

Morphological, phylogenetic and pathogenicity characterisation of *Fusarium* species associated with wilt disease of pumpkin (*Cucurbita pepo* Linnaeus)

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Abstract

Fusarium is a well-known soil-borne fungus where most species belonged in this genus is prominently phytopathogenic. Nevertheless, this pathogenic species has affected the production of pumpkin worldwide. This study underlines the morphological, phylogeny and pathogenicity characteristics of *Fusarium* for a better disease-control strategy. Twenty-six *Fusarium* isolates were collected from wilt infected pumpkin in various locations of Peninsular Malaysia. From the combinations of morphological and molecular identifications, four species were identified as *F. oxysporum* (2 isolates), *F. solani* (4 isolates), *F. proliferatum* (7 isolates) and *F. incarnatum* (13 isolates). Microscopic and macroscopic observation visualized distinct characteristics of the identified *Fusarium* species. Sequence analyses of *tefla* and β -*tub* genes inferred by maximum likelihood tree resulted in distinct section-specific characteristics. Meanwhile, pathogenicity test of *Fusarium* isolates presented by the seed inoculation produced various degrees of severities. *Fusarium solani* C2526P recorded the highest severity of 93.8% after 30 days of post inoculation (dpi). Symptoms have been identified as early as 10 dpi producing stunted growth of the plants. On the other hand, *Fusarium oxysporum* D2532P recorded 85.3% disease severity. Pathogenic *Fusarium* caused stunted growth, chlorosis, wilting and necrosis especially at the root of pumpkin plants. This study provides valuable information and methods to manage wilt infected pumpkin in the future.

Keywords: *Fusarium* wilt, *Cucurbita pepo*, Phylogeny, Pathogenicity

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Introduction

Pumpkin (*Cucurbita pepo* Linnaeus) belongs to the Cucurbitaceae family. Since the last five years, an average of 2.29% of pumpkin has been produced out

of total main crops in Malaysia (MOA, 2018). Previously known as the most variable species in the kingdom of plant, this crop is enthusiastically traded for the multifunction of seed oil (Ferriol et al., 2003; Stevenson et al., 2007; Medjakovic et al., 2016).



Commonly, pumpkin is consumed as food and applied in ethno-medicinal applications in several countries such as China, Mexico and India (Alarcon-Aguilar et al., 2002; Aggarwal and Kotwal, 2009). However, this highly demanded crop is being attacked by various fungal infections mainly in the field.

Fusarium wilt disease is recognized as a yield-limiting factor in various cucurbit productions. This disease can be perceptible by stunting, yellowing of lower leaves, progressing wilting, defoliation, necrosis of the vascular tissue and death of plants (Chehri et al., 2011; Caroline and Olubukola, 2013; Redda et al., 2018). The most important pathogens causing the disease are *Fusarium oxysporum* and *Fusarium solani*. These pathogens could survive in the soil for several years in a form of chlamydospores (Callagan et al., 2016) and have become the main limiting factor in managing disease dissemination. Thus, characterization of pathogenic *Fusarium* is essential for integrating disease management to limit the extension of its host range.

Several pathogenic species in the genus *Fusarium* are specialized with respect to their host specificity formerly known as formae speciales (*f. sp.*) (Snyder and Hansen, 1940). This specialization was recognized as the physiological capabilities of *Fusarium* strains onto one or a few species of plant. The pathogenic species is considered arduous to be controlled due to several aspects such as its ability to produce resistance structures, resistance to fungicides, manipulation on host defence responses and the ability to produce mycotoxins (Van Dam et al., 2016; Moreno-Velandia, et al., 2019). However, several species belong in the genus were considered saprophytic while others pathogenic. Identification and recognition of the primary invader could reduce the infection risks.

Therefore, the objectives of this study were to isolate and identify *Fusarium* species associated with wilt-infected pumpkin and to ascertain the pathogenicity test of the isolated *Fusarium* species.

Material and Methods

Fungal isolation

Pumpkin was obtained from five various locations throughout Peninsular Malaysia including Maran and Cameron Highland in Pahang, Tok Bali in Kelantan, Tanjung Karang in Selangor and Tangkak in Johor. Infected leaves and fruits were chosen for fungal isolation and cut into pieces of 1 cm x 1 cm. Infected

tissues were soaked in 0.5% sodium hypochlorite (NaOCl) (Chlorox, Oakland, USA) and rinsed twice with sterile distilled water (Liu et al., 2017). Tissues were dried using sterile filter paper and placed on peptone pentachloronitrobenzene agar (PPA) (Sigma-Aldrich, Missouri, USA) followed by incubation for 5 days (Summerell et al., 2003). The cultures were purified by streak plate technique onto potato dextrose agar (PDA) and incubated for 5 days at 28 ± 2 °C (Leslie and Summerell, 2006).

Morphological characterisation

Morphological characterisation was divided into microscopic and macroscopic observations on each isolate. For microscopic examination, cultures were grown on carnation leaf agar (CLA) (Leslie and Summerell, 2006) and incubated for 7 days at 28 ± 2 °C. Matured culture was observed under light microscope CX2Li (Olympus, Tokyo, Japan). With the same observation method, *Fusarium* cultures grown on water agar (WA) were also examined. Characters such as the conidia size, shape and number of septate, presence of chlamydospore, phialide and hypha were observed and recorded (Leslie and Summerell, 2006). For macroscopic examination, cultures were grown on PDA for 7 days at 28 ± 2 °C. Macroscopic characters included in this study were pigmentation, colony features and presence of sporodochia.

DNA extraction

Fusarium isolates were grown on PDA for 5 days (28 ± 2 °C). With micropipette tip, the mycelia of the culture were scratched prior to DNA extraction. The genomic DNA was extracted by Ultra Clean® Microbial DNA isolation kit (MO-BIO, Carlsbed, CA, USA) according to the procedures by manufacturer.

Amplification of *tef1a* and β -tubulin genes

The DNA fragments were amplified by T100™ Thermal Cycler (Bio-Rad, California, USA). The 20 μ L reaction mastermix (PROMEGA, Madison, WI, USA) consists of 4 μ L of 5x Green *GoTaq* buffer, 2 μ L of 0.2 mM deoxynucleotide triphosphate (dNTPs), 2 μ L of 0.2 mM magnesium chloride (MgCl₂), 0.1 μ L of *Taq* Polymerase, 1 μ L of DNA template and 8.9 μ L nuclease free water. One μ L of 0.1 mM TEF primer set containing EF1 (5'-ATGGGTAAGGAGGACAAGAC-3') and EF2 (5'-GGAAGTACCAGTGATCATGTT-3') (Geiser et al., 2004) and beta-tubulin primer set comprising T1 (5'-



AACATGCGTGAGATTGTAAGT-3) and T2 (5'-TAGTGACCCTTGGGCCAGTTG-3') (O'Donnell and Cigelnik, 1997) were used. *Tefla* gene was amplified under following cycles; initial denaturation at 94 °C for 90 s, 35 cycles of denaturation at 95 °C for 35 s, annealing at 57 °C for 55 s, extension at 72 °C for 90 s and final extension at 72 °C for 10 min. The PCR program of beta-tubulin was held through the following cycles; initial denaturation at 94 °C for 1 min, 35 cycles of denaturation at 94 °C for 35 s, annealing at 58 °C for 2 min, extension at 72 °C for 1 min, final extension at 72 °C for 10 min and soaked at 4 °C (Kumar et al., 2016).

Gel electrophoresis

Amplified DNAs were detected by gel electrophoresis. A 100 bp ladder was loaded into 1.2% agarose gel (Promega Corporation, USA) containing 20 mL of Tris-Borate-EDTA (TBE) (Hafizi et al., 2013). The gel was stained with fluorosafe dye (1st Base Company, First Base Laboratories Sdn. Bhd. Seri Kembangan, Selangor, Malaysia). Gel integration was captured by G:BOX Syngene under ultra violet (UV) radiation.

Nucleotide sequencing and phylogenetic analysis

PCR products were submitted to MyTACG Bioscience Company, Malaysia, for purification and sequence analysis. The nucleotide sequence dataset of *tefla* and β -*tub* was aligned by ClustalW using Molecular Evolutionary Genetics Analysis 7.0 (MEGA). Alignments were manually modified to exclude all ambiguous sections from analysis. Basic Local Alignment Search Tool (BLAST) gene polymorphism analysis in comparison with databases from National Centre of Biotechnology Information (NCBI) resulted in related genus species including *F. incarnatum* NRRL 31160, *F. oxysporum* NRRL 25369, *F. proliferatum* NRRL 53578 and *F. solani* FMR 8021 (Otero-Colina et al., 2010; Azor et al., 2007) as references for the alignment. *Aspergillus niger* CBS513.88 was used as an outgroup. Maximum likelihood (ML) was inferred to a phylogenetic tree displaying support value of more than 80% (Watanabe et al., 2011).

Conidial suspension

A total of 26 *Fusarium* isolates were cultured on PDA at 28 ± 2 °C for 5 days prior to inoculum preparation. Matured cultures were added with 10 mL sterile distilled water followed by tender scratch on the

filamentous mycelia using sterile micropipette tip. Fungal inoculum of 200 mL was prepared to a final concentration of 2x10⁶ conidia/ mL as adopted from Chehri et al. (2011). The mycelial sheets and residues of the media were filtered using sterile cheese cloth and transferred into a 500 mL conical flask.

Seed inoculation

The seeds of pumpkin var. Gold Butter (Green World Genetics Sdn. Bhd., Kuala Lumpur, Malaysia) were sterilised according to Zhang et al. (2012) by soaking into 10% sodium hypochlorite (NaOCl) and rinsed twice with sterile distilled water. Sterilised seeds were then inoculated by soaking into 200 mL fungal conidia suspension for 12 hours in an incubator shaker at 100 rpm in 30±1 °C (Sukanya and Jayalakshmi, 2017). Inoculated seeds were sown into 1 kg soil containing a mixture ratio of (3:2:1 = topsoil: manure: river sand) pre-autoclaved according to Mahmood et al. (2014). Plant treatments were performed in randomised complete design (RCD) in the UPM Glasshouse. Each isolate was prepared with 12 plant replicates and grown within 12/12 hrs at 32±1 °C days and 28±1°C nights with humidity of 72% for 30 days. Plant physiological parameters and disease severity index (DSI) were calculated followed by data collection.

Table-1: Disease scales for Fusarium wilt assessment.

Scales	^a Inference
0	Seed germinated, no symptoms of wilt.
1	Seed germinated, wilt symptoms, 1-24% of leaves showing slight chlorosis.
2	Seed germinated, wilt symptoms, abnormal growth with 25-49% of leaves showing chlorosis and/or curvature.
3	Seed germinated, wilt symptoms, abnormal growth with 50-74% of leaves wilting, chlorosis and/or limited necrosis.
4	Seed not germinated or seed germinated with ≥75% of the leaves showing wilt symptom.

^awilt symptoms - wilting, severe stunting and necrosis with premature defoliation that often result in the death of plants.

Disease assessment and data analysis

The cultivation was observed for 30 days of post inoculation (dpi). The progress of symptoms appearance was carried out every 5 days. After 30 dpi, the emergence of any particular wilt symptoms was assessed according to disease scale (Table 1) conducted by Schoonhoven and Pastor-Corrales



(1994) as well as Raupach et al. (1996) with slight modifications.

The disease severity index was calculated for each isolate according to the parameters in the disease scale (Mwaniki et al., 2011).

$$DSI = \frac{\sum (A \times n)}{\sum (B)} \times 100$$

A: Disease scales

n: Number of plants in specific scale

B: total number of plants

The significant disease severity among isolates was tested by the analysis of variance (ANOVA) followed by Duncan's multiple test (p<0.05) from Statistical Package for Social Sciences (SPSS).

Results

Morphological characteristics

A total of 26 isolates were recovered from Maran and Cameron Highland in Pahang, Tok Bali in Kelantan, Tanjung Karang in Selangor and Tangkak in Johor. Five species were identified as *F. oxysporum* (2 isolates), *F. solani* (4 isolates), *F. proliferatum* (7 isolates) and *F. incarnatum* (13 isolates) based on morphological characteristics according to synoptic keys for species identification.

Morphologically, all species presented primary characters, which include macroconidia, microconidia and chlamydospore. All 26 isolates were observed microscopically and macroscopically; this includes size, shape and number of septate of the macroconidia, microconidia and chlamydospore, position of conidia, pigmentation and colony features. Chlamydospores are produced in all *Fusarium* species colonies except for *F. proliferatum* (Table 2).

Table-2: Microscopic and macroscopic morphologies of *Fusarium incarnatum*, *F. proliferatum*, *F. solani* and *F. oxysporum* isolate isolated from infected-wilt disease of pumpkins

Fungal species	<i>Fusarium incarnatum</i>	<i>Fusarium proliferatum</i>	<i>Fusarium solani</i>	<i>Fusarium oxysporum</i>
Number of isolates	13	7	4	2
List of isolates	C2520P, C2522P, C2523P, C2524P, C2525P, C2527P, C2528P, C2529P, C2531P, D2533P, D2535P, D2536P, D2537P	C2521P, B1781P, J1789P, J1790P, J1791P, J1792P, J1793P	C2526P, C2530P, D2534P, B1782P	D2532P, C1788P
Microscopic characters;				
Macroconidia	Size of 29.14 -24.44 µm, wide width at the centre to slender ends, 3-5 septate.	Size of 30.73 – 70.02 µm, most straight and linear shape, 3-5 septate.	Size of 27.61-30.92 µm, almost straight curvature with rounded ends, 3-4 septate.	Size of 30.43 – 52.17 µm, almost straight curvature with rounded ends, 3-4 septate.
Microconidia	Size of 5.02– 10.11 µm, ellipsoidal with or without septate.	Size of 5.76 - 10.12 µm, long-chained microconidia.	Size of 10.22 – 20.09 µm, kidney-shaped with some is multinucleate.	Size of 6.08 – 15.14 µm, usually ellipsoidal shaped.
Chlamydospore	Size of 5.16 – 12.05 µm, usually triple-chained cell at the intermediate hypha.	absent	Size of 6.13 – 11.18 µm, Single and pair cells usually located at terminal of hypha, hold by long monophialide.	Size of 8.22 – 12.17 µm, single cell usually at intercalary of hypha, round-shaped.
Macroscopic characters;				
Pigmentation	White to cream and brown.	White to cream and violet.	White to cream.	White to pale violet and sometimes pale brown.
Colony features	Rapidly grown. Very thick, cotton-like and floccose aerial mycelia.	Sparse, floccose and not so thick.	Sparse and thin.	Floccose, sparse and abundant of aerial mycelia, cotton-like.



All species shared a variety of macroconidia shape with slightly curved and tapered towards each ends. The apical end is elongated especially for *F. proliferatum* macroconidia. The basal end is where the conidia were attached and it is slightly short. *Fusarium oxysporum* and *F. solani* shared similar features of macroconidia, but *F. solani* has bigger in size. *Fusarium proliferatum* has the only microconidia observed in chain on CLA as early as 4 days of incubation, whilst other species present detach and individual microconidia. CLA is a substrate medium produced by sterilized carnation leaf-pieces that is particularly useful in uniform conidia identification purpose (Nelson et al., 1983).

Among all the species, *F. incarnatum* colonies grown most rapidly on PDA by displayed thick and cottony aerial mycelia. As the colony grown matured of 5 days old, the pigmentation produced on media could be observed. The colours are initially white and changes as it grown matured. Even though, colonies produced quiet similar pigment but feature and thickness of the aerial mycelia differ among species. The pigmentation

produced in the media facilitates morphological distinction between species and served as the initial identification. However, the identification based on morphological characteristic was somehow affected by genotype and environmental conditions, which led to instability and complication in species identification. Thus, the identification of *Fusarium* could achieve a better accuracy as molecular identification is applied upon morphological speciation.

Phylogenetic analysis

Prior to the construction of presented phylogenetic tree, individual dataset of a single locus was first generated and found to be incongruence to each other. Apart from that, similar dataset constructed did not resolve the high confidence value as high as the stated bootstrap support value. PCR amplification of *tef1 α* and *β -tub* genes registered a single fragment on agarose gel electrophoresis ranging from 645 to 918 bp and 500 to 693 bp in size (Figure 1, Table 3).

Table-3: BLASTn analysis of all 26 isolates associated with wilt disease in pumpkins.

Isolates	Location (State, city)	Species name	BT		TEF	
			Sequence length (bp)	Accession no.	Sequence length (bp)	Accession no.
C2520P	Maran, Pahang	<i>F. incarnatum</i>	578	MK527240	668	MK519232
C2521P	Maran, Pahang	<i>F. proliferatum</i>	565	MK527241	649	MK519233
C2522P	Maran, Pahang	<i>F. incarnatum</i>	581	MK527242	670	MK519234
C2523P	Maran, Pahang	<i>F. incarnatum</i>	580	MK527243	670	MK519235
C2524P	Maran, Pahang	<i>F. incarnatum</i>	571	MK527244	810	MK519236
C2525P	Maran, Pahang	<i>F. incarnatum</i>	582	MK527245	661	MK519237
C2526P	Maran, Pahang	<i>F. solani</i>	518	MK527246	674	MK519238
C2527P	Maran, Pahang	<i>F. incarnatum</i>	576	MK527247	663	MK519239
C2528P	Maran, Pahang	<i>F. incarnatum</i>	584	MK527248	660	MK519240
C2529P	Maran, Pahang	<i>F. incarnatum</i>	564	MK527249	665	MK519241
C2530P	Maran, Pahang	<i>F. solani</i>	520	MK527250	679	MK519242
C2531P	Maran, Pahang	<i>F. incarnatum</i>	590	MK527251	662	MK519243
D2532P	Tok Bali, Kelantan	<i>F. oxysporum</i>	568	MK527252	677	MK519244
D2533P	Tok Bali, Kelantan	<i>F. incarnatum</i>	573	MK527253	669	MK519245
D2534P	Tok Bali, Kelantan	<i>F. solani</i>	509	MK527254	679	MK519246
D2535P	Tok Bali, Kelantan	<i>F. incarnatum</i>	575	MK527255	666	MK519247
D2536P	Tok Bali, Kelantan	<i>F. incarnatum</i>	567	MK527256	649	MK519248
D2537P	Tok Bali, Kelantan	<i>F. incarnatum</i>	571	MK527257	662	MK519249
B1781P	Tanjung Karang, Selangor	<i>F. proliferatum</i>	554	MK527258	680	KT211607
B1782P	Tanjung Karang, Selangor	<i>F. solani</i>	514	MK527259	695	KT211615
C1788P	Cameron Higland, Pahang	<i>F. oxysporum</i>	461	MK527260	670	KT211602
J1789P	Tangkak, Johor	<i>F. proliferatum</i>	563	MK527261	681	KT211609
J1790P	Tangkak, Johor	<i>F. proliferatum</i>	565	MK527262	677	KT211610
J1791P	Tangkak, Johor	<i>F. proliferatum</i>	564	MK527263	678	KT211611
J1792P	Tangkak, Johor	<i>F. proliferatum</i>	558	MK527264	675	KT211612
J1793P	Tangkak, Johor	<i>F. proliferatum</i>	558	MK527265	678	KT211613



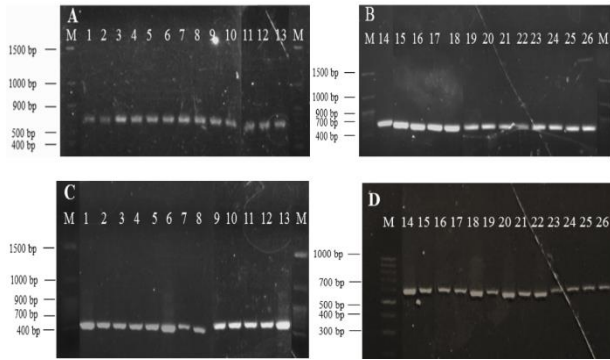


Figure-1: Banding pattern of DNA fragments. A and B: *tef1a* region, C and D: *β-tubulin* region. Lane 1-13: (C2520P, C2521P, C2522P, C2523P, C2524P, C2525P, C2526P, C2527P, C2528P, C2529P, C2530P, C2531P, D2531P), lane 14-15: (D2532P, D2533P, D2534P, D2535P, D2536P, D2537P, B1781P, B1782P, C1788P, J1789P, J1790P, J1791P, J1792P, J1793P) and M: 100 bp markers.

Based on phylogenetic analysis of combining both genes, four major clades were generated. The first clade comprises four isolates of *F. solani*. Clade II contains seven isolates of *F. proliferatum*. Clades III and IV consist of two isolates of *F. oxysporum* and 13 isolates belong to *F. incarnatum*, respectively. All isolates belong in the first clade were not diverged in nucleotide substitution represented by the horizontal branch compared to the reference sequence (Figure 2).

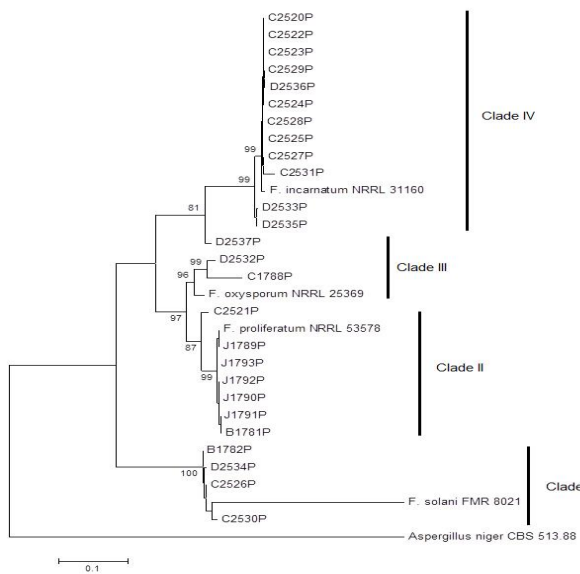


Figure-2: Maximum likelihood trees of *Fusarium* isolates and related species inferred from the combination of *tef1a* and *β-tub*

Clade I consisted of isolate with highly virulent and pathogenic *F. solani* to pumpkin. Meanwhile, Clade II represents the highest number of moderate virulent of *F. proliferatum* on pumpkin. Clade IV represented the most isolates identified as non-virulent by displaying no observable symptoms on pumpkin plants up to 30 dpi. Based on the phylogenetic topology, it could be inferred to as an indication of *Fusarium* virulence through evolutionary time.

The use of *tef1a* and *β-tub* sequences into molecular identification has brought sufficient information on *Fusarium* sp. identification. The Maximum Likelihood tree based on *tef1a* and *β-tub* genes indicated that the species in each clade has a close relationship with each other. *Fusarium solani* was diverged into a polyphyly relationship from any other species.

Pathogenicity test

This study reveals that pumpkin plants inoculated with *Fusarium* isolates produced some levels of disease scale. A total of 26 isolates produced various degree of disease severities on the plants. Within a period of 30 dpi, the pathogenesis of *Fusarium* species recorded as sufficient duration in order to cause disease invasion besides of pumpkin planting challenges in the cultivation as it requires spacious area for sprawling vines.

Pathogenic *Fusarium* infected pumpkin plants rapidly. The symptoms were observed on the plants as early as 10 dpi by *F. solani* C2526P. The first symptom appeared as stunted growth. The plants inoculated with *F. solani* C2526P presented no progression of stem height and leaves area.

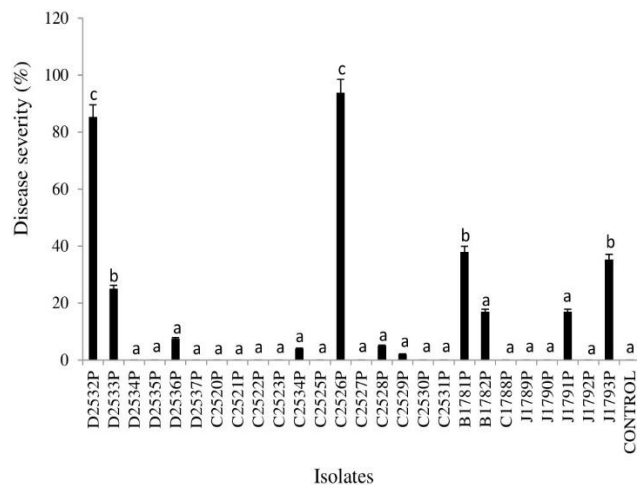


Figure-3: Disease severities of *Fusarium* isolates inoculated on pumpkin on 30 dpi



This isolate recorded the highest disease severity of 93.8% followed by *F. oxysporum* D2532P with disease severity of 85.3% (Figure 3). Three of *F. proliferatum* isolates; B1781P, J1791P and J1793P were identified as moderate virulent. All these three isolates produced average percentages of disease severity of 38.0%, 17.0% and 35.3%, respectively. Fifteen isolates were identified as non-pathogenic with no observable symptoms produced.

The symptoms were initially produced by the stunted growth of the plants. Infection starts progressing with the appearance of chlorosis on the lower leaves in which the leaves were wilted and crumpled. Cross section of the primary root structures displayed necrosis after 30 dpi. The lateral roots or the root branches were reduced on the infected plants (Figure 4) compared to the control plants. *Fusarium* infection is generally limited to the shoot area, but mainly at the root area. Therefore, severe symptoms can be observed at the root cortex.



Figure-4: Pumpkin roots and plants on 30 dpi. A and F: dH₂O (control), B and G: *F. oxysporum* D2532P, C and H: *F. solani* C2526P, D and I: *F. proliferatum* J1793P, E and J: *F. incarnatum* C2522P on 30 dpi

Discussion

Molecular identification has provided various dependable outputs of *Fusarium* identification compared to morphological characterisation. Previously, several markers including protein-coding regions and nuclear ribosomal DNA (rDNA) (Liu et al., 2015; Sanders and Rodriguez, 2016; Kusai et al., 2018; Turrini et al., 2017) were used and resulted in a high quality species identification especially for a

closely related species. Protein coding genes have showed a rapid nucleotide substitution rate and subsequently high resolution for closely related species or among conspecific strains (Watanabe et al., 2011). This study presented disagreement of morphological and molecular characterisation, which resulted in opposition to a study on *Fusarium* species by Trabelsi et al. (2017) in Tunisia.

F. solani, *F. oxysporum* and *F. proliferatum* have been previously reported to cause wilt disease in most cucurbit plants including pumpkin (Chehri et al., 2011; Najihah et al., 2017; Perez-Hernandez et al., 2017; Rezaee et al., 2018). Once the pathogen is present at the root surface, it penetrates through natural openings and grows into the root cortex. After then, the infection progresses and colonises the xylem vessels. By this stage, it is ready to invade the upper ground structure of the plants (De Sain and Rep, 2015). *Fusarium* sp. secretes a plethora of effectors that enhance colonisation in xylem vessels. These effectors are named Six (secreted in xylem) proteins that are internalised into plant cells (Francisco et al., 2018).

The extension of *Fusarium* wilt infection caused severe and dead pumpkin plants. The presence of mycelia on the soft infected stem can be observed as a severe symptom of infection. There are several factors that contribute to the severity of *Fusarium* infection. These include the environmental condition, irrigation (Chehri et al., 2011), climatic conditions (Zhang et al., 2014) temperature and oceanic air (Czembor et al., 2015).

Conclusion

The identification of *Fusarium* comprising morphological and phylogenetic analyses has revealed better species identification especially for the complex structure of taxonomic like *Fusarium*. A single piece of infected pumpkin plant has been seen inhabited by various species of *Fusarium*. These species displayed a wide degree of disease severities. *Fusarium solani*, *F. oxysporum* and *F. proliferatum* are the most important pathogens of wilt disease in pumpkin. From this study, a better *Fusarium* wilt management could be achieved by accessing the information on the factors affecting the pathogenesis of pathogenic *Fusarium*. Any biological and physiological factors of pumpkin such as growth rate should be examined upon *Fusarium* invasion.

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