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Research Article
Effects of Sodium Carbonate and Sodium Chloride on the Control of Black Rot Disease of *Mangifera indica* L. (Mango) Caused by *Aspergillus niger*

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Abstract

Background and Objective: Food loss due to diseases has been a serious problem encountered by the farmers as well as marketers and end-users of fruits in tropical regions. A study to assess the effectiveness of sodium carbonate and sodium chloride in the control of the growth of black rot disease of mango caused by *Aspergillus niger* was conducted. **Materials and Methods:** Infected mango fruits (*Mangifera indica* L.) were collected from the Faculty of Agriculture, Kogi State University, Anyigba. The identification of fungal isolates was carried out based on cultural and morphological features using standard keys, charts and illustrations. **Results:** The highest growth *in vitro* effect of sodium chloride was observed on the control on day 5 (4.00±0.10 mm) while the highest inhibitory effect (0.00±0.00 mm) was observed on day 1 and 2 at 3 and 5 g mL^{-1}, respectively with a significant difference at $p>0.05$ with the control as well as 1 g mL^{-1} (0.37±0.33) at day 2 and 5 (1.80±0.57) treatments, respectively. Sodium carbonate shows the varying degree of inhibitory effects on the growth of *A. niger* on day 1 through to day 5 at 3 and 5 g mL^{-1} concentrations, respectively while the highest mycelial growth was observed with the 1 g mL^{-1} treatments from day 1 (0.10±0.10) through to day 5 (1.00±0.17). **Conclusion:** The result recorded on the daily growth of *Aspergillus niger* rot shows that the chemicals either acted as an inhibitory or supportive constituent for the media. These salts had significant effects on the growth of black rot disease caused by *Aspergillus niger*, with sodium carbonate being the most effective. The direct and indirect effects of the chemicals on microorganisms would be discussed concerning food safety and human health.

Key words: Mango, *Aspergillus niger*, sodium chloride, carbonate, black rot

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Mango fruit *(Mangifera indica* L.) is the most popular and commonly eaten fruit among millions of people in tropical and sub-tropical areas[1]. Mango fruit contains about 81% moisture, 0.6% protein, 0.4% fat, 0.8% fibers. The fruit is also an important source of minerals such as potassium, sodium, magnesium, sulfur and phosphorus[2]. More than 100 varieties of mango fruits are produced worldwide having similar properties but specific differences common to each variety[3]. Mango is a common fruit in the tropical region and it is highly being consumed in developed countries[4].

Mango fruit can be used for various purposes. For instance, it is a very good source of vitamin A and flavonoids such as beta-carotene, alpha-carotene and beta-cryptoxanthin, pre-biotic dietary fibre, vitamins, minerals as well as poly-phenolic flavonoid compounds[2].

However, in Nigeria, most of the mango fruit produced is consumed as fresh fruit. Although, Nigeria is among the top 10 leading mango-producing countries of the world[5].

Nigeria is said to occupy the eighth position in the world mango ranking production by states[5]. The higher percentage of mango fruit is prided to be produced from the Guinea and Sudan savanna zones of Nigeria, with Benue State as the highest producer[5]. Mango is grown almost in all states of Nigeria. However, it is mainly cultivated in Benue, Niger, Bauchi, Jigawa, Adamawa, Yobe, Taraba, FCT, Kano, Plateau, Kebbi, Sokoto and Kaduna[5].

Mango fruits are perishable and susceptible to post-harvest diseases, physical injuries and high temperatures[6]. Mango fruit post-harvest diseases of important concern to producers are black rot disease caused by *Aspergillus niger*, anthracnose caused by *Colletotrichum gloeosporioides* and stem-end rot caused by *Botryosphaeria parva*[7]. Various factors affect the production of mango with post-harvest losses being one of the important constraints[8]. Various methods can be used in tackling and controlling post-harvest diseases such as the use of botanicals, fungicides and carbonic salts. The use of sodium carbonate and sodium chloride is one of the approaches for the control of plant diseases. The ability of sodium carbonate and sodium chloride to control post-harvest pathogens has been demonstrated in pepper[9] and also in melon[10]. The ripe fruits aside from being used for dessert are also utilized for producing several products like juices, syrups, nectars, jellies and jams. The green unripe mango fruits are also used in curries, pickles and sharbat[11].

Aside from the eating of mango fruit as food,[2] has highlighted the health benefits of mango leaves to contain antioxidants as well as a very good source of vitamin A and flavonoids such as beta-carotene, alpha-carotene and beta-cryptoxanthin. Mango has been reported to have diabetic medication properties as well as Lowers blood pressure as a hypotensive agent and improves digestion[3].

Recent reports have indicated that the production of mango generates high profit. In Nigeria, the production of mango is limited by both human factors (unmanned orchards) and natural factors (disease pathogens) despite the large expanse of land dedicated to its production. Fresh mango fruits from the country encountered difficulties in making it to the international market due to post-harvest infections. Various diseases of mango such as black rot disease, anthracnose, Rhizopus rot, Phoma blight, dieback and damping-off disease causes yield loss. All these diseases had been controlled in the past by planting disease-resistant varieties and having a good soil type with water infiltration rate and good aeration[12]. In Nigeria, not much has been done in tackling the black rot disease of mango in this part of the country. Black rot disease has recently transformed into a big problem for both commercial and peasant farmers in tropical countries making mango production no longer preferred due to the heavy destruction of fruits and spoilage. Fruit crop production such as mango has been low due to poor postharvest storage handling among other factors[13]. This low yield in the production of mango fruits calls for a close examination of diseases causing the low yield in mango farms and gardens. Black rot disease of mango has been reported in several regions of the globe with suitable climate preferable for mango production as one of the most important diseases of the crop of fields and post-harvest concerns[14].

There however are various methods documented for managing and or controlling black rot disease of mango caused by fungi. This concept of management of diseases that employs eco-friendly materials gains momentum as mankind became more environmentally conscious. The use of sodium carbonate and sodium chloride is one of the approaches for the control of plant diseases. Sodium carbonate is classified or generally regarded as a safe compound by the United States Food and Drug Administration[15] and it is also used as a leavening agent, pH control agent and dough strengthener[15]. The appearance of light brown circular patches at the stalk end region of the fruit, gradually a large circular spot is formed around the end of the stalk. In advanced stages, the stalk end region becomes sunken and the infected fruit becomes

yellowish at the base. The circular spot around the stalk end region of the fruit gets covered with the black conidial head of pathogens. Minor injuries could be responsible for black rot diseases in mango fruits. The fungi causes rot in any part of the fruit. The position of the black mould lesion depends upon the position of injury on the fruit. The optimum temperature for the development of the disease is 30°C. *Aspergillus* rot is a disease of mango common in all parts of Nigeria. It causes heavy economic loss of fruits. Black rot disease of mango is more serious during storage and marketing processes[16]. It has been reported to cause between 25-35% fruit losses yearly[17].

This study however, attempts that sodium carbonate and sodium chloride could be used alone or in combination with other safe treatments to act as an appropriate alternative for reducing the severity of black rot disease of mango.

MATERIALS AND METHODS

Sample collection: Infected mango fruits (*Mangifera indica* L.) were collected during the dry season of late 2020 and early 2021 (November, 2020 to January, 2021) from mango trees at the Faculty of Agriculture, Prince Abubakar Audu University, Anyigba. Samples were collected randomly from the trees by handpicking and carefully placing the collected samples into a well-ventilated basket and transported immediately to the laboratory for further analysis.

Isolation and identification of fungal isolates: Samples of infected mango fruits were washed in two changes of distilled water. This was then followed by surface sterilization using 90% ethanol. The portions from the advancing edges of lesions were obtained using a sterile blade. The cut portions were then placed in a beaker containing distilled water and finally inoculated in potato dextrose agar plates containing chloramphenicol (30 mg L^{-1}) which serves as an anti-bacterium and was incubated at 28±2°C for 7 days. Lactophenol cotton blue stain was used to stain the slide to view the morphological characteristics of isolates using the light microscope. The identification of fungal isolates was carried out based on cultural and morphological features[18]. Pure cultures were obtained from serial sub-culture as described by Aliyu and Kutama[19], were stored in agar slants for further analysis.

Pathogenicity test: The pathogenicity test was carried out[20], where each of the isolates was tested on healthy mango fruit for its ability to induce spoilage.

Assay test of chemicals

Agar dilution techniques: The Agar dilution method[21] was employed, where the effect of chemicals on mycelial growth was assayed using a concentration of 1, 3 and 5 g mL^{-1} of each chemical. A mycelial disc was taken from the peripheral region of a 5 days old culture of *Aspergillus niger* grown on PDA and transferred to the centre of a 5 cm diameter PDA plate, which had been amended by incorporating the chemicals at the required concentration into the medium before pouring.

The percentage of mycelia inhibition was calculated using the relation:

$$\text{Inhibition of radial mycelial growth (\%)} = \left(\frac{\text{C-T}}{\text{C}}\right)\times 100$$

where, C is the radial growth measurement of the pathogen in control and T is the radial growth of the pathogen in the presence of a salt.

Data analysis: Statistical analysis of inhibition of radial growth was subjected to One-way Analysis of Variance (ANOVA) using Statistical Package for Social Science (SPSS) version 17.0 and means were separated according to Duncan's New Multiple Range Test (DMRT) at a 5% probability level.

RESULTS

The results of the effect of various concentrations of sodium chloride on the mycelia growth of *Aspergillus niger* were presented in Fig. 1.

The daily *in vitro* inhibitory effect of sodium chloride against *Aspergillus niger* rot on mango fruit in Fig. 1 indicated that on day 1, there was no significant difference ($p>0.05$) between the fungal growth at 1 g mL^{-1} (0.10±0.06) and 3 g mL^{-1} (0.00±0.00) although, there was a significant difference ($p<0.05$) between the treatment 3 g mL^{-1} (0.00±0.00) and 5 g mL^{-1} (0.00±0.00) which has no fungal growth and the control (1.00±0.29) with the highest fungal growth. On day 2, there was much significant difference ($p<0.05$) between the concentration of 5 g mL^{-1} (0.00±0.00) and the control (1.53±0.15), compared to its concentration of 1 g/g/mL (0.37±0.33) and 3 g mL^{-1} (0.10±0.10). On day 3, there was a significant difference ($p<0.05$) between the treatments of 1, 3 and 5 g mL^{-1} (0.10±0.06) and the control (2.00±0.10), while the concentration of 1 g mL^{-1} (0.62±0.18) has no much significant difference with the concentration of 3 g mL^{-1} (0.30±0.10) and the concentration of 5 g mL^{-1}

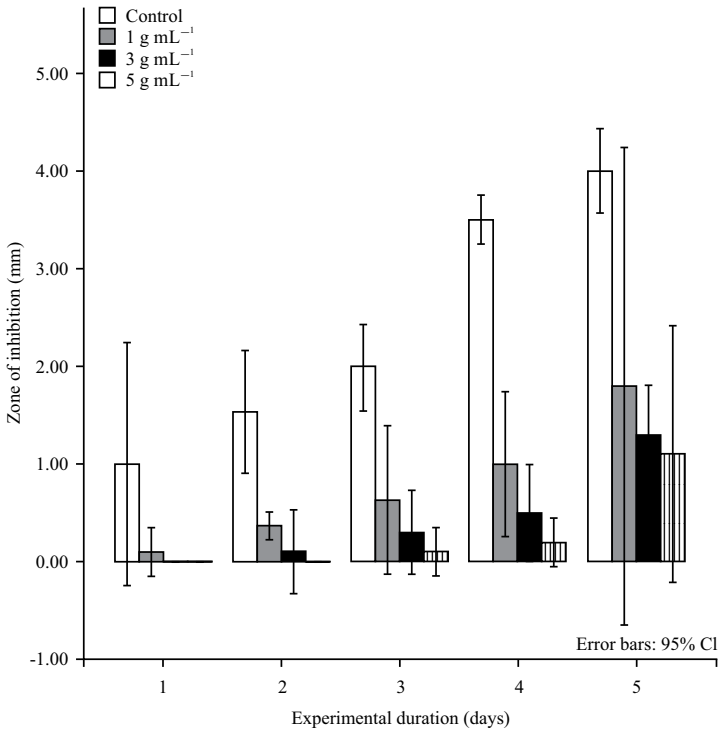

Fig. 1: Effect of NaCl on the growth of *Aspergillus niger*

Table 1: Effect of sodium carbonate concentrations on mycelia growth of *Aspergillus niger*

Treatment (g mL^{-1})	Day 1	Day 2	Day 3	Day 4	Day 5
Control	1.00±0.29^{b}	1.53±0.15^{b}	2.00±0.10^{d}	3.50±0.06^{c}	4.00±0.10^{c}
1	0.10±0.10^{a}	0.20±0.10^{a}	0.60±0.21^{c}	0.80±0.23^{b}	1.00±0.17^{b}
3	0.00±0.00^{a}	0.20±0.06^{a}	0.30±0.12^{b}	0.00±0.00^{a}	0.00±0.00^{a}
5	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

Values with the same superscript alphabets in a column are not significantly different at p>0.05

Table 2: *In vitro* inhibitory effects of NaCl and Na_2CO_3 on the growth of *Aspergillus niger*

Treatment (g mL^{-1})	Day 1	Day 2	Day 3	Day 4	Day 5
Control	1.00±0.29^{b}	1.53±0.15^{b}	2.00±0.10^{b}	3.50±0.06^{b}	4.00±0.10^{c}
Sodium chloride	0.00±0.00^{a}	0.00±0.00^{a}	0.10±0.06^{a}	0.20±0.06^{a}	1.10±0.31^{b}
Sodium carbonate	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

Values with the same superscript alphabets in a column are not significantly different at p>0.05

(0.10+0.06). On day 4, the concentration at 1 g mL^{-1} (1.00±0.17) and 3 g mL^{-1} (0.50±0.12) has little significant difference (p<0.005), as the concentration of 5 g mL^{-1} (0.20±0.06) is significantly different (p<0.05) from the control (3.50±0.06). On day 5, there was a slightly significant difference (p<0.05) between concentrations of the treatments (1, 3 and 5 g mL^{-1}) while there was a significant difference between the treatments and the control (4.00±0.10) which has the highest growth.

The *in vitro* inhibitory effect of sodium carbonate on the growth (mm) of *Aspergillus niger* rot of mango fruit is presented in Table 1.

From Table 1, there was no significant difference (p>0.05) in the fungal growth between the concentrations of the treatments, but there was a significant difference (p<0.05) between the concentration of the treatments (0.1, 0.0 and 0.0) and the control (1.00±0.29) at day 1. On day 4, there was a significant difference (p<0.05) between the control (3.50±0.06) and the concentrations of the treatment. There was no growth observed in the treatments of 3 g mL^{-1} (0.00±0.00) and 5 g mL^{-1} (0.00±0.00).

The *in vitro* inhibitory effect of highest concentrations of sodium carbonate and sodium chloride on the growth of *Aspergillus niger* rot is presented in Table 2.

From Table 2, there was a significant difference ($p<0.05$) between the concentrations of sodium chloride (0.00±0.00) and sodium carbonate (0.00±0.00) when compared to the control (1.00±0.29), while on day 2, there was a significant difference ($p<0.05$) between sodium chloride (0.00±0.00) and sodium carbonate (0.00±0.00) concentrations when compared with the control (1.53±0.15). On day 3, there was a significant difference ($p<0.05$) between the concentrations of sodium chloride (0.10±0.06) when compared that of sodium carbonate (0.00±0.00). On day 5, there was a significant difference ($p<0.05$) between the concentrations of sodium carbonate (0.00±0.00) and sodium chloride (1.10±0.31) when compared with the control (4.00±0.10). It was observed that for the highest concentration of 5 g mL^{-1}, sodium carbonate showed the highest inhibitory effect throughout the days.

DISCUSSION

The result recorded on the daily growth of *Aspergillus niger* rot shows that the chemicals either acted as an inhibitory or supportive constituent for the media. These salts have significant effects on the growth of black rot disease caused by *Aspergillus niger*, with sodium carbonate being the most effective. Rousk *et al.*[12] reported a pH of 8.3 and above for soil fungi and also noted that carbon compounds are among other factors that could affect fungi which could in turn hindermycelial growth and conidial germination of fungi. Sodium carbonate effectively hindered mycelia growth and conidial germination. As a result of this, sodium carbonate in an aqueous solution was able to maintain the fruit quality in healthy fruits by preventing infection. Similar protection has been reported using sodium carbonate for citrus[22].

Black rot is a latent infection, with the fungus gaining entry into the fruit by thereby causing decay[23]. During transportation and storage, black rot disease can spread rapidly from infected fruits to healthy fruits by direct contact[24]. The presence of wax formulation on the fruit surface would also prevent post-harvest disease transmission by acting as a physical barrier. The wax formulation has fungicidal effects by preventing decay. Coating fruits with wax is also known to reduce weight loss and shrinkage and delay over-ripening[24]. *In vivo* efficacy of these salts on the control of black rot disease of mango fruits could not be carried out. Measures should therefore, be taken to develop the use of these salts *in vivo* in controlling post-harvest diseases thereby enhancing the shelf life of fruits.

CONCLUSION

This investigation has demonstrated that sodium chloride had the highest inhibitory effect on the mycelial growth of *A. niger* at 5 g mL^{-1} concentration. In conclusion, the findings from this study show that carbon salts have the potential the protection of mango fruits against *Aspergillus niger* rot which causes black rot disease in mango fruit.

SIGNIFICANCE STATEMENT

This study discovered that sodium carbonate (Na_2CO_3) has the potential to checkmating the growth of *Aspergillus niger* that causes black rot disease, however, can be beneficial for the prolonged shelf life of mango. This study will help the researchers to uncover the critical areas of post-harvest spoilage of fruits that many researchers were not able to explore. Thus a new theory on postharvest storage of fruits may be arrived at.

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