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Research Paper

Morphological and molecular identification of fungal species associated with postharvest stem-end rot disease of avocado in Sri Lanka

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Abstract: Avocado (*Persia americana*) is one of the most popular fruits grown in Sri Lanka. Postharvest infections occur wherever the crops are cultivated. Among them, stem-end rot (SER) is the major disease reported. Several fungal species including *Lasiodiplodia* and *Dothiorella*

spp. have been reported to be associated with this disease in regions where avocado is grown. The aims of this research were to identify the fungal pathogens associated with the stem-end rot of avocado in Sri Lanka based on morphological characteristics in combination with molecular and phylogenetic analysis. Diseased avocado fruits were collected from local markets and causal agents were isolated. Colony morphology and characteristics of conidia were observed using phase-contrast microscopy. Sequence analysis was performed using internal transcribed spacers (ITS) of the ribosomal DNA followed by phylogenetic analysis. Four endophytic fungal isolates were identified and designated as SER 01, SER 02, SER 03 and SER 04 which were distinguished respectively as *Lasiodiplodia theobromae*, *Lasiodiplodia hormozganensis*, *Diaporthe nelumbonis* and *Fusarium oxysporum*. To our knowledge, this is the first evidence on the occurrence of *L. hormozganensis*, *D. nelumbonis* and *F. oxysporum* associated with SER of avocado in Sri Lanka. Identified pathogens were proven to be collectively pathogenic to avocado following demonstration of Koch's postulates. The average value of collective disease severity after seven days of inoculation of avocado cv. Pollock was 40% at 28 °C and it always fluctuated between 30% and 45%.

Keywords: *Persea americana*, Stem-end rot, Associated fungal pathogens

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Introduction

Avocado (*Persea americana* Mill.) belongs to the family Lauraceae and is one of the few commercially significant members of the genus *Persea* (Yahia, 2012). Avocado fruit is rich in fat, containing rare sugars with high carbon number and is relatively rich in certain vitamins (Vitamin A, B, C), dietary fiber, antioxidants, minerals such as potassium, phosphorus, magnesium, iron and nitrogenous substances (Yahia, 2012; Bill *et al.*, 2014). Avocado has a high oil content (3–30%) with a low sugar content (about 1%); hence it is recommended as a high energy nutritious food for diabetic patients (Kadam and Salunkhe, 1995). This fruit is consumed in all parts of the world even though it was

originated in tropical regions such as Central America and Southern Mexico (Yahia, 2012). According to market forecast, avocados are commercially produced in Mexico, Chile, Israel, Spain, South Africa, Peru, Kenya, USA and the Dominican Republic. At present, Mexico is the leading producer of avocados in the world followed by Chile and the United States (Whiley *et al.*, 2002). Majority of avocados produced in Mexico are used domestically as they are a staple food in most Mexican households (Hernandez, 2011).

In Sri Lanka, avocado is well-adapted to the wet zone of the low, mid and hill country. Presently, avocado

is successfully grown in Kegalle, Kandy, Matale districts and in certain areas of Bandarawela and Nuwara Eliya. The other potential districts where avocado could be grown are Gampaha, Colombo and Kurunegala. Apart from the wet zone, avocado is becoming popular in the intermediate zone as a home garden crop. Pollock, Hass, Purple, Fuerte, Booth -7, Simmonds and Tower 11 varieties have been recommended by the Variety Release Committee of the Department of Agriculture (Dionysius, 2000).

The highly susceptible nature of the fruit to postharvest diseases, which drastically affects the quality and quantity is a big challenge for the avocado industry (Bill *et al.*, 2014). The postharvest losses of avocado around the world have been estimated as 5-50 %, and anthracnose and stem-end rot (SER) have been reported as the most prominent postharvest diseases (Anonymous, 2016). Stem-end rot disease of avocado has been reported from all major production areas in the world (Hartill, 1991; Menge and Ploetz, 2003) affecting the shelf-life, fruit quality and marketability of the fruit. Postharvest

fungal pathogens, which survive in trees as endophytes are one of the major causes for the postharvest loss of avocado fruits during supply chain. According to Galsurker *et al.* (2018), SER-causing pathogens of avocado have been reported as *Colletotrichum gloeosporioides*, *Alternaria alternata*, *Lasiodiplodia theobromae* and *Botryosphaeria* related species such as *Dothiorella*, *Neofusicoccum* and *Phomopsis*.

To-date, the availability of detailed records on the characterization of SER causing fungal species from avocado fruits in Sri Lanka, using both morphological and molecular aspects is relatively low (Sarananda *et al.*, 2004). Hence, the main objective of the current research was to identify the fungi associated with SER of avocado in Sri Lanka using morphological and molecular characterization. An attempt was made to test the hypothesis, that a group of endophytic fungi are responsible for causing stem end rot disease in avocado in Sri Lanka.

Materials and Methods

Sample collection

Over-ripe avocado fruits of the green varieties; Pollock, Fuerte, Hass (purple) and local ones that displayed symptoms of SER, were collected from local markets in Kandy, Matale, Nuwara Eliya, Matara, Rathnapura, Gampaha and Horana areas in Sri Lanka.

Isolation and morphological identification

Diseased avocado fruit tissues (4 mm²) were cut from margins of SER-affected areas under aseptic conditions at the Fruit Research & Development Institute, Sri Lanka. The tissues were surface sterilized by soaking the sections in freshly prepared NaOCl (0.1% w/v) for 3 minutes (Anthony *et al.*, 2004). After three serial washings in sterile distilled water, avocado tissues were placed on sterile Potato Dextrose Agar (PDA) and incubated at room temperature (28 ± 2 °C). Four fungi isolated were labelled as SER 01, SER 02, SER 03 and SER 04 and were transferred to fresh, sterile PDA plates in order to obtain pure cultures. Pure cultures of fungi were first observed for their colony morphology and then observed for their morphological characteristics using a Phase Contrast Microscope (×400 magnification) (Olympus CX41 model, Tokyo, Japan) at the Department of Plant & Molecular Biology, University of Kelaniya and their

characteristics were compared with previous literature for identification (Ploetz, 2003).

DNA extraction, PCR, sequencing & phylogenetic studies

Total genomic DNA was extracted from seven-day-old pure fungal cultures (SER 01, SER 02, SER 03 and SER 04). Small amount of mycelia (~1 cm²) was taken into an eppendorf tube and 500 µl of DNA extraction buffer was added. Mycelial sample was ground with a sterile pipette tip thoroughly without any large chunks of tissue. Thereafter, phenol:chloroform: isoamyl alcohol (25:24:1, 500 µl) was added and inverted gently until a milkshake-like suspension was formed, which was centrifuged for 15 min at 12 000 rpm to obtain a clear separation. Then the supernatant (300 µl) was transferred in to a fresh eppendorf tube. Subsequently, absolute ice-cold isopropanol was added by 0.6 x volume (0.6 x 300 µl). The content in the tube was gently mixed and the resulting precipitate was centrifuged for 3 min at 12 000 rpm. The precipitate was dried and mixed gently with sterile ultrapure water and stored at -20 °C for further use (White *et al.*, 1990).

Genomic DNA was subjected to PCR amplification (White *et al.*, 1990) using primer pair ITS1 (5'TCC GTA GGT GAA CCT GCG G3') and ITS4 (5'TCC TCC

GCT TAT TGA TAT GC 3'), targeting internal transcribed spacer 1 partial sequence, 5.8S ribosomal RNA gene, internal transcribed spacer 2 complete sequence and 28S ribosomal RNA gene partial sequence at Plant Virus Index Centre, Homagama, Sri Lanka. DNA sequencing was performed at Macrogen (Pvt) Ltd, South Korea. Obtained sequences were manually edited where necessary using MEGA Sequence Alignment Editor

(Version 7.0). Using Basic Local Alignment Search Tool (BLAST), the sequences were compared with the sequences in the National Center for Biotechnology Information (NCBI) database and potential fungal species were identified. Sequences derived from this study were deposited in the GenBank (Table 1).

Table 1. Details of the locally collected stem-end rot associated fungal isolates and the highest match references taxa in NCBI database.

Sample Code	GenBank Accession Number	Highest match with NCBI database	% Identity	Query Cover	Location of previous isolate	Reference
SER 01	MK907912	<i>Lasiodiplodia theobromae</i> (HM466955.2)	100	100%	Malaysia	Sulaiman <i>et al.</i> (2012)
SER 02	MK907913	<i>Lasiodiplodia hormozganensis</i> (JX464077.1)	99	100%	Brazil	Marques <i>et al.</i> (2014)
SER 03	MK907914	<i>Diaporthe nelumbonis</i> (KT821501.1)	98	100%	Taiwan	Chen and Kirschner (2018)
SER 04	MK907915	<i>Fusarium oxysporum</i> (MF187550.1)	100	100%	China	Chai <i>et al.</i> (2018)

For the phylogenetic analysis, potential sequence homologies for the obtained sequences were identified using the BLAST search engine at NCBI. All sequences along with the reference sequences (Table 2) were aligned using MUSCLE alignment tool of the MEGA Sequence Alignment Editor (Version 7.0). *Phaemoniella capensis* (FJ372391.1) from GenBank was chosen as an outgroup according to a previous record on SER of mango (Sanchez *et al.*, 2013). Molecular phylogenetic analysis was performed using MEGA ver. 7.0 phylogenetic analysis software. The maximum likelihood trees

were obtained based on Kimura 2-parameter model (Kimura, 1980) while all alignment gaps were treated as missing data. Initial tree for the heuristic search was obtained using the Nearest-Neighbour-Interchange (NNI) method and default neighbor/BioNJ algorithms. Support for each branch in the inferred trees was evaluated based on bootstrap analysis with 1000 replicates and the best scoring tree was selected. Evolutionary distances were computed using the Maximum Composite Likelihood (MCL) approach in the form of number of base substitutions per site.

Table 2. Details of reference taxa used in the phylogenetic analysis.

Sample Code	Isolate obtained from GenBank	GenBank Number (ITS)	Origin	Query Cover	% Identity	Reference
SER 01	<i>Lasiodiplodia theobromae</i>	HM466955.2	Malaysia	100%	100	Sulaiman <i>et al.</i> (2012)
	<i>Lasiodiplodia theobromae</i>	MF671945.1	Sri Lanka	100%	100	Bandara <i>et al.</i> (2017)
	<i>Lasiodiplodia sp.</i>	JX464074.1	Brazil	100%	99	Marques <i>et al.</i> (2014)
SER 02	<i>Botryosphaeria rhodina</i>	EF423533.1	USA	100%	99	Gilbert and Webb (2007)
	<i>Lasiodiplodia sp.</i>	JX464066.1	Brazil	100%	99	Marques <i>et al.</i> (2014)
	<i>Lasiodiplodia brasiliensis</i>	NR147338.1	Brazil	100%	99	Marques <i>et al.</i> (2014)
	<i>Lasiodiplodia hormozganensis</i>	JX464077.1	Brazil	100%	99	Marques <i>et al.</i> 2014
SER 03	<i>Diaporthe nelumbonis</i>	KT821501.1	Taiwan	100%	98	Chen and Kirschner (2018)
	<i>Diaporthe ceratozamiaie</i>	KU360597.1	Spain	100%	98	Cosoveanu <i>et al.</i> (2016)
	<i>Phomopsis phoenicicola</i>	FJ889452.1	Portugal	99%	97	Santos <i>et al.</i> (2010)
	<i>Diaporthe arecae</i>	KC343032.1	Netherlands	100%	98	Gomes <i>et al.</i> (2013)
SER 04	<i>Fusarium oxysporum</i>	MF187550.1	China	100%	100	Chai <i>et al.</i> (2018)
	<i>Fusarium oxysporum</i>	MF687680.1	Netherlands	100%	100	Geisen (2017)

Development of a disease severity index to monitor SER in avocado

Eight avocado (cv. Pollock) fruits were washed with running tap water to remove dirt and debris followed by sterile distilled water. Cleaned fruits were allowed to drip dry on a laboratory bench for 30 minutes, then placed in a plastic tray at room temperature. One selected fruit showing a gradual development of SER was photographed daily. Diseased area of the fruit in each photograph was estimated by DIGIMIZER (Version 5.3.4) and the disease severity was determined as percentage SER with respect to the total area of the fruit. A disease severity index was prepared using the photographs along with their percentage SER values (Kodituwakku *et al.*, 2018).

Demonstration of Koch's postulates and pathogenicity assessment of fungi isolated from avocado with SER

Disease-free avocado (cv. Pollock) fruits were washed with running tap water followed by sodium hypochlorite (0.1% w/v) solution for surface sterilization. Subsequently, fruits were washed with sterile distilled water and allowed to drip dry for 30

minutes. Four fruits were wounded on four sides at stem end to a depth of 1.5 mm by puncturing them using a sterile pin. Each wound site was inoculated with a 7 mm diameter mycelial plug from the periphery of 7-day old pure cultures of *L. theobromae*, *L. hormozganensis*, *D. nelumbonis* and *F. oxysporum* (isolated from stem end rots of avocado). Fruits were inoculated with a mixture of all fungal isolates to test the collective contribution of four fungal isolates towards the disease, since worldwide SER is reported as a disease complex caused by few pathogens. A set of four fruits inoculated with fresh PDA plugs served as the control. Each set of fruits consisting of four replicates were placed in plastic containers disinfected with ethanol (70% v/v). A piece of sterile cotton wool soaked in sterile distilled water was placed in each container to provide moisture for fungal growth. Containers were incubated at 28 ± 2 °C (Kodituwakku *et al.*, 2018). After the appearance of the disease, pathogens were re-isolated on PDA from the inoculated fruits. Colony and microscopic criteria of the re-isolated fungi were compared with that of the originally inoculated fungi. The experiment was repeated once.

Results and Discussion

Tropical fruit crops are being affected by several postharvest diseases. Infection of fruits by postharvest pathogens can occur before harvest under field conditions or during transit, storage and marketing. Actual disease only occurs when the attacking organism starts to actively grow in the host. Postharvest diseases are caused primarily by pathogenic fungi in tropical regions. Since these diseases are common, it is necessary to identify causative pathogens to develop effective disease control strategies.

Avocado has high economic value; however, in commercial avocado production, postharvest stem-end rot development is a common observation. SER in avocado is generally caused by several pathogenic fungal genera such as *Colletotrichum*, *Alternaria*, *Dothiorella*, *Neofusicoccum* and *Phomopsis* which endophytically colonize the stem during the development of fruit in the field and remain quiescent until the fruit ripening (Galsurker *et al.*, 2018). During the endophytic stage, these pathogenic fungi colonize the vascular system of the fruit stem-end and during the ripening of fruits, the fungi convert themselves to necrotrophic lifestyle, while colonizing the fruit parenchyma, and causing SER (Galsurker *et al.*, 2018). Therefore, the first

visible symptom of SER is a shriveling around the stem base that is followed by discolouration and softening of the epicarp, eventually spreading to the entire fruit (Twizeyimana *et al.*, 2013). During the present study, the SER-affected avocado fruits have been identified according to signs and symptoms which were brown colour rotting patches on the epicarp around the point of attachment to the pedicel. Identification of causative fungal agents have been initially performed by isolation followed by morphological characterization. All together four different fungal pathogens were isolated from avocado fruits. Two types of fungal pathogens were identified from each avocado variety at all times and one of them always had a colony morphology similar to *Lasiodiplodia*. These four fungal strains have been designated as SER 01, SER 02, SER 03 and SER 04.

Colonies of the fungal isolate SER 01 grew rapidly on PDA when incubated at room temperature (28 ± 2 °C) under alternating light and dark, producing grayish colonies with a fluffy aerial mycelium that became dark with age, producing dark brown to black colour pigment (Figure 1A and 1B). Conidia produced by those colonies were initially unicellular, hyaline, granulose, ovoid to ellipsoidal,

and with time attained an average size of $24.4 \times 13.5 \mu\text{m}$. After 7 days, mature conidia became darker, uniseptate, and longitudinally striate (Figure 1C).

The isolate SER 02 grew rapidly on PDA within 3 days at room temperature. Colony on PDA was initially white to smoke-grey, turning greenish-grey on the surface and reverse, becoming dark slate blue with age (Figure 1D). No sporulation was observed under the above incubation condition.

The SER 03 isolate grew rapidly on PDA within 7 days at $28 \pm 2^\circ\text{C}$. Mycelium was initially white, later turning greyish brown in colour showing greyish brown pigmentation on the reverse of the colony. Concentric rings were visible on the surface of mature culture (Figure 1E). Mycelium was branched, septate and hyaline. Unicellular, minute conidia were observed under the above incubation condition.

Figure 1. Colony morphology on PDA of the isolate SER 01 (A), SER 02 (D), SER 03 (E) and SER 04 (F) (Upper surface and lower surface of the culture is shown by images named as U and L respectively). Phase contrast microscopic view of mycelia (B) and conidia (C) of SER 01; mycelia (G), conidia (H), macro conidia (a) and micro conidia (b) of SER 04. (Scale bars: B = $10 \mu\text{m}$, C = $10 \mu\text{m}$, G = $20 \mu\text{m}$, H = $5 \mu\text{m}$).

Colony of isolate SER 04 appeared white initially and turned pale salmon pink colour and cottony showing a violet colour on maturity (Figure 1F). Two types of spores were observable on conidiophores, namely macroconidia and microconidia. Microconidia were fusiform, allantoid, aseptate and straight or slightly curved. Macroconidia were hyaline, two to several celled, sickle shaped, mostly with an elongated apical cell and pedicellate basal cell (Figure 1 - G and H).

Accordingly, when the morphological characters were matched with those of previously published records, the isolates SER 01 and SER 02 matched with *Lasiodiplodia* sp. (Alves *et al.*, 2008) while SER 03 and SER 04 could be identified as *Diaporthe* sp.

(Udayanga *et al.*, 2011; Danggomen *et al.*, 2013) and *Fusarium* sp. (Ploetz, 2003) respectively.

Traditional identification and characterization of these SER pathogenic species have relied on cultural and morphological characteristics. However, these criteria are not always sufficient for an accurate and reliable differentiation among different fungal strains. High chance of overlapping of morphological characters and phenotypes among species makes identification difficult, especially up to species level of the organism. Hence use of molecular tools and techniques have become reliable and an effective way for more precise identification of fungi.

As the initial step of molecular authentication, DNA extraction followed by sequencing of ITS region using ITS1 and ITS4 primers have been performed. Resultant DNA fragments were about 503 bp, 531 bp, 497 bp and 476 bp, respectively, for isolates SER 01, SER 02, SER 03 and SER 04. The results of BLAST confirmed that the isolate SER 01 corresponded to *L. theobromae* (HM466955.2) showing a 100% sequence similarity while SER 02 was 99% similar to *L. hormozganensis* (JX464077.1). Further, the isolate SER 03 has presented 98% identity to *Diaporthe nelumbonis* (KT821501.1) where, the isolate SER 04 showed 100% identity with *Fusarium oxysporum* (MF187550.1) (Table 1).

Lasiodiplodia theobromae has frequently been identified as a SER-associated endophytic pathogen from fruits such as avocado (Sarananda *et al.*, 2004; Garibaldi *et al.*, 2012), mango (Sanchez *et al.*, 2013; Sathya *et al.*, 2017) and papaya (Netto *et al.*, 2014) from Mexico, Brazil and India, while *L. hormozganensis* has been associated with stem-end rot disease of papaya (Netto *et al.*, 2014) and mango (Marques *et al.*, 2013). However, *L. hormozganensis* has not been reported so far as a fungus associated with SER of avocado in Sri Lanka or elsewhere. Moreover, the first and only report about endophytic *D. nelumbonis* is from leaves of lotus (*Nelumbo nucifera*) in Taiwan (Chen and Kirschner, 2018) and has not been reported on any fruit to date. *Diaporthe spp.* such as *D. foeniculina*, *D. sterili* and *D. radis* have been reported from SER of avocado fruits by several researchers previously (Guarnaccia *et al.*, 2016; Torres *et al.*, 2016). Therefore, this is the first record of identification of *D. nelumbonis* from avocado fruits in Sri Lanka.

According to BLAST results, *F. oxysporum* is also one of the causative agents of avocado stem-end rot. This result supports the preliminary identification of the same fungus made through cultural and morphological characters during this study. A previous study by Philippine research group has reported *F. oxysporum* as non-pathogen of avocado SER while some other species such as *F. solani*, *F. equiseti* and *F. moniliforme* have been reported as SER pathogens (Suratos, 2005). Furthermore, BLASTn results are partially compatible with the information gathered by Galsurker *et al.* (2018) as *L. theobromae* is a genus included in *Botryosphaeriaceae*, and *Diaporthe* is a synonym to *Phomopsis*. However, based on the previous reports

from California, New Zealand and Italy, the most common avocado SER-associated fungi were *C. gloeosporioides*, *N. luteum*, *N. parvum* and *Phomopsis spp. (Diaporthe spp.)* (Hartill, 1991; Twizeyimana *et al.*, 2013; Guarnaccia *et al.*, 2016).

Phylogenetic analysis was performed to further identify the evolutionary relationship and to establish the phylogenetic position of each isolate by comparing with the GenBank reference sequences. The isolates and references from the GenBank have clustered in three clades: Clade *Lasiodiplodia*, Clade *Fusarium* and Clade *Diaporthe* (Figure 2). In the clade *Lasiodiplodia*, both SER 01, SER 02 and all reference sequences have clustered together with 100% bootstrap support without showing any speciation event. This can be due to the lack of information in nucleotide sequences to obtain an evolutionary difference or branching pattern in between those sequences. SER 03 isolate clustered with known isolates of *Diaporthe sp.* with a bootstrap support of 76%. However, the isolate could not be identified as a defined species since four different *Diaporthe spp.* were clustered together in that clade. At the same time, in the clade *Fusarium*, SER 04 clustered with *F. oxysporum* with a 100% bootstrap support by defining the isolate up to its species level, even though the analysis could not be used to define the species of other three isolates (SER 01, SER 02 and SER 03). However, phylogenetic analysis has proven the genus level of all four isolates as obtained from both morphological characterization and BLASTn results. This further demonstrates a clear relationship between isolates according to their evolutionary patterns.

Disease severity indices for fresh produce are important for assessing the extent of damage caused by a particular disease in order to develop suitable control strategies. By comparing the fruits subjected to pathogenicity assessment with disease severity index (DSI) data of SER pathogens on cv. Pollock (Table 3), the average value of disease severity (DS) after seven days of inoculation was noted as 40% and this value always fluctuated between 30% and 45%. This information gathered during research and DSI prepared (Figure 3) could be used in the future by agriculturists to assess the extent of damage caused to avocado (cv. Pollock) fruits by SER pathogens during pathogenicity assessment.

Figure 2. The maximum likelihood tree showing the relationships of fungal isolates with the reference sequences obtained from the NCBI data base based on the ITS region,. Numbers on branches are bootstrap values >50% in 1000 replicates. The isolate FJ372391.1 (*Phaeomoniella capensis*) was used as an out-group.

Figure 3. The disease severity index (DSI) prepared to monitor stem-end rot development in avocado (cv. Pollock).

Table 3. Details of percentage SER values on each fruit after mixed inoculation of SER associated fungal pathogens.

No of fruit	SER % (after 7 days of inoculation)
1	30
2	45
3	45
4	30-40
5	30
6	45
7	30-40
8	45

The combination of all pure cultures of fungal isolates identified as *L. theobromae*, *L. hormozganensis*, *D. nelumbonis* and *F. oxysporum* were pathogenic to avocado (cv. Pollock), resulting in visible lesions after the re-inoculation of test fruits while all the control fruits inoculated only with PDA were healthy. The symptoms included brown colour lesions with mycelial growth initiating around the inoculation sites (Figure 4).

Comparing with the DSI prepared at the laboratory, at the time of inoculation, the disease severity was 0% indicating the healthiness of fruits. After four days of inoculation, the disease severity had been increased to 12% and at the 7th day of inoculation it was increased up to 45% in certain fruits showing the collective pathogenicity contribution of the four fungal isolates. The respective four fungal species were re-isolated from the diseased avocado fruits and their morphological characteristics were identical to the original pure cultures establishing the pathogenicity of fungal strains isolated from SER affected avocado. Meanwhile, SER disease severity

on cv. Hass in California has been described by Twizeyimana *et al.* (2013). According to this research, Botryosphaeryaceae isolates of *N. luteum* and *N. parvum* caused the highest stem-end rot severity (84.4% and 77.3% respectively) and *Phomopsis sp.* the lowest disease severity (18.8%) after 12 days of inoculation at approximately 25 °C and >80% relative humidity.

In comparison to above reported data, we could assume that the isolate *L. theobromae* and *L. hormozganensis* in the fungal mixture used for the re-inoculation in this study (which were always isolated from over ripe avocado) could have a high SER virulency on cv. Pollock since these two isolates are members of Botryosphaeryaceae family and the isolate *D. nelumbonis* could have a low virulency on same cultivar since *Diaporthe* is a type of *Phomopsis sp.* However, the SER severity on cv. Pollock during the present research was lower when compared to SER severity on cv. Hass reported by Twizeyimana *et al.* (2013).

Figure 4. Stem-end rot in avocado (cv. Pollock) after inoculation with a mixture of isolate of *Lasiodiplodia theobromae*, *L. hormozganensis*, *Diaporthe nelumbonis* and *Fusarium oxysporum*. (A) Inoculation of test fungi. (B) Development of SER on fruit after four days of inoculation (C) Development of SER on fruit after seven days of inoculation

Conclusion

The present work is the first comprehensive study of fungi associated with avocado stem-end rot in Sri Lanka. A new SER disease complex with an average

disease severity of 40% was identified including four fungal pathogens as *Lasiodiplodia theobromae* (MK907912), *L. hormozganensis* (MK907913), *Diaporthe nelumbonis* (MK907914) and *Fusarium oxysporum* (MK907915).

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