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THE POTENTIAL OF FUNGAL ENDOPHYTES IN SHELF LIFE EXTENSION OF TOMATO (*Solanum lycopersicum* L.) FRUITS

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ABSTRACT
Tomato (Solanum lycopersicum L.*) is one of the most popular and widely consumed vegetables worldwide. However, tomato fruits are of a highly perishable nature, with a short shelf life of between 12 to 72 hours. The aim of this study was to investigate the potential of fungal endophytes in shelf-life extension of tomato fruits. Fungal endophytes were isolated from healthy leaves and fruits of tomato and identified. Shelf life extension of the tomato fruits was carried out by soaking the fruits in the broth cultures of the test endophytes for an hour. The control treatment was soaked in sterile distilled water. The isolated fungal endophytes were identified as Aspergillus ochraceus (from fruit) and Aspergillus niger (from leave). The results obtained show that the endophytes have potential in shelf life extension of the treated fruits. At day 2, all the fruits treated with the test endophytes were very marketable compared to the control that had 66.6 % moderately marketable and 33.3 % of the fruits marketable. At day 4, all (100 %) of the fruits treated with Aspergillus niger were very marketable and very firm compared to the other treatments. The findings from this study suggest that the isolated endophytes have potential in the shelf life extension of tomato fruits. Sample B (Aspergillus niger) seems to be more effective than Sample A (Aspergillus ochraceus) in extending the shelf life of the fruits. The mode of action of the test endopohytes should be further investigated. Quality and safety assessment of fruits preserved using endophytes should be further investigated. Future research should aim at extracting bioactive compounds from endophytes that can be used in shelf life extension of fruits.*

Key words: *Aspergillus*, Food security, Preservation, Postharvest

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INTRODUCTION
Tomato (*Solanum lycopersicum* L.) is one of the most popular and widely consumed vegetables worldwide (Dias, 2012). Over 120 million metric tons are grown annually (Kimura and Sinha, 2008). It belongs to the extremely large and diverse family, Solanaceae, comprising more than 90 genera (Bohs and Olmstead, 1997). The culinary, industrial and ornamental values of tomato make it very famous (Shittu and Robb, 2011). Nigeria is ranked the second tomato producing country in Africa after Egypt and 13th globally (Ebimieowei *et al*., 2013a). Tomato has several health benefits and it is often regarded as a natural medicine because of its health benefits. It is rich in vitamin A, B and C; it also contains lycopene which is a strong antioxidant that helps to fight against cancerous cell formation. Phytosterols which help to keep the cholesterol level low are also present in tomato (Bhowmik *et al*., 2012).

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However, tomato fruits are of a highly perishable nature, with a short shelf life of between 12 to 72 hours (Ejale and Abdullah, 2004). According to the Food and Agricultural Organization, more than 800 million people still suffer from hunger, yet one-third of food (including tomato) produced is either lost or wasted globally each year. Postharvest losses are considered a major component of food loss and waste. Losses occur due to improper handling, storage, transport, preservation techniques and infection by microorganisms. Apart from loses due to postharvest pathogens, toxins produced by spoilage agents could also harm humans and spoilage product could serve as vehicles for pathogens (Barth *et al*., 2009; Islam *et al*., 2018). There have been attempts to control postharvest pathogens and preserve perishable fruits, most especially with the use of chemicals. Synthetic fungicide treatment has been the main method of controlling postharvest diseases and shelf life extension of fruits. However, there is increasing concern over the indiscriminate use of synthetic fungicides because of the possible harmful effects on human health. The hazards associated with chemicals beckons for the need to source for environmentally friendly methods. Therefore, the aim of this preliminary study was to investigate the potential of fungal endophytes in shelf life enhancement of tomato fruits.

Endophytes are microorganisms that dwell within plant tissues without causing any apparent harm to the host plants (Gao *et al*., 2010). They are mainly bacteria and fungi. Endophytes confer protection to plants and increase plant growth and productivity; the plants provide food and shelter in return. They can also be referred to as hidden beneficial associates of plants (Okungbowa *et al*., 2019). They serve as biocontrol agent for controlling plant disease and are good antagonist against pathogens. Endophytes are important in producing drugs because they possess bio-active compounds and antimicrobial substances that are useful in controlling diseases (Grayattori *et al*., 2017). Examples of fungal endophytes include *Pestalotiopsis versicolor* and *Pestalotiopsis neglecta* that live within *Taxus cuspidate* (Kumaran *et al*., 2010). There seem to be limited reports on the use of endophytes in shelf life extension of perishable fruits and in the control of postharvest pathogens.

MATERIALS AND METHODS

Materials used: Conical flask, Petri dishes, measuring cylinder, inoculation loop, syringe, bunsen burner, beaker, sieve among others.

Experimental methodology: The experimental methodologies were as follows:

Collection of samples: Healthy leaves of tomato were collected from a home garden and healthy tomato fruits were purchased from New Benin market, Benin City, Edo State, Nigeria.

Preparation of samples: Preparation of the plant samples for the isolation of fungal endophytes was carried out following the modified method of Obiazikwor *et al*. (2021). The healthy leaf and fruit samples were prepared by washing, surface sterilization using 70% ethanol. The part of the leaf and tomato used were teased out and then rinsed with sterile distilled water.

Preparation and sterilization of medium: The medium used was Potato Dextrose Agar (PDA) and it was prepared according to the manufacturer's instructions. Weight of 39 g of PDA powder was obtained using a weighing balance and this was dissolved in 1 litre of distilled water. The medium was sterilized using autoclave for 15minutes at 121 ^{0}C. It was aseptically dispensed into petri dishes after it was left to cool down. Two hundred and fifty milligram (250 mg) of

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chloramphenicol was added to 250 ml of the medium before pouring to inhibit bacteria growth.

Isolation and sub-culturing of fungal endophytes

Isolation was carried out using direct plating method. After the medium solidified, the prepared plant samples were inoculated on the correctly labeled plates. Cultures were incubated at room temperature (28+2°C) for 72 hours and observed for fungal growth.

Sub-culturing was carried out to obtain pure cultures. The mycelia of the fungal culture obtained from both samples (leave and fruit) were picked up using a sterilized inoculation loop and were inoculated into fresh potato dextrose agar medium. The cultures were incubated at room temperature (28±2°C) for 72hours

Description and identification of fungal isolates: The identification of the fungal isolates was done using macroscopy and microscopy. The morphological characteristics of the fungal isolates were described. For the microscopy, the fungal isolates were stained with lactophenol blue dye on clean and sterilized glass slide. The mycelia were teased to have a homogenous mixture. The mixture was gently covered with cover slips and allowed to stay for a few seconds. The slide was viewed under the microscope at x40 magnification. This was then compared with a laboratory manual for fungal identification.

Preparation and sterilization of broth medium to establish broth culture

Broth medium was prepared by boiling 30 g of potato in 150 ml of distilled water for 20 minutes; 3 g of dextrose sugar was dissolved in the culture and 0.0075 g of chloramphenicol was added to inhibit bacteria growth and was sterilized for 15 minutes using a conical flask and it was sealed with paraffin. After sterilization, it was left to cool down. The pure cultures of endophytes were inoculated into the broth using a syringe to establish broth culture (Plate 1) and they were incubated at room temperature (28±2°C) for 72hours.

Treatment of tomato using test fungal endophytes

This was carried out using the broth cultures of the test endophytes. The culture was agitated to have a homogenous mixture. An aliquot of 15 ml of the broth culture was poured into a clean beaker. Freshly purchased tomatoes were placed inside the beakers and soaked for an hour (Plate 2), after which, the tomatoes were brought out. Three tomato fruits were used for each treatment (pseudo replicate). The tomato fruits were then placed in flat plate labelled Sample A, B, and control plate. The control treatment was tomato fruits soaked in sterile distilled water.

Determination of firmness and marketability

The firmness of the treated tomato fruits was determined on a daily basis. This was carried out by following a 1-4 scale, where 1, 2, 3 and 4 were taken to be least firm, moderately firm, firm and very firm respectively. The marketability was also determined following a similar scale where 1 was taken to be least marketable, 2 was moderately marketable while 3 was marketable and 4 was taken to be very marketable respectively.

Statistical analysis:

Each treatment was represented in three replicates (pseudo replicates) and data obtained were subjected to descriptive and inferential statistics were appropriate. Statistical Package for Social sciences (SPSS), version 20 software was employed for the statistical analysis.

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RESULTS

The fungal endophytes isolated from healthy tomato leave and fruit. The endophytes were identified as *Aspergillus niger* and *Aspergillus ochraceus* (Plate 3 and Plate 4).

The firmness of tomato fruits subjected to endophytic treatments is shown in Table 1. At day 0, 100% of the fruits subjected to the different treatments were very firm. Duncan multiple range test shows that there was no significant difference observed at day 0. At day 2, all the tomato fruits subjected to endophytic treatments were all very firm while 66.6% of the control was moderately firm and 33.3, firm.

The frequency distribution chart showing firmness of tomato fruits subjected to different endophytic treatments after 7 days of storage (Figure 1) shows that 100 % of the fruits treated with *Aspergillus niger* were still very firm after seven days of storage, while about 66.7 % of the control treatment were least firm. Figure 2 shows the frequency distribution chart showing firmness of tomato fruits subjected to different endophytic treatments after 9 days of storage. After 9 days of storage, all the control fruits were least firm while 66.7% of fruits treated with *Aspergillus niger* were firm. However, none of the treatment produced fruits that were very firm after nine days of storage.

Table 2 shows the marketability of tomato fruits subjected to endophytic treatments. At day 0, all the tomato fruits subjected to the different treatments were all very marketable. At day two, 100% of the tomato fruits subjected to endophytic treatment were very marketable while 66.6% of the control was moderately marketable, with 33.3% marketable. (Plate 5) At day 4, 100 % of the tomato fruits subjected to isolate B (*Aspergillus niger*) were very marketable.

Frequency distribution chart showing marketability of tomato fruits subjected to different endophytic treatments after 9 days of storage (Figure 3) shows that all the fruits treated with *Aspergillus niger* were very marketable after 7 days of storage while 66.7% of the control fruits were least marketable and deteriorated (Plate 7). After 9 days of storage, 66.7 % of the fruits treated with *Aspergillus niger* were marketable while 100 % of the untreated fruits (control) were least marketable and deteriorated (Figure 4). None of the treatments produced fruits that were very marketable after 9 days of storage.

DISCUSSION

In this study, the two endophytes isolated from healthy tomato fruit and leaves were coded as isolate A and isolate B and they were identified as *Aspergillus ochraceus* and *Aspergillus niger* respectively. *Aspergillus ochraceus* is a mild species in the genus *Aspergillus* and is known to produce the toxin ochratoxin A, one of the most abundant food contaminating mycotoxins, and citrinin. It also produces the dilydroisocoumarin mellein. It is a filamentous fungus in nature and has characteristics hibernate conidiophore. *Aspergillus niger* is a filamentous ascomycete fungus that is ubiquitous in the environment and has been implicated in the opportunistic infections in humans. There are previous studies on the isolation of *Aspergillus* from plants as endophytes. Rajeswari *et al*., 2016 had isolated *Aspergillus ochraceus* and *Aspergillus niger* from the medicinal plant, *Moringa oleifera*. *Aspergillus japonicuss* has also been isolated from the wild plant, *Euphorbia indica* (Ismail *et al*., 2018). Therefore, the isolation of *Aspergillus* as endophytes in the current study agrees with previous studies. However, there seems to be no reports on the use of the test organisms in shelf life extension of fruits.

Preservation of fruit and vegetables is of great importance because it makes provisions for

sustainable use and eliminates wastage. Tomato fruits are of a highly perishable nature, with a short shelf life of between 12 to 72 hours (Ejale and Abdullah, 2004). According to the Food and Agricultural Organization, more than 800 million people still suffer from hunger, yet one-third of food (including tomato) produced is either lost or wasted globally each year. Postharvest losses are considered a major component of food lost and waste. Losses occurred due to improper handling, storage, transport, preservation techniques and infection by microorganisms. Apart from loses due to postharvest pathogens, toxins produced by spoilage agents could also harm humans and spoilage product could serve as vehicles for pathogens (Barth *et al*., 2009). The current study demonstrated the potential of fungal endophytes in the shelf life extension of tomato fruits.

Table 1 shows the firmness of tomato fruits subjected to endophytic treatments. There was no significant difference in the tomato fruits subjected to the different treatments at day 0. At day 4, all the tomato fruits subjected to the endophytic treatments were still very firm, compared to the control (Plate 6). This shows that the test endophytes were effective at maintaining the firmness of the treated fruits. However, one of the fruits treated with *Aspergillus ochraceus* had growth on it.

At day 9, the control fruits were all deteriorated, compared to the fruits subjected to endophytic treatments; 66.7 % of the fruits treated with isolate B (*Aspergillus niger*) were firm while 33.3 % of the fruits were moderately firm (Figure 2). There was significant difference in the firmness of the fruits subjected to the different treatments at day 9, with sample B (*Aspergillus niger*) proving to be more effective compared to Sample A (*Aspergillus ochraceus*) (Table 1). Previous studies have reported endophytes as bio-control agents of plant pathogens (Obiazikwor *et al*., 2021; Jayawardena *et al*., 2016). There seems to be limited reports on the use of fungal endophytes in shelf life extension of fruits. However, Alsoufi and Aziz, 2017 reviewed the extension of shelf life of fruits using some microorganisms biological products. Masumbilla *et al*., 2020 reported the shelf life expansion of guava using antagonistic bacteria. The observations from the current study agree with previous studies that have demonstrated the use of endophytes in shelf life extension of fruits.

The marketability of tomato fruits subjected to endophytic treatments (Table 2) shows that all the fruits tested were very marketable at day 0. At day 2, all the fruits subjected to endophytic treatments were very marketable with 100% frequency, compared to the control that had 66.6% of the fruits moderately marketable and 33.3% marketable. This shows that the endophytes enhanced the marketability of fruits during storage indicating the extension of shelf life. At day 7, 100% of the fruits treated with sample B (*Aspergillus niger*) were marketable, compared to the other treatments. There was significant difference ($p \leq 0.05$) in the marketability of the treated fruits compared to the control using Duncan multiple range test (Table 2). This shows that Sample B (*Aspergillus niger*) was more effective in extending the shelf life of the treated fruits compared to Sample A (*Aspergillus ochraceus*). This difference could be attributed to the difference in the antagonistic nature of the test organisms. However, this should be further investigated.

CONCLUSION AND RECOMMENDATION

The findings from this study indicate that the isolated endophytes have potential in the shelf life extension of tomato fruits. Sample B (*Aspergillus niger*) was more effective than

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Sample A (*Aspergillus ochraceus*) in extending the shelf life of the fruits. The mode of action of the test endopohytes in extending the shelf life of fruits should be further investigated. Quality and safety assessment of fruits preserved using endophytes should be further investigated. Future research should be geared at towards extracting bioactive compounds from endophytes that can be used in the shelf life extension of fruits. This will help to reduce postharvest loses thus ensuring food security.

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APPENDICES

Isolate B Isolate A

Plate 1: Broth cultures of isolated fungal endophytes after 48 hours

Legend: Isolate B= *Asperigillus niger;* Isolate A= *Aspergillus ochraceus*

C A B

Plate 2: Treatment of fresh tomato fruits with broth cultures of test fungal endophytes

Legend: C= Control (tomato fruits soaked in distilled water); B= Tomato fruits soaked in *Aspergillus niger* broth; A= Tomato fruits soaked in *Aspergillus ochraceus* broth

ISOLATE A ISOLATE B

Plate 3: pure cultures of isolated fungal endophytes grown on PDA

Legend: Isolate A= *Aspergillus ochraceus*; Isolate B= *Aspergillus niger*

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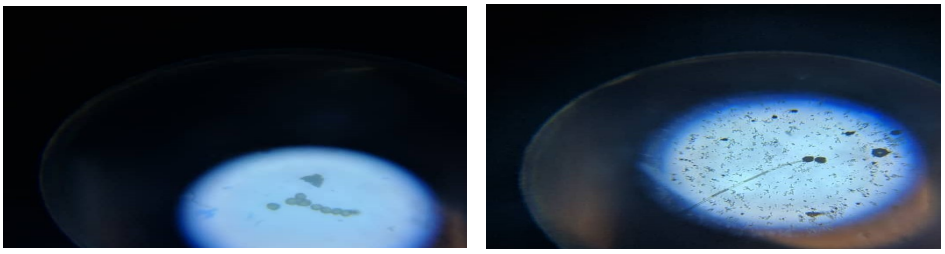

A B

Plate 4: Photomicrograph of the isolated endophytes

Legend: Isolate A = *Aspergillus ochraceus;* Isolate B = *Aspergillus niger*

Table 1: Firmness of tomato fruits subjected to endophytic treatments

	Frequencies (%)					
	Day0	**Day2**	**Day4**	**Day7**	**Day9**	**Day13**
Control						
Least firm	0	0	66.7	66.7	100	100
Moderately firm	0	66.7	33.3	33.3	0	0
Firm	0	33.3	0	0	0	0
Very Firm	100	0	0	0	0	0
Duncan Letter	**A**	**A**	**A**	**A**	**A**	**A**
Isolate A						
Least firm	0	0	0	33.3	66.7	100
Moderately firm	0	0	33.3	33.3	33.3	0
Firm	0	0	66.7	33.3	0	0
Very Firm	100	100	0	0	0	0
Duncan Letter	**A**	**B**	**B**	**AB**	**A**	**A**
Isolate B						
Least firm	0	0	0	0	0	66.7
Moderately firm	0	0	0	0	33.3	33.3
Firm	0	0	0	0	66.7	0
Very Firm	100	100	100	100	0	0
Duncan Letter	**A**	**B**	**C**	**B**	**B**	**A**

Values obtained from treatments bearing similar letters on the same column are not significantly different from each other using Duncan multiple range test at 0.05 level of significance

Legend: Isolate A = *Aspergillus ochraceus;* Isolate B = *Aspergillus niger*

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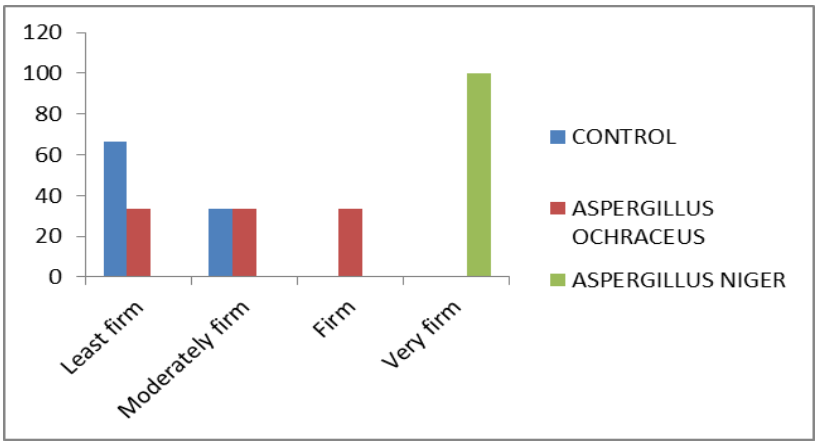

Figure 1: Frequency distribution chart showing firmness of tomato fruits subjected to different endophytic treatments after 7 days of storage

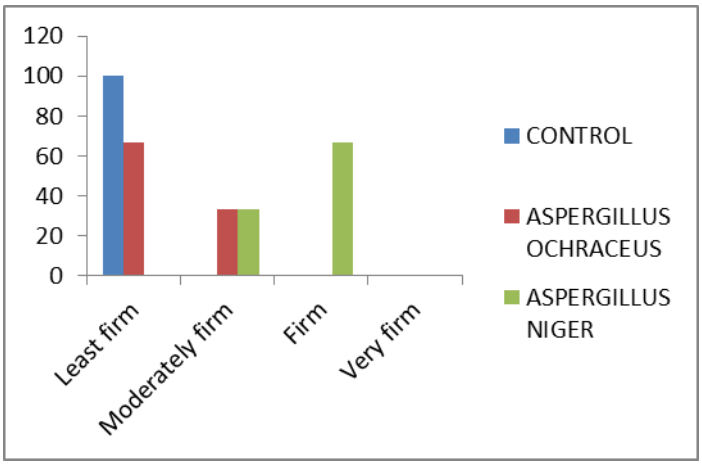

Figure 2: Frequency distribution chart showing firmness of tomato fruits subjected to different endophytic treatments after 9 days of storage

Table 2: Marketability of tomato fruits subjected to endophytic treatments

	Frequencies (%)					
	Day0	**Day2**	**Day4**	**Day7**	**Day9**	**Day13**
Control						
Least marketable	0	0	66.7	66.7	100	100
Moderately marketable	0	66.7	33.3	33.3	0	0
Marketable	0	33.3	0	0	0	0
Very marketable	100	0	0	0	0	0
Duncan Letter	**A**	**A**	**A**	**A**	**A**	**A**

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Isolate A						
Least marketable	0	0	0	33.3	66.7	100
Moderately marketable	0	0	33.3	33.3	33.3	0
Marketable	0	0	66.7	33.3	0	0
Very marketable	100	100	0	0	0	0
Duncan Letter	**A**	**B**	**B**	**AB**	**A**	**A**
Isolate B						
Least marketable	0	0	0	0	0	66.7
Moderately marketable	0	0	0	0	33.3	33.3
Marketable	0	0	0	0	66.7	0
Very marketable	100	100	100	100	0	0
Duncan Letter	**A**	**B**	**C**	**B**	**A**	**A**

Values obtained from treatments bearing similar letters on the same column are not significantly different from each other using Duncan multiple range test

Legend: Isolate A = *Aspergillus ochraceus;* Isolate B = *Aspergillus niger*

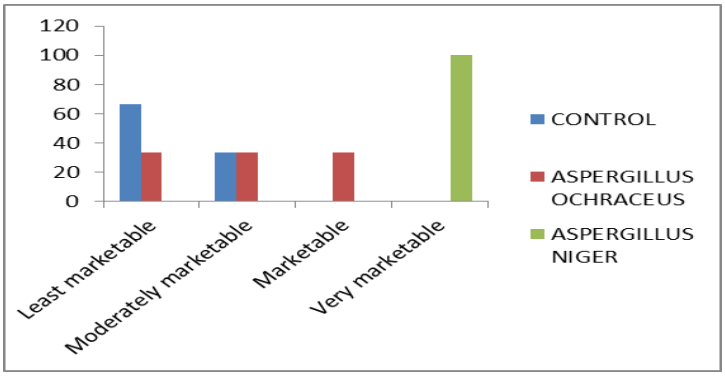

Figure 3: Frequency distribution chart showing marketability of tomato fruits subjected to different endophytic treatments after 7 days of storage

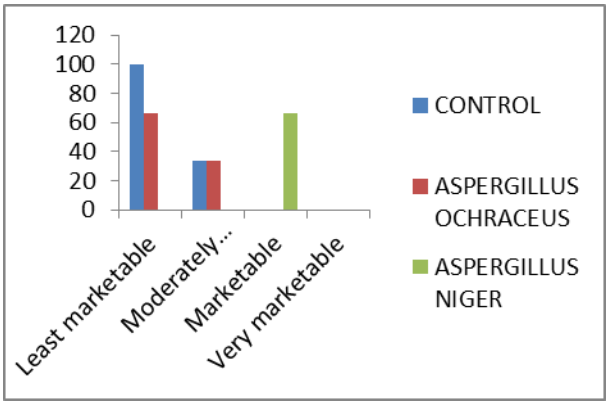

Figure 4: Frequency distribution chart showing marketability of tomato fruits subjected to different endophytic treatments after 9 days of storage

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C **A** **B**

Plate 5: Tomato fruits treated with fungal endophytes after two days of storage
Legend: C= Control; A= Tomato fruits treated with *Aspergillus ochraceus;* B= Tomato fruits treated with *Aspergillus niger*

C **A** **B**

Plate 6: Tomato fruits treated with fungal endophytes after four days of storage
Legend: C= Control; A= Tomato fruits treated with *Aspergillus ochraceus;* B= Tomato fruits treated with *Aspergillus niger*

C **A** **B**

Plate 7: Tomato fruits treated with fungal endophytes after seven days of storage
Legend: C= Control; A= Tomato fruits treated with *Aspergillus ochraceus*
B= Tomato fruits treated with *Aspergillus niger*