

Phytophthora disease on durian

- In Vietnam, Dang Vu Thi Thanh *et al* (2004) reported that *Phytophthora palmivora* caused a wide range of diseases in durian.
- The integrated management of *Phytophthora* diseases of durian has been reported by Guest *et al* (2004).
- The bioassay method was used on durian to evaluate the susceptibility of different durian varieties to *Phytophthora* (Verma and Anwar, 2000).



Recently, Problem of quick decline on Durian





Dark brown trips in
xylem and the hole
due to beetle

***Phytophthora citricola*: New finding
pathogen on durian in Vietnam, control
models and preliminary results on varieties
screen against *Phytophthora* spp.**

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2. Materials and Methods

Materials

- Ready made questionnaires
- Equipment for isolation: Laminar flow, incubator, autoclave, microscope, Petri disks, tubes, micro pipet, etc.
- Media used: WA, PDA, PCA, V8,...
- Samples: infected trunk parts, roots from diseased trees.
- Rootstocks: Kho qua xanh, Sua hat lep Chin hoa, La Queo vang, Chuong bo, D6, Ri6 and Chanee.

Methodology

Survey on current situation of quick decline disease on durian at Tiengiang, Vinhlong and Lam Dong provinces: Survey was conducted following the methods of McMaugh (2005), Dang Vu Thi Thanh and Ha Minh Trung (1999).

Observation for spores color and morphology: The isolated spores and mycelia were observed under the light microscope based on the method written by Nguyen Van Tuat (1997) and classification by Donalt and Olaf (2005) for *Phytophthora* and *Pythium*.

***Koch's postulates for confirmation of the pathogen:** The experiment was conducted by CRD on Kho qua xanh variety with 8 treatments: (I) *Fusarium* + *Xyleborus* sp.; (II) *Phytophthora* sp. + *Xyleborus* sp; (III) *Phytophthora* sp. + *Fusarium* sp. + *Xyleborus* sp; (IV) *Phytophthora* sp.+ *Fusarium* sp; (V) Only *Xyleborus* sp; (VI) Only *Phytophthora* sp.; (VII) *Phytophthora* sp. + *Phytophthora palmivora* and (VIII) control, five replicates were used per treatment, one tree each replicate.

* **Sequence of 28S rRNA of *Phytophthora* sp.**

- Cultures of *Phytophthora* sp were sent to NK-BIOTEK lab, at Hochiminh city for sequencing using 28S rRNA gene.
- Using BLAST N software for comparison of *Phytophthora* sp DNA sequence with other relative sequences from gene bank for identification of species.
- **Determination of temperature range for development of *P. citricola*.**
- **Demonstration:** Six demonstrating sites were conducted in six different orchards from three districts at Lamdong province using integrated disease management and the control as farmer method.

* **Screening for tolerant rootstock to *Phytophthora* spp.**

- The experiment was **complete random designed** with 6 treatments of **six cultivars** eg. such as Sua hat lep Chin hoa, La queo vang, Chuong bo, D6, Ri6 and Chanee, four replicates each and three seedlings per replicate. The test was followed the method described by O'Gara *et al* (2004).
- **Inoculum preparation:** cultures of *Phytophthora citricola* and *Phytophthora palmivora* were prepared on PCA medium at 7 days in advance for inoculation.
- **4 months old** seedlings were inoculated (10^6 spores/ml) and then kept in the net house for symptoms development.
- Data recorded at 3 weeks after inoculation:
 - Disease Severity (%)
 - Diameter of the lesion (cm)
- All the data was analysed by SPSS software (version 13.0).

3. Results and discussion

** Survey on current situation of quick decline on durian at Tiengiang, Vinhlong provinces*

Location	Variety	Knowlegde on disease (%)	Diseased orchards/ surveyed Orchards (%)	Related to beetle	Treatment
Ngu Hiep - Cailay	Kho Qua Xanh	- Yes: 54.5 - No: 45.5	38.2	Beetle appear	Cut off the tree
	Chuong bo	- Yes: 12.4 - No: 87.6	16.0	Yes	Cut off the tree, plant new tree
Tam Binh - Cailay - TienGiang	Mon thong	- Yes: 10.3 - No: 89.7	28.6	Yes	Remove diseased part & paste with chemical
	Sua hat lep Chin Hoa	- No: 100.0	16.0	Yes	Remove diseased part & paste with chemical
Tam Binh- Vinh Long	Kho Qua Xanh	- Yes: 25.0 - No: 75.0	32.4	Yes	Cut off the tree
Long Ho – Vinh Long	Kho qua Xanh	- Yes: 13.3 - No: 86.7	22.6	Yes	Remove diseased part & paste with chemical

- In Lam Dong province, the survey in 2012 shown that the ratio of patch canker and quick decline infection were high respectively (21.8; 81.14 %).

Many farmers still have not known how to control it, it ranged from 40 to 77% number of interviewed growers from different locations.

The other ones used Agrifos 400, Aliette, Coc 85 (copper) and Ridomyl (Metalaxyl) for controlling of the disease, but less effective.

Field observation on susceptibility of varieties

Table 3: Result on field surveyed orchards

Location	Variety	Diseased tree/ surveyed trees (%)	Disease severity (%)
Ngu Hiep - Cailay	Kho Qua Xanh	73.3	80.3
	Chuong bo	32.7	30.4
Tam Binh-Cailay -TienGiang	Mon thong	49.1	40.1
	Sua hat lep Chin Hoa	36.3	29.7
Tam Binh-Vinh Long	Kho qua Xanh	67.7	63.2
Long Ho – Vinh Long	Kho qua Xanh	65.2	55.4

Results of pathogen isolation from quick decline trees
Table 4. Fungi isolated from diseased parts of durian trees (%)

Collected location		<i>Phytophthora</i> sp.	<i>Phytophthora</i> <i>palmivora</i>	<i>Fusarium</i> sp.
Tiengiang (Cai lay district)	Ngu Hiep	51.43	62.34	33.17
	Tam Binh	48.60	43.59	38.76
Vinh Long	Tam Binh	55.25	49.78	57.41
Lam Dong	Dahuoai	66.00	83.00	76.00

Results of pathogen isolation from beetles

Table 5: Frequently appearance of fungi isolated from beetle *Xyleborus* sp. (%) (2012)

Location	<i>Fusarium</i> sp.	<i>Phytophthora</i> <i>palmivora</i>	<i>P. sp.</i>	Other
Da Huoai	66.40	53.30	64.04	33.6
Di Linh	78.20	65.00	56.20	45.8
Bao Lam	40.50	30.00	42.50	19.3

Table 6: Koch's postulations of different fungi on Monthong variety under net house conditions (SOFRI, 2012)

Treatment	Dis. severity (%)		Length of lesion (cm)		Symptoms expression
	7 DAI	14 DAI	7 DAI	14 DAI	
Fus + Xyl	32.0c	38.7cd	1.8bc	4.637d	Dry black lesion, leaves started with yellow and dropped off at 7 days after inoculation, holes due to <i>Xyleborus</i> presented.
Phyt + Xyl	59.7ab	89.7a	3.4ab	10.7a	Long dry dark brown lesion with brown trips along the stem, leaves fall, holes due to <i>Xyleborus</i> presented.
Phyt+ Fus+ Xyl	33.3b	69.7b	1.9bc	8.4ab	Long dry dark brown lesion with brown trips along the stem, leaves fall, holes due to <i>Xyleborus</i> presented.
Phyt + Fus	79.7a	43.7cd	4.5a	4.9cd	Long dry dark brown lesion with brown trips along the stem, leaves fall, water soaking on the lesion in the morning.
Only Xyl	37.7b	22.7d	1.3c	2.7d	Only holes due to <i>Xyleborus</i>
Only Phyt	90.3a	63.0bc	5.1a	7.5bc	Long dry dark brown lesion with brown trips along the stem, leaves fall.
Phyt + Phy –pal	40.0b	38.3cd	2.3bc	4.6d	Dry dark brown to black lesion, with brown trips along the stem, leaves fall.
Control	-	-	-	-	Wound dry and started to heal after 7 days
CV (%)			57,26	51,77	

Note:

Fus + Xyl : Fusarium sp. + Xyleboruss sp.

Phyt + Xly: Phytophthora sp + Xyleborus sp.

Phyt + Fus+ Xyl: Phytophthora sp + Xyleborussp. + Xyleborus sp

Phyt + Fus : Phytophthora sp. + Fusarium sp.

Phyt + Phy –pal: Phytophthora sp + Phytophthora palmivora

Leaves fall, holes on the trunk of the seedling
and brown strips



Characteristics of *Phytophthora* sp.

- The **mycelium** developed directly on medium like cotton at the centre of the disk where it was inoculated.
- **Sporangial morphology:** There were ellipsoid to pear shape sporangia with unclear or without papilla, the ratio of long and wide of sporangium was 2:1.
- They had **round and smooth zoospores**.
- The sporangial morphology of *Phytophthora* sp. was the same as what described by Donalt and Olaf (2005) for *Phytophthora citricola* such as ellipsoid to pear like sporangium, unclear papilla and mycelium like cotton on medium.



Sequence of 28S rRNA gene of *Phytophthora* sp.

- The sequences of 28S rRNA gene of *Phytophthora* sp. revealed 184 bp rDNA sequence and when we made BLAST SEARCH from the gen bank on NCBI, the result revealed the *Phytophthora* sp. rDNA was identity with that of *Phytophthora citricola* up to 99%. The identities with *P. citricola* started bp of 640 to bp of 824 and only one bp different at bp number 816 (disappear). Results as following

```
Query    1      GGT TGG GACT GAG GTG CCT ACA ACG TGC TTT TGA GTG GG TTT GTG TCT CCG TGT GCG CCG      60
          |||
Sbjct    640    GGT TGG GACT GAG GTG CCT ACA ACG TGC TTT TGA GTG GG TTT GTG TCT CCG TGT GCG CCG
          699

Query    61      TGT GCG GAT AGC TTG CTAT GCG TGT GTG GTT GTG TGT GG ATG GAT GCG GGC CTT AACT TG
          120
          |||
Sbjct    700    TGT GCG GAT AGC TTG CTAT GCG TGT GTG GTT GTG TGT GG ATG GAT GCG GGC CTT AACT TG
          759

Query    121     TCG CCG TTC GGG ACG TTG ACG AAA TGG AGC GAT CCG ACC CGT CTT GAA ACAC GGA C-AAG
          179
          |||
Sbjct    760     TCG CCG TTC GGG ACG TTG ACG AAA TGG AGC GAT CCG ACC CGT CTT GAA ACAC GGA CCAAG
          819

Query    180     GAG TC      184
          ||||
Sbjct    820     GAG TC      824
```

- Therefore, the results from **sporangial morphology**, **Koch's postulates** and **rDNA sequence** of 28S rRNA gene of *Phytophthora* sp. has **confirmed** that the **quick decline** of durian in Tiengiang, Vinhlong and Lamdong provinces caused by *Phytophthora citricola*.
- This is an important pathogen on citrus trees at many different regions of the world, including Vietnam (Dang Vu Thi Thanh and Ngo Vinh Vien, 2004). Jung and Burgess (2009) reported that this fungus developed and caused much damage at temperature of 25°C, it had wide host range of 39 woody trees including coffee and cacao, our result showed that *P. citricola* has a new host that is durian.

*** Optimum temperature for development of *P. citricola***
Table 7: Development of *P. citricola* at different temperature ranges

Temp. (°C)	Mycelium diameter of <i>P. citricola</i> after inoculation (cm)					
	24 h	48 h	72 h	96 h	120 h	144 h
15	0.82 d	2.07 d	2.46 d	4.07 c	5.26 d	6.25 c
20	1.01 d	2.22 cd	2.70 d	4.68 bc	6.17 d	7.33 ab
25	1.33 c	2.54 bcd	3.04 cd	5.62 ab	8.02 a	8.50 a
26	1.41 c	2.84 bc	3.45 bc	6.13 a	7.34 abc	8.50 a
28	1.60 bc	2.92 b	3.40 c	5.12 b	6.61 bc	8.50 a
30	1.86 ab	3.55 a	4.05 ab	5.46 ab	7.42 abc	7.97 a
35	2.08 a	4.08 a	4.58 a	5.61 ab	7.84 ab	8.34 a
40	1.36 c	2.34 bcd	2.84 cd	3.16 d	3.92 e	4.30 d
CV(%)	20.38	22.94	19.73	18.54	19.52	17.59

Note:- In one column, those numbers have the same character is not significant different under Duncan test.

Table 8: Response of some durian varieties against *Phytophthora* spp. under net house conditions

Varieties	<i>P. citricola</i>		<i>P. palmivora</i>	
	Disease severity (%)	Lesion Diameter (cm)	Disease severity (%)	Lesion Diameter (cm)
Sua hat lep Chin				
Hoa	44.59 c	1.44 c	57.10 ab	1.12
La queo vang	61.24 b	2.07 b	37.58 c	1.31
Chuong bo	44.74 c	2.47 b	50.15 b	1.40
D 6	83.64 a	3.19 a	66.01 a	1.46
Ri 6	59.97 bc	2.65 ab	59.97 ab	1.65
Chanee	71.83 ab	2.59 ab	29.74 c	1.43
Significant different	*	*	*	ns
CV%	16.74	17.08	15.67	22.61

Note: - In one column, those numbers have the same character is not significant different under Duncan test.

Demonstration sites for *Phytophthora* sp. Disease management on durian at Lamdong province



Treated the tree base with lime, Agrifos injection



Lime and fertilizer application



Effective control of *Phytophthora* sp. using integrated disease management on durian in comparison with the control

Data to be recorded	Time to record	DaHuoai							
		Farm 1		Farm 2		Farm 3		Farm 4	
		Control	Demo	Control	Demo	Control	Demo	Control	Demo
Ratio of gummosic symp. (%)	Before	66.55	65.02	67.05	65.50	65.44	66.01	64.77	63.89
	5 months after	69.60	52.50	76.56	45.30	72.34	49.12	77.02	34.77
Difference (%)		3.05	-12.52	9.51	-20.2	6.9	-16.89	12.25	-29.12
Death ratio (%)	Before	4.00	4.23	7.13	7.00	6.25	6.50	5.44	6.12
	5 months after	5.35	0.00	8.45	3.25	8.45	0.00	6.12	3.44
Difference (%)		1.35	-4.23	1.32	-3.75	2.2	-6.50	0.68	-2.68
Yield (ton/ha)	In 2011	8.8	11.31	10.01	9.55	9.98	13.42	9.11	8.44
	In 2012	9.01	16.82	8,98	15.5	11.12	18.77	9.6	13.99
Difference (%)		1.22	19.58	-5.42	23.75	5.40	16.62	0.49	24.74

Effective control of *Phytophthora* sp. using integrated disease management on durian in comparison with the control (cont.)

Data to be recored	Time to record	Bao Lam district		Di Linh district	
		Farm 5		Farm 6	
		Control	Demo	Control	Demo
Ratio of gummosic symp. (%)	Before	67.12	63.44	66.34	64.01
	5 months after	77.87	45.24	79.55	35.98
Difference (%)		10.75	-18.2	13.21	-28.03
Death ratio (%)	Before	3.89	4.07	5.89	6.05
	5 months after	6.33	1.55	7.55	2.55
Difference (%)		2.44	-2.52	1.66	-3.5
Yield (ton/ha)	In 2011	9.99	9.34	8.88	9.51
	In 2012	9.87	14.92	12.02	18.34
Difference (%)		-0.6	23	26.38	31.70

Average cost and revenue from managing pilot and control plot for 1 ha of durian

Criteria	Cost and revenue (VN dong)	
	Control	Demo
Revenue (VN dong)	191,900,000	311,410,000
Total cost (VN dong)	93,380,000	97,618,800
<i>1. Expense</i>	<i>55,380,000</i>	<i>62,228,800</i>
<i>2. Labour cost</i>	<i>25,500,000</i>	<i>27,600,000</i>
<i>3. Yield lost</i>	<i>12,500,000</i>	<i>7,790,000</i>
Profits (VN dong)	98,520,000	213,791,200
Difference (VN dong)		115,271,200
Ratio of profits (%)		21.9

4. Conclusions

- In this study, at Tiengiang, Vinhlong and Lamdong provinces, the quick decline was the new disease occurred recently.
- Most of interviewed farmers said that durian trees died due to disease attacked on the trunk base, but they did not know causal agent or any control measures, so that they only removed infected trees and replaced with new seedlings.
- Also under this survey, there were four durian varieties such as Kho qua xanh, Chuong bo, Monthong and Sua hat lep infected by this disease, but the two Chuong bo and Sua hat lep were less susceptible to this one.
- Results from isolation showed that there were three fungi named *Phytophthora palmivora* and *Phytophthora* sp., *Fusarium* associated with the disease.

- Result from Koch's postulates revealed that *Phytophthora* sp. was the causal agent which had sporangia sometimes arranged in chains, the sporangium with the papilla was not clear, sometimes no papilla. The rDNA sequence of 28S rRNA gene was indicated this was *Phytophthora citricola*.
- This fungus could grow well in the range of temperature from 25 to 35°C.
- Results from varieties' evaluation, the two La queo vang and Chanee were tolerant to *P. palmivora* while the two Sua hat lep and Chuong Bo were tolerant to *P. citricola*.
- The demonstration farms showed good control of the disease and also better yield in comparison with the farmer farms.



CÁM ƠN SỰ CHÚ Ý THEO DÕI
THANK YOU FOR YOUR ATTENTION