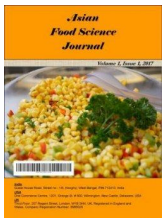

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Efficacy of Crude Extract from Candle Brush (*Senna didymobotrya*) Leaves against *Aspergillus niger* in Reduction of Post-harvest Losses in Tomatoes

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Authors' contributions

This work was carried out in collaboration between both authors. Author CAO designed the study, performed the analysis and wrote the first draft of the manuscript. Author LGN supervised the study and analysed the data. Both authors managed the literature search and writing of the final manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Background: *Senna didymobotrya* grows naturally in East Africa and is commonly used to treat microbial infections by African communities due to the presence of various phytochemicals such as alkaloids, terpenoid, anthraquinones, tannins, saponins, phenols and flavonoids.
Objective: This study was conducted to evaluate the effectiveness of the crude extract of candle brush *(Senna didymobotrya)* leaves against *Aspergillus niger* in the reduction of post-harvest losses in tomatoes.
Methods: A completely randomised design with two treatments, each replicated six times was used. In this study. Dried leaves were ground into a fine powder and extracted using the Soxhlet apparatus with 100% methanol. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the extract were determined against *Aspergillus niger*. The shelf life of tomatoes was determined by spraying the extract reconstituted in water at 0.4 g/ml and

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further analysed the total viable counts at three-day intervals and checking for the growth of mould, colour changes and odours as indicators of spoilage.
Results: After extraction 22 g of the crude extract was obtained from the dried leaves The MIC and MFC of the methanol crude extract against *Aspergillus niger* were 0.04 g/ml. The shelf life of the treated tomatoes was 14 days while for the untreated was 9 days. T-test results at ($P \leq 0.05$) showed a significant difference between the treated and untreated samples based on the total viable counts.
Conclusion: The crude extract of the candle brush inhibited the growth of *Aspergillus niger* in fresh tomatoes and also prolonged the shelf life of tomatoes for 14 days. Further studies should be done to evaluate the market acceptability of the tomatoes.

Keywords: Methanol; food security; shelf life; Soxhlet.

1. INTRODUCTION

Every year Kenya loses up to a third of its produce through post-harvest losses and wastage. According to the Kenya National Bureau of Statistics, Ksh 150 billion worth of food was lost due to post-harvest spoilage in the year 2017. Ksh 2.4 billion worth of tomatoes were lost, thus tomatoes accounted for about 1.6% of the total post-harvest losses in the country [1]

Tomatoes (*Lycopersicum esculantum*) are the major source of antioxidant lycopene [2] which has been linked to many health benefits, including reduced risk of heart disease and cancer. They are a great source of vitamins (C and K), minerals such as potassium, calcium, sodium, phosphorus [3]. It is one of the most widely consumed fresh vegetables in Africa and used by the food industries as raw material for the production of derived product such as purees or ketchup. The major phytochemicals in tomato are the carotenoids consisting of lycopene, neurospene and carotenes [4].

The use of synthetic fungicides in the pre-harvest treatment of fruits like tomatoes is a common practice in farming as they reduce crop losses [5], however, residues of these synthetic fungicides have been proved to be hazardous upon consumption. Fungicide resistance has also developed among some pathogens rendering the efforts to spray tomatoes pre-harvest futile. Some residues still end up in food and pose as hazards upon human consumption. The synthetic antimicrobials cannot also be used in the post-harvest treatment of produce thus creating the need for the production of post-harvest fungicides and antimicrobials. The synthetic fungicides also interfere with the ecosystem when they leach into the soil and destroy the beneficial microorganisms [6]. The production of natural antimicrobials will, therefore, help to promote environmental conservation and help farmers maximise profits from the sale of their produce.

Senna didymobotrya is a medicinal plant (Fig. 1) growing naturally in East Africa and is commonly used medicinally to treat bacterial and fungal infections among African communities [7]. It contains various phytochemicals that include alkaloids, terpenoids, anthraquinones, tannins, saponins, phenols and flavonoids which have antimicrobial capabilities [8]. The *Ameru* and *Mbeere* communities in Kenya boil the plant leaves and apply it on the skin to treat fungal infections, ringworms and treat malaria [9]. Extracts of the candle brush have been found to also contain antimicrobial properties against bacteria such as *Staphylococcus aureus* and *Bacillus cereus* [10]. This study aims at testing the anti-fungal properties of the candle brush leaf extract in prolonging the shelf life of tomatoes thus reducing post-harvest losses. The fungicidal properties of the leaf extracts have not been tested on tomatoes.

2. MATERIALS AND METHODS

2.1 Sample Collection

Fresh samples of ripe tomatoes were purchased from Kangemi market, Kiambu County, Kenya. These samples were carried in ventilated wooden boxes to the Food microbiology laboratory, Department of Food Science, Nutrition and Technology. The leaves of the *Senna didymobotrya* were obtained from trees in Muthure, Upper Kabete, Kiambu County. The leaves were put in clean polythene sacks sterilised by ultraviolet light overnight.

2.2 Extraction

The leaves were spread and left to dry for one week at room temperature. Dry plant material

gives free bonding of solvents with the phytochemicals. The dried leaves were ground into a fine powder with a grinder, weighed on an electric beam balance and stored in polythene bags. One hundred (100 g) portions of the powder were extracted by continuous extraction on the Soxhlet apparatus using 100% methanol. The extraction proceeded for 12 hours. The extract was evaporated on the rotary vacuum evaporator at 50°C to evaporate part of the methanol. The remaining extract was evaporated on a sand bath for 4 days to evaporate the remaining methanol and obtain 22 g of the crude extract. The presence of methanol was tested by freezing the extract at -18°C for 5 hours, methanol freezes at -97°C thus will remain liquid.

2.3 Experimental Designs

A completely randomised design with two treatments, in one treatment the extract was applied on the tomato samples and the other set of tomatoes had no extract, each treatment was replicated six times.

2.4 Determination of MIC and MFC

A pure colony of *Aspergillus niger* was inoculated into five test tubes containing Mueller Hinton broth. To each test tube, different concentrations of the extract reconstituted in sterile distilled water were added. The concentrations were 0 g/ml, 0.02 g/ml, 0.04 g/ml, 0.06g/ml and 0.08g/ml, respectively. The test tubes were incubated at 30°C for 48 hours and were observed for turbidity as a sign of mould growth. The contents from all the tubes were plated in Mueller Hinton agar in duplicates, incubated for 48 hours at 30°C and observed for fungal growth. The amount of extract in the tube that did not show any growth after plating was considered to be the minimum fungicidal concentration.

2.5 Shelf Life of Tomatoes Treated with the Methanol Extract

2.5.1 Preparation of the extract spray

MFC determined in section 2.3 was 0.04 g/ml. This amount was reconstituted proportionally in 20 ml of sterile distilled water. This formed the stock and was dispensed in a sterile glassy sprayer of 20 ml volume.

2.5.2 Shelf life determination

Each treatment had six tomatoes. These tomatoes were sprayed with the extract while placed on a sterile aluminium foil. They were then placed on a clean tray at room temperature. The shelf life of each treatment was analysed. The parameters analysed were the total viable count for microorganisms on the tomatoes, the weight loss with time, colour changes, odour. The results for the different parameters were obtained over a period of 14 days at 3-day intervals. Every three days a tomato was randomly picked from each tray and analysed for the total viable count by serial dilution. The results were tabulated and graphically analysed on MS excel. The control tomatoes were not sprayed with the extract.

2.6 Chemicals and Reagents

All chemicals and reagents used in this work were of analytical grades.

100% methanol (Oxoid)
Mueller Hinton agar (Oxoid)
Mueller Hinton broth (Oxoid)
Distilled water
Plate count agar (Oxoid)

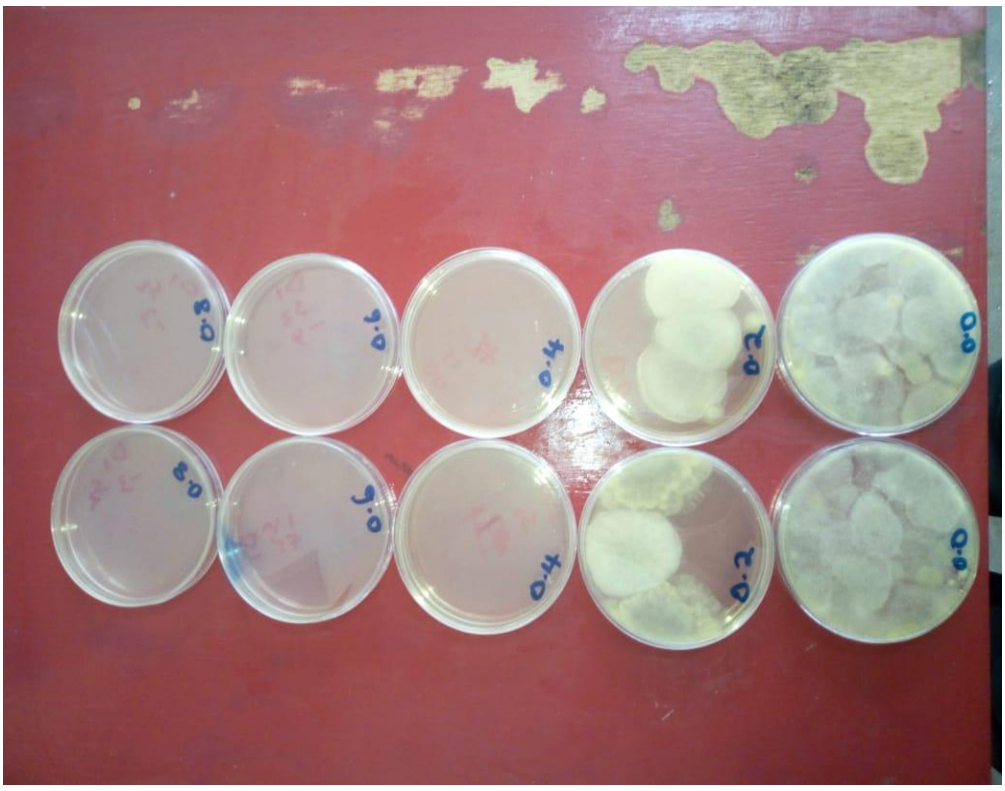
2.7 Statistical Analysis

A T-Test was performed at the $P<0.05$ level of significance. The T-test was done on data collected total viable counts (TVC). The graphical representation of three data was done using MS excel.

3. RESULTS AND DISCUSSION

The tubes containing 0.04 g/ml of the extract had no turbidity and was considered as MIC (Photgraph 1). The tube containing 0.04 g/ml of extract did not indicate any fungal growth and was therefore considered as MFC (Photgraph 2). Research by [11] found the inverse MICs of extracts of the stem and roots of *Senna didymobotrya* to be 0.16 µg/100µl against *Staphylococcus aureus*. The concentration of the tubes with no growth or tubes where 99.9% of the microorganisms were killed was considered to be the one having MFC [12] The *Senna didymotrya* extract inhibited the growth of *Aspergillus niger*, which is one of the major causes of spoilage in tomatoes [13], at different concentrations. This concurs with the findings of [10] whereby plant extracts of different Plectranthus species inhibited the growth of *Aspergillus niger.*

Photograph 1. Represents concentrations of extract for the determination of MIC of the extract against *Aspergillus niger* which was found to be 0.4 g in 10 ml i.e. 0.04 g/ml

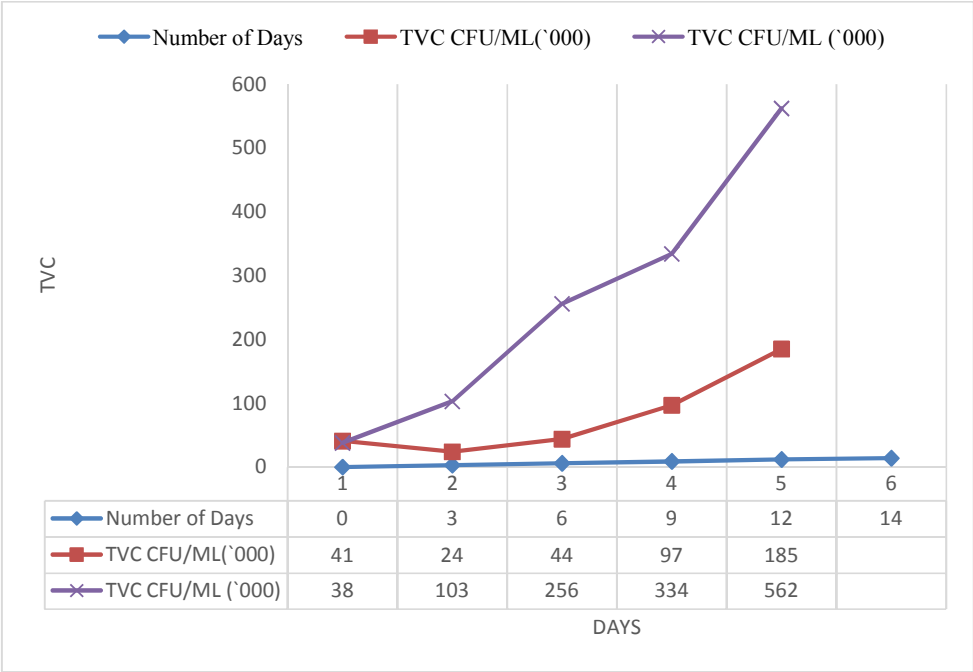


Photograph 2. Represents the MFC of the extract of *Senna didymobotrya* leaves against *Aspergillus niger* found to be 0.04 g/ml

Tables 1 A and B shows the total viable count (TVC), colour, weight, loss and odour. The trend in the total viable count of the treated tomatoes showed antifungal activity since the TVC had reduced on the 3rd day. The untreated tomatoes however continuously increased in fungal count over time. The differences between the two treatments were tested at ($P \leq 0.05$)significance. There was a significant difference between the two treatments. T-test results at ($P \leq 0.05$) showed a significant difference between the treated and untreated samples based on the total variable counts with error margins of ± 6.7 and $\pm$ 11.9, respectively. The difference was attributed to the application of the plant extract on the treated tomatoes. The antifungal activity of the extract is attributed to the presence of phytochemical compounds in the extract and their antifungal capabilities [7]. The extract not only inhibits the growth of fungi but also bacteria [11] thus a total viable count becomes relevant [14]. The application of herbal extracts on fruits and vegetables depends on the specific micro-organism being targeted since microorganisms have different levels of resistance to anti-microbials [15].

Tomatoes treated with the extract differed with those that were untreated in the intensity of the measured parameters due to the plant extracts fungicidal activity. The storage life of the untreated tomatoes was determined to be 9 days. After day 9, the tomatoes exhibited signs of deterioration such as mould growth [16] and bad odour which are indicators of spoilage [17]. The treated tomatoes did not show any signs of spoilage up to the 14th day of the experiment when observations were concluded. The prolonged shelf life of the tomatoes can be attributed to the activities of the phytochemical compound in the leaf extract [18]. The outcome concurs with the findings of Bhagwat and Datar [19] in that on the 14th day of treatment of their tomatoes with *Garnica indica* extracts, the tomatoes were still acceptable (Fig. 1).

The loss in the weight of the tomatoes was as shown in Fig. 2. The rate of weight loss in fruits may be due to loss of substrate or water loss through transpiration [20]. The tomatoes did not exhibit much difference in their rates of weight loss owing to the fact that the storage conditions were similar considering that the storage method has a great effect on the shelf life of tomatoes [21]. Thus, the extract had no effect on the rate of weight loss of the tomatoes.



	1	2	3	4	5	6
Number of Days	0	3	6	9	12	14
TVC CFU/ML(`000)	41	24	44	97	185	
TVC CFU/ML (`000)	38	103	256	334	562	

Fig. 1. Graphical representation of the total viable count of the tomatoes

Table 1A. Results for the total viable count (TVC), colour, weight loss and odour

Treated samples						
Number of days	**O**	**3**	**6**	**9**	**12**	**14**
TVC CFU/ML (000)	41	24	44	97	185	-
Colour change	None	None	None	None	None	None
Weight loss (%)	0	1.22	3	12	14.1	17.6
Odour	None	None	None	None	Slight	Slight

Table 1B. Results for the total viable count (TVC), colour, weight loss and odour

Untreated samples						
Number of days	**O**	**3**	**6**	**9**	**12**	**14**
TVC CFU/ML (000)	38	103	256	334	562	-
Colour change	None	None	None	none	Dark red	Moldy
Weight loss (%)	0	3.00	5	9	15	16
Odour	None	None	None	Slight	Bad	Very bad

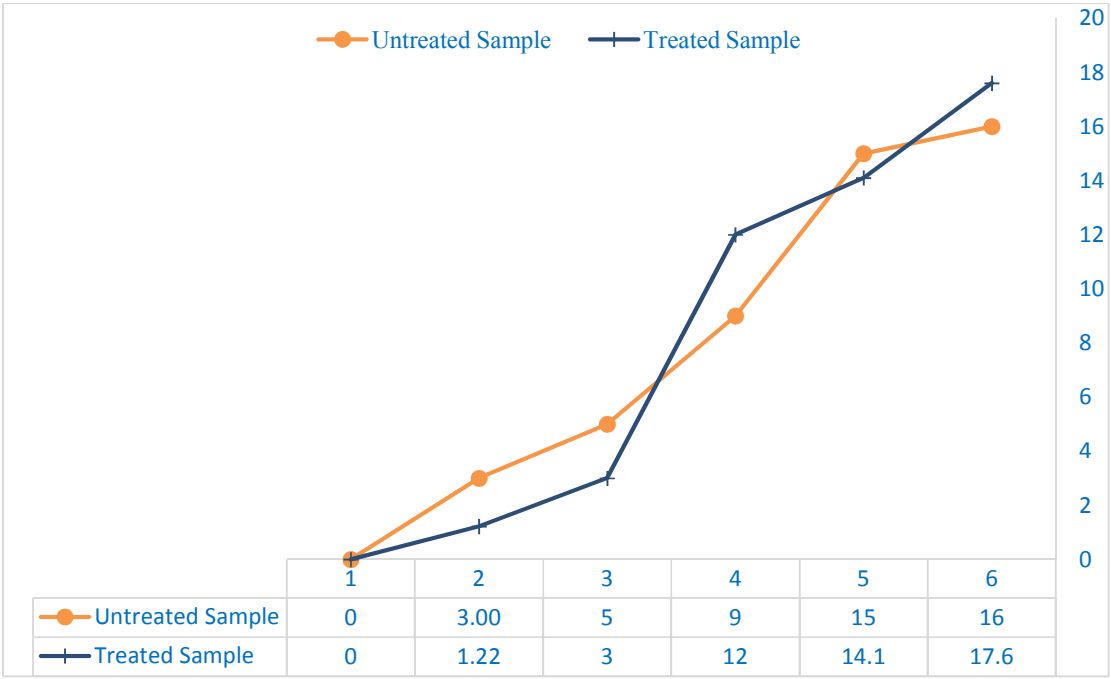


Fig. 2. Percentage weight loss of the tomatoes with time

The untreated tomatoes developed off odours after the ninth day of storage. Off odours in tomatoes are as a result of fungal rots that develop due to continuous multiplication of fungi within the fruit as it spoils [22]. The tomatoes treated with the extract only had a slight odour by the twelfth day of storage. The difference is attributed to the reduced rate of fungal growth and spoilage upon application of the extract.

4. CONCLUSIONS

Extract from candle brush *(Senna didymobotrya)* leaves was effective against the growth of fungi (*Aspergillus niger*) in tomatoes. The extract also prolonged the shelf life of the treated tomatoes for 14 days while the control spoilt at 9 days. The extract can, therefore, be used effectively in the reduction of post-harvest losses of tomatoes and

eventually contribute to overall food security in the country.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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