

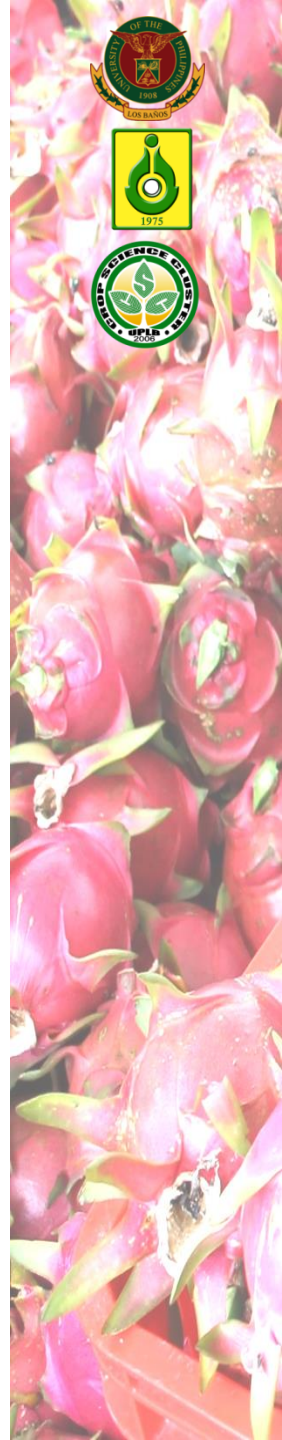
ETIOLOGY OF DESTRUCTIVE DRAGON FRUIT FUNGAL PATHOGENS IN THE PHILIPPINES BY CONVENTIONAL AND MOLECULAR TECHNIQUES

Cecilia B. Pascual
Jamie Ann B. Tumolva
Rizalina L. Tiongco



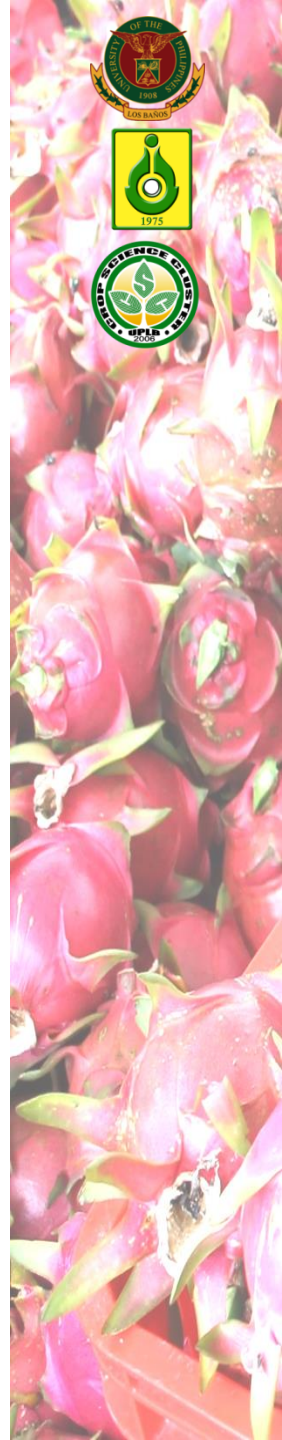
Dragon Fruit

- Dragon cactus (*Hylocereus spp.*), locally known as “saniata” (a lovely maiden derived from its beautiful flowers), is an introduced crop in the country.
- The first commercial variety in the Philippines was grown in 1992 in 6 ha farm of a Taiwanese businessman in Tambong Balagbag, Indang, Cavite.
- commonly grown now in Cavite, Ilocos region, Davao, Laguna, Batangas, Nueva Ecija, Nueva Viscaya and Bataan among other places.
- Cultivation in the Philippines started in small scale in early 90's and the areas expanded into about 300 hectares ranging from backyard, small and medium scale production.



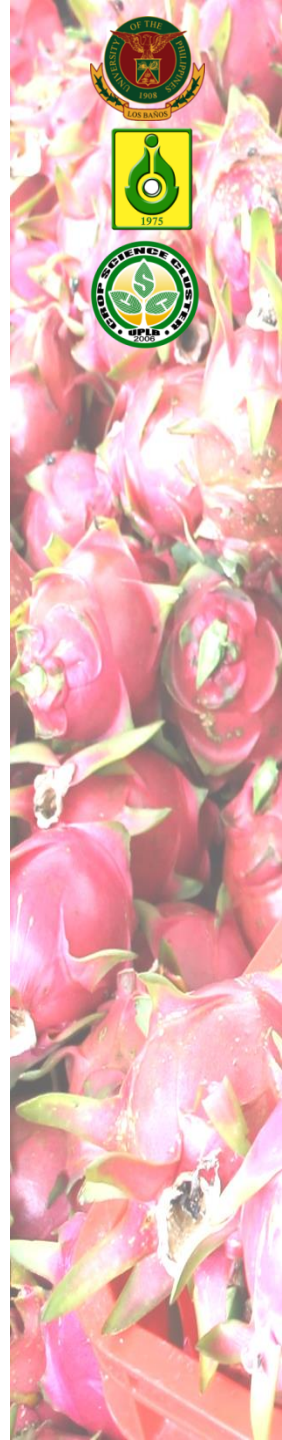
Dragon Fruit

- popular across the Philippines due to its claimed health benefits and commercial value
- white dragon fruit has anti-proliferative agent for cancer cells while the red ones has a potential use in the cosmetic industry due to its high tyrosinase inhibition activity and %radical scavenging activity (College of Medicine, UP Manila,2016)
- Major constraints were identified such as low yield, prevalence of insect pests and **DISEASES**, short shelf life of fruits, no standardization of fruit quality, no continuous supply of fruits, problems on marketing among others.



Objective

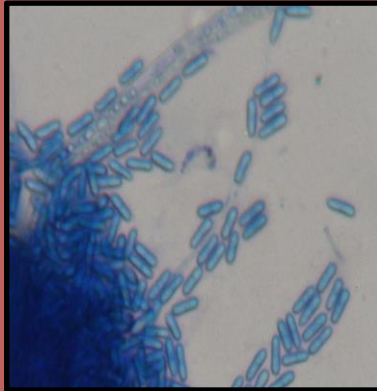
- To diagnose and identify the causal organisms of collected disease specimens of dragon fruit in growing areas by phenotypic and molecular assays.



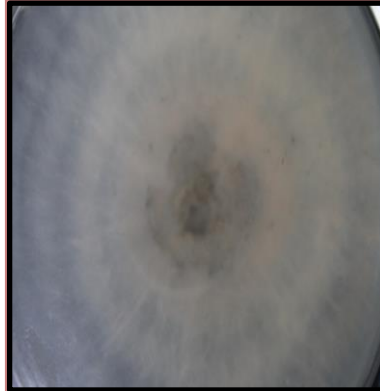
Methodology



**Sample
Collection**



**Microscopic
Examination**



Isolation



**Pathogenecity
test**



Methodology

Fungal DNA Extraction



Polymerase Chain Reaction

Using species specific primer and primer from ITS rDNA region



Sequencing and homology test



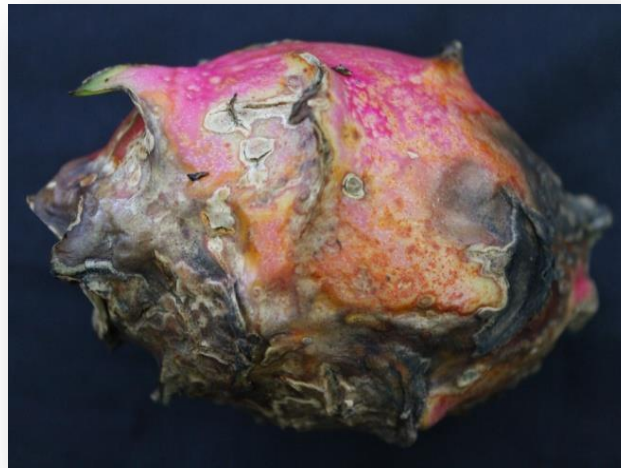
Results

Pathogen 1: Causes stem canker



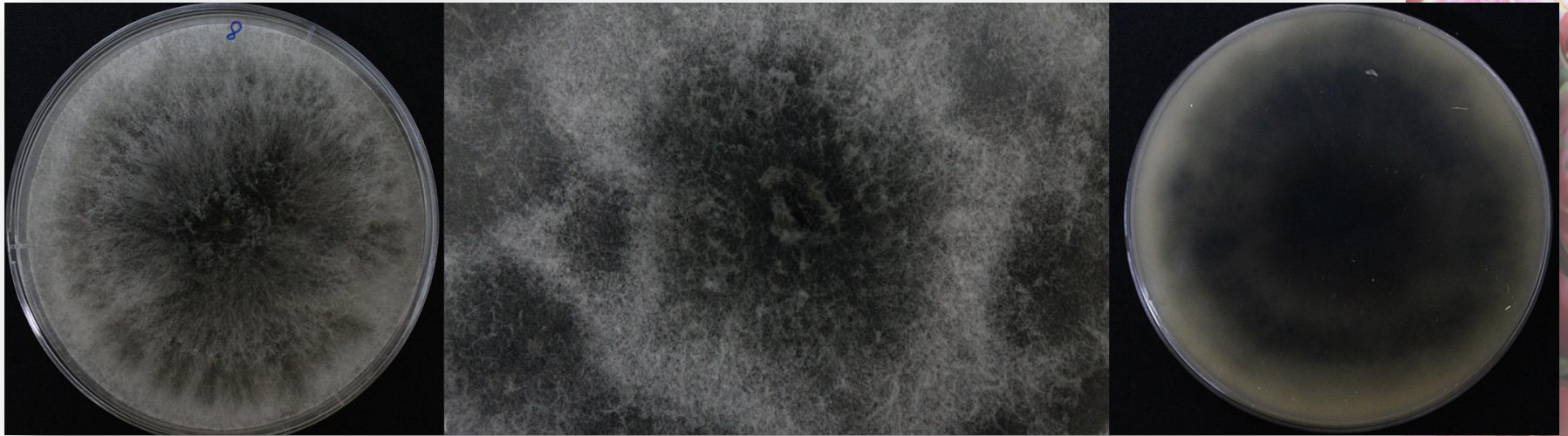
Results

Pathogen 1: Fruit canker

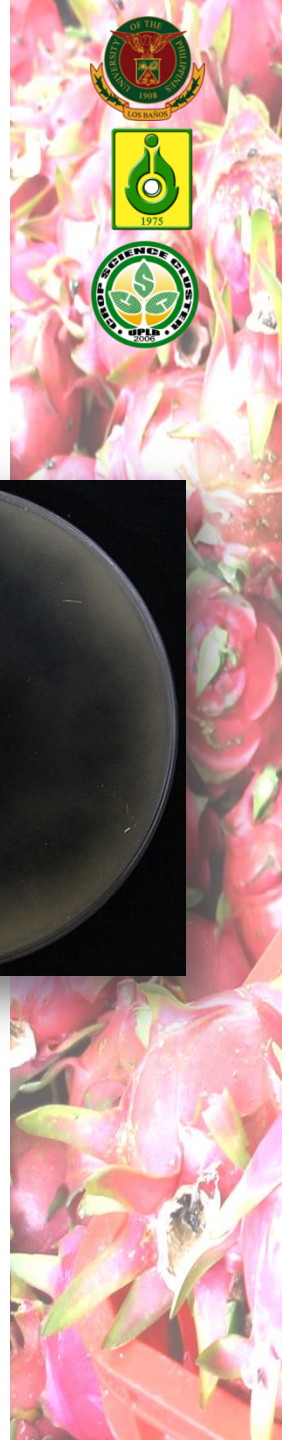


Results

Pathogen 1: Cultural growth

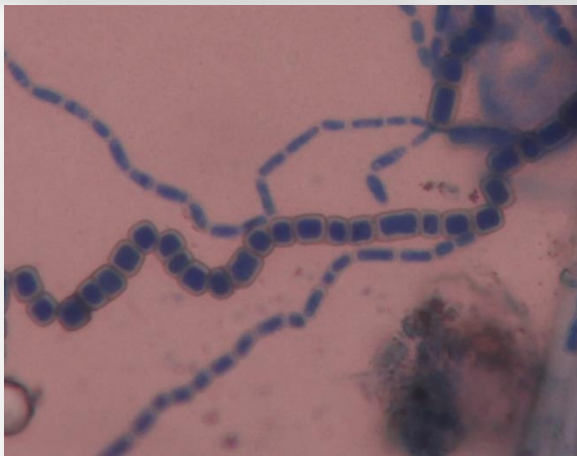
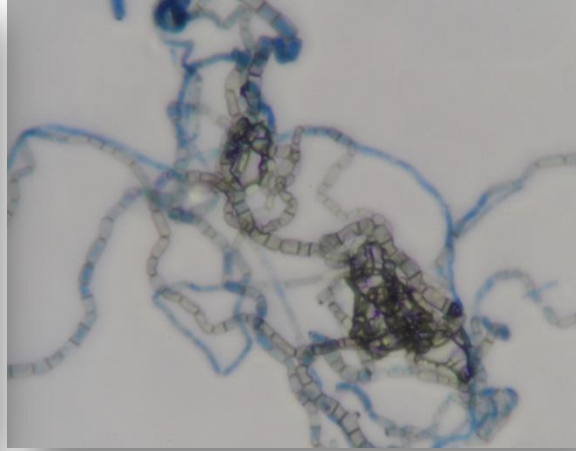
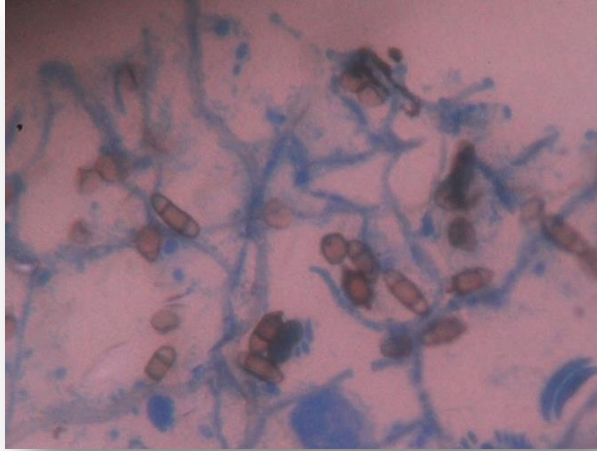


Fungal isolate 1 on PDA plate producing gray to black aerial mycelia



Results

Pathogen 1: Morphological characteristic



Microscopic examination of isolate 1 on 40x magnification: (upper left) pycnidial conidia observed on fruit , young arthroconidia grown in PDA (upper right) and mature arthroconodia (below).



Results

Pathogen 1: *Neoscytalidium dimidiatum*

- First report causing stem canker on stem and fruit
- Reported in different Asian countries like Taiwan, Vietnam, Thailand, Malaysia and China where growers import planting materials.
- Most prevalent now-a-days, even in the market.
- On other countries, mango is also a host of this pathogen causing rot, canker and die-back on fruit (Ray et.al 2010)



Results

Pathogenicity test



Neoscytalidium dimidiatum
Inoculated dragon fruit stem at 12
DAI (Days after inoculation) causing
stem canker





PCR using ITS 1 (TCC GTA GGT GAA CCT GCG G) and ITS 4 (TCC TCC GCT TAT TGA TAT GC) region

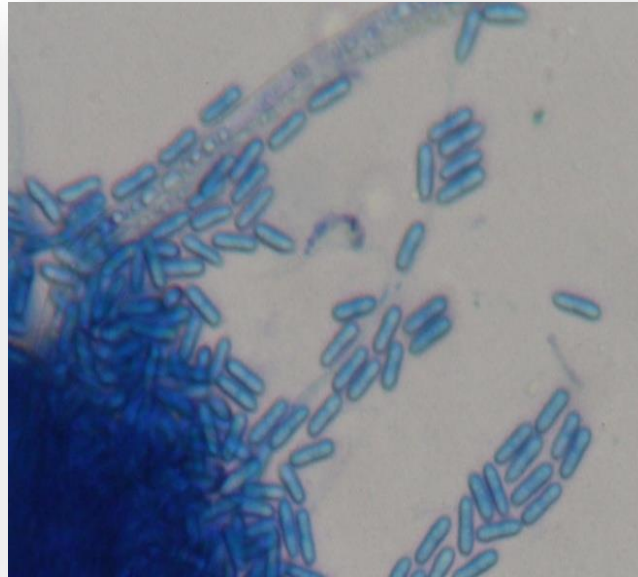
Description	Max score	Total score	Query cover	E value	Ident	Accession
Neoscytalidium dimidiatum strain HLN5 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequen	822	822	98%	0.0	94%	KF612310.1
Neoscytalidium dimidiatum 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, cor	819	819	98%	0.0	94%	JX524168.1
Neoscytalidium dimidiatum isolate BWH-TS1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcri	817	817	98%	0.0	94%	JX473739.1
Neoscytalidium dimidiatum 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, cor	815	815	96%	0.0	94%	KF000372.1
Neoscytalidium dimidiatum isolate Nd3 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed s	815	815	96%	0.0	94%	HQ439174.1
Neoscytalidium dimidiatum voucher ZJHLG-1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcr	813	813	96%	0.0	94%	KF812550.1
Neoscytalidium dimidiatum strain HLN4 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequen	813	813	96%	0.0	94%	KF612309.1
Neoscytalidium dimidiatum strain HLN2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequen	813	813	96%	0.0	94%	KF612308.1
Neoscytalidium dimidiatum strain HLN1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequen	813	813	96%	0.0	94%	KF612307.1

Neoscytalidium dimidiatum
 Query cover : 98%
 Similarity: 94%



Results

Pathogen 2: Anthracnose on stem



Stem Anthracnose of dragon Fruit (A) Brown lesion on stem of dragon fruit and (B) hyaline conidia.



Results

Pathogen 2: Cultural growth



Fungal isolate 2 on PDA plate producing whitish mycelia
with orange masses of conidia in concentric rings





PCR using species-specific CgInt and ITS 4

	Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/>	Colletotrichum gloeosporioides isolate FJCG-1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal	762	762	86%	0.0	99%	gi 725812549 KM463758.1
<input type="checkbox"/>	Fungal sp. strain 2 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, compl	756	756	86%	0.0	99%	gi 1043540065 KX271284.1
<input type="checkbox"/>	Colletotrichum fruticola isolate LJ20 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcri	756	756	86%	0.0	99%	gi 918567040 KP748211.1
<input type="checkbox"/>	Colletotrichum gloeosporioides isolate OORC5 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal	755	755	86%	0.0	99%	gi 333123090 JF710560.1
<input type="checkbox"/>	Glomerella cinquilata isolate P099 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer	755	755	85%	0.0	99%	gi 126131233 EF423544.1
<input type="checkbox"/>	Colletotrichum sp. T21 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S	753	753	86%	0.0	99%	gi 952937560 KT351620.1
<input type="checkbox"/>	Colletotrichum gloeosporioides strain CG30 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete	753	753	86%	0.0	99%	gi 347550450 JF796300.1
<input type="checkbox"/>	Colletotrichum gloeosporioides isolate CrP8 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete	753	753	86%	0.0	99%	gi 498922934 KC920834.1
<input type="checkbox"/>	Colletotrichum gloeosporioides strain CrP3 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribe	753	753	86%	0.0	99%	gi 456371619 KC503498.1

	Score	Expect	Identities	Gaps	Strand
	762 bits(844)	0.0	428/432(99%)	0/432(0%)	Plus/Plus
Query 1	GATAACAACCTCTCTGATTTAACGACGTTTCTTCTGAGTGGTACAAGCAAATAATCAAAAC 60				
Sbjct 123	GATAACAACCTCTCTGATTTAACGACGTTTCTTCTGAGTGGTACAAGCAAATAATCAAAAC 182				
Query 61	TTTTAACACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGT 120				
Sbjct 183	TTTTAACACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGT 242				
Query 121	AATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAG 180				
Sbjct 243	AATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAG 302				
Query 181	CATTCTGGCGGGCATGCCTGTTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTTGGTGTG 240				
Sbjct 303	CATTCTGGCGGGCATGCCTGTTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTTGGTGTG 362				
Query 241	GGGCCCTACAGCTGATGTAGGCCCTCAAAGGTAGTGGCGGACCTCCCGGAGCCTCCTTT 300				
Sbjct 363	GGGCCCTACAGCTGATGTAGGCCCTCAAAGGTAGTGGCGGACCTCCCGGAGCCTCCTTT 422				
Query 301	GCGTAGTAACCTTTACGCTCGCACTGGGATCCGGAGGGACTCTTGCCGTAACCAACCCCA 360				
Sbjct 423	GCGTAGTAACCTTTACGCTCGCACTGGGATCCGGAGGGACTCTTGCCGTAACCAACCCCA 482				
Query 361	ATTTTCAAAGGTTGACCTCGGATCAGGTAGGAATACCGCTGAACCTAAGCATATCATA 420				
Sbjct 483	ATTTTCAAAGGTTGACCTCGGATCAGGTAGGAATACCGCTGAACCTAAGCATATCATA 542				
Query 421	AAGCCGGAGGAA 432				
Sbjct 543	AAGCCGGAGGAA 554				

Colletotrichum gloesporioides
Query cover :86%
Similarity :99%



Results

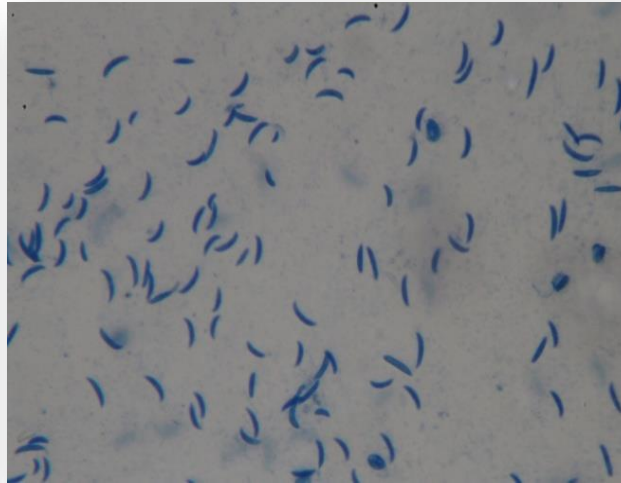
Pathogen 2: *Colletotrichum gloesporoides*

- Reported on Malaysia (Masratul Hawa et al. 2008; Masyahit et al. 2009; Suzianti et al. 2014)., and China.



Results

Pathogen 3: Anthracnose on stem



Stem Anthracnose of dragon Fruit (A) Brown lesion on stem of dragon fruit and (B) hyaline conidia of *C. truncatum*.



Results

Pathogenicity test



C. truncatum inoculated dragon fruit stem
at 12 DAI (Days after inoculation) observed
having minor symptoms of anthracnose



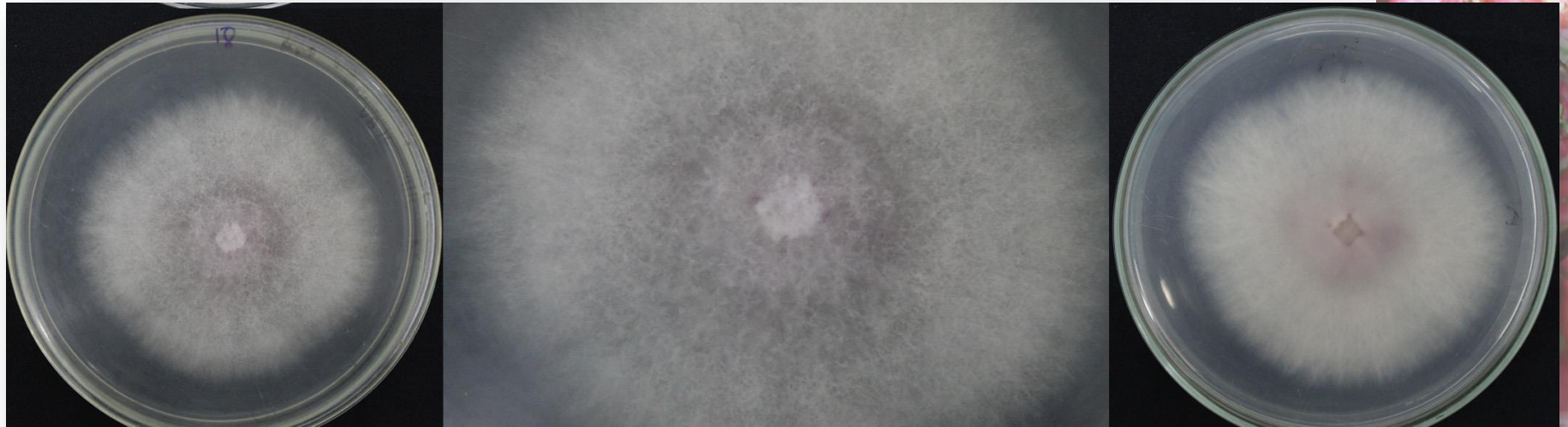
Results

Pathogen 4: Stem rot



Results

Pathogen 4: Cultural growth

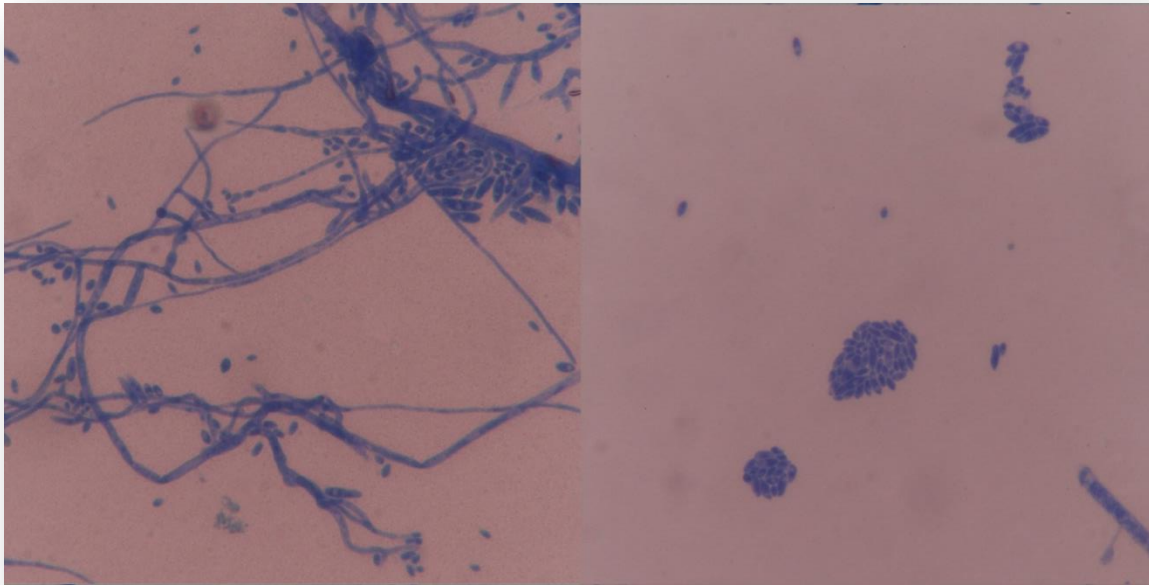


Isolated *Fusarium* spp., on PDA plate producing whitish mycelia with pink to purple pigmentation



Results

Pathogen 4: Morphological characteristic



Microscopic examination of *Fusarium* spp., on 40x magnification: (left) microconidia formed from monophialides and microconidia in clusters



Results

Pathogenicity test



Fusarium spp., inoculated dragon fruit stem at 12 DAI (Days after inoculation) observed having minor





PCR using genus EF1 and EF2

	Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/>	Fusarium oxysporum f. sp. radicis-lycopersici strain MN-0724 translation elongation factor 1 alpha gene, partial sequence	1146	1146	97%	0.0	99%	gi 297660735 HM057324.1
<input type="checkbox"/>	Fusarium oxysporum isolate 4511 translation elongation factor 1-alpha gene, partial cds	1146	1146	97%	0.0	99%	gi 165967732 EU246567.1
<input type="checkbox"/>	Fusarium oxysporum partial tef1a gene for translation elongation factor 1 alpha, strain LEMM 110231	1144	1144	96%	0.0	99%	gi 809278141 LN828010.1
<input type="checkbox"/>	Fusarium oxysporum voucher RIFA 92-R1 translation elongation factor 1 alpha gene, partial cds	1144	1144	98%	0.0	99%	gi 699059049 KF624780.1
<input type="checkbox"/>	Fusarium oxysporum isolate Fo95024 translation elongation factor 1 alpha (EF-1alpha) gene, partial cds	1144	1144	96%	0.0	99%	gi 512764477 KC622305.1
<input type="checkbox"/>	Fusarium oxysporum f. sp. radicis-lycopersici strain HE-0610 translation elongation factor 1 alpha gene, partial sequence	1144	1144	96%	0.0	99%	gi 297660739 HM057328.1
<input type="checkbox"/>	Fusarium oxysporum f. sp. radicis-lycopersici strain CL-0601 translation elongation factor 1 alpha gene, partial sequence	1144	1144	96%	0.0	99%	gi 297660738 HM057327.1
<input type="checkbox"/>	Fusarium oxysporum strain GXR-1 translation elongation factor 1-alpha (tef) gene, partial cds	1142	1142	97%	0.0	99%	gi 1042811027 KX253982.1
<input type="checkbox"/>	Fusarium oxysporum isolate PG translation elongation factor 1-alpha gene, partial cds	1142	1142	97%	0.0	99%	gi 959098666 KP964878.1
<input type="checkbox"/>	Fusarium oxysporum strain CPO 3.011 translation elongation factor 1-alpha gene, partial cds	1142	1142	97%	0.0	99%	gi 943577903 KR935895.1
<input type="checkbox"/>	Fusarium oxysporum strain CPO 3.010 translation elongation factor 1-alpha gene, partial cds	1142	1142	97%	0.0	99%	gi 943577888 KR935894.1

Fusarium oxysporum
 Query cover : 97%
 Similarity : 99%



Conclusion

- Dragon fruit in the Philippines was infected with several economically important diseases namely anthracnose (*C. gloesporoides* and *C. truncatum*) , stem canker (*N. dimidiatum*) and stem rot (*Fusarium spp.*).
- No published paper on the study on plant pathogens affecting dragon fruit has been reported. This is the first report on the occurrence these three diseases caused by 4 distinct pathogens.





Thank you!