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Pestalotia: CROWN ROT DISEASE OF STRAWBERRY 1ST REPORT IN IRAQ

HUDA HAZIM WAFI AL-TAAE* AND ALI KAREEM AL-TAAE
Department of Plant Protection, College of Agriculture and Forestry, University of Mosul, Mosul, Iraq [HHWAT, AKAT].
[*For Correspondence: E-mail: dr.huda@uomosul.edu.iq]

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ABSTRACT

Strawberry (*Fragaria ananassa* Duch.) considered as mostly locally important consummated fruit crop in Iraq. Infected plants collected from strawberry farm of Horticulture Department, Agriculture & Forestry College, University of Mosul, Ninevah governorate, North Iraq, during the spring 2018. From tissues being infected, fungus being isolated and identified initially according to its morphological features utilizing hyphal and conidial structures. According to cultural and morphological features, the Pestalotia sp. was detected as strawberry pathogen. Fungus pathogenicity was confirmed also via postulates of Koch. This was confirmed by the molecular identification test, methodology confirmed it. Molecular fungal identification pathogen was performed via implementing transcribed internal spacer (ITS) conserved ribosomal region of DNA. All sequences as ITS proved homologous to isolates of *Pestalotia rhododendri* in database of Gen-Bank at similarity % of 100. At Gen-Bank, Iraqi isolate was assigned No. MN128595.1 as Accession. Up to our best knowledge, this is *P. rhododendri* 1st molecular strawberry record in Iraq.

Keywords: Strawberry; *Fragaria ananassa*; *Pestalotia rhododendri*; crown rot.

INTRODUCTION

Strawberry worldwide is significant crop in regarding its values of nutrition, medicine, and commercial. Strawberries are worldwide appreciated due to its flavor being unique, important macronutrients source and dietary compounds being beneficial [1].

Strawberry fruits are grown in many Arab countries, especially, Syria, Egypt, Palestine, Lebanon and the countries of the Maghreb [2]. Because of its high nutritional and therapeutic value, strawberry enters Iraq in the middle of the last century [3]. Various strawberries pathogens were recorded worldwide. Diseases commonly existed basically in stem, root, crown and fruit. Particularly, crown disease rot might be caused via many pathogens types i.e., *Colletotrichum gloeosporioides, C. fragaria, C. acutatum, Phytophthora cactorum,*. In South America, diseases of root and crown in commercial crops as

strawberry are endemic N. Amer. [4], Asia [5], Europe [6] and [7]. These diseases might result via single fungal or combinations and/or pathogens of oomycete, include one *Fusarium* species or more [8], *Rhizoctonia* and *Pythium* [9], *Macrophomina* [4], *Phytophthora* [10], *Cyclindrocarpon* [11]. In Iraq, *Pestalotia* sp. recording on Date Palm leaves as pathogen was of 1st time record [12,13] isolated the same fungus from plants of Thuja in Ninevah province. Such pathogen was reported to cause crown rot on strawberry in Morocco, Vietnam, Spain, Latvia, Holland, India and Italy [14,15,16,17,18,19] but not reported in Iraq so far. The goal of the current research was identifing the causative crown rot agent disease noticed in strawberry in Nineveh governorate, according to mycological features, and pathogenicity test and identify it using spacer as internal transcribed (ITS) conserved ribosomal region of DNA utilizing primers ITS4 and ITS1.

MATERIALS AND METHODS

Isolation and Diagnosis

At spring 2018, crown rot symptoms were observed on strawberry farm of Horticulture Department, Collage of Agriculture & Forestry, Mosul University. From strawberry being infected, samples had been collected. Samples of stem infested had been sterilized via dipping at solution of NaOCl 1% for three–five minutes and then washed with sterile H_2O for three times. Stems were cut by a blade and four diseased tissue pieces (*ca*. 5 × 5 mm) were put on dextrose potato agar surface (PDA, Mumbai, Himedia) medium. With streptomycin sulfate, PDA was amended to reduce bacterial growth chances. Incubation for plates was done at 28 ±2°C in which noticed periodically; purification was done for growth of fungi was for definition of subsequent experiments and utilization. For identification morphologically, isolates of single spore were grown for ten–fifteen days on PDA media. Features of culture were detected from ten–fifteen days old PDA cultures. Microscopic conidia conidiophores features were determined also according to [20,21].

Test of Pathogenicity

Test of pathogenicity was conducted utilizing a method of suspension spore. Autoclaved soil placed in five plastic pots along strawberry runners were directly sown to every pot. Inoculation (foliar) was performed plants of 1 month old via suspension of spore (1×10^7 spore's ml^{-1}) performed from *Pestalotia* sp. seven days old through culture homogenization in sterile D. H_2O. Pots were irrigated untill saturation. Controls by only sterile distilled water spray were kept. With bags of polythene for humidity maintaining, plants were covered.

Extraction of Genomic DNA and Amplification of PCR

Pure Pestalotia cultures were grown at 25–28°C in dextrose potato broth (PDB) for ten days in dark. Harvesting for mycelia was done via filtration by filter paper Whatman 1. Mycelia harvested were immediately utilized for extraction DNA utilizing Yeast of Fungal/Bacterial/ DNA MiniPrep™, Catalog # D6005 based on factorty Protocol methods below:

1. Addition of wet weight of 50 – 100 mg bacterial cells1 or fungal which were re-suspended in two hundred µl of H_2O or buffer being isotonic (i.e., PBS) or to tissue of 200 mg to Lysis Tube ZR BashingBead™ (0.1 mm & 0.5 mm). To tube 2, addition of solution of 750 µl Lysis.
2. Securing in a fitted beater bead along holder of 2 ml assembly tube and processing at speed of maximum for ≥ five min.
3. Centrifugation the Lysis Tube of ZR Bashing Bead™ in a microcentrifuge at 10,000 x *g* for 1 minute.
4. Transferring to four hundred µl supernatant to Spin Filter Zymo-Spin™ IV (Orange Top) in Tube of Collection and centrifugation at 7,000 x *g* for 1 minute.
5. Addition of DNA for Fungal/Bacterial Binding Buffer 1,200 µl to filtratation in tube of collection (Step 4).
6. Transferring mixture of eight hundred from Step 5 to IIC Zymo-Spin™ Column 3 in tube of collection and centrifugation at 10,000 x *g* for 1 minute.
7. Discarding through tube of collection, repeating Step 6.
8. Addition µl Pre-Wash DNA Buffer of 200 to IIC Column of Zymo-Spin™ in new

collection tube and centrifugation at 10,000 x *g* for 1 minute.

9. Addition of Fungal/Bacterial DNA Wash Buffer 500 µl to Column of IIC Zymo-Spin™ and centrifugation at 10,000 x *g* for 1 min.
10. Transferring the Column of IIC Zymo-Spin™ to a clean microcentrifuge 1.5 ml tube and adding directly 100 µl (35 µl minimum) Elution DNA Buffer to matrix column. Centrifugation for 30 sec. at 10,000 x *g* for DNA elution.

DNA Agarose Gel Electrophoresis

Electrophoresis was done for DNA pieces determination following extraction process or detecting PCR interaction result through standard DNA presence for distinguishing PCR interaction outcome bundle size on gel of Agarose.

Alignment Sequence and Sequencing

Products of PCR on a 2% agarose electrophoresis gel were separated in which they were visualized via UV light exposure (302 nm) following staining Red. Sequencing of gene was done through national centre of instrumentation for management of environment online at (http://nicem.Snu.ac.kr/main/? En _skin=index.html), biotech. Lab., DNA sequencer machine is 3730XL, Applied Biosystem), search of Homology was done utilizing program of Basic Local Tool Alignment Search (BLAST) that is available at (NCBI) online at (http://www.Ncbi.nlm.nih.gov) and Bio Edit program.

RESULTS AND DISCUSSION

Isolation and Diagnosis

Following incubation, various acervuli were particularly yielded in strawberry chlorosis leaves. On PDA, mycelium as aerial was of white color, branched more and brown golden in reverse attaining diameter of 70 mm following seven at 24°C days in dark and in old culture acervuli were yielded. Conidia (20 – 28 × 6-8.3 µm) were straight or curved slightly fusiform and 5 celled. Median darker cells were 3 with a wall being thick. Typically, the 2 upper cells being brown along band being darker at septa between cells, whereas the lowest one colored lighter. Cells at basal and apical were of shape as conical, colorless thin and walled. Appearing appendages were at base and apex. It can be noticed that appendages (2-4) of length (19.5 – 31.5 to 37 µm), commonly 3. Appendage of basal (6.5 - 9.5 µm) was centric and single, according to morphology, the fungus *Pestalotia* sp. was identified [22].

Pathogenicity Test

First symptoms appeared on leaves at edges after eight days of inoculation on plants, as leaves blightening. Such disease spread then from plant top to its bottom and spread then down to region of crown. Stems and leaves are becoming black and dried, crown rot symptoms were noticed following inoculation of 19 days. No disease symptoms on strawberry plant sprayed with sterilized D. H_2O on uninjured produced surface of leaf. Re-isolation from infected strawberry parts produced *Pestalotas* sp. That proved postulate of Koch in which symptoms of disease were resulted from the same fungus which was isolated originally. Crown strawberry rot caused by *Pestalotas* sp. has been reported previously in different regions worldwide [14,15,16,23,24,17,18,19]. In Iraq, to best of the knowledge such is *Pestalotia* sp. 1st report on strawberry.

Sequencing and Sequence Alignment

For identification morphological confirmation, Pestalotia isolate (ITS) region was implicated of primers as universal ITS4 and ITS1. The isolates were especially diagnosed following copies conformity with at the Gene-Bank at (NCBI) where genes of 100% accuracy of diagnosis matching with MH857478.1 isolation: Query sequence cover of 100% (Fig. 1).

Table 1. Conformity ratio between especially diagnosed copies and other copies at NCBI

Score	Expect	Identities	Gaps	Strand
875 bits(970)	0.0	485/485(100%)	0/485(0%)	Plus/Plus

Results showed the relationship close genetically among isolated *Pestalotia* as black prism) and those deposited worldwide in database of Gen-Bank. Fig. 2 represents comparison between local Iraqi isolate strain of *P. rhododendri* with the strain *P. rhododendri* recorded in the National Center Biotechnolgy Information (NCBI) and isolated from different countries showed compatibility 100% with *P. rhododendri* strains accession Nos: MH857478.1 from Netherlands and 92% with accession No AF377294.1 from and Italy (Fig. 2). Nucleotide ITS sequence from isolate of Iraq were Gen-Bank assigned as No MN128597.1 Accession.

Query 1
ATGTGAACTTACCTTTTGTTGCCTCGGCAGAAGTTATAGGTCTTCTTATAGCTGCTGCCG 60
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 64
ATGTGAACTTACCTTTTGTTGCCTCGGCAGAAGTTATAGGTCTTCTTATAGCTGCTGCCG 123

Query 61
GTGGACCATTAAACTCTTGTTATTTTATGTAATCTGAGCGTCTTATTTTAATAAGTCAAA 120
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 124
GTGGACCATTAAACTCTTGTTATTTTATGTAATCTGAGCGTCTTATTTTAATAAGTCAAA 183

Query 121
ACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAA
180
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 184
ACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAA
243

Query 181
GTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCATT 240
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 244
GTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCATT 303

Query 241
AGTATTCTAGTGGGCATGCCTGTTCGAGCGTCATTTCAACCCTTAAGCCTAGCTTAGTGT 300
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 304
AGTATTCTAGTGGGCATGCCTGTTCGAGCGTCATTTCAACCCTTAAGCCTAGCTTAGTGT 363

Query 301
TGGGAATCTACTTCTTTTATTAGTTGTAGTTCCTGAAATACAACGGCGGATTTGTAGTAT 360
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 364
TGGGAATCTACTTCTTTTATTAGTTGTAGTTCCTGAAATACAACGGCGGATTTGTAGTAT 423

Query 361 CCTCTGAGCGTAGTAAtttttttCTCGCTTTTGTTAGGTGCTATAACTTCCCAGCCGCTA
420
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 424
CCTCTGAGCGTAGTAATTTTTTTCTCGCTTTTGTTAGGTGCTATAACTTCCCAGCCGCTA 483

```
Query  421
AACCCCCAATTTTTTGTGGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCA  480
       ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  484
AACCCCCAATTTTTTGTGGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCA  543

Query  481  TATCA  485
       |||||
Sbjct  544  TATCA  548
```

Fig. 1. Sense flanking sequencing of partial *ITS* gene in comparison to gene standard of MH857478.1, from Gene Bank. Sample query represents; Subject represent of database of (NCBI)

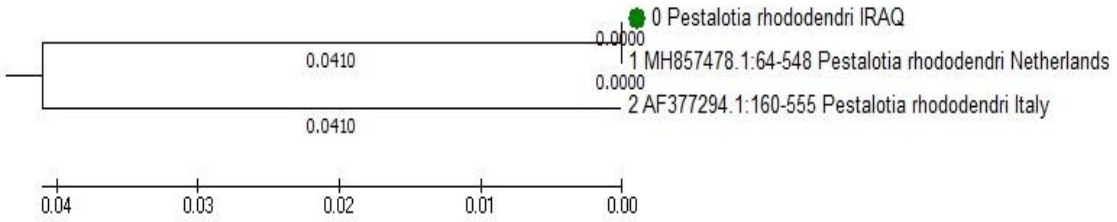

Fig. 2. Constructed tree as phylogenetic by method of neighbor-joining illustrating phylogenetic *Pestalotia rhododendri* relationships in comparison to the sequences reference of Gene-Bank

CONCLUSION

The results showed that the *Pestalotia rhododendri* was detected as the causative crown strawberry rot agent in Iraq. To best of knowledge such is *P. rhododendri* 1st molecular strawberry record in Iraq.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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