International Food Research Journal 26(1): 123 - 131 (February 2019)

Journal homepage: *http://www.ifrj.upm.edu.my*

Morphological and PCR characterisation of fungi isolated from tomato postharvest, and potential control of fruit spoilage by antifungal plant extracts

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Article history

Received: 9 January, 2017
Received in revised form: 19 April, 2017
Accepted: 21 June, 2018

Keywords

Solanum lycopersicum
Fungal infection
Thymus vulgaris
Natural fungicide

Abstract

Fungal infection causes significant postharvest loss in tomato production. Few studies have evaluated natural products *in vivo* as alternative to unsafe synthetic chemical fungicides. The present work evaluated three plants with known antifungal activity namely; *Cymbopogon citratus* (lemon grass), *Ocimum gratissimum* (African basil) and *Thymus vulgaris* (thyme), against fungi isolated from tomato postharvest *in vitro*. The most active extract was tested on healthy fruits for preliminary assessment of its effect. Fungi isolated from tomato postharvest in Buea, South West Cameroon, were characterised using morphological techniques followed by molecular identification using polymerase chain reaction. Four distinct fungal isolates were detected with *Fusarium* and *Colletotrichum* spp. being the predominant isolates. In antifungal disc diffusion and microdilution bioassays, the plant extracts exhibited moderate to high inhibition of fungal growth. The methylene chloride extract of *T. vulgaris* (TV_C) was the most active with a minimum inhibitory concentration of 12.5 mg/mL. TV_C suppressed artificial infection with *F. oxysporum* isolate and inhibited natural infection of fruits with 100% inhibition at 0.15 and 0.31% of TV_C at 4°C after 30 days. *T. vulgaris* extract can potentially prevent postharvest infection and should be further investigated on tomato fruits as a suitable alternative to synthetic fungicides for extension of tomato shelf life postharvest.

Introduction

Tomato (*Solanum lycopersicum*; *Solanaceae* family) which is grown and consumed worldwide has very important nutritional and health benefits and economic value (Raiola *et al*., 2014).Tomato is cultivated in Cameroon on a large scale (Food and Agriculture Organisation, 2012), but the industry records significant pre- and postharvest losses which are due to several factors. Several diseases attribute up to 50% of the total losses with fungal infection being the major cause (Fontem *et al*., 1999). Fungi also produce mycotoxins capable of inducing mycotoxicoses in humans following ingestion or inhalation (Wagacha and Muthomi, 2008).

Several tools have been employed in the prevention and control of postharvest fungal spoilage which include recombinant DNA engineering of resistant varieties (Redenbaugh *et al*., 1992), synthetic chemical fungicides, biological control and physical methods (Etebu *et al*., 2013).These methods are limited by cost to various extents, safety to humans and the environment, resistance and limited spectrum in the case of chemicals (Tandi *et al*., 2014). Natural products are a potential alternative to the above control methods. According to Etebu *et al*. (2013), such products from higher plants have relatively broad spectrum, bio-efficacious, economical and environmentally safe. Crude extracts of *Cymbopogon citratus* (Poaceae), *Ocimum gratissimum* (Labiatae) and *Thymus vulgaris* (Lamiaceae) have been shown to possess high antifungal activity (Centeno *et al*., 2010; Galani *et al*., 2013). Furthermore, these plants are widely consumed as vegetables and spices (Idris *et al*.,2011; Nambiar and Matela, 2012; Anzlovar *et al*., 2014), and are largely safe at low doses (European

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Medicine Agency, 2013; Sinha *et al*., 2014). However, few studies have investigated the effects of antifungal plants on tomato fruits postharvest. The present work therefore attempted to assess the *in vitro* and *in vivo* antifungal activity of these plant extracts. These extracts have not been extensively tested against the fungi isolated in the study area. The Buea municipality has a long tradition of tomato cultivation (Tandi *et al*., 2014) but the postharvest fungi in this area remain undocumented. In view of effective control and prevention of fruit loss, these postharvest fungi were targeted. Essentially, isolation, cultivation and morphological techniques were used to obtain the fungi. Polymerase chain reaction (PCR) targeting the internal transcribed spacer (ITS) ribosomal DNA (rDNA) was used to molecularly identify the isolates.

Materials and methods

Study design

To investigate the shelf life extension effect of the three antifungal plants on tomato, fungi were isolated from tomato fruits under storage conditions and characterised using morphological and PCR techniques. The most frequently isolated fungi were targeted. Crude extracts of the plants were prepared in appropriate solvents and their antifungal activity against the target isolates confirmed *in vitro*. Pathogenicity of the most frequent fungi in tomato fruits was evaluated. Next, preliminary assessment of the antifungal effect of the most active extract (*in vitro*) was done *in vivo* against artificial and natural fungal infection. The data collected were analysed for statistical significance.

Isolation and cultivation of fungi from tomato

The study area, the municipality of Buea, South West Cameroon, has a mild and humid equatorial climate. A selection of fresh visibly healthy tomatoes were purchased from local farms immediately after harvesting, and used in all experiments. For fungal isolation, ten fruits each were kept similarly like what the farmers practice, in weaved baskets to allow for aeration, from three locations (> 2 km apart) in Buea, under shade, for five to 30 d to allow for natural microbial contamination. The fruits were examined daily for tissue disruption and rot; pathological changes were examined and a time profile for tomato postharvest infection was established. Six incubation experiments were conducted. Rotten fruits were washed with clean water and surface sterilised by immersion in 1.0% sodium hypochlorite for 10 min, and rinsed four times in sterile distilled water. Isolation was done as described (Joshi *et al*., 2013) with a slight modification. Two cut peripheral thin sections were rinsed in sterile distilled water once, and aseptically placed in previously prepared potato dextrose agar (PDA) with 0.05 g/mL chloramphenicol (Sigma-Aldrich Inc., Germany). The plates were incubated at ambient temperature (27± 2°C) for 3-7 d. Fungal growth associated with rotten tissue was analysed, and the frequency of occurrence noted (Okigbo and Emeka, 2010). Pure cultures were obtained by several aseptic transfers of the colony growth to fresh PDA plates. A fresh culture of each fungal isolate was used in each procedure or experiment described below.

Morphological and molecular identification of fungal isolates

Fungal isolates were characterised principally on the basis of morphological features followed by molecular identification for validation (Colak and Bicici, 2013; Joshi *et al*., 2013). Each pure isolate was examined macroscopically and identified based on physical features and growth patterns i.e., shape, obverse and reverse colour, elevation, texture, mycelium, density and edge (Sharma and Pandey, 2010). Pictures of the mature fungi were compared with those of previously identified fungi grown on PDA (Alexopoulos *et al*., 1996). Microscopic examination was done using sporulated fungi on PDA. Tease mounts (McGinnis, 1980) or coverslip preparations in Lactophenol Cotton Blue were observed, and the microscopic characteristics noted i.e., hyphae and conidial features, spore (Alexopoulos *et al*., 1996). Pictures were taken and compared with standard pictures of previously identified fungi.

For PCR, fungal species that frequently attack tomato postharvest were listed from literature, and species-specific primers from the ITS region of the rDNA gene were obtained (Table 1). Five fungal isolates obtained from pure 15-day-old cultures were analysed. Total genomic DNA was extracted from mycelia using the cetyltrimethyl ammonium bromide (CTAB) method (Lacap *et al*., 2003) with modifications. Approximately 2.5 mg of fungal tissue in a sterile microcentrifuge tube was frozen at -80°C overnight, homogenised and then transferred into 1.5 mL microcentrifuge tubes. To each tube, 500 µL of 1.5× CTAB extraction buffer (0.1 M Tris–HCl of pH 7.5, 1% CTAB, 0.7 M NaCl, 10 mM EDTA, 1% 2-mercaptoethanol, 0.3 mg/mL proteinase K added prior to use) was added. The tubes were heated in a water bath (65°C for 30 min), cooled and an equal volume of chloroform/isoamyl alcohol (24:1) was added. The tubes were vortexed and centrifuged (10 min at 2,000 g), (Eppendorf, Germany). The DNA was precipitated with an equal volume of isopropanol,

rinsed with 70% ethanol, air dried and checked by electrophoresis. The DNA was kept at 4°C overnight or stored at -20°C. Two rounds of amplifications were performed, a first round amplification with fungus-specific universal primers ITS-3 and ITS-4 designed within the 5.8S rDNA region (amplicon length polymorphism), and the second round with species-specific primers.

The PCR master mix recipe per reaction was: 10 µL ReadyMix (Sigma-Aldrich, Germany), 1 µL upstream primer, 1 µL downstream primer and 6 µL filter-sterilised autoclaved water. Genomic DNA was added to make up 20 µL of the reaction mixtures. The thermal cycling profiles were carried out using a Peltier Thermal Cycler (Model MG 96+, Medical Equipment Co. Ltd., China) for each species according to literature (Table 1). The PCR product was electrophoresed, and the gels analysed and photographed using an automated electrophoresis station (Bio-Rad, Hercules, CA). The detection was based on molecular weight of products.

In vitro assessment of antifungal activity of plant extracts

The plants were collected and identified by Mr Ndive Elias, a botanist at the Limbe Biodiversity and Conservation Centre, Cameroon, using voucher specimens: *Cymbopogon citratus* (SCA2841), *Ocimum gratissimum* (SCA4764), and *Thymus vulgaris* (SCA3242). Extracts were prepared as described (Nguefack *et al*., 2007); 100 g of processed plant material was macerated separately for 72 h in 1 L methanol and methylene chloride. The filtrate (via Whatman filter paper No. 1; pore size 60 µA°) was concentrated by rotary evaporation (BUCHI Rotavapor RE-111, Switzerland), and the extracts air-dried, weighed and stored at -20°C until tested. Antifungal activity was assessed against the two most frequent fungal isolates, according to the Clinical and Laboratory Standards Institute recommended method (CLSI, 2012). Mueller-Hinton agar plates containing chloramphenicol (0.05 g/mL) were seeded with a suspension (McFarland 0.5-1×10^6 – 5×10^6 cells/mL at 530 nm in 0.85% saline) of a mycelial portion of fungal culture (5-10 d old). Discs (Whatman filter paper No. 1) of extracts (50 to 200 mg in 100% dimethyl sulfoxide, DMSO) were gently fixed on the agar surface. A positive (50 µg clotrimazole) and negative control (DMSO) were also included. The plates were incubated at room temperature (27 ± 2°C) for 48-72 h. The experiment was conducted in duplicate. The diameter of inhibition per disc was

Table 1. Polymerase chain reaction primers and conditions for the detection of fungal species.

Species	Primer Sequences	References
Alternaria alternata	AAF2 (5'-TGCAATCAGCGTCAGTAACAAAT AAR3 (5'-ATGGATGCTAGACCTTTGCTGAT	(Konstantinova *et al*., 2002)
Alternata solani	AS1F (5′-GCTCCCACTCCTTCCGCGC) AS2R(5′-GGAGGTGGAGTTACCGACAA)	(Kumar *et al*., 2013)
Botrytis cinerea	BC108F(5'ACCCGCACCTAATTCGTCAAC) BC563R(5'-GGGTCTTCGATACGGGAGAA)	(Rigotti *et al*.,2006)
	C729F (5'-AGCTCGAGAGAGATCTCTGA) C729R (5'- CTGCAATGTTCTGCGTGGAA)	
Colletotrichum	Col-F (5′-AACCCTTTGTGAACATACCT) Col-R (5′-CCACTCAGAAGAAACGTCGTT)	(Cano *et al*., 2004)
Fusarium	TEF-Fu3F (5'-GGTATCGACAAGCGAACCAT TEF-Fu3R (5'-TAGTAGCGGGGAGTCTCGAA	(Arif *et al*., 2012)
	ITS-Fu1F(5'-ACAACTCATAACCCTGTGAACAT ITS-Fu1R (5′-CAGAAGTTGGGTGTTTTACGG	
Mucor puriformis	MucL1F 5′-TGATCTACGTGACATATTCT 3′) MR1R 5′-AGTAGTTTGTCTTCGGKCAA 3′)	(Machouart *et al*., 2006)
Phytophtora infestans	ITS3F (5'-GCATCGATGAAGAACGCAGC-3') ITS4R (5'-TCCTCCGCTTATTGATATGC-3') PISP-1R (5'-AATGCCAAGCTAAAGAGCCA-3')	(Kim and Lee, 2001)
Rhizopus	RpL1F (5′-TGATCTACGTGACAAATTCT 3′) MR1R (5′-AGTAGTTTGTCTTCGGKCAA 3′)	(Machouart *et al*., 2006)

measured at 24 and 48 h along two perpendicular diameters. Percentage inhibition (% I) relative to positive control was calculated as follows:

$$\% I = \frac{\text{(diameter of inhibition with the treatment sample)}}{\text{(diameter with the positive control sample)}} \times 100$$

Determination of Minimum Inhibitory Concentration (MIC) of extracts

The MIC was determined for the two most active crude extracts; methylene chloride extract of *T. vulgaris* and methanol extract of *O. gratissimum,* on the two most frequent fungal isolates, *Fusarium oxysporum* and *Colletotrichum* spp., according to the European Committee for Antimicrobial Susceptibility Testing guidelines (EUCAST, 2014). Extract solution (100 µL of 200 mg/mL DMSO) was diluted serially with Mueller-Hinton broth in duplicate wells to 0.7813 mg/mL. Two negative controls (NC) were included; medium/inoculum and medium/extract; and a positive control (200 µg/mL fluconazole). The plates were set up in duplicate. A conidial spore suspension (100 µL of McFarland 0.5) of a 5-day culture was added to all required wells, then incubated at room temperature and observed visually. Absorbance (A) at 595 nm was read at 24, 48 and 72 h (Emax-Molecular Devices Corporation, California, USA). Percentage inhibition was calculated as follows:

$$\% \text{ inhibition of growth} = [\{A(NC) - A(test)\} / A(NC)] \times 100.$$

MICs were considered as the lowest extract concentration that inhibited at least 50% of growth when compared with the negative control after 48 h incubation.

Pathogenicity test for Fusarium spp.

A pathogenicity test was conducted for the most frequent isolate detected; *F. oxysporum* in tomato fruits. This was done as described (Okigbo and Emeka, 2010) with modifications. Ten fruits were surface-sterilised as mentioned above using 2% sodium hypochlorite. Wounds of approximately 4 mm in depth were made at the equator of the air-dried tomatoes with a sterile syringe. The fruits were inoculated with 10 µL vortexed conidial suspension (5×10^6 spores/mL sterile distilled water) using a micropipette; distilled water was used as control. Fruits were incubated separately in sterilized sealed containers at room temperature for 12 d and observed for infection and depreciation. Two days later, the lesion diameters were measured daily in two perpendicular directions and the average noted.

Assessment of antifungal activity on fruits under artificial infection

This was done for the most active antifungal extract, the methylene chloride extract of *T. vulgaris* against *Fusarium oxysporum*. Fruits were surface-sterilised as in the pathogenicity test, air-dried and the whole fruits' surface inoculated (0.2 mL of 5×10^6 spores per mL). After 30 min, 0.4 mL of 200 mg/L extract was injected over one half of the fruits' surface with a 1 mL sterile syringe. The samples were incubated at room temperature (27±2°C) and observed daily for 7 d. Control fruits were inoculated with distilled water (Nashwa and Abo-Elyousr, 2012).

Preliminary test for antifungal effect against natural infection

Forty grams (40 g) of TV_C was dissolved in 800 mL 60% ethanol, filtered through Whatman filter paper No.1 to remove undissolved particles, thereby giving 5% (w/v) stock solution. The stock was diluted serially in 20% ethanol giving seven final concentrations (0.078% to 5% with maximum 40% ethanol in the highest concentration). Fruits were soaked in tap water for 1 h, gently wiped with tissue paper and sterilised (2% sodium hypochlorite). Six tomatoes were treated per concentration by dipping each into the TV_C test solution for 1 min, air-dried for 30 s (Ibrahim and Al-Ebady, 2014) and placed separately in labelled perforated cardboard boxes. A solvent treatment (20% ethanol) and a negative control (untreated fruits) were also included. The boxes were kept aerated at room temperature. A second experiment with similar set up but placed in plastic containers and loosely covered was refrigerated at 4°C. Experiments were run for 30 d while noting daily the number of uninfected and infected fruits (surface colonies of fungal growth and rot).

Data analysis

Data on fungal isolation frequency and tomatoes treated with extract were analysed using Microsoft Excel 2013. *P* values for significance at 0.05 were calculated using the Paired student t-test. Computer analysis of PCR data was performed by using the software Gel Analyser on the images of electrophoresis agarose gel to obtain the molecular weights of the amplicons. The effect of extract in preliminary test

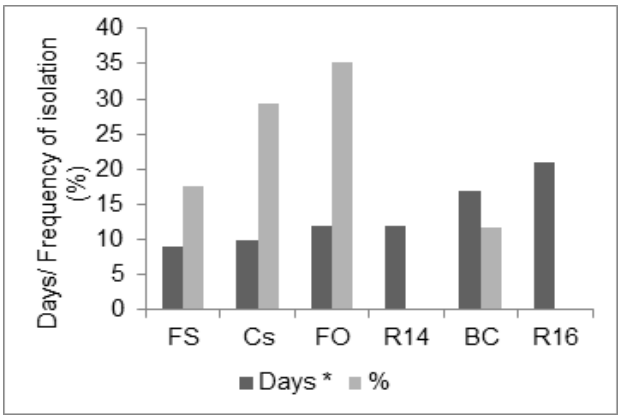


Figure 1.Time profile of fungal colonisation of tomato postharvest (days) and frequency of isolation of fungi (%) in Buea, South West Cameroon.*Number of days before fungal colony becomes visible to the naked eye. FS: *Fusarium solani*, Cs: *Collectotrichum* spp., FO: *Fusarium oxysporum* R14: Unknown, BC: *Botrytis cinerea,* R16: Unknown.

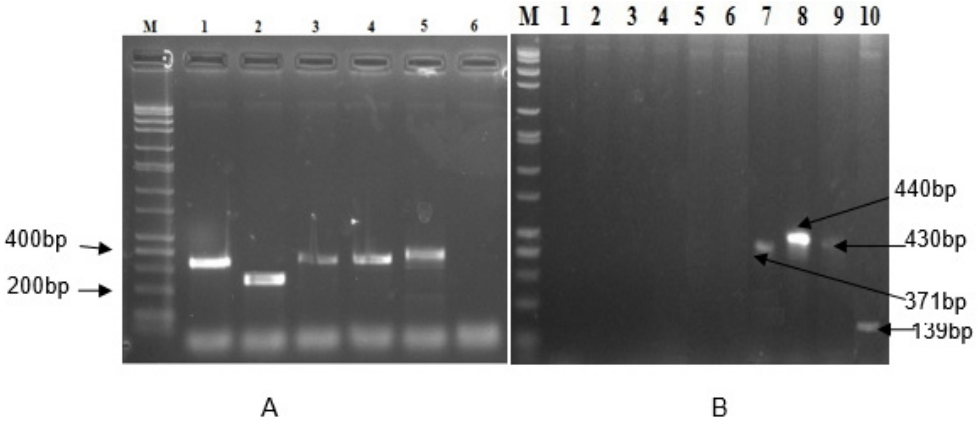


Figure 2. A: Electrophoregram of amplified ITS II regions (fungi specific marker) obtained using ITS-3F/ITS-4R primer pair. Lane M is a wide range DNA ladder. Lane 1: *Fusarium solani,* lane 2: *Botrytis cinerea*, lane 3: *Colletotrichum coccodes*, lane 4: *Fusarium oxysporum* while lane 6 is the negative control (no DNA).
B: Electrophoregram of DNA of fungal isolates amplified using species-specific primers. Lane M: DNA ladder, lane 1: negative control (PCR product with no DNA), lanes 2-6: PCR results (negative) for RI4 for different primer pairs (lane 2: *Altenaria alternata,* lane 3: *Alternaria solani*, lane 4: *Rhizopus* spp., lane 5: *Mucor piriformis*, Lane 6: *Phytophthora infestans*). Lanes 7-10: positive results (lane 7: *Fusarium oxysporum*, lane 8: *Fusarium solani,* lane 9: *Botrytis cinerea*, lane 10: *Colletorichum coccodes*).

against natural infection of tomatoes was calculated in terms of percentage healthy whole fruits with no sign of infection per treatment concentration after 30 d.

Results

Fungal identification by morphological and molecular methods

A total of 17 fungal isolates were obtained and five distinct phytopathological fungal isolates were identified macroscopically. From the time profile of infection (Figure 1) the isolate with the shortest infection period of 9 d was *F. solani* while an unidentified isolate (RI6) had the longest (21 d). *F. oxysporum* was the most frequently isolated fungus (35.29%) followed by *Colletotrichum* spp. (29.41%), *F. solani* (17.65%) and *Botrytis cinerea* (11.76%). One isolate which did not show identifiable features was classified as unknown (5.88%).

Probing the isolates with the first pair of primers (universal ITS-3F/ ITS-4R primers) yielded products from approximately 200 to 450 bp and four species were detected as moulds. The ITS region of the rDNA of the five isolates showed some amplicon length polymorphism after electrophoresis (Figure 2). Amplification with species-specific primer pairs for the ITS region generated bands ranging from 139 to 440 bp (Figure 2) and four species were again detected. There was no amplification in presence

Table 2. Antifungal activities of plant extracts against cultured fungal isolates.

Code	*Fusariun oxysporum*				*Colletotrichum* spp.			
	Zone in mm[a] (%)[b]			MIC*	Zone in mm[a] (%)[b]			MIC*
	50 mg	100 mg	200 mg		50 mg	100 mg	200 mg	
TV_C	15 (88.2)	27 (158.8)	33 (194)	12.5	18 (105.9)	22 (129.4)	25 (148.5)	12.5
TV_M	0	0	0	–	12 (70.6)	12 (70.6)	12 (70.6)	–
OG_C	18 (105.8)	20 (117.6)	20 (117.6)	–	15 (88.2)	15 (88.2)	17 (100)	–
OG_M	12 (70.6)	13 (76.4)	23 (135.3)	50	22 (129.4)	18 (105.8)	23 (135.3)	50
CC_C	0	0	0	–	0	0	0	–
CC_M	12 (70.6)	12 (70.6)	13 (76.4)	–	14 (82.4)	19 (111.7)	21 (123.5)	–

Extract code: TV_C, TV_M = methylene chloride and methanol extracts of *Thymus vulgaris*, respectively; OG_C, OG_M = methylene chloride and methanol extracts of *Ocimum gratissimum*, respectively; CC_C, CC_M = methylene chloride and methanol extracts of *Cymbopogon citratus*, respectively.

[a]Zone diameter and [b]corresponding %inhibition relative to positive control (50 µg clotrimazole) following 24 hours incubation. MIC = Minimum Inhibitory Concentration.

of primers of five other fungal species (*Alternaria alternata*, *Altenaria solani*, *Rhizopus* spp., *Mucor puriformis* and *Phytophtora infestans*). Based on both morphological analysis and PCR detection, the detected species were *F. oxysporum*, *F. solani*, *Colletotrichum* spp. and *Botrytis cinerea*.

Antifungal activity of plant extracts

Extracts of the three plants produced visible zones against the two most frequent fungal isolates after 24 h incubation; the zones after 48 h were not significantly higher, hence are not reported. Methylene chloride extracts of *T. vulgaris* (TV_C) was the most active with the highest inhibition zone of 33 mm against *F. oxysporum* and 25 mm against *Colletotrichum* isolates. The least active plant was *C. citratus* (Table 2). The MICs read visually corresponded to those obtained from absorbance readings at 595 nm. TV_C recorded the lowest MIC of 12 mg/Ml, and the methanol extract of O. gratissimum gave a MIC of 50 mg/mL against both isolates (Table 2).

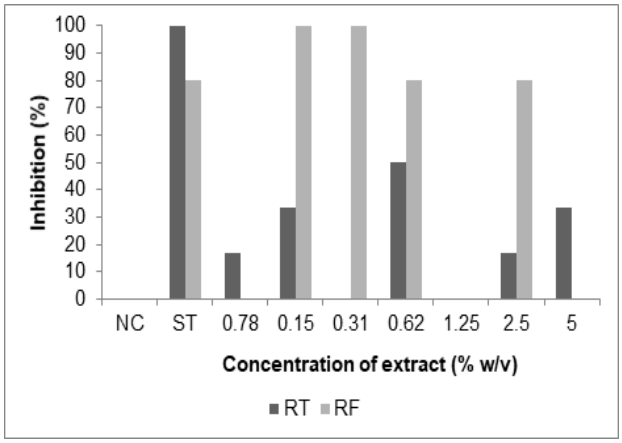
Pathogenicity of Fusarium oxysporum

F. oxysporum, the most frequent isolate, infected the inoculated fruits and produced the same pathological features as in the natural infection, causing softening and rotting radiating outward from the sites of injection to an average diameter of 17.5 mm and 13 mm for the control.

Activity of T. vulgaris against artificial and natural infection

TV_C (200 mg/mL) suppressed the growth of rot pathogen following artificial infection with the most frequent fungal isolate and fruits were effectively protected for 5 d. After 4 d incubation, there were 21 visible large colonies on the untreated half of the fruit with only 9 visible small colonies on the half treated with extract. In the experiments to assess antifungal activity of TV_C against natural infection based on the proportion of physically intact healthy fruits (no sign of infection) after 30 d, it was observed that at room temperature (RT) 0 to 50% (average 23.8%) of the fruits were uninfected over the concentration range of TV_C tested; and under refrigeration at 4°C (RF), 0 to 100% (average 51.4%) of the fruits were uninfected. Untreated tomatoes (negative control) were completely infected for both RT and RF (0%). Generally, higher inhibition was observed for the RF fruits compared to RT but not statistically significant ($P > 0.05$). The highest inhibition (100%) was recorded at 0.15 and 0.31% of extract for RF. The solvent (20% ethanol in distilled water) showed comparable inhibition (RF) or higher inhibition (RT), but its effect was not consistent across the various treatments and not significant ($P > 0.05$), (Figure 3).

Figure 3. Inhibition of natural infection of tomato fruits by methylene chloride extract of *T. vulgaris* over 30 days.



NC = negative control; ST = solvent treatment; RT = room temperature; FR = refrigeration at 4°C.

Discussion

The present work attempted to use natural products from plants as a control tool to limit postharvest loss of tomatoes. Two key findings were observed. Firstly, four postharvest fungal species were detected, two of which (*F. oxysporum* and *Colletotrichum* spp.) were more frequently isolated. Secondly, the pathogenicity of *F. oxysporum* was demonstrated, and artificial and natural infections of tomato fruits were inhibited using crude extracts of antifungal plants. Based on published literature, this is the first report on the activity of *T. vulgaris* against fungi on tomato postharvest. The present work therefore demonstrated the proof of concept that the antifungal plant *T. vulgaris* is a potential natural control tool for the prevention of tomato rot postharvest, and a possible suitable alternative to synthetic fungicides with less harmful side effects.

Fusarium and *Colletotrichum* spp. were the most likely cause of tomato postharvest loss in the study area which could likely be reduced if the four species detected are effectively targeted by antifungal natural products. Some recent studies have similarly detected and reported on fungal species including *Fusarium* species (Colak and Bicici, 2013; Joshi *et al*., 2013). Considering that fungal isolation was spread out to different sites, the time profile of infection (Figure 1) might reflect the infectivity of each fungus. The five undetected species might have a relatively lower infectivity. The pathological features produced in the pathogenicity test were further evidence that the isolate was *F. oxysporum* and on this basis it was used in subsequent experiments.

The extracts inhibited growth of the fungal isolates against which they have not been extensively tested *in vitro*. This confirms previously reported antifungal activity against other fungi (Centeno *et al*., 2010; Galani *et al*., 2013). Since TV_C demonstrated the highest activity *in vitro* (Table 2), it was further investigated on the possible application as antifungal agent. Though TV_C prevented artificial infection for a relatively short time, the proof of concept was demonstrated for *T. vulgaris* against fungi on tomato fruit postharvest for the first time. The inhibition of postharvest natural infection by TV_C in treated fruits as against total (100%) infection in the negative control further demonstrated the potential control of postharvest loss using antifungal natural products. This preservative and shelf life extension effect of TV_C on fruit was not significant ($P > 0.05$). This could be attributed to multi-factorial causes of tomato damage which include principally several species of fungi and bacteria (Fontem *et al*., 1999; Etebu *et al*., 2013) not all of which are inhibited by the TV_C extract. This could also account for the non-dose dependent effect observed. This further suggests that a cocktail consisting of two or more natural antimicrobial agents with broad spectrum activity targeting different microbes might be required. One study recorded similar results with high *in vitro* activity alongside insignificant non-dose dependent reduction of tomato infection by ethanolic extract of *Rosemarinus officinalis* L. and essential oil of *Origanum vulgare* L. (Ibrahim and Al-Ebady, 2014). On the contrary, in the cited study, ethanolic *T. vulgaris* extract was not active *in vitro* against *Fusarium* spp. and other fungal species probably due to the lower dose used (100 mg/mL versus 200 mg/mL in the present work). In another study, essential oils of O. vulgare L. showed dose-dependent inhibition of tomato inoculated on the surface with *B. cinerea* (Vitoratos *et al*., 2013).

The antifungal activity of crude extracts and essential oils of the plants of interest though previously reported (Centeno *et al*., 2010; Galani *et al*., 2013), has not been extensively investigated *in vivo* on tomato fruit. The findings of the present work therefore provide grounds for a detailed study to fully assess the efficacy, physical, chemical and sensory effects on tomato quality of the crude extract, essential oils and chemical component(s) of *T. vulgaris* as natural alternative antifungal agents for prevention of microbial destruction of tomatoes (Islam *et al*., 2016) and other crops postharvest.

Conclusion

The present work has demonstrated the pathogenicity of isolated fungi in tomato fruits and also shown that *T. vulgaris* crude extract could inhibit natural infection of tomato fruits postharvest caused by fungi. These findings justify further investigation on the potential and suitability of this extract or other products from this plant as an alternative to synthetic chemical fungicides.

Competing interests

The authors declare no competing interests on the present work and its subsequent publication.

Acknowledgement

The work was financially supported by the Special Fund for the Modernisation of University Research in Cameroon, Presidential decree No 2009/121 of 8th April, 2009. The authors acknowledge the University

of Buea (UB) for the resources in the Biotechnology Unit, Faculty of Science. The tomato farmers interviewed in the present work, and Mr. Mbaabe Felix of the Life Sciences Teaching Laboratory, UB, are duly acknowledged for their technical assistance and contribution.

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