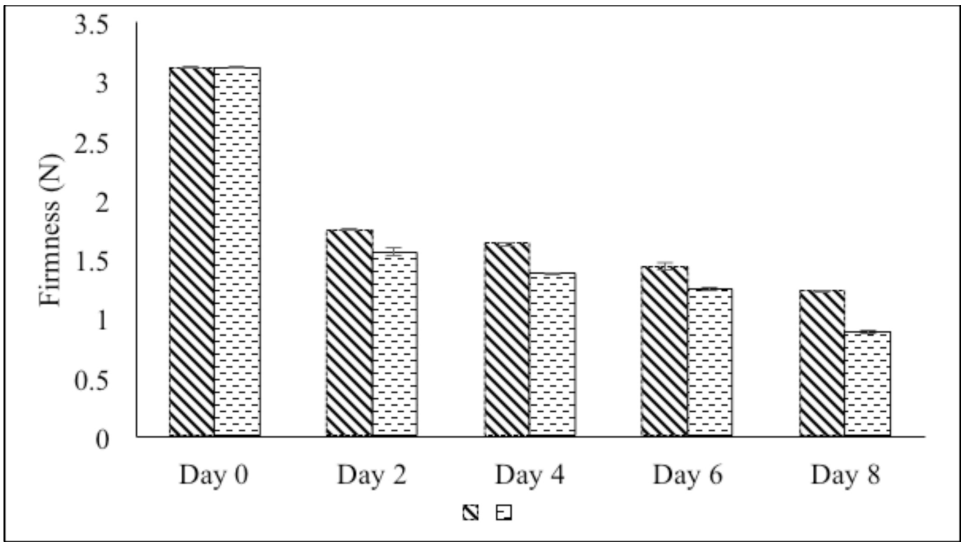

Figure 3: Firmness of treated and untreated (control) tomato fruits.

In terms of firmness, the treated tomato fruits recorded higher firmness from Day 2 until Day 8 when stored at room temperature, 27±2°C. These results contradicted with the percentage of weight loss recorded in this study. It shows that although tomato fruits undergo higher transpiration process, dipping of tomato fruits in 10% aqueous kaffir lime extract could help to retain the firmness of the fruits. Negative results obtained in percentage of weight loss in this study might be due to low concentration of plant extract and different preparation method which only used aqueous extract. The results of this study were in line with the findings of the study conducted by Ullah (2009) that reported that tomato fruits dipped in plant extracts will retard the respiration and transpiration rate which help to retain their firmness. The ability of the fruit flesh to withstand compressive force is essential in determining the fruit quality that relates to sense of touch during purchasing (Tijskens & Evelo, 1994). In addition, higher firmness values in treated tomato fruits may be due to lower hydrolytic enzymes activity at the cell wall which helps to retain the intercellular adhesion and eventually maintain the firmness of tomatoes.

Conclusion

As a conclusion, *R. stolonifer* is a common fungus that causes profit loss in tomato fruits during storage. Treatment of fresh tomato fruits with 10% kaffir lime aqueous extract did not produce positive results in reducing the weight loss. However, it helped in maintaining the firmness of the fruits. Further study should be conducted at higher concentration of aqueous kaffir lime extract in order to improve its ability in maintaining the post-harvest quality of the tomato fruits.

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