

STUDY ON PATHOGENICITY OF *COLLETOTRICHUM GLOEOSPORIOIDES* ON COFFEE IN NORTH OF VIETNAM

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ABSTRACT: Coffee is an important crop in Vietnam and it has recently brought a lot of benefit for the country through export. After coffee leaf rust, anthracnose is the second destructive disease for coffee production and is caused primarily by *Colletotrichum gloeosporioides*. In order to investigate the pathogenicity of *C. gloeosporioides* on coffee in the North of Vietnam, we carried out isolation of *C. gloeosporioides* species from different coffee plantations and found that *C. gloeosporioides* presents in different parts of coffee including leaf, twig, ripe berry and green berry. The pathogenicity of a total of twenty eight *C. gloeosporioides* isolates was tested on green berry in laboratory. Interestingly, two most pathogenic isolates were originated from green berry. In detail, the rate of green berry infection by *C. gloeosporioides* isolates varied from 4.44% to 76.67% and the variation of infection rate was also observed clearly among *C. gloeosporioides* isolates originating from leaf (14.40% to 45.56%), twig (4.44% to 58.89%), ripe berry (22.20% to 61.10%) and green berry (24.40% to 76.67%). Three selected pathogenic isolates representing for *C. gloeosporioides* isolates originated from twig, ripe berry and green berry were further tested for pathogenicity on hypocotyl seedling in greenhouse. The rate of hypocotyl seedling infection by *C. gloeosporioides* isolates originated from twig, green berry and ripe berry is 48.89%, 37.78 and 23.33% respectively. Our data suggests that pathogenicity of *C. gloeosporioides* is variable and specific among isolates, parts of coffee.

Key words: *Colletotrichum gloeosporioides*, pathogenicity, coffee anthracnose disease.

Colletotrichum gloeosporioides is the most predominant *Colletotrichum* pathogen, causes anthracnose disease in more than 197 species of plants including crops, weeds, and trees. This fungal pathogen causes serious disease on mango [10], avocado [5], citrus and apple [4], pasture legume... Especially, *C. gloeosporioides* is post-harvest and pre-harvest pathogen on avocado, mango and citrus. *C. gloeosporioides* is highly variable pathogen and considered as a group species [8]. However, there are few reports of damage caused by this pathogen. On coffee, there are three *colletotrichum* species cause anthracnose disease including *C. kahawae*, *C. gloeosporioides* and *C. acutatum* [6]. *C. kahawae* has so far only been found in Africa where it can cause yield losses up to 80% and can infect all of stages of crop from flowering to ripe berries. *C. gloeosporioides* is considered as a weakly pathogen compare to *C. kahawae*. However, *C. gloeosporioides* and *C.*

acutatum were recently reported to cause anthracnose disease on Arabica coffee in New Guinea, only attacks to ripe berries and can causes yield losses up to 40% [6]. Some reports showed that *C. gloeosporioides* is secondary parasite following *C. kahawae* and is capable of overwhelming *C. kahawae*. This activity may enhance the CBD infection process under field conditions. In the field, from CBD infected berries, some isolates of *C. gloeosporioides* were isolated and determined as saprophytic [2].

In Vietnam, anthracnose disease observes at all coffee plantations and in differences part of coffee including leaf, Twig, ripe berry and green berry. The disease usually appears in the beginning of the rainy season (May) and causes damage in September during berry development and ripening. During recent years, it has mostly been observed on the Arabica cultivar which is now commonly grown in Vietnam. The yield losses caused by this disease ranged between

15-60% and varied among geographical zones. Among *Colletotrichum* species, *C. gloeosporioides* was the most commonly found from all anthracnose disease samples collecting difference zones (7, 9). In previous study, we have reported that *C. gloeosporioides* has been isolating from coffee anthracnose disease expressed diversity of morphology character [7]. In this study, we present virulence of two collection of *C. gloeosporioides* isolates from Nghe An and Ba Vi coffee plantations.

I. MATERIALS AND METHODS

1. Fungal isolates

Anthracnose disease samples from leaf, Twig, ripe berry and green berry were collected from Nghe An and Ba Vi coffee plantations. Isolation of *C. gloeosporioides* isolates were made from leaf, twig, ripe berry and green berry and identification was based on morphology and molecular characteristic (8).

2. Preparation of fungus conidia suspension for inoculation

A total of 28 *C. gloeosporioides* isolates originated from Nghe An and Ba Vi coffee plantations (table 1) and CBD56 isolate (*C. kahawae*) for positive control supported by CBS centre (Holland) were used for inoculation. Conidia suspensions were made from 7- to 10-day-old PDA cultures grown at 25°C under 12h fluorescent light and adjusted to 1×10^6 conidia/ml in sterile dionized water.

Material for inoculation: Green coffee berries, healthy twigs with coffee berry, coffee seedling were harvested from Nghe An and Ba Vi. These materials did not express any disease symptom and were considered as sanitary material.

Pathogenicity test: Coffee berries were washed with running water, surface-sterilized in alcohol 70% for 7 minutes, than rinsed by sterilize water three times. Berries were dispensed into plastic boxes with sterilize sand or humidity tissue (10 berries per box). One hundred microlites of inoculums was dropped on each fruit surface wounded slightly with sterilized pins. For the control, berries was

infected by sterilize water. Tray was covered with white nylon for keep optimal humidity, put in the chamber with 12h fluorescent light. The test was conducted with 3 boxes, repeat three times for each isolate.

Health twigs with coffee berries, twigs were also sterilized in running water and plunge three twigs into each sterilize water in flask. Berries in twigs were made wounding by syringe needle, sprayed conidia suspension every day in a week and covered with white nylon. Control was made as the same except using distilled water as inoculums.

Arabica coffee seedling was cultivated on sterilized sand in plastic tray (30 coffee seedlings per tray). Using needle wound on stem of 8 week-old coffee seedling. The cotton was dipped in the conidia suspension of *C. gloeosporioides* and wrapped strips of the cotton around the seedling. Covering the inoculation point by parafilm. Tray were covered by white nylon for blocking the lost of humidity and cross inoculation. After 24h of incubation, drop 1 ml of conidia suspension on cotton again. Watering every day. Each isolate was infected on three trays.

II. RESULTS AND DISCUSSION

1. Isolation of *C. gloeosporioides* isolates

We conducted the survey and sample collection at coffee farms belonging Arabica Coffee Research Centre - Ba Vi and Coffee and Rubber Company - Nghe An. The anthracnose disease samples were collected and keep in newspaper at 4°C until isolation. From 19 and 37 anthracnose disease samples origination from green and ripe berry, leaf and twig from Nghe An and Ba Vi (fig. 1), we isolated and identified 8 and 20 *C. gloeosporioides* isolates, respectively. In a total of 28 *C. gloeosporioides* isolates; 6, 12.5 and 5 isolates originated from leaf, twig, green berry and ripe berry respectively (table 1). Because sample collection period at Nghe An is not fruiting time, so disease samples only were leaf and twig. These *C. gloeosporioides* isolates were cultured on slant agar in the tube and keep at 4°C for conservation.



Fig. 1. Anthracnose symptom on coffee
a. on ripe berries; b. on green berries; c. on twig; d. on leaf.

Table 1

Source of isolate of *C. gloeosporioides* from coffee at Nghe An and Ba Vi and percentage of infected berry with *C. gloeosporioides* after 6 days of inoculation

N ^o	Isolate	Host tissue	Geographical location of isolates	Percentage of infected berries (%)
1	TH1.8(L)11	Leaf	Nghe An	45.56 ± 0.98
2	TH1.6(L)11	Leaf	Nghe An	35.56 ± 1.73
3	TH1.15(L)4	Leaf	Nghe An	42.22 ± 1.23
4	TH1.4(T)15	Twig	Nghe An	12.22 ± 0.49
5	TH1.14(T)19	Twig	Nghe An	57.78 ± 1.97
6	TH1.17(L)3	Leaf	Nghe An	41.1 ± 2.47
7	TH1.8(L)17	Leaf	Nghe An	27.78 ± 1.97
8	TH1.18(L)2	Leaf	Nghe An	14.4 ± 1.97
9	BVQ4R5	Ripe berry	Ba Vi	38.89 ± 1.73
10	BVQ2G	Green berry	Ba Vi	74.4 ± 2.69
11	BV1G	Green berry	Ba Vi	35.56 ± 1.73
12	BVT22	Twig	Ba Vi	58.89 ± 3.2
13	BVQ6G4	Green berry	Ba Vi	76.67 ± 2.95
14	BVT16	Twig	Ba Vi	42.2 ± 3.2
15	BVQ4R7	Ripe berry	Ba Vi	55.6 ± 1.73
16	BVT7	Twig	Ba Vi	14.4 ± 1.23
17	BVT10	Twig	Ba Vi	15.56 ± 1.97
18	BVT14	Twig	Ba Vi	14.4 ± 0.98
19	BVT19	Twig	Ba Vi	51.1 ± 3.2
20	BVT1	Twig	Ba Vi	33.3 ± 1.48
21	BVT20	Twig	Ba Vi	12.2 ± 1.97
22	BVT4	Twig	Ba Vi	16.67 ± 1.48
23	BVT11	Twig	Ba Vi	4.44 ± 1.23
24	BVQ6R1	Ripe berry	Ba Vi	22.2 ± 1.23
25	BVQ10R7	Ripe berry	Ba Vi	61.1 ± 1.97
26	BV2G	Green berry	Ba Vi	37.8 ± 2.7
27	BVQ5G8	Green berry	Ba Vi	24.4 ± 1.23
28	BVQ12R4	Ripe berry	Ba Vi	55.6 ± 1.73
29	Control			0

2. Pathogenicity test

Pathogenicity test on green berry showed that all 28 *Colletotrichum* isolates attacked and

produced typical symptoms of anthracnose disease on inoculated green berries. Therefore, these isolates were considered to be pathogenic

to coffee green berries. However, the rate of green berry infection by among *C. gloeosporioides* isolates was varied from 4.44% to 76.67% and the variation of infection rate was also observed clearly among *C. gloeosporioides* isolates originated from leaf (14.40% to 45.56%), twig (4.44% to 58.89%), ripe berry (22.20% to 61.10%) and green berry (24.40% to 76.67%). Of 12 *C. gloeosporioides*

isolates from twig, 3 have infection rate more than 50%. Of 5 *C. gloeosporioides* isolates from ripe berry, 3 have infection rate more than 50%. Especially, of 5 *C. gloeosporioides* isolates from green berry, 2 have infection rate more than 70% (table 1). Analysis data reveal that *C. gloeosporioides* isolates from green berry have highest pathogenicity, then ripe berry, twig and leaf (fig. 2).

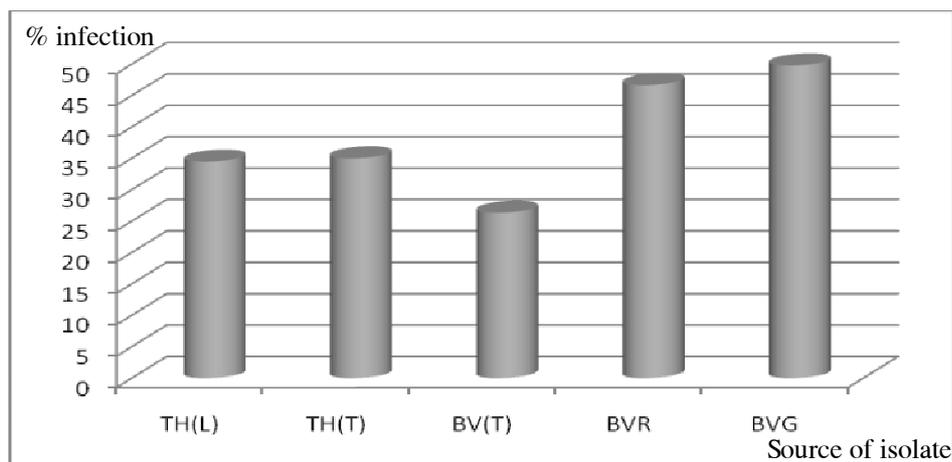


Fig. 2. Infection level on green berry of *C. gloeosporioides* isolates

The symptom on infected green berries appeared as typical anthracnose symptom on the field. The symptom begins with brown spot,

than spread and become black. The lesions are wet, sunken, and some berries appear orange conidia mass (fig. 3).

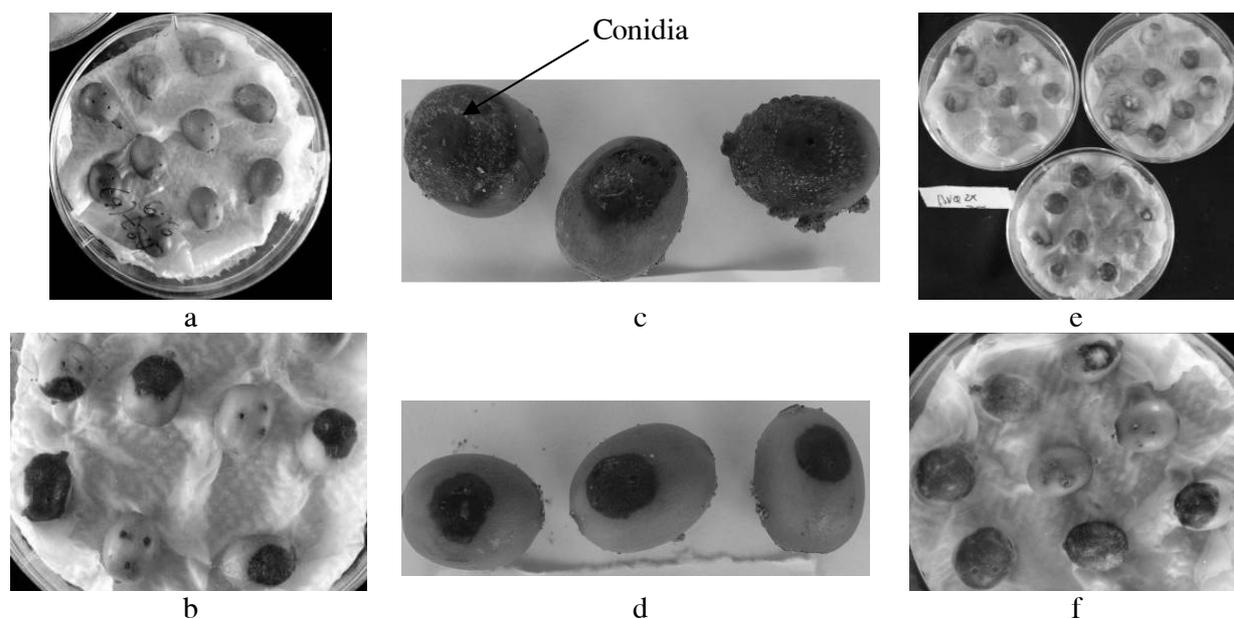


Fig. 3. Anthracnose symptom on coffee berry after 6 days of inoculation
a. Control; b. BVT22; c. TH1.14 (T) 19; d. TH1.8 (L) 11; e and f. BVQ2X

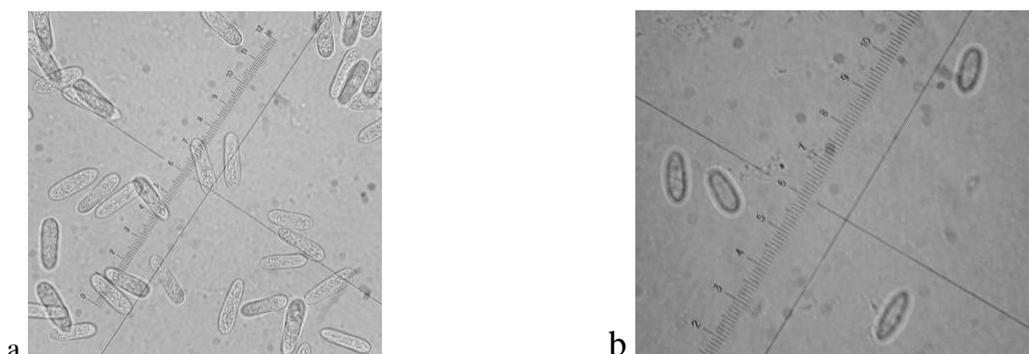


Fig. 4. Conidia character of *C. gloeosporioides* re-isolated from green berries

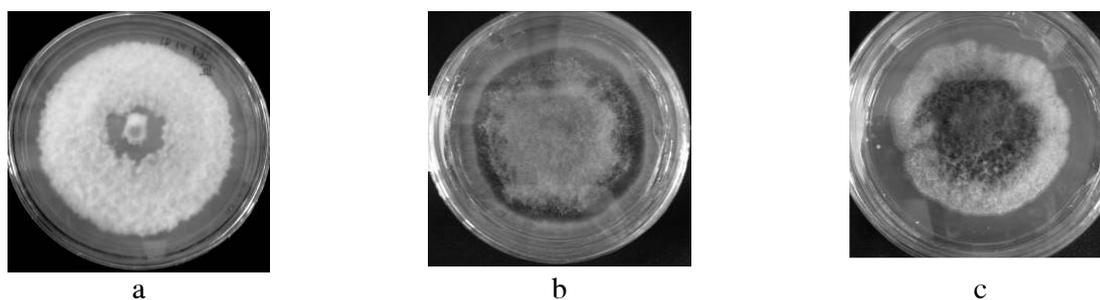


Fig. 5. Colony morphology of *C. gloeosporioides* isolates using for pathogenicity test on coffee seedling. a. BVQ12R4; b. BVQ2G; c. TH1.14(T)19

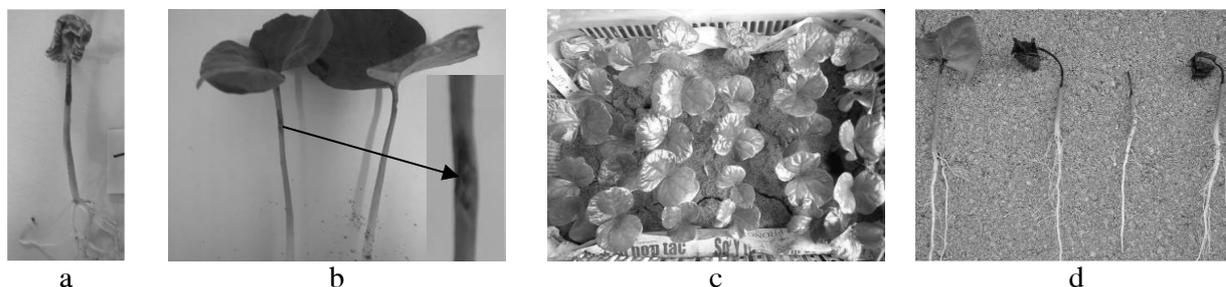


Fig. 6. Pathogenicity test on coffee seedling with TH1.14(T)19
a. after infection 7 days; b and c. after 15 days; d. coffee seedling infected with CBD56

In order to confirm the infection of *C. gloeosporioides* on green berries, we re-isolated fungal from infected green berries that have typical anthracnose symptom based on morphology character of conidia. The result shows that all of *C. gloeosporioides* isolates have been recovered from these infected green berries (fig. 4).

From the result of pathogenicity test on green berry, we screened three selected pathogenic isolates named TH1.14(T)19, BVQ2G, BVQ12R4, representing for *C. gloeosporioides* isolates originated from twig, ripe berry and green berry respectively for pathogenicity test on the healthy twigs with

coffee berry and coffee seedling (fig. 5). These isolates had severely pathogenic on berries (57.8%, 74.4% and 55.6%, respectively).

We first did Pathogenicity test of *C. gloeosporioides* isolates on the healthy twigs with coffee berry. After 7 days, the disease symptom was not appeared clearly. The berries on all of twigs become yellow and fall out; this is also observed with control twigs. It is possible that coffee berries on twig have good vitality to overcome infection of pathogen (data is not shown).

On the coffee seedling, *C. gloeosporioides* showed weak virulence as compared to green berries. Infected seedling appeared slight

anthracnose symptom, but the infection did not affect significantly its growth, as a result, it was able to grow to mature coffee. In contrast, upper

part from wounded stem of infected seedling by CBD isolate was severely infected and consequently, a lot of seedling was dead (fig. 6).

Table 2

Percentage of coffee seedling appearing anthracnose symptom

Item	Isolate			
	CBD56	TH1.14(T)19	BVQ2G	BVQ12R4
Percentage of seedling with symptom (%)	78.89 ± 1.97	48.89 ± 1.97	37.78 ± 1.97	23.33 ± 2.22
Level of virulence	+++	++	+	+

Note: (+++). Very strong virulence; (++) Medium virulence; (+). Weakly virulence.

It has been reported that *C. gloeosporioides* from coffee is a weakly pathogen, only attacks to ripe berry in the field condition. It has not infected on detached green coffee berries, while penetration of *C. kahawae* occurred. However, *C. gloeosporioides* is capable of penetration when green berries were subjected to previous heat shock of 55°C for 30s. In addition, inoculation of *C. gloeosporioides* on ripe berries was occurred, but only was limited to the inoculated area [1, 2, 6]. In our research, all *C. gloeosporioides* isolates from leaf, twig, ripe berry and green berry are pathogenic to detached green coffee berries coffee. Especially, two *C. gloeosporioides* from green berry are very pathogenic with infection rate very high (76.67%), suggesting that a potential threat of *C. gloeosporioides* isolates for Vietnam coffee production has been developed.

It has also reported that *C. gloeosporioides* is able to infected on coffee hypocotyl seedling (3). *C. gloeosporioides* isolates from difference coffee plantations of Vietnam has possibility of infection and induced the severely anthracnose symptom on hypocotyl seedling. However, their virulence on hypocotyl seedling is not severity.

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NGHIÊN CỨU ĐỘC TÍNH NẤM *COLLETOTRICHUM GLOEOSPORIOIDES* GÂY BỆNH TRÊN CÀ PHÊ Ở MIỀN BẮC VIỆT NAM

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TÓM TẮT

Cà phê là cây trồng quan trọng ở Việt Nam, gần đây mang lại rất nhiều lợi nhuận cho quốc gia thông qua việc xuất khẩu. Sau bệnh đốm nâu, bệnh thán thư cà phê gây ra bởi nấm *C. gloeosporioides* có mức độ nguy hiểm thứ hai. Để nghiên cứu độc tính của *C. gloeosporioides* trên cà phê ở các tỉnh phía Bắc Việt Nam, chúng tôi tiến hành phân lập các chủng *C. gloeosporioides* ở các vùng trồng cà phê và phát hiện nấm *C. gloeosporioides* gây hại trên các bộ phận khác nhau của cây bao gồm lá, cành, quả chín và quả xanh. Độc tính của 28 chủng nấm *C. gloeosporioides* được kiểm tra trên quả cà phê xanh ở điều kiện phòng thí nghiệm. Điều thú vị là hai chủng có độc tính cao nhất có nguồn gốc từ quả cà phê xanh. Cụ thể, tỉ lệ lây nhiễm quả xanh của các chủng *C. gloeosporioides* dao động từ 4,44% đến 76,67% và độ giao động về tỉ lệ lây nhiễm cũng được quan sát rất rõ giữa các chủng nấm *C. gloeosporioides* có nguồn gốc từ lá (14,40% to 45,56%), cành (4,44% to 58,89%), quả chín (22,20% to 61,10%) và quả xanh (24,40% to 76,67%). Ba chủng độc tính trên quả xanh đại diện cho các chủng nấm *C. gloeosporioides* có nguồn gốc từ cành, quả chín và quả xanh được tiếp tục nghiên cứu độc tính trên cây con ở giai đoạn vườn ươm trong điều kiện nhà kính. Tỉ lệ lây nhiễm cây con của các chủng nấm *C. gloeosporioides* có nguồn gốc từ cành, quả xanh và quả chín tương ứng là 48,89%, 37,78% và 23,33%. Kết quả nghiên cứu của chúng tôi chứng tỏ độc tính của các chủng *C. gloeosporioides* thay đổi và đặc hiệu giữa các chủng nấm và bộ phận của cây.

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