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Molecular Identification and Evaluation of Fungi Associated with Sooty Canker Disease on Albizia lebbeck in Karbala, Iraq

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ABSTRACT

Sooty canker disease poses a significant threat to *Albizia lebbeck* trees, an important ornamental and shade tree species in Karbala province, Iraq. The study aimed to investigate the canker disease *A. lebbeck* at several places in the province of holy Karbala in Iraq. Al-Atishi, fields of the College of Agriculture / University of Kerbala the infection rate was recorded at 100% with three degrees of infection, then Al-Atishiand Al-Qudah followed Al-Hussein. The infection rate was 80 and 60 and 55 respectively, the lowest infection rate was recorded 45%in Al-Naqeeb neighborhood, the degree of infection for all surveyed regions was two. The frequency ratio for the *Fusarium incarnatum* was 82%, and the fungus *Trichoderma harzianum* had a frequency of 10%. The two fungi were identified molecularly by PCR search of the *Fusarium* and *Trichoderma*-ID database showed 100% nucleotide similarity with the *F. incarnatum* and *T. harzianum.* This study highlights the fungi linked to sooty canker in *A. lebbeck* trees in Karbala, Iraq, emphasizing integrated disease management, monitoring, cultural practices, fungicides, and the need for resistant tree varieties, this is the first report about *F. Incarnatum* of canker disease on *A. lebbeck* in Karbala province of Iraq.

Keywords: *Fusarium incarnatum*, *Trichoderma harzianum*, PCR, *Albizia. lebbeck*

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INTRODUCTION

Albizia. lebbeck (L.) Benth., The Albizia genus has around 160 species found in Asia, Africa, Australia, and tropical and subtropical regions of America. Multiple Albizia species are grown for their visual appeal or tannin extraction. Albizia is a significant plant species valued for its use as feed, lumber, and medicinal purposes. Several species are grown for their attractive flowers, as shown by [1, 2]

In Iraq, the Albizia is cultivated as ornamental trees, they have medicinal importance as an antioxidant [3] and anti-inflammatory [4] and are used for making furniture [5]. The Albizia tree is infected with Many fungal diseases such as dieback of large branches which is caused by *Scytalidium dimidiatum* [6]. and stem canker diseases by *Botryodiplodia theobromae* and *Pestalotiopsis guepinii* [7]. The Fusarium incarnatum-equiseti species complex (FIESC) comprises a limited number of officially recognized species that are distinguished by the overall dorsiventral curvature of their macroconidia and the presence of numerous chlamydospores, which can occur singly, in chains, or clusters [8, 9]. However, there is still uncertainty regarding the identification of further isolates in this group due to substantial genetic diversity, *F. incarnatum*- species complex infects a wide variety of food crops [10] secondary invaders of environmental habitats humans and animals [11]. Due to the spread of the disease on Albizia trees in several regions of Karbala province and the scarcity of studies on fungal diseases that affect Albizia trees in Iraq, where studies that dealt with diseases specific to Albizia trees are very limited. Therefore, the study aimed to identify the damage to Albizia trees that led to the economic, commercial, and industrial value as a result of the fungal attack, which caused several infections that led to the damage.

MATERIALS AND METHODS

Survey of Sooty Canker Disease

A field survey of deteriorating *A. lebbeck* trees was conducted in different regions of holy Karbala province (Al-Atishi, fields of the College of Agriculture / University of Kerbala, Al-Qudah neighborhood, Al-Hussein neighborhood, and Al-Naqib neighborhood) 2024. The infestation was determined through the cankers found on the tree trunks to restrict the extent of the infestation. According to the available evidence of the severity of retrogradation [16] the damaged trees were classified according to the extent of damage to the trees randomly selected from each area into five categories.

$$Severity = \frac{No.of\ trees\ caregory\ 1\ X\ frequency\ ....+No.of\ trees\ categort\ 5\ X\ frequency}{Total\ No.of\ trees\ tested\ X\ highest\ category\ index}\ X\ 100$$

The percentage of infection of *A. lebbeck* trees in the surveyed areas was calculated from the following equation:

$$Infection\ \% = \frac{No.of\ Infected\ tress}{Total\ No.of\ trees}\ X\ 100$$

Table 1 Disease severity of sooty canker disease on *A. lebbeck* tree

Categories	Evidence of deterioration	Percentage of infected part of the tree
1	0	Uninfected
2	1	1-25
3	2	26-50
4	3	51-75
5	4	76-100

The severity of infection in trees was calculated from the following equation:

Isolation

A. Lebbeck which was infected by sooty canker taken from it as a random sample, the damaged parts were washed with flow water for not less than half an hour, and parts of the areas adjacent to the canker were taken for a sterile scalpel. The pieces were subjected to surface sterilization by immersing them in a 1% solution of sodium hypochlorite for three minutes, the traces of the sterilizing agent were removed by washing the pieces several times with distilled water and then using two sterile Whatman No. 10 filter papers to dry the specimen. The pieces were placed in sterile Petri dishes with a diameter of 9 cm containing sterile nutrient medium, dextrose agar (PDA), genitive the antibiotic chloramphenicol is added at a rate of "250 mg/L" before hardening, five pieces per dish, after sterilizing them in an autoclave for 20 minutes at 121 °C and a pressure of 1.5 pounds/inch2, to prevent bacterial growth, the dishes were incubated at 25-27 °C for four to five days, The colony was purified using a container containing a medium of potato extract, glucose and agar using the hyphae tip technique after that test tubes containing potato extract, glucose and sterile agar were used to store the isolated fungi until they were used in subsequent experiments.

Phenotypic and Molecular Diagnosis

For morphological identification, color, shapes, mycelia, and types of conidia were characterized and compared with previous descriptions [12]. The percentage frequency of the two fungi in isolation was determined. The two fungi were identified using molecular detection using PCR, and their nucleotide sequence was determined in the laboratory of Scientific Progress /Baghdad. The genomic DNA (gDNA) of the fungus colony was extracted using the approved DNeasy Plant Mini Kit from QIAGEN NV (Hilden, Germany). The primers ITS1 and ITS4 were utilized to get ITS DNA.

A set of ready-to-use PCR beads was broken into a 25 µl solution containing beads and 1 µl of each primer at 5 pmol concentration plus 2 µl (50-100 ng) of template DNA (GE Healthcare, Illinois, USA). The obtained sequences were compared with other existing ITSr DNA sequences of fungus in the GenBank database, using the BLAST tool provided by NCBI. Subsequently, the genetic analysis of the sequences was conducted using MEGA6 [13] The produced sequence was deposited to the GenBank database with a unique accession number

RESULTS AND DISCUSSION

Survey of Sooty Canker Disease

The survey results showed deterioration of *Albizia* trees *A. lebbeck* at several places in the province Karbala (Al-Atishi, fields of the College of Agriculture / University of Kerbala, Al-Qudah neighborhood, Al-Hussein neighborhood and Al-Naqib neighborhood (Fig. 1).

Fig. 1 Symptoms of natural canker on a tree trunk *A. lebbeck*

It is clear from Table (2) that the average infection rate in the fields of the College of Agriculture / University of Karbala was higher than the rest of the regions under study by 100%, the percentage of infection ranged between 45-100%, then Al-Atishiregion, which recorded 80%, then Al-Qudah neighborhood had a 60%, followed by Al-Hussein neighborhood with a 55% infection rate, while the lowest infection rate was recorded in Al-Naqeeb neighborhood, amounting to 45%, regarding the degree of infection, the fields of the College of Agriculture / University of Kerbala excelled with an infection rate of three the degree of infection ranged between two -three, while the rest of the regions did not differ from each other in the degree of infection, which reached two.

During the field survey, symptoms appeared on the main stem, and shield canker infections were observed, which is prevalent in the surveyed regions of *A. lebbeck* trees.

Table 1 Percentage and degree of natural infection in a number of regions of the Holy Karbala province for the year 2024

Treatment	% of infection	Degree of infection
Al-Atishi	80	2
fields of the College of Agriculture / University of Kerbala	100	3
Al-Qudah neighborhood	60	2
Al-Hussein neighborhood	55	2
Al-Naqib neighborhood	45	2
Average	68	2.2

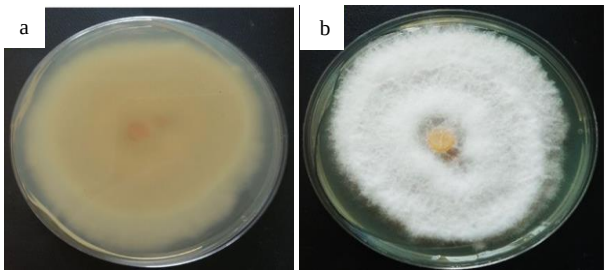

Fig. 2 Colony on PDA; a. Surface; b. Reverse

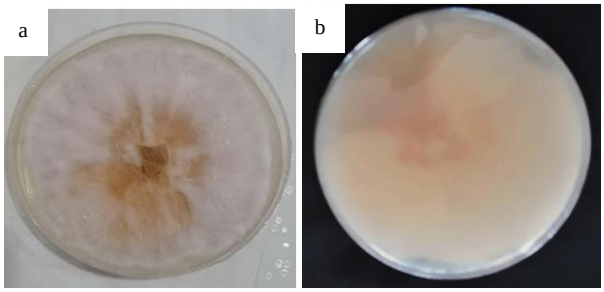

Fig. 3 *F. incarnatum* Colony on PDA: a. Surface; b. Reverse

In addition to the presence of some signs of gumming the dimensions of the cankers vary depending on the age of the trees and the influence of environmental factors these factors have an impact on the infection as well as the extent of the damage caused, the deterioration may be attributed to misexploitation and the lack of controls to protect it or take care of it by protecting it from pests. Thus, it led to the spread of fungal diseases. Thus, it led to the spread of fungal diseases [14] mentioned that broadleaf trees suffer from ulcers on their stems caused by fungi.

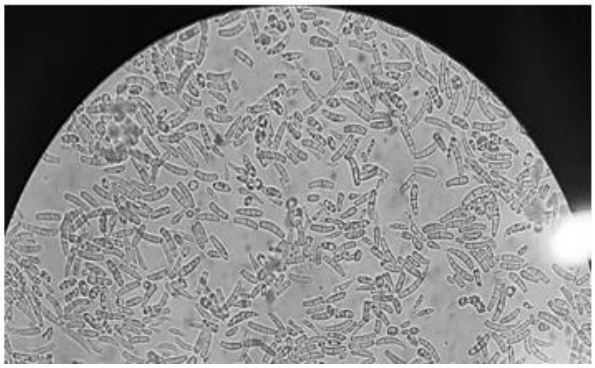

Fig. 4 *F. incarnatum* various types of the conidia on PDA

Isolation

The results of isolation from stems of Albizia trees infected with canker showed in the surveyed regions, the fungi *F. incarnatum* and *T. harzianum* appeared *F. incarnatum* was isolated with a frequency of 82% and the fungal colony was characterized by colonies growing rapidly; aerial mycelium floccose, the first impression is a whitish figure (2), which eventually changes to a color ranging from avellaneous to buff-brown. The reverse side starts pale and gradually turns into a peach-colored figure (3). The conidiophores are dispersed throughout the aerial mycelium and have a loosely branching structure.

There is a plentiful presence of polyblastic conidiogenous cells. The sporodochial macroconidia are slightly curved and have a foot cell. They are three to seven. The chlamydospores are sparse, spherical, and have a diameter of 10-12 µm. As they mature, they become brown. The chlamydospores are intercalary and can be found either singly or in chains. The microconidia were non-septate, ovoid, hyaline, single-celled, and 9 to 12 × 1 to 3 µm figures (4 and 5) . The morphological characteristics of the isolate were indicative of the *F. incarnatum* fungus complex as described by Leslie and Summerell (2006). These results agreed with [15].

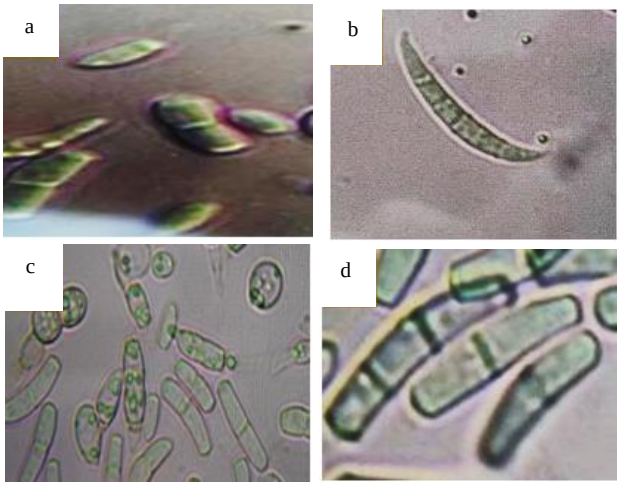

Fig. 5 *F. incarnatum*, a. Microconidia; b. Macroconidia; c-Chlamydospores; d-Macroconidia two to three septate

Morphological of *T. harzianum*

The colony of *T. harzianum* initially appeared white, then transitioned to a dark green color with a cottony texture. The growth of *T. harzianum* was predominantly effuse, covering the entire surface of the plates. After a specific incubation period of three days at a temperature maintained between 25-27 °C, the colony's diameter reached approximately 9 cm.

Microscopic examination revealed that the conidia of *T. harzianum* are smooth-walled, subglobose to short oval in shape, and primarily green, although they can also appear hyaline. These conidia are clustered at the tips of the phialides. The conidiophores are branched in tufts, featuring divergent and often irregularly bent, flask-shaped phialides (Fig. 8). The colony of *T. harzianum* was firstly white then turned to dark green with, a cottony texture, a condition in the *T. harzianum* was predominantly effuse covering the entire surface of the plates.), The colony's diameter has reached approximately 9 cm after three The incubation period lasts for a specific number of days.

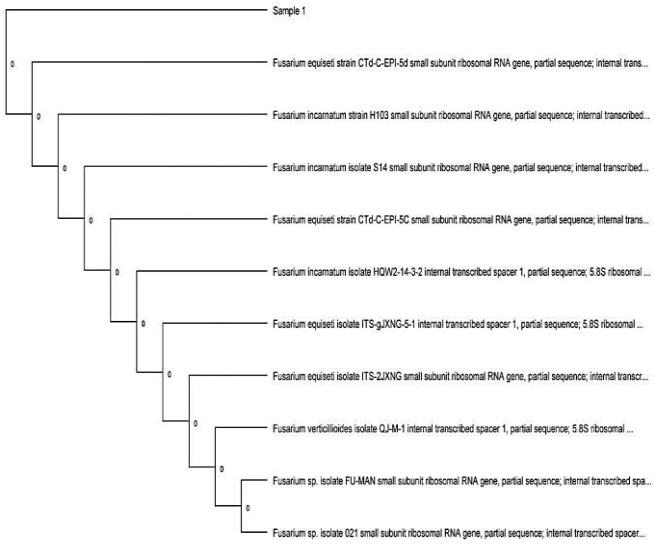

Fig. 6 Genetic tree for *F. incarnatum*

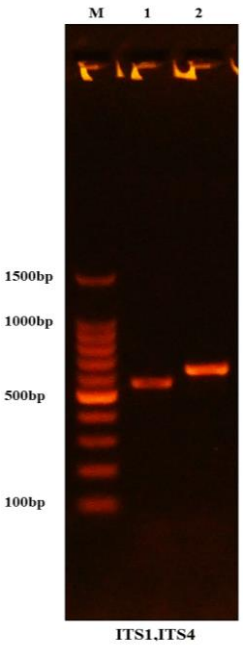

Fig. 7 Agarose gel electrophoresis of amplified PCR product (550 bp) 1. *F. incarnatum* 2. *T. harzianum*

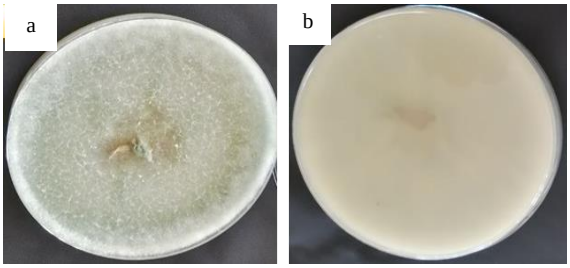

Fig. 8 *T. harzianum* colony on PDA:a. Surface; b. Reverse.

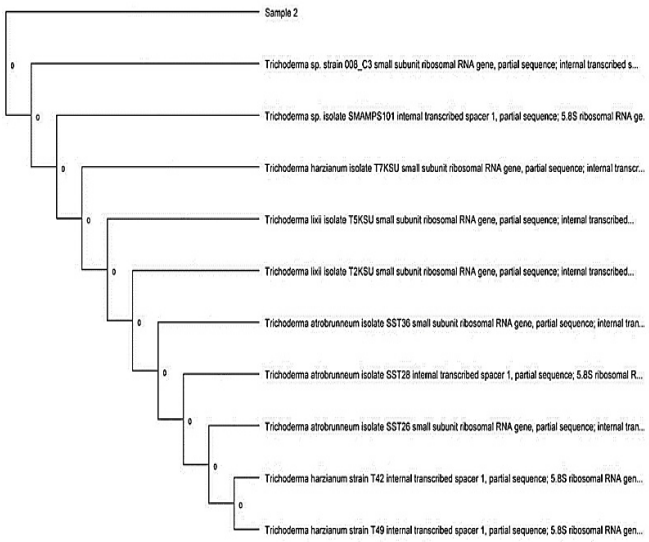

Fig. 9 Genetic tree for *T. harzianum*

The results indicated that DNA could be successfully extracted from the isolates of the two fungi, *F. incarnatum* and *T. harzianum*, and subsequently amplified using polymerase chain reaction (PCR).

The PCR process yielded nucleic acid products with sizes ranging from 500 to 600 base pairs (bp), utilizing both forward and reverse primers (ITS1 and ITS4).

By analyzing the sequences of the nitrogenous bases of the multiplexed DNA products from the isolates of two fungi *F. incarnatum* and *T. harzianum* and using the BLAST program it was found that the two isolates of the two fungi *F. incarnatum* and *T. harzianum*, they showed a 100% match between the nitrogenous base sequences of the multiplication products of DNA, The results also proved that these two isolates one and two gave a similarity rate of 100% with most other isolates belonging to the same two fungi(*F. incarnatum* and *T. harzianum*) Previously it has registered with the National Centre for Biotechnology Information (NCBI) it has been uploaded to the GenBank database. It has been assigned an accession number for identification the number is PP892187.1 for *F. incarnatum* and PP892188.1 for *T. harzianum*. to identify ITS (Intragenic transcriptional spacer) Discovering a precise method for identifying fungi at the species or strain level and understanding the genetic relationships (phylogeny) between different species is highly significant. Variations in the internal transcribed spacer (ITS) region, particularly in ribosomal DNA (rDNA), play a crucial role in diagnosing eukaryotic organisms, including fungi such as *Aspergillus versicolor*, *Trichoderma* spp., and *F. incarnatum* [14] which the temperature is maintained at 25-27 °C. Under microscopic examination, the conidia of *T. harzianum* are smooth-walled with a subglobose to a short oval shape, and are mostly green, sometimes hyaline, clustered at the tips of the phialides. Conidiophores are branched in tufts with divergent, often irregularly bent, flask-shaped phialides (Fig. 8).

CONCLUSION

This study provides valuable insights into the fungi associated with sooty canker disease on *A. lebbeck* trees in Karbala province, Iraq. The findings underscore the need for integrated disease management approaches that include regular monitoring, proper cultural practices, and the use of fungicides where necessary. Further research is needed to explore the interactions between these fungal pathogens and the host tree, as well as to develop resistant *A. lebbeck* varieties.

Funding

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Conflict of Interest

There is no conflict of interest to declare.

REFERENCES

1. Maroyi A. Albizia adianthifolia: Botany, Medicinal Uses, Phytochemistry, and Pharmacological Properties. Scientific World Journal. 2018;7463584. doi: 10.1155/2018/7463584.
2. Balkrishna A., Sakshi Chauhan M., Dabas A., Arya V. A Comprehensive Insight into the Phytochemical, Pharmacological Potential, and Traditional Medicinal Uses of *Albizia lebbeck* (L.) Benth. Evidence-Based Complementary and Alternative Medicine. 2022; 5359669. doi: 10.1155/2022/5359669.
3. Tasneem R., Khan H.M.S., Zaka H.S., Khan P. Development and cosmeceutical evaluation of topical emulgel containing *Albizia lebbeck* bark extract. Journal of Cosmetic Dermatology. 2022; 21(4): 1588-1595. doi: 10.1111/jocd.14244.
4. Mani S., Duraipandian C., Chidambaram S.B. Analgesic, anti-inflammatory and acute oral toxicity profile of leaf and bark extracts of Albizia procera. BMC Complementary Medicine and Therapies. 2022; 22(1): 50. doi: 10.1186/s12906-021-03497-7.

5. Latif A., Malik S.A., Saeed S., Zaka S.M., Sarwar Z.M., Ali M., Azhar M.F., *et al.* Pollination biology of *Albizia lebbeck* (L.) Benth. (Fabaceae: Mimosoideae) with reference to insect floral visitors. Saudi Journal of Biological Sciences. 2019; 26(7): 1548-1552. doi: 10.1016/j.sjbs.2018.12.005.
6. Derviş S., Özer G. Plant-Associated Neoscytalidium dimidiatum - Taxonomy, Host Range, Epidemiology, Virulence, and Management Strategies: A Comprehensive Review. Journal of Fungi (Basel). 2023; 9(11): 1048. doi: 10.3390/jof9111048.
7. Al Raish S.M., Saeed E.E., Sham A., Alblooshi K., El-Tarabily K.A., AbuQamar S.F. Molecular Characterization and Disease Control of Stem Canker on Royal Poinciana (Delonix regia) Caused by Neoscytalidium dimidiatum in the United Arab Emirates. International Journal of Molecular Sciences. 2020; 21(3): 1033. doi: 10.3390/ijms21031033.
8. Hou Y.H., Hsu L.H., Wang H.F., Lai Y.H., Chen Y.L. Calcineurin Regulates Conidiation, Chlamydospore Formation and Virulence in Fusarium oxysporum f. sp. lycopersici. Frontiers in Microbiology. 2020; 11: 539702. doi: 10.3389/fmicb.2020.539702.
9. ÇAT A. Fusarium incarnatum Causing Fusarium Wilt on Protea (Protea cynaroides L.) in Turkey. Turkish Journal of Agricultural Research. 2022; 9(1): 34-40. https://doi.org/10.19159/tutad.1033335.
10. Ghosal D., Datta B. Characterization, phylogenetic analysis and toxigenic potential of Fusarium incarnatum-equiseti species complex isolates associated with root rot disease in vegetables. Journal of Phytopathology. 2024; 172 (4): e13362. https://doi.org/10.1111/jph.13362.
11. Sandoval-Denis M., Guarnaccia V., Polizzi G. Symptomatic Citrus trees reveal a new pathogenic lineage in Fusarium and two new Neocosmospora species. Persoonia. 2018; 40: 1–25. doi: 10.3767/persoonia.2018.40.01.
12. Kubicek C.P., Harman G.E. Trichoderma and Gliocladium Volume 1, Basic biology, Taxonomy and Genetics. Taylor and Francis e-Library. 2002; 278. https://doi.org/10.1201/9781482295320.
13. Tamura K., Stecher G., Peterson D., Filipski A., Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology and Evolution. 2013; 30: 2725–2729. doi: 10.1093/molbev/mst197.
14. Al-Tememe Z.A.M., Al-Sweedi T.M.M., Abdullah K.M., Jawid Ch.K., Hamzah R.M. Diagnosis of causes of cankers on apricot trees Prunus armeniaca L. using PCR technique in some of the AlQantara, Al-Baydaa orchards in Karbala- Iraq. IOP Conference Series: Earth and Environmental Science. 2019; 388(1): 012008. DOI:10.1088/1755-1315/388/1/012008.
15. Villani A., Moretti A., De Saeger S., Han Z., Di Mavungu J.D., Soares C.M.G., Proctor R., et al. A polyphasic approach for characterization of a collection of cereal isolates of the Fusarium incarnatum-equiseti species complex. International Journal of Food Microbiology. 2016; 234: 24–35. doi: 10.1016/j.ijfoodmicro.2016.06.023.
16. Boland J.M., Woodward D.L. Impacts of the invasive shot hole borer (Euwallacea kuroshio) are linked to sewage pollution in southern California: the Enriched Tree Hypothesis. 2019;7:e6812. doi: 10.7717/peerj.6812. eCollection 2019.