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Research Article
A Novel Isolate of *Phyllosticta capitalensis* Causes Black Spot Disease on Guava Fruit in Egypt

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Abstract

Background and Objective: Guava black spot (GBS) disease is a quiescent infection, that infect immature fruit prior to harvest. Visible symptoms of the disease on guava fruit showed sunken lesions with concentric development, variation in color ranging from greenish black to black and spread in severity affected fruit. An unrecorded disease of guava fruit (*Psidium guajava* L.) cv. White Balady, was observed in this study during postharvest disease survey in Egypt. **Materials and Methods:** Tissues of guava fruit spot used to isolate the pathogenic fungal. To perform the phylogenetic analysis, the internal transcribed spacer (ITS) region amplified by Polymerase chain reaction (PCR). To amplify the ITS, the primer ITS-1 and reverse primer ITS-4 used to amplify rDNA-ITS regions of the fungus. The fungal identification was done by molecular analysis as *Phyllosticta capitalensis* novel isolate ARAFAT-GF5 according to the GenBank (Accession number–LC269950.1; GI: 119461242) with the synonym: *Guignardia mangiferae*. **Results:** The isolate ARAFAT-GF5 (626 bootstrap) used and the Basic Local Alignment Search Tool (BLAST) program used to search for nucleotide sequence homology in GenBank. The computational analysis of the synonymous DNA sequence was useful for predicting the codon profiling. Pathogenicity test performed to complete Koch's postulates. Typical black spot symptoms developed and the pathogen recovered from the inoculated fruit after 10 days and found as *P. capitalensis*. **Conclusion:** This is the first report of black spot disease on guava fruits in Egypt, caused by a novel isolate of *P. capitalensis*. The results presented here may enable enhancements in the program of integrated disease management.

Key words: Guava fruits, black spot disease, *Phyllosticta capitalensis*, *Guignardia mangiferae*, codon profiling

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Competing Interest: The author have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Guava fruit (*Psidium guajava* L.) cultivated and widespread in many tropical and subtropical regions. Egypt is a subtropical country which existing between 22° and 32° north latitude. In recent years, several studies have focused on the incidence of postharvest diseases that decrease the value of the fruits and alter their physical and chemical properties and contribute to their reduced shelf life[1-3]. The major postharvest diseases of guava are including anthracnose, black spot and astylar end rot[3,4]. The GBS disease is a quiescent infection, that infect immature fruit prior to harvest. Fruits with quiescent infections stay asymptomatic until maturity, when structural and physiological changes trigger the onset of the disease[5]. However, there have been few studies in the literature reporting GBS disease. The *G. psidii* reported in the first study, which causing black spot on guava fruits, during in the field and transportation in India[6]. While *G. pisidii* infection occurs in young fruits and stays quiescent until maturity in Brazil[7]. Similarly, *P. psidiicola*, reported as a potential cause of GBS disease in Taiwan[8] and in Venezuela[9]. The results to date, has been extensive experiments on *Guignrdia* species, where 10 isolated from asymptomatic tissues on different hosts, that were *G. mangiferae* classification by rDNA ITS1-5.8S-ITS2 sequence methods, caused symptoms in guava fruits[10]. Visible symptoms of the disease showed sunken lesions with concentric development, variation in color ranging from greenish black to black and spread on severity affected fruit[9]. The genus *Phyllosticta* Pers. Ex Desm. confirmed[11]. It includes endophytes, plant pathogens and saprobes[12-17]. Species in the genus *Phyllosticta* are mostly plant pathogens of a wide range of hosts and responsible for diseases, including black spots on leaf and fruits[13,18-24]. *Phyllosticta* species are also potential biocontrol agents[25] and has reported to produce novel mycotoxin viz. phyllostine and phyllostoxin[26]. Recently, the name *Phyllosticta* Pers. Ex. Desm. (asexual state) and *Guignardia* Viala and Ravaz (sexual state) have used separately following the dual classification system used by mycologist over several decades[27-30]. ITS rDNA sequences often used to infer phylogeny relationships in many groups of fungi, including *Phyllosticta*[10,14,15,31]. Nevertheless, researchers using molecular methods suggested that the fungi isolates found as *G. psidii* could be in fact *G. mangiferae* or also could be conspecific to this cosmopolitan species[32]. The objective of this study was to identify and characterization of *P. capitalensis* novel isolate ARAFAT-GF5 associated with GBS a new disease on guava fruit in Egypt. Identification of the isolate performed using DNA sequence data of the rDNA ITS1-5.8S-ITS2-28S.

MATERIALS AND METHODS

Samples collection and isolation of the pathogenic fungal: *P. capitalensis* obtained from naturally guava fruit (*Psidium guajava* L.) cv. White Balady at an immature and mature stage. Fruits collected for each three seasons (2015-2017) from different local markets in El-Kharga city (25.4390 N, 30.5586 E), New Valley Governorate, Egypt. The collected samples kept in sterilized polyethylene bags and brought to the laboratory of the Plant Pathology Department, Faculty of Agriculture, New Valley University, Egypt. Fruit samples cut into (5 mm) and immersed in NaOCl (0.5%) for 5 min rinsed in sterile distilled water, then transfer to blotted dry in sterile paper towels for drying. Samples transferred into 9 cm Petri dishes containing potato dextrose agar (PDA) and incubation at 25±3°C under a 12 h photo period for 10-15 days[33]. The fungus characterized by initially gray and turned black with overripe.

Identification of pathogenic fungus: Identification of pathogenic fungus based on morphological methods, using characters of the phenotype of the fungus culture, i.e., colony or hyphae, the characters of the spore or reproductive structure if these features were discernible[34, 35].

Molecular characterization of pathogenic fungus: The fungus was grown in the cultivation media and incubated at 25°C for 15 days, then the growth of fungal was scraped and suspended in 100 µL of distilled water and boiled at 100°C for 15 min and stored at -80°C. DNA was extracted from fungal cultures using the genomic DNA Prep kit (SolGent, Daejeon, Korea) according to SDS/CTAB lysis and phenol/chloroform extraction method[15]. The ITS region, including ITS1, 5.8S and ITS4, 28S rRNA amplified via PCR using primer pair ITS1 (5'–CTTGGTCATTTAGAGGAAGTAA-3') and ITS4 (5' TCCTCCGCTTATTGATATGC-3')[36]. The ITS sequence obtained through the commercial service offered by Macrogen (Macrogen Comp., South Korea). The sequence compared with known homologous sequences of *Phyllosticta* and *Guignardia* in databanks (National Center for Biotechnology Information (NCBI)-(http: //www.ncbi.nlm.nih.gov/Genbank) and The European Molecular Biology Laboratory (EMBL) (https://www.ebi.ac.uk/) by the BLSAT program. DNA sequences deposited in the GenBank database (GenBank Accession No. LC269950).

Sequence analysis: The BLAST search program at NCBI (http://blast.ncbi.nlm.nih.gov/BLAST.cgi) used to analyze the obtained sequence. CLUSTALW program (http://clustalw.ddbj.

nig.ac.jp/top-ehtml) applied to achieve the sequence alignment and phylogeny. Phylogenetic analysis performed by a neighbor joining method to infer the relationships between the fungus isolate and sequences available for *Phyllosticta* and *Guignardia* in the NCBI and The EMBL nucleotide databases using Kimura 2-parameter distances[37]. For analysis, 100 and 33 bootstrap replicates performed to assess the statistical support for the tree.

Nucleotide distributions and frequencies: The DNA sequence analysis used free software (http://www.bioinformatics.org/sms2/index.html) usefully to attain the coding usage of DNA stats. DNA stats returns the number of occurrences of each residue in the sequence entered. Codon usage accepts a DNA sequence and returns the number and frequency of each codon type. Since the program also compares the frequencies of codons that code for the same amino acid (synonymous codons). So, it used to assess whether a sequence shows a preference for certain synonymous codons[38].

Pathogenicity test: Guava fruits obtained from a local supermarket and immediately transferred into mycological laboratory. Fruits in similar shape and size certain and treated with 96% ethanol, soaked with sterilized distilled water and drained at room temperature 25+2°C. Two wounds (5 mm diameter and 3 mm deep) made through at different equatorial lines of each fruit using the tip of a sterile cork-borer. Each one of guava fruits inoculated with a mycelial plug (5 mm in diameter) of the fungus culture into each wound. Other guava fruits having artificial wounds of only plugs of PDA culture used as control. Twenty-five fruits used for each treatment and then the fruits air dried and placed in the plastic boxes (with wetted sterilized cotton pieces to maintain high-level of humidity). The experiment frequent twice. The virulence of the tested fungus identified by observing the development of GBS disease, after 10 days on infested guava fruits[39].

RESULTS

Samples collection and isolation of the pathogenic fungal: Samples of guava fruit at immature and mature stage, collected according to GBS disease symptoms, from the different local market in El-Kharga city, New Valley Governorate, Egypt. The first visible symptoms of the infected guava fruit were small, slightly sunken on mature fruits. Symptom developer showed in Fig. 1, sunken lesions with concentric development, variation in color ranging from greenish black to black and spread in severity affected fruit. As shown before, these symptoms showed that, a strong relationship between symptoms and *P. capitalensis* as the pathogenic fungal of GBS disease of guava fruit.

Identification of the pathogenic fungus: Morphological characters of the isolated fungus from guava fruit showed that closed to *P. capitalensis* in colony appearance, although the hyphal growth of PDA culture, produced gray mycelium at the early stage of growth followed by black colored conidia (Fig. 2). Conidia is hyaline, unicellular, obovate, ranged 6-11 X 5-7 µm (Fig. 3). The pathogen found associated with the GBS disease based on morphological features identified as *Phyllosticta capitalensis*.

Molecular characterization of pathogenic fungus: The fungus found by molecular analysis as *P. capitalensis* according to the GenBank (Accession number-LC269950.1; GI: 119461242) with synonym: *Guignardia mangiferae*. Analysis of ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence. The DNA from *P. capitalensis* from guava fruit amplified when the PCR region performed using primers ITS1 and ITS4. The corresponding PCR region amplified the ITS rDNA sequence of region 1 and 4, which also include 5.8S rRNA and 28S rRNA gene. The PCR produce was 626 bp.

Sequence analysis: ITS sequence of *P. capitalensis* isolate (ARAFAT-GF5) aligned with different *Phyllosticta* isolates available from the GenBank nucleotide database. The aligned sequences visually inspected and minor adjustments made to improve alignment. Phylogenetic analysis performed by a neighbor joining method to infer the relationships between the *Phyllosticta* isolate (ARAFAT-GF5) and sequences available for Phyllosticta and Guignardia in the NCBI (https://www.ncbi.nlm.nih.gov/nuccore/?cmd=historysearch&querykey=1) and EMBL-EBI (https://www.ebi.ac.uk/ena/data/view/LC269950) nucleotide databases (Table 1) using Kimura 2-parameter distances. For analysis, 100 bootstrap replicates performed to assess the statistical support for the tree. *Phyllosticta capitalensis* (LC 269950.1) sequence No. 1 homologous with all sequences, ranged score between (98.08-100%). Furthermore, the isolate LC269950 compared with the other sequences (33 bootstrap) published for *Phyllosticta* and *Guignardia* isolates obtained in the NCBI. Alignment of the available sequences of *Phyllosticta* and *Guignardia* exposed both similarity in the ITS sequences (Table 2).

Table 1: Nucleotide length of 100 isolates compared with *P. capitalensis* and GenBank accession numbers of their characters data

Sequence No.	GenBank (Accession number)	Bootstrap (bp)	Isolates	Pairwise alignments	Score
1	*LC269950.1	626 bp	*Phyllosticta capitalensis*	Sequences 1	-
2	KR016633.1	626 bp	*Fungal endophyte*	Sequences (1:2)	100.00
3	KR015491.1	626 bp	*Fungal endophyte*	Sequences (1:3)	100.00
4	KR015441.1	626 bp	*Fungal endophyte*	Sequences (1:4)	100.00
5	HM537040.1	626 bp	*Fungal endophyte*	Sequences (1:5)	100.00
6	GU066692.1	626 bp	*Guignardia vaccinii*	Sequences (1:6)	100.00
7	GU066670.1	626 bp	*Guignardia* sp.	Sequences (1:7)	100.00
8	GQ352495.1	626 bp	*Guignardia* sp.	Sequences (1:8)	100.00
9	EU686803.1	626 bp	*Fungal endophyte*	Sequences (1:9)	100.00
10	EU167584.1	626 bp	*Phyllosticta elongata*	Sequences (1:10)	100.00
11	EU273524.1	626 bp	*Guignardia mangiferae*	Sequences (1:11)	100.00
12	AM403717.1	626 bp	*Guignardia mangiferae*	Sequences (1:12)	100.00
13	AY601899.1	626 bp	*Fungal endophyte*	Sequences (1:13)	100.00
14	NR_147316.1	625 bp	*Phyllosticta fallopiae*	Sequences (1:14)	100.00
15	KR015859.1	625 bp	*Fungal endophyte*	Sequences (1:15)	100.00
16	AB731125.1	625 bp	*Guignardia mangiferae*	Sequences (1:16)	100.00
17	AB454332.1	625 bp	*Guignardia mangiferae*	Sequences (1:17)	100.00
18	AB454307.1	625 bp	*Phyllosticta fallopiae*	Sequences (1:18)	100.00
19	AB454279.1	625 bp	*Guignardia* sp.	Sequences (1:19)	100.00
20	AB454270.1	625 bp	*Guignardia philoprina*	Sequences (1:20)	100.00
21	KR016813.1	624 bp	*Fungal endophyte*	Sequences (1:21)	100.00
22	MF076618.1	627 bp	*Phyllosticta elongata*	Sequences (1:22)	99.20
23	KR015511.1	623 bp	*Fungal endophyte*	Sequences (1:23)	100.00
24	KP743018.1	626 bp	*Phyllosticta capitalensis*	Sequences (1:24)	99.84
25	JN791606.1	626 bp	*Guignardia mangiferae*	Sequences (1:25)	99.84
26	JN791605.1	626 bp	*Guignardia mangiferae*	Sequences (1:26)	99.84
27	FR863606.1	626 bp	*Uncultured fungus*	Sequences (1:27)	99.84
28	HM807531.1	626 bp	*Guignardia mangiferae*	Sequences (1:28)	99.84
29	HM537020.1	626 bp	*Fungal endophyte*	Sequences (1:29)	99.84
30	FJ462743.1	626 bp	*Guignardia camelliae*	Sequences (1:30)	99.84
31	GU066668.1	626 bp	*Guignardia camelliae*	Sequences (1:31)	99.84
32	GQ352496.1	627 bp	*Guignardia* sp.	Sequences (1:32)	99.20
33	AB454364.1	625 bp	*Phyllosticta* sp.	Sequences (1:33)	99.84
34	AB454264.1	625 bp	*Guignardia alliacea*	Sequences (1:34)	99.84
35	AB454263.1	625 bp	*Guignardia alliacea*	Sequences (1:35)	99.84
36	AB454262.1	625 bp	*Guignardia philoprina*	Sequences (1:36)	99.84
37	KP743020.1	626 bp	*Phyllosticta capitalensis*	Sequences (1:37)	99.68
38	KC218454.1	627 bp	*Guignardia* sp.	Sequences (1:38)	99.04
39	JQ809680.1	627 bp	*Guignardia* sp.	Sequences (1:39)	99.36
40	JQ086349.1	626 bp	*Guignardia camelliae*	Sequences (1:40)	99.68
41	HM537060.1	626 bp	*Fungal endophyte*	Sequences (1:41)	99.68
42	EU747726.1	627 bp	*Guignardia mangiferae*	Sequences (1:42)	99.04
43	EU747725.1	627 bp	*Guignardia mangiferae*	Sequences (1:43)	99.04
44	KJ883595.1	619 bp	*Phyllosticta capitalensis*	Sequences (1:44)	100.00
45	KF435651.1	619 bp	*Fungal endophyte*	Sequences (1:45)	100.00
46	AB731124.1	622 bp	*Guignardia mangiferae*	Sequences (1:46)	99.83
47	GU066689.1	622 bp	*Guignardia* sp.	Sequences (1:47)	99.83
48	AB454315.1	625 bp	*Guignardia mangiferae*	Sequences (1:48)	99.68
49	AB454291.1	625 bp	*Phyllosticta miurae*	Sequences (1:49)	99.68
50	DQ377879.2	619 bp	*Guignardia* sp.	Sequences (1:50)	100.00
51	KP998485.1	628 bp	*Phyllosticta capitalensis*	Sequences (1:51)	99.04
52	GU066723.1	618 bp	*Guignardia* sp.	Sequences (1:52)	100.00
53	GU066719.1	621 bp	*Guignardia camelliae*	Sequences (1:53)	99.83
54	GU066675.1	628 bp	*Guignardia* sp.	Sequences (1:54)	98.56
55	GU066669.1	618 bp	*Guignardia* sp.	Sequences (1:55)	100.00
56	FJ037766.1	618 bp	*Guignardia* sp.	Sequences (1:56)	100.00
57	AY816311.1	621 bp	*Guignardia mangiferae*	Sequences (1:57)	99.83
58	KR015490.1	617 bp	*Fungal endophyte*	Sequences (1:58)	100.00

Table 1: Continue

Sequence No.	GenBank (Accession number)	Bootstrap (bp)	Isolates	Pairwise alignments	Score
59	KC816052.1	626 bp	*Guignardia mangiferae*	Sequences (1:59)	99.52
60	GU066700.1	620 bp	*Guignardia* sp.	Sequences (1:60)	99.83
61	AY277709.1	617 bp	*Guignardia mangiferae*	Sequences (1:61)	100.00
62	KX908973.1	616 bp	*Dothideomycetes* sp.	Sequences (1:62)	100.00
63	KR056285.1	616 bp	*Phyllosticta capitalensis*	Sequences (1:63)	100.00
64	JQ759968.1	616 bp	*Dothideomycetes* sp.	Sequences (1:64)	100.00
65	JQ759953.1	616 bp	*Dothideomycetes* sp.	Sequences (1:65)	100.00
66	JQ759952.1	616 bp	*Dothideomycetes* sp.	Sequences (1:66)	100.00
67	AY277712.1	619 bp	*Guignardia mangiferae*	Sequences (1:67)	99.83
68	KU671305.1	615 bp	*Phyllosticta capitalensis*	Sequences (1:68)	100.00
69	KR015693.1	618 bp	*Fungal endophyte*	Sequences (1:69)	99.83
70	KF435717.1	615 bp	*Fungal endophyte*	Sequences (1:70)	100.00
71	KC686598.1	628 bp	*Guignardia mangiferae*	Sequences (1:71)	98.08
72	KF381072.1	625 bp	*Guignardia mangiferae*	Sequences (1:72)	99.04
73	JX436789.1	615 bp	*Guignardia mangiferae*	Sequences (1:73)	100.00
74	JQ936158.1	618 bp	*Guignardia vaccinii*	Sequences (1:74)	99.83
75	GQ352474.1	621 bp	*Guignardia* sp.	Sequences (1:75)	99.67
76	DQ377880.2	622 bp	*Guignardia* sp.	Sequences (1:76)	98.87
77	EF419973.1	615 bp	*Fungal endophyte*	Sequences (1:77)	100.00
78	AY277714.1	618 bp	*Guignardia mangiferae*	Sequences (1:78)	99.83
79	KX424992.1	619 bp	*Phyllosticta elongata*	Sequences (1:79)	98.54
80	KR016683.1	617 bp	*Fungal endophyte*	Sequences (1:80)	99.18
81	KR016182.1	614 bp	*Fungal endophyte*	Sequences (1:81)	100.00
82	KR015353.1	618 bp	*Fungal endophyte*	Sequences (1:82)	99.02
83	KR014948.1	620 bp	*Fungal endophyte*	Sequences (1:83)	98.70
84	KF435727.1	614 bp	*Fungal endophyte*	Sequences (1:84)	100.00
85	HQ622105.1	614 bp	*Guignardia* sp.	Sequences (1:85)	100.00
86	HM595514.1	620 bp	*Phyllosticta* sp.	Sequences (1:86)	99.35
87	EU821358.1	614 bp	*Guignardia mangiferae*	Sequences (1:87)	100.00
88	EU821356.1	614 bp	*Guignardia mangiferae*	Sequences (1:88)	100.00
89	AY277716.1	617 bp	*Guignardia mangiferae*	Sequences (1:89)	99.83
90	AY277713.1	617 bp	*Guignardia mangiferae*	Sequences (1:90)	99.83
91	AY277711.1	621 bp	*Guignardia mangiferae*	Sequences (1:91)	98.87
92	KU663502.1	613 bp	*Phyllosticta capitalensis*	Sequences (1:92)	100.00
93	KR016814.1	613 bp	*Fungal endophyte*	Sequences (1:93)	100.00
94	KR016812.1	613 bp	*Fungal endophyte*	Sequences (1:94)	100.00
95	KR016695.1	613 bp	*Fungal endophyte*	Sequences (1:95)	100.00
96	KR015487.1	613 bp	*Fungal endophyte*	Sequences (1:96)	100.00
97	KR056282.1	613 bp	*Phyllosticta capitalensis*	Sequences (1:97)	100.00
98	KF128847.1	619 bp	*Guignardia* sp.	Sequences (1:98)	99.19
99	JQ759989.1	616 bp	*Dothideomycetes* sp.	Sequences (1:99)	99.83
100	JQ759948.1	616 bp	*Dothideomycetes* sp.	Sequences (1:100)	99.83

Fig. 1: Natural GBS disease symptoms of guava fruits

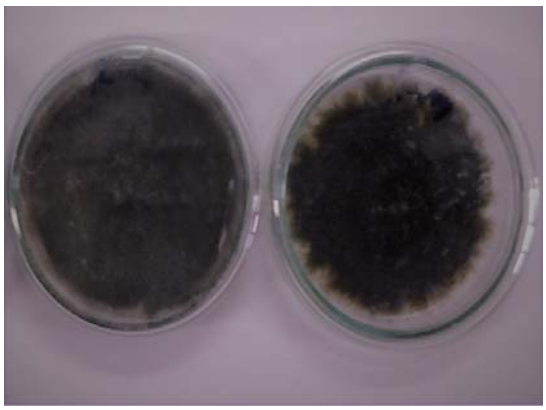

Fig. 2: Hyphal growth of *P. capitalensis* on PDA medium

Table 2: Nucleotide length of 33 isolates of *Phyllosticta* or *Guignardia* compared with *P. capitalensis* and GenBank accession numbers of their characters data

Sequence No.	GenBank (Accession number)	Bootstrap (bp)	Isolates	Pairwise alignments	Score
1	*LC269950.1	626 bp	*Phyllosticta capitalensis*	Sequences 1	-
2	KU671305.1	615 bp	*Phyllosticta capitalensis*	Sequences (1:2)	100.00
3	KU663502.1	613 bp	*Phyllosticta capitalensis*	Sequences (1:3)	100.00
4	KP998485.1	640 bp	*Phyllosticta capitalensis*	Sequences (1:4)	99.04
5	KR056285.1	641 bp	*Phyllosticta capitalensis*	Sequences (1:5)	98.40
6	KR056282.1	623 bp	*Phyllosticta capitalensis*	Sequences (1:6)	98.39
7	KP743020.1	639 bp	*Phyllosticta capitalensis*	Sequences (1:7)	99.68
8	AM403717.1	638 bp	*Guignardia mangiferae*	Sequences (1:8)	100.00
9	AY816311.1	632 bp	*Guignardia mangiferae*	Sequences (1:9)	99.04
10	EU273524.1	663 bp	*Guignardia mangiferae*	Sequences (1:10)	100.00
11	AY277709.1	624 bp	*Guignardia mangiferae*	Sequences (1:11)	98.87
12	AY277712.1	625 bp	*Guignardia mangiferae*	Sequences (1:12)	98.88
13	AY277711.1	630 bp	*Guignardia mangiferae*	Sequences (1:13)	98.08
14	AY277714.1	625 bp	*Guignardia mangiferae*	Sequences (1:14)	98.72
15	AY277716.1	626 bp	*Guignardia mangiferae*	Sequences (1:15)	98.40
16	EU747726.1	643 bp	*Guignardia mangiferae*	Sequences (1:16)	99.04
17	EU747725.1	642 bp	*Guignardia mangiferae*	Sequences (1:17)	99.04
18	EU821358.1	635 bp	*Guignardia mangiferae*	Sequences (1:18)	98.08
19	EU821356.1	637 bp	*Guignardia mangiferae*	Sequences (1:19)	98.08
20	JN791605.1	663 bp	*Guignardia mangiferae*	Sequences (1:20)	99.84
21	AB454332.1	1207 bp	*Guignardia mangiferae*	Sequences (1:21)	99.84
22	AB454315.1	1207 bp	*Guignardia mangiferae*	Sequences (1:22)	99.52
23	HM807531.1	641 bp	*Guignardia mangiferae*	Sequences (1:23)	99.84
24	JN791606.1	663 bp	*Guignardia mangiferae*	Sequences (1:24)	99.84
25	KF381072.1	646 bp	*Guignardia mangiferae*	Sequences (1:25)	98.88
26	KC816052.1	639 bp	*Guignardia mangiferae*	Sequences (1:26)	99.52
27	JX436789.1	658 bp	*Guignardia mangiferae*	Sequences (1:27)	98.24
28	AB731125.1	628 bp	*Guignardia mangiferae*	Sequences (1:28)	99.84
29	AB731124.1	624 bp	*Guignardia mangiferae*	Sequences (1:29)	99.51
30	KC686598.1	882 bp	*Guignardia mangiferae*	Sequences (1:30)	98.08
31	KP743018.1	639 bp	*Phyllosticta capitalensis*	Sequences (1:31)	99.84
32	AY277713.1	625 bp	*Guignardia mangiferae*	Sequences (1:32)	98.56
33	KJ883595.1	655 bp	*Phyllosticta capitalensis*	Sequences (1:33)	98.88

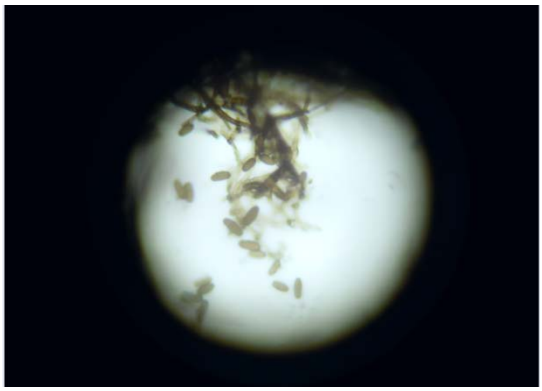

Fig. 3: Conidia spores of *P. capitalensis* (X400)

Nucleotide distributions and frequencies: The results of *P. capitalensis* (LC 269950.1) showed that sequences length, consisting of 626 bases. Distributions and frequencies of bases found (C) base repeated 160 times with 25.56%, followed by (G, T, A) bases repeated 157, 157, 152 times with 25.08, 25.08, 24.28%, respectively. Dinucleotide frequencies found the highest bases were (AA and GC) repeated 48 and 45 times with 7.68 and 7.20%, respectively. Moreover, G, C and A, T dinucleotide bases found repeated 317 and 309 times with 50.64 and 49.36%, respectively. Trinucleotide frequencies found the highest bases (GAA) repeated 16 times with 2.56% and the latest trinucleotide was (CAC) repeated 3 times with 0.48% (Table 3).

Codon usage: The analysis and simulations of *P. capitalensis* results for 626 sequences indicated that prediction of amino acids was the highest (leucine) frequency 26 times with 124.99 times/1000, followed by (serine, arginine, alanine, glycine, cysteine, isoleucine, asparagine, Valine, phenylalanine, glutamine, throine, proline, Tyrosine, glutamic acid, lysine, aspartic acid, tryptophane, histidine and methionine) frequencies "26, 18, 16, 15, 14, 11, 11, 11, 11, 9, 9, 9, 8, 7, 6, 6, 5, 5, 4 and 0" with "107.02, 76.93, 72.12, 67.32, 52.88, 52.88, 52.89, 79.86, 43.27, 28.85, 76.67, 38.48, 28.75, 28.85, 28..84, 24.04, 17.57 and 19.23/1000", respectively (Table 4).

Table 3: Frequencies and percentage nucleotides of *P. capitalensis*

Codon	Frequencies	Percentage
Nucleotide		
G	157	25.08
A	152	24.28
T	157	25.08
C	160	25.56
Dinucleotide		
GG	40	6.40
GA	41	6.56
GT	30	4.80
GC	45	7.20
AG	32	5.12
AA	48	7.68
AT	41	6.56
AC	31	4.96
TG	41	6.56
TA	31	4.96
TT	44	7.04
TC	41	6.56
CG	44	7.04
CA	32	5.12
CT	42	6.72
CC	42	6.72
Trinucleotide		
AAA	10.00	1.60
AAC	12.00	1.92
AAG	12.00	1.92
AAT	14.00	2.24
ACA	5.00	0.80
ACC	9.00	1.44
ACG	9.00	1.44
ACT	8.00	1.28
AGA	5.00	0.80
AGC	8.00	1.28
AGG	10.00	1.60
AGT	9.00	1.44
ATA	9.00	1.44
ATC	11.00	1.76
ATG	7.00	1.72
ATT	14.00	2.24
CAA	11.00	1.76
CAC	3.00	0.48
CAG	9.00	1.44
CAT	9.00	1.44
CCA	6.00	0.96
CCC	10.00	1.60
CCG	11.00	1.76
CCT	15.00	2.40
CGA	12.00	1.92
CGC	12.00	1.92
CGG	15.00	2.40
CGT	5.00	0.80
CTA	6.00	0.96
CTC	11.00	1.76
CTG	12.00	1.92
CTT	13.00	2.08
GAA	16.00	2.56
GAC	11.00	1.76
GAG	6.00	0.96
GAT	8.00	1.28
GCA	7.00	1.12
GCC	15.00	2.40
GCG	14.00	2.24
GCT	9.00	1.44
GGA	11.00	1.76
GGC	14.00	2.24
GGG	5.00	0.80
GGT	9.00	1.44
GTA	11.00	1.76
GTC	6.00	0.96
GTG	7.00	1.12
GTT	6.00	0.96
TAA	11.00	1.76
TAC	5.00	0.80
TAG	5.00	0.80
TAT	10.00	1.60
TCA	14.00	2.24
TCC	7.00	1.12
TCG	10.00	1.60
TCT	10.00	1.60
TGA	13.00	2.08
TGC	11.00	1.76
TGG	10.00	1.60
TGT	7.00	1.12
TTA	5.00	0.80
TTC	13.00	2.08
TTG	15.00	2.40
TTT	11.00	1.76

Fig. 4: Artificial GBS disease symptoms of guava fruit inoculated with *P. capitalensis*

Taxonomy

***Phyllosticta capitalensis* (*Guignardia mangiferae*):** Eukaryota, Fungi, Dikarya, Ascomycota, Pezizomycotina, Dothideomycetes, Dothideomycetes incertae sedis, Botryosphaeriales, Phyllostictaceae, Phyllosticta.

Pathogenicity tests: Pathogenicity of the characteristic *P. capitalensis* novel isolate ARAFAT-FG5 confirmed by inoculating guava fruits at mature stage. The artificially inoculated guava fruits developed black spot symptoms after 10 days of the inoculation. These symptoms similar to those of the naturally infected guava fruits (Fig. 4). All control fruits

Table 4: Frequencies amino acids probability of *P. capitalensis*

Amino acid	Codon	Number	Sum	Per thound	Sum
Ala	GCG	11	47	17.57	75.07
Ala	GCA	10		15.97	
Ala	GCT	16		25.56	
Ala	GCC	10		15.97	
Cys	TGT	14	26	22.36	41.53
Cys	TGC	12		19.17	
Asp	GAT	17	25	27.16	
Asp	GAC	8		12.78	
Glu	GAG	6	14	9.58	22.36
Glu	GAA	8		12.78	
Phe	TTT	10	18	15.97	28.75
Phe	TTC	8		12.78	
Gly	GGG	14	48	22.36	76.67
Gly	GGA	13		20.77	
Gly	GGT	10		15.97	
Gly	GGC	11		17.57	
His	CAT	7	11	11.18	20.76
His	CAC	4		9.58	
Ile	ATA	8	31	12.79	49.53
Ile	ATT	10		15.97	
Ile	ATC	13		20.77	
Lys	AAG	9	18	14.38	28.76
Lys	AAA	9		14.38	
Leu	TTG	10	59	15.97	94.25
Leu	TTA	7		11.18	
Leu	CTG	13		20.77	
Leu	CTA	7		11.18	
Leu	CTT	9		14.38	
Leu	CTC	13		20.77	
Met	ATG	10	10	15.97	15.97
Asn	AAT	7	23	11.18	36.74
Asn	AAC	16		25.56	
Pro	CCG	8	26	12.78	41.52
Pro	CCA	6		9.58	
Pro	CCT	6		9.58	
Pro	CCC	6		9.58	
Gln	CAG	19	29	30.35	46.32
Gln	CAA	10		15.97	
Arg	AGG	9	44	14.38	70.28
Arg	AGA	3		4.79	
Arg	CGG	11		17.57	
Arg	CGA	7		11.18	
Arg	CGT	6		9.58	
Arg	CGC	8		12.78	
Ser	AGT	11	67	17.57	107.02
Ser	AGC	11		17.57	
Ser	TCG	10		15.97	
Ser	TCA	12		19.17	
Ser	TCT	15		23.96	
Ser	TCC	8		12.78	
Thr	ACG	12	48	19.17	76.67
Thr	ACA	14		22.36	
Thr	ACT	15		23.96	
Thr	ACC	7		11.18	
Val	GTG	10	50	15.97	79.86
Val	GTA	11		17.57	
Val	GTT	15		23.96	
Val	GTC	14		22.36	
Trp	TGG	11	11	17.57	17.57
Tyr	TAT	8	18	12.78	28.75
Tyr	TAC	10		15.97	

persisted healthy. The pathogen of the inoculated fruit was re-isolated, cultivated and confirmed as ARAFAT-FG5 isolate based on fungal morphology. Pathogenicity tests revealed the presence of *P. capitalensis* as the pathogen for GBS in El-Kharga city, New Valley Governorate, Egypt. The morphological characteristic identification of the pathogen confirmed with a molecular and phylogenetic approach.

DISCUSSION

This was the first study of the GBS disease in Egypt, with *P. capitalensis* novel isolate ARAFAT-GF5 of guava fruit. The postharvest diseases caused by fungi handle biodeterioration of tropical fresh fruits pulp[40,41]. Postharvest fungal pathogens cause severe losses on guava during postharvest storage and marketing. The most aggressive pathogen is *P. capitalensis* on guava fruits under environmental conditions in El-Kharga city, New Valley Governorate-Egypt. The presence GBS disease had received little attention and not well documented in Egypt, hence, this study focused more attention to this disease. During investigation of postharvest fungal diseases, GBS disease of novel symptoms seen, comprehension of disease symptoms on plant hosts is important for field identification by taxonomists as well as a plant pathologist interested in disease incidence, management and distribution[42]. After infection by *P. capitalensis* the guava mature fruit may become sunken lesions with concentric development, variation in color ranging from greenish black to black and spread on severity affected fruit and pycnidia on fruits is usually black. The fungus isolated and found using a combination of morphological and molecular (ITS region sequences) methods. The morphological characteristics of the fungus *P. capitalensis* isolated from guava fruit, helped to show the fungus on PDA medium[16,42,43]. In the recent decade, results of molecular biology have progressed the systematic classifications of different multiplex groups of plant pathogenic fungi, including *Phyllosticta* species that have helped to facilitate the identification of species and resolution of species complex's[44-46]. The ITS phylogram supported the identify of *P. capitalensis* (or Guignardia mangiferae) as a common foliar endophyte and pathogen with wide range of hosts[15,47]. Most of endophytic fungi belong to the ascomycetes and asexual fungi[48]. *P. capitalensis* was recorded 1543 times in GenBank to 31 August 2018 (https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=121624). However, there are powerful proofs that *G. psidii* and *G. mangiferae* are either the same species[10]. Moreover, no data available about the biology and ecology of the *G. endophytes* except for the production of chemically novel and pharmaceutically useful

secondary metabolism of some isolates in Brazil[49]. Codon usage plays a significant role in the efficiency of the gene expression system. Studies have shown that the presence of rare codons influences gene expression levels and the solubility and amount of the expressed protein[50,51]. Therefore, synonymous codons not only specify protein sequences and translation dynamics, but also help determine gene expression levels[52]. However, this review emphasizes the significant role of determining codon usage to gene expression levels.

CONCLUSION

The novel isolate of *P. capitalensis* which isolated from guava fruit, found caused GBS as a new disease in Egypt. This is the first report of *P. capitalensis* causing GBS in Egypt. The results presented here may enable enhancements in the program of integrated disease management.

SIGNIFICANCE STATEMENT

This study discover the *Phyllosticta capitalensis* that can be beneficial for identification the causal agent of guava black spot disease This study will help the researcher to uncover the critical areas of postharvest diseases that many researchers were not able to explore. Thus a new theory on fungal taxonomy may be arrived at.

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